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## Changes of Petal Colors during Senescence in *Hibiscus syriacus*

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The blueing effect of purplish petals during senescence in *Hibiscus syriacus* L. was investigated. Absorption spectra of old petals of purple flowers always produced small shoulders at 640 nm. Anthocyanin content was decreased in old petals, while flavone content was not changed. Resulting increased flavone/anthocyanin ratio enhanced the copigmentation effect. This effect was further accelerated by the increase of cell sap pH.

### INTRODUCTION

Although color fading is a common phenomenon in many flowers during aging, the pigment level stays stable in some flowers (Pecket, 1966 ; Stead and Moore, 1977), declines drastically in others (Stickland, 1972), whereas in some flowers a drastic synthesis of anthocyanins is evident (Arditti and Knawft, 1969).

The red flowers of *Hibiscus syriacus* show color fading and the so-called blue flowers show no color changes during senescence. The purple flowers of *H. syriacus*, however, exhibit a well marked color change turning to purplish blue as they age.

With a view to understanding the pigmental physiology of the sequence in color change, a detail examination of the flowers of *H. syriacus* was conducted. Another purpose of this experiment is to clarify whether some qualitative changes in anthocyanin compositions occur or not during the process of flower senescence, especially regarding the aliphatic acylated anthocyanins which were newly identified (Kim et al., 1989).

### MATERIALS AND METHODS

All the experiments were conducted between July 15 and September 30, when the flowering periods were at the peak in this area. Flowers of 9 cultivars were collected at 12-hour intervals after anthesis (0 hr ; 6 : 00 a. m.) from the plants grown outdoors in the experimental field of Kyushu University, and immediately transported to the laboratory. Floral characters of each cultivar examined were previously described (Kim, 1988).

Absorption spectra of fresh petals were measured with Shimadzu MPS-500 multipurpose spectrophotometer. Three petals with uniform size and color were selected from different 3 flowers of each cultivar. Disks of 1.0 x 3 cm<sup>2</sup> were punched out from the same position of upper body part of each petal. Fresh weight of them was

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measured and then they were extracted with 5% MeOH-HCOOH for anthocyanin analyses at 5°C. Anthocyanins were resolved by an HPLC system as described previously (Kim et al., 1989). Determination of the pH of expressed cell sap was made with a compact pH meter (Horiba C-1). Estimation of the flavone/anthocyanin ratio was conducted with the method of Ishikura (1978). The optical spectra of the 0.1% MeOH-HCl extracts were measured with a Beckman DU 7 HS spectrophotometer.

## RESULTS AND DISCUSSION

Flowers of *H. syriacus* opened in early morning (5 : 00-6 : 00 a. m.) on the day 0 and remained open until late night (12 : 00 p. m.) of the same day. At midnight the petals began to roll inward and lose their turgor. Flower senescence was associated with a large decrease of fresh weight of the petals (Table 1).

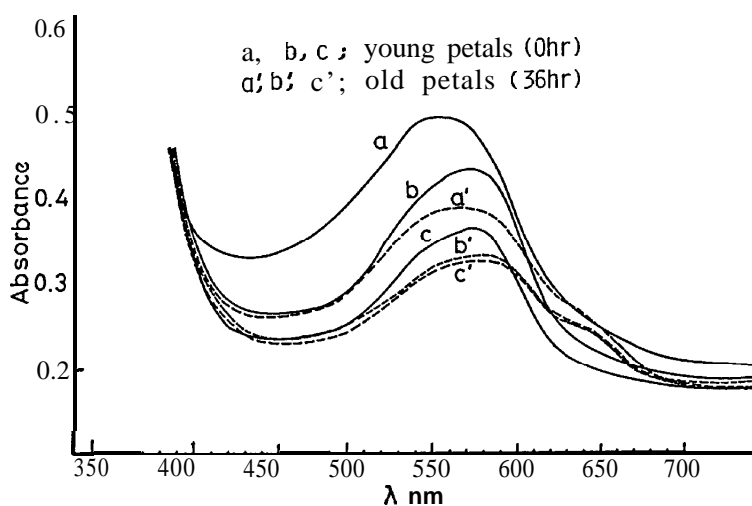
Figure 1 shows the differences of absorption spectra between young and old petals of purplish cultivars. Young petals have one absorption peak, while old petals produce one main peak and an additional small shoulder at 640 nm. In the previous work of

**Table 1.** Changes of the petal fresh weight with aging.

Cultivar	Fresh weight (mg/cm <sup>2</sup> ) ± standard error			
	0 hr	12 hr	24 hr	48 hr
Hikarihanagasa	15.2f0.04	13.5±0.03	12.5f0.03	10.1±0.02
Ardens	10.9±0.04	11.9±0.04	9.7±0.02	8.3f0.03
Akagionmamori	14.3f0.02	12.9f0.03	12.1±0.02	11.5t0.04
Coelestis	12.9±0.02	12.5f0.02	12.0t0.01	11.4±0.01

\*Values are the average of five measurements.

