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## Anthocyanin Pigmentation Patternings in Petals of *Hibiscus syriacus*

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Petals of *Hibiscus syriacus* are characterized by the various colored main part 'body' and an intense red to mauve 'eye' in the base of each petal. The concentrations of each anthocyanin varied greatly in the body part by the colors of the petals, while the predominant pigments were the cyanidin 3-glucoside and 3-malonylglucoside in the eye regions of all examined cultivars. These pigmentation patternings were further investigated in several flowering stages; the anthocyanin pathways were distinctly different in the two regions of the same petals. Aliphatic acylation was the last step in the pathways of anthocyanin synthesis.

### INTRODUCTION

Pigment patternings in flowers have been known in many plants and were recognized as honey guides or guide markers (Harborne, 1982). They take the form of colored dots or lines on corolla tubes. The visible color differences between the petal body and the petal blotch regions have been thought to be quantitative in nature (Dorn and Bloom, 1984), but in the case of poppy it has been reported that the pigment in the blotch is different from that present in the rest of the petal (Acheson et al., 1962).

Flower petals of almost all sections of *Hibiscus* are characterized by a yellow, ivory or cyanic main part 'body', and an intense red to mauve blotch 'eye' in the base of each petal. The differences of anthocyanin pigmentation between the body and eye were readily observable in *Hibiscus* (Lowry, 1976), but their pigmental nature has not been fully discussed.

This study was performed to characterize the pigment patternings in flowers of some selected cultivars of *Hibiscus syriacus*. Experiments also comprise detailed qualitative and quantitative analyses of the progress of anthocyanin formation in body and eye regions during flower development emphasizing the malonated anthocyanin (Kim et al., 1989) formation.

### MATERIALS AND METHODS

Three 1.959 cm<sup>2</sup> disks of bodies and eyes of five *H. syriacus* cultivars were punched out from the uniform and fully developed petals at their peaks of color at about 10 : 00 a. m. before any fading or change in color with age. Anthocyanins were extracted with 2 ml of 5% MeOH-HCOOH for 2 days at 5°C. The extract was passed through a 0.45 µm Millipore filter and analyzed by HPLC as described previously (Kim et al., 1989). Anthocyanin content in µg/cm<sup>2</sup> of petal area was calculated by integration of the area

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of each peak with a Shimadzu Chromatopac-EIA integrator.

To investigate the anthocyanin accumulation during flower development in bodies and eyes, three cultivars were chosen; 'Natsuzora' is distinct with blue body and deep-red eye, 'Elegantissimus' has pink body and deep-red eye, and 'Suminokurayae' represents deep-red eye and the most redish body in the cultivars. All clones of each cultivar were derived from stem cuttings of each single plant. Flower buds were classed in stages corresponding to 1 or 2 day intervals under summer growing conditions. The length of individual flower bud was measured from the base of the calyx to the edge of the corolla. Since flowers which had opened were flattened, the length was measured to the edge of the longest corolla lobe. Fresh weight of the excised petals was measured, and then they were separated into two parts, the eyes and the bodies, with a razor blade. Anthocyanins were analyzed identically with the method described above. Three samples were analyzed in each stage of flower development.

## RESULTS AND DISCUSSION

Table 1 shows the anthocyanin distribution in the bodies and eyes of the petals. Five cultivars are the representatives in colors as described previously (Kim, 1988). 'Kobata' and 'Heikeyama' analyzed as controls. The former is a white flower having red eye and the latter represents purple color having similar anthocyanin composition to 'Natsuzora'. The visible color differences between the two petal regions were quantitative and simultaneously qualitative in nature, resulting from elevated levels of anthocyanins in the area of the eye, and from the differences of accumulating anthocyanins in the two petal regions. Cyanidin derivatives are the main pigments in the eyes of all examined cultivars, whereas the petal bodies have different anthocyanin compositions by the cultivars. Such partial differences of pigmentation in petals were further studied in relation to the bud development in three cultivars.

