원예과학기술지 16(3):381-384. 1998

Distribution of Floral Anthocyanins in the Species of Genus *Hibiscus*

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ABSTRACT Intersectional differences in anthocyanin composition were observed in a survey of floral anthocyanins of 27 species in genus *Hibiscus* (Malvaceae). The most common suits of floral anthocyanins were 3-xylosylglucosides and 3-glucosides of delphinidin and cyanidin in species of section Trichospermum, Fucaria, Trionum, Abelmoschus, and Ketmia. Cyanidin 3-sophoroside was the predominant anthocyanin in species of section Lilibiscus. Six common anthocyanidin 3-glucosides and corresponding malonates were detected only in the species of section Bombycella. These intersectional variation coincided generally with proposed sectional boundaries based on morphological characteristics. Anthocyanin composition was more complicated in self-incompatible species than in self-compatible species. The systematic significance of diverse anthocyanin profile was discussed in the aspect of pollination ecology.

Additional key words: acylated, anthocyanin survey, anthocyanins, intersectional differences, Malvaceae, pollination ecology, self-compatibility

Introduction

Genus *Hibiscus* (Malvaceae) is a heterogeneous array of at least 250 species, most of which are tropical or subtropical (Wise and Menzel, 1971; Bates, 1965). This genus has been divided into twelve sections (Hochreutiner, 1900) or thirteen series (Bates, 1965) based on morphological characteristics. The East Asian group, section Bombycella, includes the cold hardy Rose of Sharon, *Hibiscus syriacus*. Tachibana (1984) studied genetic relationships within this group and placed *H. mutabilis* in the section. This assignment differs from Hochreutiner (1900), who placed *H. mutabilis* in the section Trionum. Thus, some disagreement exists as to the assignment of a species to a particular section.

Interspecific crossability studies have shown that *H. syriacus* was compatible with species of section Bombycella (Tachibana, 1984). In this section, *H. sinosyriacus*, *H. paramutabilis,* and *H. syriacus* were included. An understanding of anthocyanin composition is important not only as a breeding potential in delimiting the potential for transfer of desirable genes from related species but also as a chemosystematic tool. However, anthocyanin composition of these species were not fully understood except for *H. syriacus* (Kim et al., 1989).

In a survey of Malesian *Hibiscus*, Lowry (1976) reported the widespread occurrence of cyanidin 3-sambubioside, cyanidin 3-glucoside and some delphinidin derivatives. Others (Kuwada, 1960; Shibata and Furukawa, 1969; Lowry, 1971; Du and Francis, 1973; Ishikura, 1973; Pomilio and Sproviero, 1973) also reported delphinidin and cyanidin derivatives in this genus fragmentarily. However, Egolf and Santamour (1975) reported 3-glucosides of delphinidin, cyanidin, petunidin, and malvidin in *H. syriacus*. During the cultivar survey in *H. syriacus* (Kim and Fujieda 1991), authors found that *H. syriacus* contained six common anthocyanidin 3-glucosides and their corresponding malonates(Kim et al., 1989).

These facts are of possible interest for two reasons. Firstly, *H. syriacus* has different anthocyanidin composition compared with other *Hibiscus* species, and earlier studies suggested possible intersectional variation in anthocyanin profile. Secondly, neither aromatic nor aliphatic acylation was reported in other species of this genus except for *H. syriacus* (Kim et al., 1989).

The objectives of this study were to identify and compare the relative percent of anthocyanins in cultivated species of *Hibiscus*, and to compare these results with those obtained from current classifications and pollination ecological studies.

Materials and Methods

Plant materials

Cuttings or seeds of several species were obtained from Dr. Tachibana of the Botanical Garden of Osaka City University, Japan. Tropical species were grown in open field during summer and in a greenhouse during winter. Fresh petals were collected during their optimum bloom season. Petals were separated into basal part, 'eye' and upper main part, 'body'. Anthocyanins were analyzed in both parts or only in the 'eye' because yellow flowers of many species in this genus have anthocyanins only in the eye region. Twenty flowers of each species were self-pollinated to inspect self- compatibiliy. Each flower was enveloped a day before anthesis, and pollination was conducted at early morning. Self-compatibility was judged based on seed setting after pollination.