\*0 hr means the time of anthesis: about 6 a.m.



**Fig. 1.** Light absorption spectra of the young and old petals. a, a' ; 'Kijibato', b, b' ; 'Ardens', c, c' ; 'Coelestis'

flower color in *H. syriacus* (Kim, 1988), cultivars were classified into four groups according to the absorbance and  $\lambda$  max of the fresh petals. Purplish cultivars (group C) have an absorption peak at their fully developed stage before senescence while so-called blue cultivar group which was named group D has one main absorption peak and an additional shoulder at 640 nm of the absorption curves even at the peak of the color. Therefore, the appearance of the shoulder on the absorption curves of old petals of purple cultivars (Fig. 1) may be closely related to bluing of the flowers as were observed in blue cultivars (Kim, 1988), and the mechanism of blue color production in the old petals of purple cultivars may be similar to that of the blue cultivars of group D (Kim, 1988).

Changes of total anthocyanin content with aging in four selected cultivars are shown in Fig. 2. Anthocyanin content increased until afternoon of the day 0 in all cultivars examined, and then declined rapidly. The declining rates of anthocyanin content varied depending upon cultivars.

Qualitatively, the anthocyanin profiles in different stages showed relatively constant constitutions as shown in Fig. 3. Absorption maxima of the 0.1% MeOH-HCl extracts at 300-360 nm are chiefly due to the presence of concomitant phenolic compounds, which have a bathochromic effect on intact cell spectra (Pecket, 1966; Harborne, 1967 ; Ishikura, 1978). The optical density ratio of E uv max. to E vis max. of the 0.1% MeOH-HCl extracts was calculated to estimate the approximate flavone/anthocyanin ratio (Ishikura, 1978).

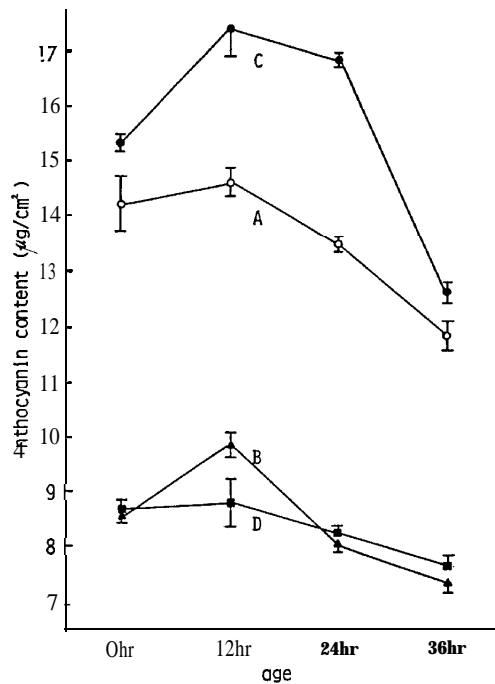
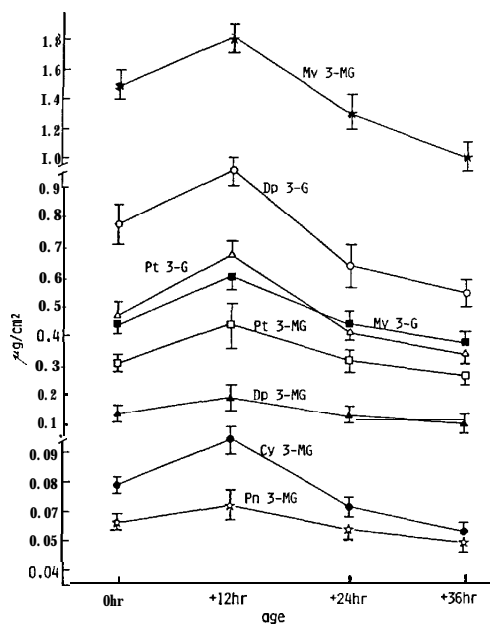
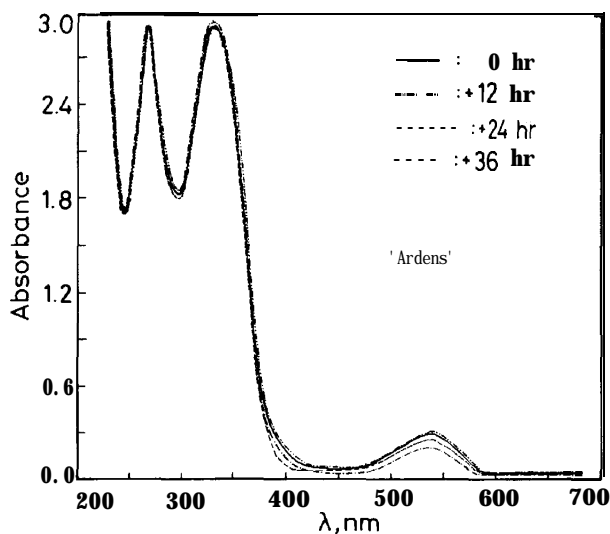