It was substantiated by growth measurements that bud length provides a convenient and reliable morphological index of flower development. The representative nature of bud development is shown in Fig. 1, in which the logarithm of bud length is plotted against times and exponential features. The data were divided into two groups, one including measurements made from 15 to 4 days before anthesis, and another from -3 days to anthesis. Two regression lines were fitted to each set of data ( $r=0.9138$ ,  $p<0.01$ ;  $r=0.9899$ ,  $p<0.01$ ). The high linear relationship between the logarithm of bud lengths and the stages increases the usefulness of log L as a development index, since any bud with different length can easily be converted into an absolute time scale of bud development.

The other useful data are the relationship between the bud length and the fresh weight of the petals. Fig. 2 shows a double logarithmic plots of fresh weight (mg) and length (mm) which seems best to be described by a pair of straight lines. Straight lines were fitted to each set of data ( $r=0.9607$ ,  $p<0.01$ ;  $r=0.9767$ ,  $p<0.01$ ). Similar relationships were obtained in the other two cultivars.

Young buds were apparently absent of anthocyanins. A trace of red color was firstly shown at the bud stage -7 days (Fig. 1), but anthocyanins were firstly detectable by HPLC at the stage -9 days. At the less colored bud stage (-9 days), the anthocyanin content in the two petal regions were very low (Fig. 3), and then they

Table 1. Differences in anthocyanin composition between the body and eye parts of petals.

Cultivar	Petal part	% of individual anthocyanin"												Total anthocyanin ± S.D. ( $\mu\text{g}/\text{cm}$ )
		Dp3G	Cy3G	Pt3G	Pg3G	Pn3G	Mv3G	Dp3MG	Cy3MG	Pt3MG	Pg3MG	Pn3MG	Mv3MG	
Elegantissimus (pink)	body	3	21	t	39	2	t	t	6	t	23	2	4	0.54f0.18
	eye	3	47	2	1	2	2	2	36	2	t	1	2	31.56f1.32
Suminokurayae (violet red)	body	10	20	9	t	1	10	3	9	9	1	3	25	5.77±0.87
	eye	5	45	4	2	3	1	5	27	2	1	3	2	20.71f1.31
Heikeyama (purple)	body	9	2	11	t	1	15	3	t	6	t	1	52	2.25±0.24
	eye	4	49	2	1	2	2	2	35	1	t	1	1	16.93±0.98
Natsuzora (blue)	body	3	1	3	t	t	20	t	t	1	t	3	69	2.05±0.43
	eye	2	48	2	1	3	1	t	28	1	t	10	4	31.63±1.33
Kobata (white)	body	0	0	0	0	0	0	0	0	0	0	0	0	0
	eye	3	56	2	1	2	1	2	29	1	t	2	1	10.96f0.94

• Color of the body part.

••t; trace, Dp; delphinidin, Cy; cyanidin, Pt; petunidin, Pg; pelargonidin, Pn; peonidin. Mv; malvidin, 3G; 3-glucoside, 3MG; 3-malonylglucoside.

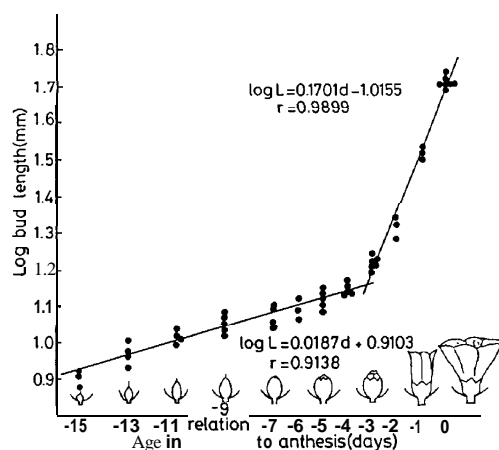


Fig. 1. Increase in log bud length with bud development in 'Elegantissimus'.

rapidly increased to the maximum values at 2 days before anthesis. The maximum values were different depending upon the regions in petals and cultivars, and turned to decrease thereafter in the bodies. However, decrease in anthocyanin formation was not distinctly observed in the eyes of all the cultivars examined here.