Anthocyanin identification

Anthocyanin identification was carried out by standard procedure as described previously (Kim et al., 1989). Petals were extracted with MeOH-HCOOH (95:5) at 5℃ for 24. Small volume of extract was analyzed by HPLC before and after saponification (Kim et al., 1989) to check the presence of acylated anthocyanins. In

Table 1. Rf values and retention times (Rt) of representative anthocyanins of genus *Hibiscus.*

Pigments	Rf(× 100) in[z]				Rt (min)	Authentic markers
	BAW	BuHCl	1% HCl	HOAc-HCl		
Dp 3-GX	25	17	26	54	9.1	Dp 3-xylosylglucoside from *Hibiscus sabdarifa* (Du and Francis, 1973)
Dp 3-G	26	11	4	17	9.6	Dp 3-glucoside from *Hibiscus syriacus* (Kim et al., 1989)
Cy 3-Sop	47	24	28	55	9.8	Cy 3-sophoroside from *Hibiscus rosa-sinensis* (Lowry, 1976)
Cy 3-GX	49	27	28	55	11.5	Cy 3-xylosylglucoside from *Hibiscus mutabilis* (Lowry, 1976)
Cy 3-G	38	24	7	26	12.1	Cy 3-glucoside from *Hibiscus syriacus* (Kim et al., 1989)

[z]Rf values were measured on microcrystalline cellulose TLC in the solvent of BAW [n-BuOH-HOAc-H_2O(4:1:5)], BuHCl[n-BuOH-2 M HCl(1:1)], 1% HCl[conc HCl-H_2O (3:97)], and HOAc-HCl[H_2O-HOAc-HCl(82:15:3)].

※ This paper was in part supported by overseas research grant of Kangwon National University, 1996.

Table 2. Sectional distribution of anthocyanins and some ecological traits in genus *Hibiscus*.

Species	Petal part	Color	Distribution of anthocyanins(%)[z]						Habit	Self compatibility	Acylated anthocyanin
			Dp 3-GX	Dp 3-G	Cy 3-S	Cy 3-GX	Cy 3-G	Others			
Section Azanza											
H. tiliaceus L.	body	yellow	-	-	-	-	-	-	tree	com	-
	eye	dark	-	-	-	60	40	-			-
H. hamabo Sieb. & Zucc.	body	yellow	-	-	-	-	-	-	tree	com	-
	eye	dark	-	-	-	60	40	-			-
Section Bombycella											
H. paramutabilis Bailey	body	white	-	-	-	-	-	-	shrub	incom	-
	eye	red	-	-	-	-	90	10[y]			minor[v]
H. sinosyriacus Bailey	body	mauve	-	10	-	-	2	88[x]	shrub	incom	main[u]
	eye	red	-	-	-	-	80	20			minor
H. syriacus L.	body	purple	-	10	-	-	5	85[w]	shrub	incom	main
	eye	red	-	10	-	-	60	30			minor
Section Trichospermum											
H. callyphyllus Cav.	body	yellow	-	-	-	-	-	-	berb	com	-
	eye	dark	50	10	-	30	10	-			-
H. rokii Deg. & Deg.	body	yellow	-	-	-	-	-	-	herb	com	-
	eye	dark	40	20	-	25	15	-			-
H. calycinus Will.	body	yellow	-	-	-	-	-	-	berb	com	-
	eye	dark	15	5	-	70	10	-			-
Section Fucaria											
H. costatus Rich.	body	purple	50	10	-	30	10	-	herb	com	-
	eye	red	t	t	-	60	40	-			-
H. cameronii K. W.	body	pink	t	t	-	60	40	-	herb	com	-
	eye	red	t	t	-	60	40	-			-
H. brackenridgei A.	body	yellow	-	-	-	-	-	-	berb	com	-
	eye	dark	70	25	-	3	2	-			-
H. heterophyllus Vent.	body	brown	t	t	-	90	10	-	herb	com	-
	eye	dark	t	t	-	85	15	-			-
H. acetocella Wel.	body	crimson	80	15	-	5	t	-	herb	com	-
	eye	dark	85	5	-	10	t	-			-
H. radiatus Cav.	body	crimson	30	60	-	3	7	-	herb	com	-
	eye	dark	70	5	-	25	t	-			-
Section Lilibiscus											
H. rosa-sinensis L.	body	red	t	-	90	2	8	minor[t]	shrub	incom	-
	eye	red	t	-	85	2	13	minor			-
H. schizopetalus Hook	body	red	t	-	95	t	5	minor	shrub	incom	-
	eye	red	3	-	95	t	2	minor			-
H. liliflorus Cav.	body	red	t	-	35	t	65	minor	shrub	com	-
	eye	red	t	-	75	t	25	minor			-
H. storkii Seaman	body	red	t	-	90	t	10	minor	shrub	com	-
	eye	red	t	-	95	t	5	minor			-
H. archerii Watson	body	red	t	-	98	2	t	-	shrub	incom	-
	eye	red	t	-	95	t	5	-			-
H. kokio Wara	body	red	t	-	95	t	5	minor	shrub	com	-
	eye	red	t	-	98	2	t	minor			-
Section Trionum											
H. trionum L.	body	white	-	-	-	-	-	-	herb	com	-
	eye	dark	40	30	-	10	20	-			-
H. mutabilis L.	body	pink	5	t	-	75	20	-	shrub	com	-
	eye	red	5	3	-	80	12	-			-
H. cisplantinus St.-Hill	body	purple	10	78	-	2	10	-	herb	com	-
	eye	dark	50	30	-	10	10	-			-
Section Abelmoschus											
H. manihot L.	body	yellow	-	-	-	-	-	-	herb	com	-
	eye	dark	50	25	-	20	5	-			-
H. esculentus L.	body	yellow	-	-	-	-	-	-	berb	com	-
	eye	dark	50	20	-	20	10	-			-
H. moschatus Medic.	body	yellow	-	-	-	-	-	-	berb	com	-
	eye	dark	50	20	-	20	10	-			-
Section Ketmia											
H. ludwigii Ekl. & Zey.	body	yellow	-	-	-	-	-	-	berb	com.	-
	eye	dark	25	t	-	65	10	-			-

