

*Studies on "Bluing Effect" in the Petals
of Red Rose VII.
Cytological Observation on the Epidermal Cells
of Bluing Petals Incorporated
into the Miscellaneous-type.*

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Summary

The miscellaneous-type of bluing in rose petals (YASUDA and KIKUCHI, 1978) was re-examined using the following five cultivars: Cardinal de Richelieu, Blue Boy, Reine des Violettes, Shigyoku, and Samurai. The massive structures appearing in the epidermal cells of their petals showed striking similarity to the anthocyanophore-like structure in some rose petals which was reported by YASUDA in 1974b, 1976 and 1979 at three points: 1) "staining features both with ruthenium red and safranin", 2) "developmental process", 3) "behaviors against weak acids".

From these results, it was proposed that the bluing pattern exhibited in such roses as five cultivars mentioned above should be separated from the miscellaneous-type as a new group of bluing, an anthocyanophore-type.

Introduction

In the previous paper YASUDA *et al* (1978) grouped various bluing patterns of red rose petals into three types, on the basis of the survey which was performed on one hundred and forty five cultivars including some old roses.

1. Cell sap-type: Central vacuole of the upper epidermal cells is uniformly blue without any apparent blue structures.
2. Tannin body-type: The tannin bodies, which are spherical in shape and blue in color, appeared in the central vacuole of the upper epidermal cells.
3. Miscellaneous-type: Some blue structures other than tannin bodies are recognized in the central vacuole of the upper epidermal cells.

In the same paper YASUDA *et al* explained developmental mechanism of cell

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sap-type, applying the theory that ASEN *et al* (1971) gave to the cause of bluing in Better Times rose. That is, the bluing of cell sap-type is due to the color action of the anthocyanin-flavonol co-pigment complex and to the effects of the decrease in acidity associated with aging of the petals.

YASUDA in 1970 and 1974a presented an opinion that the formation of tannin body is one of the important mechanism of bluing effects in the petals of a rose cultivar, cl-Crimson Glory. Same opinion was adopted as general explanation about the cause of bluing effect of the tannin body-type by YASUDA *et al* in 1978.

As indicated in the previous paper, multitudes of bluing patterns, in which the epidermal cells having some structures other than tannin body, are lumped together into miscellaneous-type. The evidence to be presented in this paper shows that a pattern, in which the massive structure similar to the anthocyanophore-like body as reported by YASUDA in 1976 and 1979 appears in the upper epidermal cells, separated from the miscellaneous-type of bluing, brought up for new one type, anthocyanophore-type.

Materials and Methods

The plants used in the present observation were five cultivars of rose : Cardinal de Richelieu, Blue Boy, Reine des Violettes, Shigyoku, and Samurai. Those cultivars were grown in the garden of Keisei Rose Nurseries (Yachio, Chiba, Japan) outdoors.

For the purpose of observations, two kinds of microscopic specimens were prepared from the petals of the cultivars above mentioned, one being fresh sections of petals cut at 30 μ in thickness by a freezing microtome, and others paraffin sections of the small pieces of fresh petals (approximately 5×5 mm square) fixed with 10 % neutral buffered formalin or Kaiser's solution. The compositions of individual fixative and the durations of fixations are shown in Table 1.

Table 1 The compositions of fixatives, the durations of fixations and the manners of washings in preparing the paraffin sections.

Fixative	Composition	Duration of fixation	Manner of washing
10 % Neutral buffered formalin	40 % Formaldehyde	10 ml	Three times with phosphate buffer (pH 7)
	Distilled water	90 ml	
	Anhydrous sodium dihydrogen phosphate	350mg	
	Anhydrous disodium hydrogen phosphate	650mg	
Kaiser's solution	Mercuric chloride	10 g	70 hrs. With running water over-night
	Glacial acetic acid	3 ml	
	Distilled water	300 ml	

After completion of fixation, to remove any trace of the fixatives, the pieces fixed were thoroughly washed in the manners given in Table 1.

The sections, which were fixed with 10 % neutral buffered formalin, were stained with safranin (30 minutes) and ruthenium red (1-2 seconds), respectively. On the other hand sections fixed with Kaiser's solution were stained with toluidine blue for an hour. This procedure is referred to as "Kaiser's solution-toluidine blue method" in the present paper.

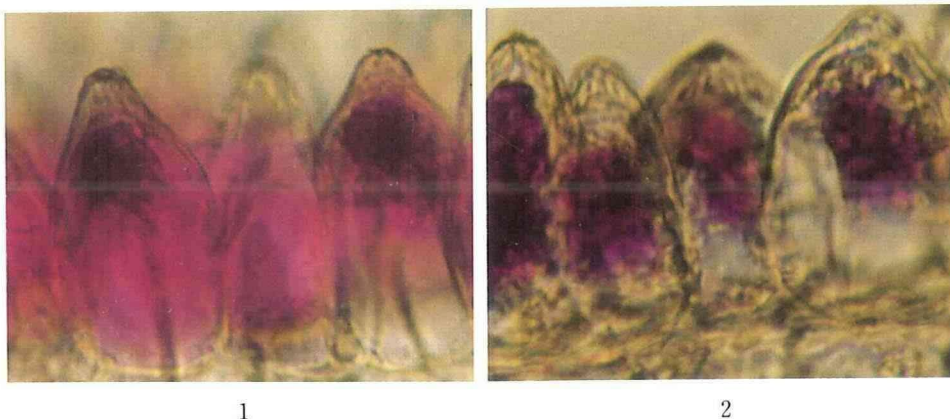
Observations

Developmental observations

In the fresh sections prepared from the younger petals which showed slight degree in the bluing effect, some massive structures were recognized at the upper portions of their epidermal cells in the indeterminate forms (Fig. 1).