The decreases of the relative pigment content were the reflections of petal growth. The length and fresh weight of the petals were increased rapidly from the stage -3 days, but the rate of anthocyanin formation did not reach to that of petal growth. The decreasing rates of the relative anthocyanin contents after the maximum point in the

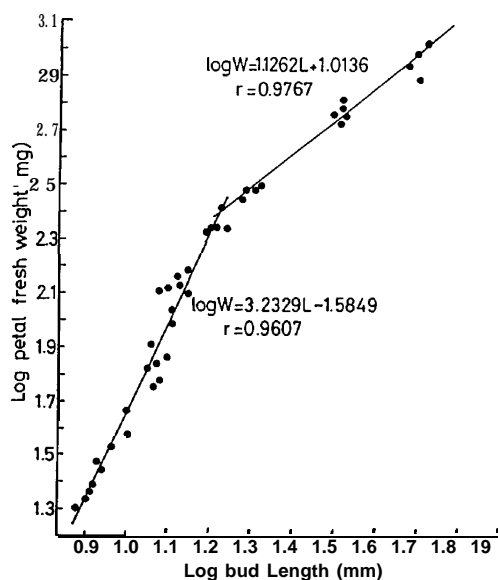


Fig. 2. Log petal fresh weight versus log bud length in 'Elegatissimus'.

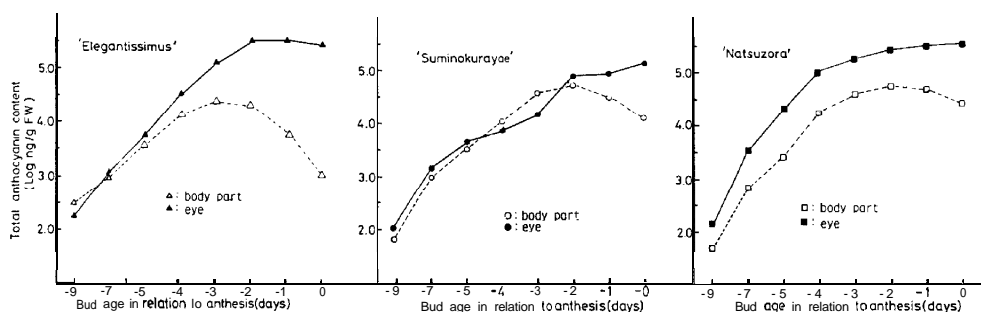


Fig. 3. Changes in total anthocyanins with bud development in two different petal parts.

body parts were different by cultivars. The rate was bigger in 'Elegatissimus' than in other two cultivars, and the anthocyanin content of this cultivar at the maximum point was low compared with that of the others. It may be said from the results showing the relationship between anthocyanin formation and petal growth that the body color of pinkish cultivars becomes pink.

Pelargonidin, cyanidin and delphinidin are generally considered as primitive pigments, and peonidin and malvidin as stable pigments (Harborne, 1967). Petunidin is considered as it is in the middle of the chain of transformations from cyanidin to malvidin (Peket, 1966 ; Roggero *et al.*, 1986). In the body parts the main accumulating anthocyanins were pelargonidin 3-glucoside and 3-malonylglucoside in 'Elegatissimus', malvidin 3-malonylglucoside and cyanidin 3-glucoside in 'Suminokurayae', and 3-malonylglucoside and 3-glucoside of malvidin in 'Natsuzora', while cyanidin deriva-

