

*Studies on "Bluing Effect" in the Petals of
Red Rose, V. A Survey of the
Various Bluing Types.*

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Abstract

One hundred and forty five rose cultivars, including old roses, were used to determine the various bluing patterns of red rose petals. The upper epidermis of fresh petals, which had exhibited the bluing phenomenon, were peeled and examined microscopically.

The bluing patterns observed were grouped into three types. The first was the cell sap-type where the central vacuole of upper epidermal cells was uniformly blue without any apparent blue structures. The second was a tannin body-type in which blue spherical tannin bodies appeared in the vacuoles. The third was a miscellaneous-type which included blue structures in the vacuole other than tannin bodies.

Old roses derived from *Rosa chinensis* usually exhibited the cell sap-type of bluing while those derived from *Rosa gallica* had a tendency to exhibit a combination of the tannin body-miscellaneous bluing types. Combinations of two or even all three of the bluing types were found in some cultivars. With the long history of rose breeding, the various bluing types and their combination could have evolved through the segregation and recombination of the bluing factors, specifically when *Rosa chinensis* or *Rosa gallica* were used as parents.

Introduction

BAYER¹⁾ observed that many red flowers, whose principal pigments were anthocyanins, had a tendency to become bluer as they aged. This phenomenon,

usually referred to as "bluing" or "bluing effect", is prevalent among the many red rose cultivars, and growers and breeders attach great importance to this problem.

The genetic make-up of roses is extremely complex with more than fifteen thousand cultivars. However, investigations of the causes for bluing have been limited to only a few rose cultivars, namely Lay Maureen Stewart and Hadley by CURREY²⁾, Better Times by WEINSTEIN³⁾ and ASEN *et al.*⁴⁾, and cl-Crimson Glory by YASUDA^{5,6,7)}. The causes cited for bluing are: 1) a lack of tannic matter in the cells of petal tissue; 2) an increase in pH of the cell sap with aging and its effect on the color of anthocyanin-copigment complexes; and 3) an appearance of some particular structure mainly composed of tannic substances in the upper epidermal cells of aging petals.

Since roses are genetically so complex (HURST⁸⁾ and WYLIE^{9,10)} and only a relatively few cultivars have been examined, it is at present difficult to draw any general conclusions for bluing. As the first step for a more complete understanding, we will present data obtained from one hundred and forty five cultivars, including old roses, of what we consider to be the various types of bluing found in these flowers.