Fig. 2. Changes of anthocyanin content during flower senescence. A ; Hikarihanagasa, B. Ardens, C ; Akigionmamori, D ; Coelestis, Bars ; standard error



**Fig. 3.** Change of individual anthocyanins during flower senescence in 'Ardens'. Bars ; standard error, Cv : cyanidin, Dp : delphinidin, Pt ; petunidin, Pn ; peonidin, Mv ; malvidin, 3-G ; 3-glucoside, 3-MG ; 3-malonylglucoside



**Fig. 4.** Changes in absorption curves of the 0.1% MeOH-HCl extracts from the petals of different ages.

Figure 4 shows the absorption curves of different stages in 'Ardens'. Optical density of UV max. (at 330 nm) shows constancy, while that of Vis max. (at 538 nm)

shows small but distinct differences. Since the relative content of anthocyanins to flavone is very small, the minute decrease in anthocyanin content leads to the result of an increase in the flavone/anthocyanin ratio.

The changes of E uv max./E vis max. ratios in several cultivars are presented in Table 2 which shows distinct increase of the ratios in old petals. ‘Tottorihanagasa’ and ‘Shiguruma’ which show no color shift during senescence were analyzed as control. The former is a representative cultivar of redish and the latter is one of so-called ‘blue’ colors (Kim, 1988). Although the E uv max./E vis max. ratio increased in older petals of ‘Shiguruma’, the bathochromic shift was not increased. This fact may indicate the limit of bathochromic shifts in *H. syriacus*. Other purplish cultivars showed color shifts in all aged petals and the E uv max./E vis max. ratios increased significantly. Thus, the results (Table 2) confirm that the older petals differ from the younger petals in having a higher quantity of flavones relatively to anthocyanins. The differences are

Table 2. Changes of the optical density ratio of E uv max. to E vis max. of the 0.1% MeOH-HCl extract during senescence.

Cultivar	Petal color	E uv max./E vis max.± S. E.* at flower age of				in 0.1% MeOH-HCl	
		0hr	+12hr	+24hr	+36hr	UV max.	Vis max.
Tottorihanagasa	violet red	2.05 ±0.04	2.05 f0.11	2.10 f0.02	2.22 ±0.02	323	533
Ardens	purple	10.16 f0.1	10.49 f0.21	11.47 ±0.15	13.37 zko.13	332	539
Coelestis	purple	10.56 ±0.29	10.58 ±0.14	10.88 ±0.14	11.88 k0.32	333	539
Kijibato	purple	6.99 f0.20	7.27 f0.35	8.39 ±0.18	8.36 f0.19	333	539
Iriomote-1	purple	17.23 ±0.19	18.21 ±0.26	21.56 f0.42	23.58 f0.53	333	538
Shiguruma	violet blue	8.43 ±0.37	8.60 ±0.57	9.33 ±0.09	9.35 ±0.06	333	538

\*Standard error. Each value was obtained as the mean of five replications.

Table 3. pH values of the expressed sap from the petals of different ages.

Cultivar	pH± standard error	
	0 hr	+36 hr
Tottorihanagasa	5.07±0.02	5.32f0.05
Ardens	5.63f0.08	5.81f0.03
Coelestis	5.58±0.03	5.85±0.03
Kijibato	5.46f0.08	5.95f0.04
Shiguruma	5.99f0.02	6.09±0.03

not large but are likely to be important (Pecket, 1966).

