# *Studies on the Expression of Color Tone in Rose Petals III*

## *The Role of Anthocyanin in the Expression of BlackTone in the Petals of Velvety Dark Red Roses.* *

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The author suggested that the appearance[1] and disappearance[2] of black tone in the petals of velvety dark red roses were attributable presumablely to the shadow cast by the long nipple-like epidermal cells situated at some intervals on the red petal surface, without dependence on the quantity and quality of anthocyanin in these petal tissues. But this suggestion was made by some preliminary experiments, and therefore it was expected to estimate practically the pigments in these tissues in more detail.

Since WILLSTÄTTER et al.[3] found cyanin in red petals of *Rosa gallica*, many researchers have reported that cyanin was distributed widely in rose petals. For instance, WEINSTEIN[4] identified cyanin in cultivar BETTER TIMES, AHUJA et al.[5] in PINK CORONET and HAPPINESS and YASUDA[6] in ENA HARKNESS respectively.

While WEINSTEIN and AHUJA et al. emphasized that cyanin was the only anthocyanin detected in their materials, TAKAKUWA[7], investigating the pigments in the petals of thirty nine rose cultivars, stated that at least four kinds of anthocyanin were proved in rose petals, among which cyanin and chrysanthemin were contained in the red and dark red petals. ROBINSON et al.[8] identified pelargonidin-3:5-dimonoside as the anthocyanin in the orange-red polyantha rose cultivars GLORIA MUNDI and PRINCE of ORANGE.

As to the variation of anthocyanin content among rose cultivars and the change in quantity of the pigment during the flower development, there were reports published by AHUJA et al.[5][9] They indicated that there was a difference in anthocyanin contents between cultivars PINK CORONET and HAPPINESS, and that in both cases the pigment was decreased in quantity during flower development.

In the present paper the quantity and quality of anthocyanin in the petals of velvety dark red and red rose cultivars and the change of pigment content during the flower development were investigated.

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* A part of this work was delivered at the 30th Annual Meeting of the Botanical Society of Japan, 1965.

## Materials and Methods

*Materials*: The plants used in the present investigation were a velvety dark red and a red rose cultivars grown out-doors in our garden at MATSUMOTO, the names of which were mentioned in the following idems respectively. Their insect- and disease-free petals were collected at the flowering seasons in 1964-5.

*Quantitative tests of anthocyanin*: BONNE NUIT and HAPPINESS were used as a velvety dark red and red cultivars respectively for these tests.

Paperchromatographies of anthocyanin, anthocyanidin and sugar were carried out according to the technique recommended hy SHIBATA et al.[10] The samples, prepared by the procedure described below, were developed by the solvents tabulated in **Table 1**.

**Table 1.** Compositions of developing solvent.

| Abbreviated designation | Applied for | Composition of developing solvent and its ratio (v/v) |
|---|---|---|
| Bu. H | anthocyanin | n-butanol + conc. hydrochloric acid + water (7:2:5) |
| AA. H | anthocyanidin | acetic acid + conc. hydrochloric acid + water (5:1:5) |
| Bu. AA-1 | sugar | n-butanol + acetic acid + water (4:1:2) |
| Bu. AA-2 | sugar | n-butanol + acetic acid + water (4:1:5) |

10 g of fresh petals of each cultivar was immersed in 20 ml of cold methanol containing 1 per cent. hydrochloric acid over night and filtered. Masspaperchromatography of this extract was done using Bu. H, and a red color band obtained was cut off from the filter paper, and extracted by methanol containing 5 per cent. acetic acid. This red solution was applied to the anthocyanin sample and its Rf value was measured. At the same time the absorption spectrum of this solution was observed with BECKMAN spectrophotometer.

5 ml of this anthocyanin sample was boiled with equal volume of conc. hydrochloric acid for three minutes, cooled in a tap water, and after addition of distilled water the resultant aglycone was extracted with a small quantity of iso-amylalcohol. The alcohol layer was used as anthocyanidin sample, and the aqueous layer was applied to sugar sample after being concentrated under reduced pressure.

In these experiments cyanin, cyanidin and glucose were cochromatographed respectively as specimen.

For the detection of sugar spot, ammoniacal silber nitrate solution was sprayed onto the filter paper.