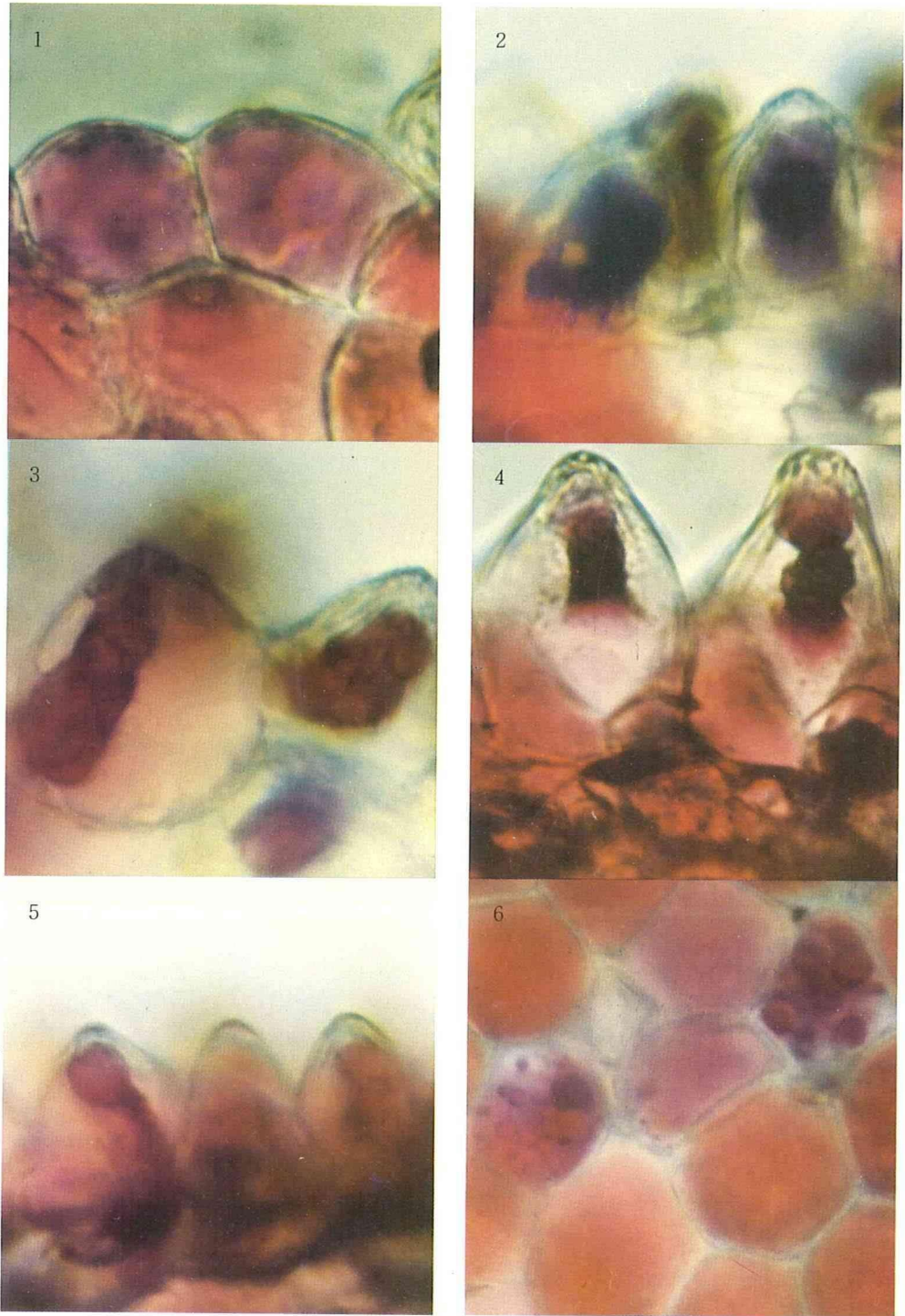
Materials and Methods

Both old and modern rose cultivars were examined. They were grown outdoors at the Keisei Rose Nursery Company (Owada Shinden, Yachio, Chiba Prefecture, Japan).

Fresh petals which blued were harvested as previously described¹¹⁾. The upper epidermis of a flower petal was peeled off and pieces approximately 3×5mm were placed in a drop of distilled water on a microscope slide. A cover glass was placed over the tissue and it was observed microscopically. In some instances observations also were made of paraffin sections, seven micron thick, prepared by two different procedures. The epidermis was fixed with KAISER's solution (10g mercuric chloride +3ml glacial acetic acid +300ml distilled water) for approximately 70 hours, and the sections then were stained with toluidine blue for 30 minutes. With the other procedure, the epidermis was fixed with 10% neutral buffered formalin (10ml 40% formaldehyde +350mg anhydrous sodium dihydrogen phosphate +650mg anhydrous disodium hydrogen phosphate +90ml distilled water), and the section was stained with safranin for 30 minutes.

Observation

General observations revealed various patterns of bluing, as shown in Figs. 1-6. Some had upper epidermal cells whose cell sap was uniformly blue while



Figs. 1-6 Micrographs of upper epidermis peeled off from the bluing petals of roses. Various patterns of bluing are demonstrated.

others exhibited various bluish structures within the vacuole. Some of the bluish vacular inclusions were found to be tannin bodies as previously reported by YASUDA^{13,14}) in cl-Crimson Glory. The patterns of bluing found could be classified into three categories. The first was the cell sap-type where the central vacuole of upper epidermal cells was uniformly blue without any apparent blue structures. The second was a tannin body-type in which blue spherical tannin bodies appeared in the vacuoles. The third was a miscellaneous-type which included blue structures in the vacuole other than tannin bodies. Some roses grouped as this type, when stained with 10% buffered formalin-safranin method, showed the characteristic staining features as the massive vacular inclusions of the so-called black rose 'Charles Mallerin'^{13,14}). Some cultivars exhibited a combination of two or all three of the categories of bluing.

The frequency of the occurrence of the three types of bluing, in various groups of garden roses, is shown in Table 1. Old roses derived from *Rosa chinensis* tended to exhibit the cell sap-type of bluing while those derived from *Rosa gallica* exhibited a combination of both the tannin-body and miscellaneous bluing types. With modern roses, such as hybrid teas, floribundas and grandifloras, the cell sap-type of bluing occurred most frequently followed by the tannin body-type. Also found was the combination of the cell sap-tannin body bluing type. The combination of the cell sap-miscellaneous type was rarely found.

As shown in Table 2, the cell sap-type of bluing occurred most frequently

Table 1 Frequency appearance of bluing type in bluing petals of various roses.