The most important factor determining the color change in aging petals have been suggested to be a change in the pH of the vacuole (Stewart et al., 1975). An increase in pH has been demonstrated in roses (Asen et al., 1971; Borochoy et al., 1976), *Lathyrus* (Pecket, 1966), geranium and other flowers (Stewart et al., 1975). The increase of pH was attributed to the breakdown of proteins and release of free ammonia (Weinstein, 1951; Paulin, 1971). In some other flowers an opposite trend was found. The blue, violet or purple petals of cornflowers (Asen, 1967), morning glory (Asen et al., 1977) and fuchsia (Stewart et al., 1975; Yazaki, 1976) became more red as they age. A concurrent decrease in pH has been demonstrated in these cases, which was attributed to an increase in the content of organic acids, such as aspartic, malic and tartaric acids (Yazaki, 1976). The pH values of expressed cell sap in young and old petals are presented in Table 3. Small but distinct differences were observed in all examined cultivars and the pH increased in old petals.

In *H. syriacus* (Kim, 1988) as well as in most flowers, however, the decisive factor determining the intensity of the color and its bluing is the copigmentation with other flavonoids and their related compounds. This copigmentation effect may explain the infinite variations in the color of flowers which exists in the pH range of 4 to 6, and it accelerated by the increase of pH (Brouillard, 1983). This pH range is most prevalent in petals, where anthocyanins per se are virtually colorless. The pH range in petals of *H. syriacus* during senescence was relatively small and the values of it in young petals were high (Table 3). Therefore, the most important factor affecting the bluing of the purplish petals during senescence seems to be the copigmentation effect, resulting from the increased flavone/anthocyanin ratio. The increases of pH in old petals are likely to be an acceleration of the copigmentation effect.

#### REFERENCES

- Arditti, J. and R. L. Knauft 1969 The effect of auxin, actinomycin D, ethionine, and puromycin on post-pollination behavior in *Cymbidium* (Orchidaceae) flowers. *Amer. J. Bot.*, 65 : 620-629
- Asen, S. 1967 Anthocyanin and other flavonoids in red cornflower. *Proc. Amer. Soc. Hort. Sci.*, 91 : 653-659
- Asen, S., K. H. Norris and R. N. Stewart 1971 Effect of pH and concentration of the anthocyanin-flavonol co-pigment complex on the color of 'Better time' roses. *J. Amer. Soc. Hort. Sci.*, 96 : 770-773
- Asen, S., R. N. Stewart and K. H. Norris 1977 Anthocyanin and pH involved in the color of 'Heavenly Blue' morning glory. *Phytochemistry*, 16 : 1118-1119
- Borochoy, A., S. Mayak and A. H. Halevy 1976 Combined effects of acid and sucrose on growth and senescence of rose flowers. *Physiol. Plant.*, 36 : 221-224
- Brouillard, R. 1983 The *in vivo* expression of anthocyanin color in plants. *Phytochemistry*, 15 : 1311-1323
- Harborne, J. B. 1967 *Comparative Biochemistry of the Flavonoids*, Academic press, London
- Ishikura, N. 1978 Light absorption patterns of anthocyanin-containing cells. *Plant & Cell Physiol.*, 19 : 887-893
- Kim, J. H. 1988 Relation of flower colors to optical spectra, anthocyanins and co-pigmentation. pp. 56-84. Ph D thesis, Kyushu University, Fukuoka, Japan
- Kim, J. H., G. Nonaka, K. Fujieda and S. Uemoto 1989 Anthocyanidin malonylglucosides from flowers of *Hibiscus syriacus*. *Phytochemistry*, (in press)
- Paulin, A. 1971 Influence de la composition de la solution nutritive sur la teneur en divers acides

- amines libres et en ammoniac des petales de fleurs coupees. *Ann. Technol. Agric.*, **20**: 283-303
- Pecket, R. C. 1966 Color changes in flowers of *Lathyrus birstus* senescence. *Nature*, 211: 1215
- Stead, A. D. and K. G. Moore 1977 Flower development and senescence in *Digitalis purpurea* L., cv. Foxy. *Ann. Bot.*, 41: 283-292
- Stewart, R. N., K. H. Norris and S. Asen 1975 Microspectrophotometric measurements of pH and pH effect on color of petal epidermal cells. *Phytochemistry*, **14**: 937-942
- Stickland, R. G. 1972 Change in anthocyanin, carotenoid, chlorophyll, and protein in developing florets of the chrysanthemum. *Ann. Bot.*, **36**: 459-469
- Weinstein, L. H. 1951 Senescence of roses I. Chemical changes associated with senescence of cut 'Better times' roses. *Contrib. Boyce Thompson Inst.*, 19 : **33-48**
- Yazaki, Y. 1976 Co-pigmentation and the color change with age in petals of *Fuchsia hybrida*. *Bot. Mag.*, Tokyo 89 : 45-57