[z]Abbreviations - Dp:delphinidin; Cy:cyanidin; Pt:petunidin; Pg:pelargonidin; Pn:peonidin; Mv:malvidin; G:glucoside; GX:xylosylglucoside; S:sophoroside; com:compatible; incom:incompatible; t:trace

[y, x, w]See Figure 1.

[v, u]Mean malonyl anthocyanins in Fig. 1.

[t]Unknown minor anthocyanins in HPL chromatograms. Data were not presented.

preliminary TLC surveys, five representative anthocyanins were detected in the species of section Trichospermum, Fucaria, Trionum, Lilibiscus, Abelmoschus and Ketmia. These representative anthocyanins were confirmed by co-chromatography with authentic markers on microcrystalline cellulose plates using 4 different solvents (Table 1). Rf values of five representative anthocyanins were identical with those of authentic markers. Each TLC spot of the five anthocyanins was extracted with 5% MeOH-HCOOH and reconfirmed by HPLC with authentic markers. Relative percentage of the five representative anthocyanins were analyzed by HPLC on a Shimpak CLC-ODS reverse-phase column (0.45×15 cm) using 10% formic acid as a solvent system. Anthocyanins of *H. paramutabilis* and *H. sinosyriacus* were determined by co-chromatography on HPLC with authentic markers which were identified in H. syriacus before (Kim et al., 1989) using a stepwise gradient method. Further details were mentioned previously (Kim and Fujieda, 1991). The flow rate was 1 mL/min and the detector wavelength was 530 nm.

Results and Discussion

Five representative anthocyanins were

identified from 24 species except for section Bombycella (Table 2) by co-chromatography with authentic markers on TLC and HPLC (Table 1). Anthocyanins detected were in agreement with those reported by earlier investigators (Kuwada, 1960; Shibata and Furukawa, 1969; Lowry, 1971; Du and Francis, 1973; Ishikura, 1973; Pomilio and Sproviero, 1973; Rakhimkhanove et al., 1973). Discrepancies with earlier reports centered around the presence of additional minor pigments as summarized in Table 2.