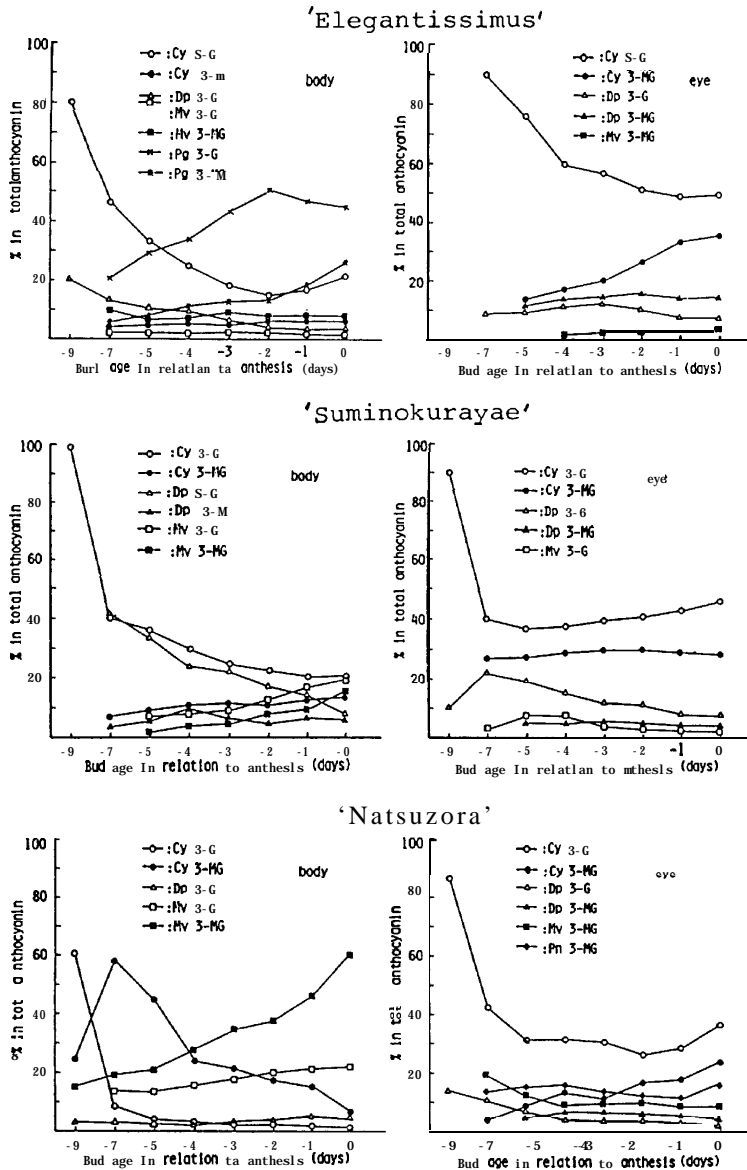


Fig. 4. Changes in main anthocyanins with bud development in different petal parts.

tives were the main accumulating anthocyanins in the eye parts of these cultivars (Fig. 4).

The differences in pigment accumulation may be due to differential gene expression in these two regions as previously pointed (Dorn and Bloom, 1984). Although the data serve to establish the magnitude and timing of production of anthocyanins which are relevant to the further consideration of the origin of visible differences in the two

regions, the developmental data did not allow precise definition of the biosynthetic pathways. In the body parts the pathways seem to be more complicated than those in the eye parts. They may be further complicated by the polyploid and aneuploid nature of *H. syriacus* ( $2n = 4x = 80, 82, 84, 86, 90$ , etc., unpublished data of the authors).

Cyanidin and delphinidin 3-glucosides were the firstly appearing anthocyanins at the stages of -9 or -7 days in all cultivars, and then they sharply decreased (Fig. 4). A sharp decrease of them may be an indication of a convenient transformation into stable pigments. Other anthocyanins (see Table 1) seem to act as intermediates in the anthocyanin pathways. The pigment pathways in 'Elegantissimus' seem to be blocked in several places by unknown genes. Although the acylated anthocyanins appear from relatively early bud stages, it is clear from the serial changes of each anthocyanin (Fig. 4) especially in 'Natsuzora', that acylation is the last step in the pathways of anthocyanin syntheses.

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