Rose	Frequency appearance (%)							Number of survey
	Cell sap type (I)	Tannin body type (II)	Miscellaneous type (III)	I, II combined type	I, III combined type	II, III combined type	I, II, III combined type	
The old rose derived from <i>R. chinensis</i>	100	0	0	0	0	0	0	9
The old rose derived from <i>R. gallica</i>	0	0	0	0	0	100	0	3
The rose derived from <i>R. rugosa</i>	81.8	9.1	0	9.1	0	0	0	11
Hybrid perpetual	33.3	0	33.3	0	0	33.3	0	3
Tea	0	100	0	0	0	0	0	2
Miniature	80	20	0	0	0	0	0	5
Climing rose	75	0	0	12.5	0	0	12.5	8
Hybrid tea	57.7	14.1	2.8	21.1	2.8	0	1.4	71
Rambler	100	0	0	0	0	0	0	2
Hybrid polyantha	50	0	16.7	16.7	0	16.7	0	6
Floribunda	45.4	18.2	4.5	27.3	4.5	0	0	22
Glandiflora	33.3	0	0	33.3	0	0	33.3	3

Table 2 Frequency appearance of bluing type in bluing petals of various flower colors.

Flower color	Frequency appearance (%)							Number of survey
	Cell sap type (I)	Tannin body type (II)	Miscellaneous type (III)	I, II combined type	I, III combined type	II, III combined type	I, II, III combined type	
Red	47.0	10.6	4.5	25.8	4.5	3.0	4.5	66
Deep pink	81.8	9.1	6.1	0	0	3.0	0	33
Weak pink	71.0	12.9	3.2	9.7	0	3.2	0	31
Orange	46.7	20.0	0	33.3	0	0	0	15

Table 3 Appearance of some structure in the upper epidermal cells of the petals showing various colors.

Flower color	Tannin body	Pectin body	Others
Red	+	+	+
Deep pink	+	+	+
weak pink	+	+	+
Orange	+	+	+
White	-	-	-
Yellow	-	-	-

with red, weak pink, deep pink or orange roses, but there was little or no relationship between color and the various bluing types.

Vacuolar inclusions associated with bluing were not found in epidermal cells from sixteen yellow or four white rose cultivars (Table 3).

Discussion

Data obtained from the present survey revealed that the bluing of red rose petals is not always a simple process. Three types of bluing were distinguished: cell sap, tannin body and miscellaneous, together with various combinations of all three.

According to our survey, the bluing of Better Times rose was due to the cell sap-type. ASEN et al.⁴⁾ spectrally showed that the bluing of Better Times rose was due to a decrease in acidity associated with aging and its effect on the color of the anthocyanin-flavonol copigment complex found in the vacuole. We should like to propose, tentatively, the same explanation for the developmental mechanism of the cell sap-type of bluing.

The tannin body-type of bluing was confirmed by the KAISER's solution-toluidine

blue method which is recommended as a specific reaction of the histochemical detection of tannic substances in plant cells^{5,12}). YASUDA^{5,6}) suggested a possible explanation for the mechanism of the tannin-type bluing found in cl-Crimson Glory rose. The tannin bodies in the rose may be similar to tannin found in the vacuole of *Mimosa* by TORIYAMA¹²). These tannin bodies absorb the anthocyanin in vacuoles of epidermal cells to cause bluing, being involved in iron probably accumulated by the transpiration stream.

The miscellaneous-type of bluing was apparently due to many factors. The massive structure similar to the anthocyanophor-like body^{13,14}) was evident, as indicated by the characteristic staining obtained from 10% neutral buffered formalin-safranin. Further studies are in progress to elucidate the relationship between this massive structure and the other factors responsible for this type of bluing.

Old roses derived from *Rosa chinensis* or *R. gallica* exhibited a tendency to blue. The bluing of those roses derived from *Rosa chinensis* usually was the cell sap-type while the bluing of those roses derived from *R. gallica* usually was the tannin-miscellaneous combined type. Thus, during the long history of rose breeding, the tannin body-miscellaneous combined type of bluing associated with *R. gallica* presumably segregated and thereafter recombined with the cell sap-type of bluing associated with *R. chinensis* which resulted in the various types of bluing now noted.

Vacuolar inclusions, usually composed of tannic or pectic substances, were found only in cells that contained anthocyanins (Table 3). Therefore, it is suggested that the development of these structures may be involved in the metabolism of plant polyphenolic compounds.

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