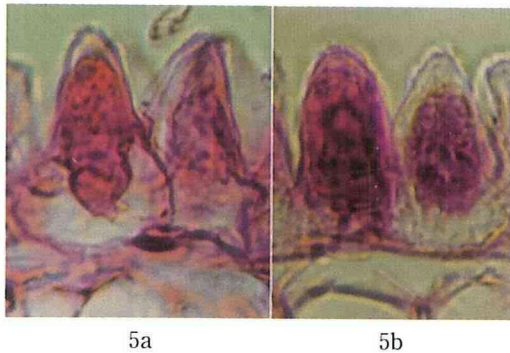
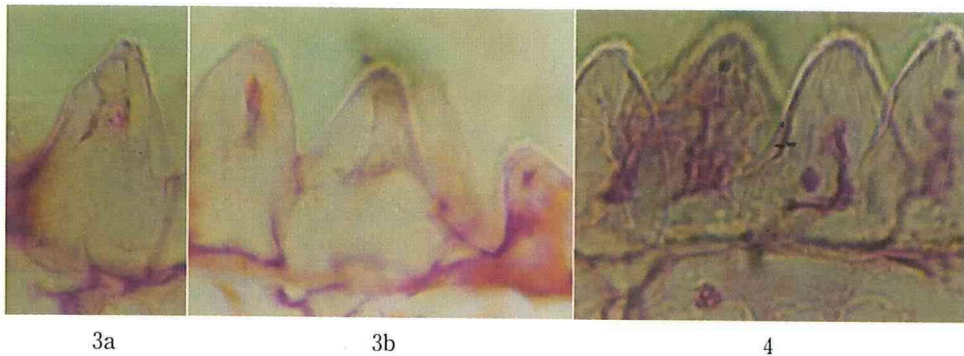
In the epidermal cells which were seriously advanced in the bluing effect, massive structures occupied large portion of the central vacuole of the cells (Fig. 2).

The detail observations of the developments of the massive structures were accomplished by using the paraffin sections of the petals exhibiting bluing effects in various intensities. The series of the structural changes in the developments are fully explained divided into three phases as presented below.



Figs. 1 and 2 The fresh sections of the upper epidermal cells prepared from the petals showing the slight degree in the bluing effect (1), and in the seriously advanced effect, (2).

Cultivar used : Samurai



Figs. 3-5 The upper epidermal cells including the massive structures in the various developing phases.

3a and b: The early phase

4: The middle phase

5a and b: The later phase

Fixative: 10 % Neutral buffered formalin

Dye: Safranin in 50 % alcohol

1. The early phase Massive structures began to appear in various shapes, for instance, thread-like, star-like, T-shaped, arched or meteoric at the upper portions of the epidermal cells (Fig. 3 a, b).
2. The middle phase Massive structures underwent a marked increase both in length and thickness, occasionally divided into two branches (Fig. 4).
3. The later phase The massive structures showed rod-like or in certain cases, branched appearances, and they became longer and thicker to occupy the large portion of the central vacuoles of the epidermal cells (Fig. 5 a, b).

The microchemical and histochemical results

1. The treatment with diluted hydrochloric acid.

The microscopic examination showed massive structures losing their own forms expanding over the central vacuoles as soon as the fresh sections of the petals were treated with diluted hydrochloric acid.

2. Staining with ruthenium red.

In the paraffin sections fixed with 10 % neutral buffered formalin, massive structures were stained with ruthenium red, showing the characteristic tinge of pectic substances.

3. Kaiser's solution-toluidine blue method.

As mentioned above, massive structures were broken down with weak acids. Accordingly, they did not show good staining with toluidine blue after the use of acidic fixatives such as Kaiser's solution in many cases. However, there were some cases where the clear blue color was observed about the areas corresponding to the massive structures by this method. There appeared to be no significant differences in the images of this dyeing between the present massive structure and the tannin body (YASUDA ; 1970, 1974a).

Discussion

From the observation presented here, it is evident that the structures dealt with in this investigation (hereinafter referred as the present structure) have striking resemblance to the structure reported by YASUDA in 1974b, 1976 and 1979 (hereinafter referred as anthocyanophore-like structure) in the following three points :

- 1) The present structure showed staining feature both with ruthenium red and safranin to indicate the presence of pectic substances.
- 2) The development of the present structures followed similar process as that of anthocyanophore-like structure from start to finish.
- 3) Present structure precisely resembled anthocyanophore-like structure in the behavior toward the weak acids, losing its original form by the treatments with acids.

From these similarities it seems highly probable that the present structure bears homologous characters to the anthocyanophore-like structure.

Thus, we would like to separate the present structure from the miscellaneous-type as a new group of bluing, giving the name of "an anthocyanophore-type".

Kaiser's solution-toluidine blue method has been reported as the recommendable procedure in the detections of tannic substances in the motor cells of *Mimosa pudica* L. (TORIYAMA, 1954), and applied in the recognition of tannin body in the bluing rose petals (YASUDA ; 1970, 1974a, and YASUDA *et al* 1978). Accordingly, the occasional observations of the positive results with this method presumably be interpreted as existence of some cases where the anthocyanophore-type is mixed with tannin body-type.

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