*Quantitative determination of pigment*: The petals of velvety dark red cultivars, BONNE NUIT, CHARLES MALLERIN and JOSEPHINE BRUCE, and those of red cultivars,

INDEPENDENCE, KARL HERBST, HAPPINESS, ENA HARKNESS and *cl*-CRIMSON GLORY, were used for this estimation. Concerning to the petals of BONNE NUIT the pigment content at different flowering stages was estimated.

A disk, being 2.5mm in radius, was cut out from the petal with a cork-borer, submerged in 10ml of methanol containing 0.3 per cent. hydrochloric acid for about five hours, and then filtered. The disk was soaked again in 10ml of the same solvent for twenty hours and filtered. After washing several times the disk with small amount of the solvent by means of filtration, all filtrates were collected and diluted with the solvent to 30ml exactly. According to the procedure adopted here, the pigment in the petal could be extracted completely. When the optical density of the pigment solution thus prepared was below 0.3, its absorption was found to follow BEER's law strictly. (**Fig. 1**)

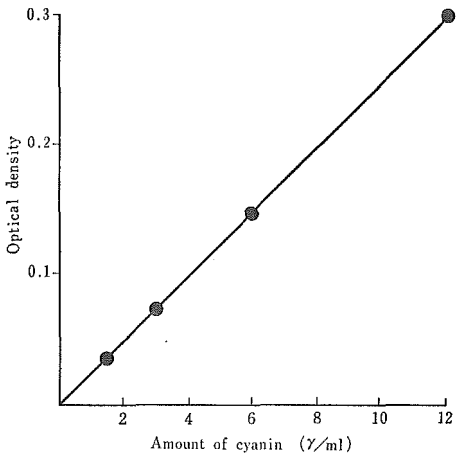



**Fig. 1.** Straight line relationship between the amount of cyanin and optical density.

*Measurement of spectro reflectance*: For this practice a SHIMAZU's reflection accessory set, connected with a BECKMAN spectrophotometer, was employed for the petals of the black roses, BONNE NUIT and CHARLES MALLERIN, and those of red roses, KARL HERBST and HAPPINESS.

## Results

*Qualitative test of anthocyanin*: In the anthocyanin samples of the petals of BONNE NUIT and HAPPINESS, only one spot was recognized in each paperchromatogram.

**Table 2.** The Rf values in Bu.H and the wave lengths of maximum absorption of anthocyanins.

| Anthocyanin | Rf value in Bu.H | Wave length of maximum absorption (in methanol containing 5% acetic acid) mμ. |
|---|---|---|
| from BONNE NUIT | 0.15~0.17 | 530 |
| from HAPPINESS | 0.15~0.17 | 530 |
| cyanin | 0.16 | 530 |

**Table 3.** The Rf values of anthocyanidins.

| Anthocyanidin | Rf value in AA.H |
|---|---|
| from BONNE NUIT | 0.31~0.33 |
| from HAPPINESS | 0.31~0.33 |
| cyanidin | 0.32 |

**Table 4.** The Rf values of sugars.

| Sugar | Rf value | |
|---|---|---|
| | in Bu. AA-1 | in Bu. AA-2 |
| from BONNE NUIT | 0.26~0.29 | 0.21~0.23 |
| from HAPPINESS | 0.26~0.29 | 0.20~0.24 |
| glucose | 0.28 | 0.22 |

**Table 5.** Anthocyanin contents in the petals of velvety dark red cultivars.

| Name of cultivar | Anthocyanin content ($\gamma$/cm$^2$) | |
|---|---|---|
| | in the black area | in the red area |
| BONNE NUIT | 839 | 860 |
| CHARLES MALLERIN | 788 | 562 |
| JOSEPHINE BRUCE | 592 | 528 |

**Table 6.** Anthocyanin contents in the petals of red cultivars.

| Name of cultivar | Anthocyanin content ($\gamma$/cm$^2$) |
|---|---|
| INDEPENDENCE | 250 |
| KARL HERBST | 294 |
| HAPPINESS | 611 |
| ENA HARKNESS | 697 |
| *cl*-CRIMSON GLORY | 321 |

Their Rf values were 0.15–0.17 coincident with that of authentic cyanin indicating 0.16 (**Table 2**).

The paperchromatograms of anthocyanidin samples prepared by hydrolyzing the anthocyanin samples of both cultivars indicate that their Rf values were 0.31–0.33 which corresponded with the value 0.32 of authentic cyanidin. (**Table 3**).