The most common suit of floral anthocyanins among examined species can be seen in the sections of Trichospermum, Fucaria, Trionum, Abelmoschus and Ketmia (Table 2). Although no qualitative variation in anthocyanin profile existed in species of these sections, quantitative differences in anthocyanin arrays were evident. With the exception in few species, 3-xylosylglucosides (sambubiosides) of delphinidin and cyanidin were major pigments, and 3-glucosides of cyanidin and delphinidin were minor pigments in these sections.

Cyanidin 3-xylosyglucoside and 3-glucoside were detected in species of section Azanza. As previously discussed (Lowry, 1976), the lone presence of cyanidin derivatives and the vegetative character of tree (Table 2) in section Azanza could be interpreted as the primitive characteristics.

Cyanidin 3-sophoroside was the predominant anthocyanin in species of section Lilibiscus. Previous work (Lowry, 1976) reported only cyanidin 3-sophoroside in this section, however, cyanidin 3-glucoside and other minor pigments were detected in this study (Table 2).

The most interesting result was the finding of acylated anthocyanins in section Bombycella. The HPLC separation of anthocyanins of three species are presented in Fig. 1. Cyanidin 3-glucoside was the main pigment, and others were only traces in *H. paramutabilis*. This simplicity of anthocyanin composition may be explained by the flower color of *H. paramutabilis* which has only white with red eye flower. *H. syriacus* and *H. sinosyriacus* have very diverse anthocyanin composition including malonates compared with those found in other *Hibiscus* species (Table 2).

The visible color differences between the petal body and eye regions as shown in *H. sinosyriacus* (Fig. 1) seemed to be quantitative and also qualitative, especially in some cyanic flowers of section Bombycella and Fucaria (Table 2), resulting from the elevated levels of anthocyanins and different anthocyanin pathway in the eye (Dorn and Bloom, 1984). These pigmentation patterns were generally known as honey guide (Harborne, 1988).

Detailed studies of the pollination ecology were generally lacking in species of *Hibiscus*. However, floral biology in the species showed special mechanism which seemed to be related with the anthocyanin distribution. Field observation showed that many species in the genus *Hibiscus* have a specialized pollination mechanism (Buttrose et al., 1977). Almost all of the *Hibiscus* species have flowers with prominent styles which become reflexed so that the stigmas come into contact with dehisced anthers. This self-pollination was interrupted if stigmas were pollinated before or during bending. This phenomenon was interpreted as a mechanism giving first preference to outcrossing, but facilitating selfing if outcrossing failed (Buttrose et al., 1977).

An interesting observation we made in this experiment was the relationship between the anthocyanin composition and the self-incompatibility (Table 2). Self-incompatible species were observed only in sections Bombycella and Lilibiscus (Table 2). The sections containing self-incompatible species showed more complicated anthocyanin composition, viz., section Bombycella contained all of the six common anthocyanidin 3-glucosides with corresponding malonates and Lilibiscus contained many unknown pigments (Table 2), while the self-compatible species showed relatively simple anthocyanin composition, viz., sections Azanza, Trichospermum, Fucaria, Trionum, and Abelmoschus contained only cyanidin and delphinidin derivatives. Since the most common system to enforce outbreeding is incompatibility (Crowe, 1964), species of section Bombycella and Lilibiscus might have been received higher recombination and mutational pressure than others. Furthermore, acylation of anthocyanins represents an advanced feature in angiosperm (Harborne and Smith, 1978) and acylated anthocyanins were detected only in section Bombycella in this study. Thus, the occurrence of diverse aglyconic and acylated anthocyanins in section Bombycella or minor unknown anthocyanins in section Lilibiscus may be interpreted as an evolved character by the result of pollination mechanism.