The Rf values of sugar samle were 0.26–0.29 in Bu. AA–1 and 0.21–0.23 in Bu. AA–2, coinciding with the Rf values 0.28 and 0.22 of glucose itself. (**Table 4**).

*Quantitative determination of pigment* : Anthocyanin contents in the petals of velvety dark red cultivars are shown in **Table 5**. From this table it is obvious that in the petals of BONNE NUIT the anthocyanin content of red area was slightly higher than those of black portion.

Comparing these data with the pigment quantities in the petals of five red cultivars indicated in **Table 6**, the author can point out that velvety dark red rose, JOSEPHINE BRUCE, is lower than both red cultivars, HAPPINESS and ENA

**Table 7.** Anthocyanin contents in the petals of BONN NUIT at the various flowering stages.

| Flowering stage* | Anthocyanin content ($\gamma$/cm$^2$) |
|---|---|
| unopened stage | 893 |
| half-opened stage | 1004 |
| mostly-opened stage | 734 |
| fully-opened stage | 562 |

*The explanation of the stages was described in the previous paper[2].

HARKNESS, in anthocyanin content of the petals. On the contrary BONNE NUIT and CHARLES MALLERIN are higher than red cultivars in this conection.

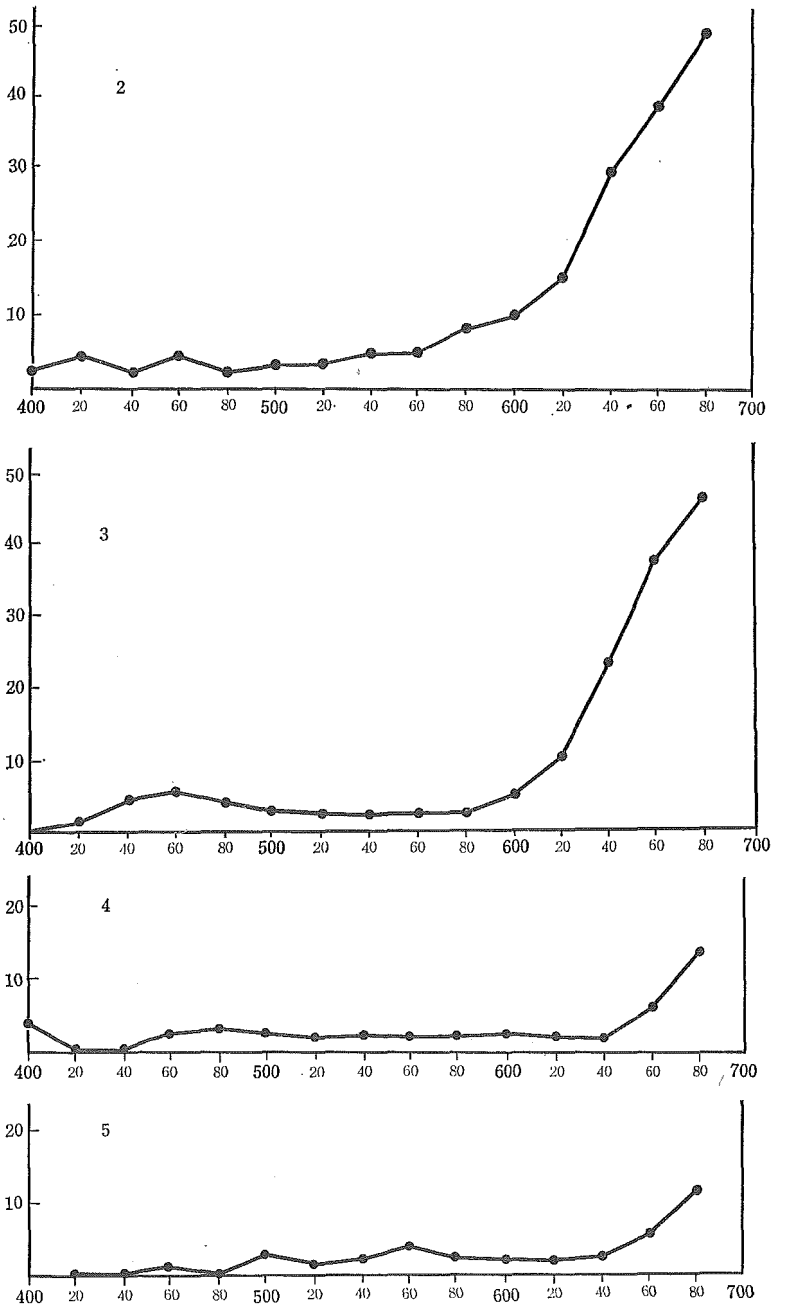
Anthocyanin contents in the petals of BONNE NUIT at different flowering stages are presented in **Table 7**. This table shows that the pigment quantity in the petals increased from unopened upto half-opened stage, and then decreased thereafter.