On the other hand, the occurrence of pelargonidin derivatives in the species of section Bombycella requires reconsideration of breeding potential concerning flower colors. Although pelargonidin derivatives were invariably minor components

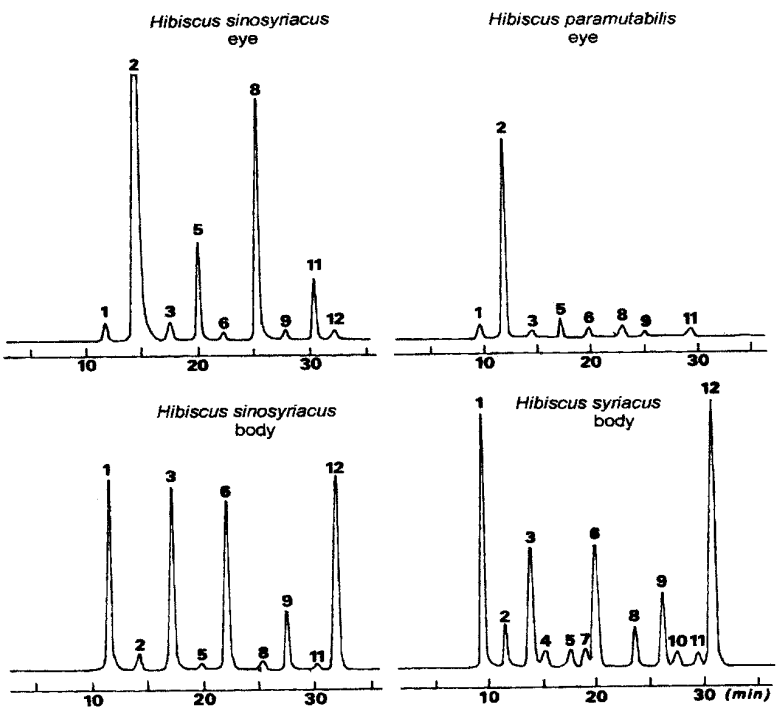

Fig. 1. HPLC resolution of petal anthocyanins of three *Hibiscus* species which are included in section Bombycella. Eye and body mean basal blotch and upper main part of petal, respectively. 1:Delphinidin 3-glucoside; 2:Cyanidin 3-glucoside; 3:Petunidin 3-glucoside; 4:Pelargonidin 3-glucoside; 5:Peonidin 3-glucoside; 6:Malvidin 3-glucoside; 7:Delphinidin 3-malonylglucoside; 8:Cyanidin 3-malonylglucoside; 9:Petunidin 3-malonylglucoside; 10:Pelargonin 3-malonylglucoside; 11:Peonidin 3-malonylglucoside; 12: Malvidin 3-malonylglucoside.

in cultivars of *H. syriacus*, the percentage of these pigments increased in cultivars of violet red and pink groups (Kim and Fujieda, 1991). Since pelargonidin derivatives have been recognized as important pigments in horticultural plants for their spectral properties of orange-red and scarlet, the presence of pelargonidin in section Bombycella which showed interspecific crossability within section (Tachibana, 1980) gave us an incentive for breeding new colors in this section.

Acknowledgement

We are grateful to Dr. Y. Tachibana (Botabical Garden of Osaka City University) for providing several plants and his helpful advice.

*Hibiscus*속 종내의 anthocyanin 분포

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초 록

*Hibiscus*속 (아욱과)의 27개 종에 대한 안토시아닌분포를 조사한 결과 절(section)간 안토시아닌조성의 차이를 관찰하였다. 가장 대표적으로 발견되는 안토시아닌은 delphinidin과 cyanidin의 3-xylosyglucoside와 3-glucoside로, Trichospermum, Fucaria, Trionum, Abelmoschus, Ketmia절에 속하는 종들에서 나타났다. Cyanidin 3-sophoroside는 Lilibiscus절의 종들에서 발견되는 대표적인 안토시아닌이었다. Bombycella절에서는 6가지의 common anthocyanidin의 3-glucoside와 3-malonylglucoside가 검출되었다. 이러한 안토시아닌의 절간 분포 차이는 기존의 형태적 자료에 기초를 둔 section의 구분 범주와 잘 일치하였다. 또한 자가불화합성을 나타내는 section에서 다양한 안토시아닌의 변이를 나타내었다. 이러한 안토시아닌의 변이결과들을 수분생태학과 관련지어 논의하였다.

추가주요어 : 아실화 안토시아닌, 안토시아닌 분포, 자가불화합성, 절간 차이, 아욱과, 수분생태

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