*The spectro reflectance on the petal surface* : The reflectances on the petal surfaces of velvety dark red cultivars, BONNE NUIT and CHARLES MALLERIN, and of red cultivars, KARL HERBST and HAPPINESS, are shown in **Figs. 2-5**. In the petals of red cultivars a steep rise of reflectance was noticed at the range of wave length longer than 580m$\mu$, and in the velvety dark red a less noticeable reflectance was found at the range of wave length longer than 630m$\mu$. At the other range of wave length, any essential distinction in the reflectance between two kinds of cultivars was not appreciated.

## Discussion

The paperchromatography adopted here was the technique initiated by BATE-SMITH[11], improved by HAYASHI[12], HAYASHI et al.[13] and ABE et al.[14], and recommended by BHIBATA et al.[10], for the identification of anthocyanins and related substances in the petal extract. On the basis of the fact that with this technique the Rf values of anthocyanin, anthocyanidin and sugar in the petal extract or its hydrolyzing product, agreed well with those of cyanin, cyanidin and glucose respectively, the author in this paper intends to propose that the anthocyanin contained in the petals of both cultivars, BONNE NUIT and HAPPINESS, is cyanin. This fact indicates that anthocyanin is not implicated in the expression of black tone in the velvety dark red petals.

Generally speaking, deep red color gives an impression of a blackish tone. But in the black rose petal it can be said that the expression of the black tone may not be imputed imediately to the abundance of pigment in the petal from following three reasons: (1) in the petals of BONNE NUIT the black portion is




**Figs. 2~5.** Spectro reflectances on the petals of red cultivars, KARL HERBST (**Fig. 2**) and HAPPINESS (**Fig. 3**), and of velvety dark red cultivars, BONNE NUIT (**Fig. 4**) and CHARLES MALLERIN (**Fig. 5**).
Vertical axis : reflectance (%)
Horizontal axis : wave length (m$\mu$)

lower in the pigment content than that of the red portion; (2) the anthocyanin content of the petals of a velvety dark red cultivar, JOSEPHINE BRUCE, is lower than that of the petals of red cultivar, HAPPINESS and ENA HARKNESS; (3) the petals of half-opened flower had the highest quantity of pigment among those of any other stages, though the black tone in the petals of velvety dark red cultivars was gradually reduced in contrast with the red coming out slowly during the flower development.[2]

Many investigators have reported that the anthocyanin may not necessarily assume the same color tone depending on the physical or chemical states of cell sap. But the results of spectrophotometric measurements of reflectance on the petal surface indicate that the color expressions of cyanin in cell saps of both velvety dark red and red petals are essentially equal.

From above circumstances, the author in this paper would conclude as follows: so far as these cultivars are concerned the quantity and quality of anthocyanin in the rose petals may have no direct responsibility for the expression of the black tone in the velvety dark red petals.

## Summary

The anthocyanin in the petals of a velvety dark red cultivar BONNE NUIT, and a red cultivar HAPPINESS, was identified as cyanin according to the paper-chromatography recommended by SHIBATA et al.[10]

Comparative determinations of cyanin contents in the petals were made between velvety dark red and red cultivars, between the black and red portions within the same petal and among the different stages of flower development respectively. It was found that cyanin contents of rose petals were not necessarily higher in the dark red cultivar nor in the dark red portion of a single petal.

Furthermore it was also evidenced that the cyanin content in the petals had no causal relation to more blackish appearance of the opened flowers.

Spectrophotometrical determinations indicated that there was no significant difference in reflectances between the velvety dark red and red petals.

According to these findings, it may be concluded that the quantity and quality of anthocyanin in the petals of velvety dark red cultivars are not concerned directly in the expression of black tone.

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