

*Studies on "Bluing Effect" in the Petals
of Red Rose, III.*

*The Histochemical Detection of Iron in the
Bluing Petals of Rose.*

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Abstract

The bluing petals of red rose cultivar, cl-Crimson Glory, were fixed with 10% neutral buffered formalin. In the paraffin sections, the region corresponding to the blue spherule present in the upper epidermal cells, was clearly stained blue with the Prussian blue reaction. The same regions showed a brownish red color of ferric oxide with incineration.

These facts are suggestive of that iron exists within the blue spherule, making the previous possibility more likely.

YASUDA¹⁾ reported that the bluing effect of rose petals is mainly due to the blue spherule present in the upper epidermal cells of the petals, and foresaw a possibility that the bluish color of the spherule may be exhibited essentially by the conjunction of the following three components: anthocyanin, tannic substance and iron. As for two of these components, anthocyanin and tannic substance, it was previously expected that they would participate in the components of the spherule (YASUDA^{1,2)}). But it remains uncertain whether iron is located in the blue spherule or not, while this metallic component was found in the crude solid pigment obtained from the red rose petals by the methanolic hydrochloric acid-ether method (YASUDA³⁾).

The present paper reports the histo-

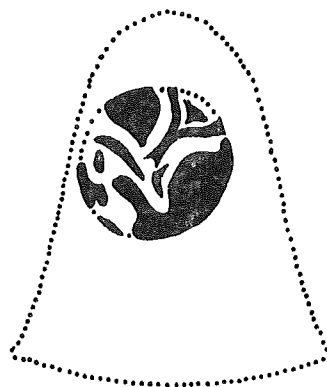


Fig. 2 A schematic diagram illustrating the spodiogram of upper epidermal cells of the bluing petals. The black area indicates the exhibition of brownish red color of ferric oxide.

chemical detection of iron in the bluing petals of rose.

Fig.1 is a photomicrograph representing the features of the upper epidermal cells treated with the Prussian blue reaction. Blue staining is visible in the region corresponding to the blue spherule reported previously^{1,2)}. As shown schematically in Fig.2, the incinerated sections show the characteristic brownish red color of ferric oxide in the same distribution as the Prussian blue staining.

Thus, it seems highly probable that iron is a component of the blue spherule. This makes the previous possibility^{1,2)} more likely, namely that the blue color of the spherule is exhibited by the co-operation of anthocyanin, tannic substance and iron.

Experimental

The plant used in the present investigation was the same as the previous one¹⁾, a red rose cultivar, cl-Crimson Glory, grown outdoors.

Petals fully exhibiting the bluing effect were collected from the plant materials, and fixed with 10% neutral buffered formalin^{4,5)} for 48 hours. After completion of fixation they were dehydrated through the butylalcohol series and embedded in paraffin. Paraffin blocks were cut 20 micra in thickness.

The detection of iron in the paraffin sections was accomplished in two ways, one being the Prussian blue reaction^{4,5)} and the other being the incineration method⁵⁾.

Observations were made only upon the upper epidermis.

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References

- 1) YASUDA, H. (1970) *Bot. Mag. Tokyo* 83 : 233.
- 2) YASUDA, H. (1973) *Cytologia* 36 : in press.
- 3) YASUDA, H. (1968) *Bot. Mag. Tokyo* 81 : 377.
- 4) BANCROFT, J.D. *An Introduction to Histochemical Technique*, p.58, 168 (BUTTERWORTHS, London, 1967).
- 5) TARAO, S. (1957) *Jour. Fac. Sci. Hokkaido Univ.* 13 : 326.

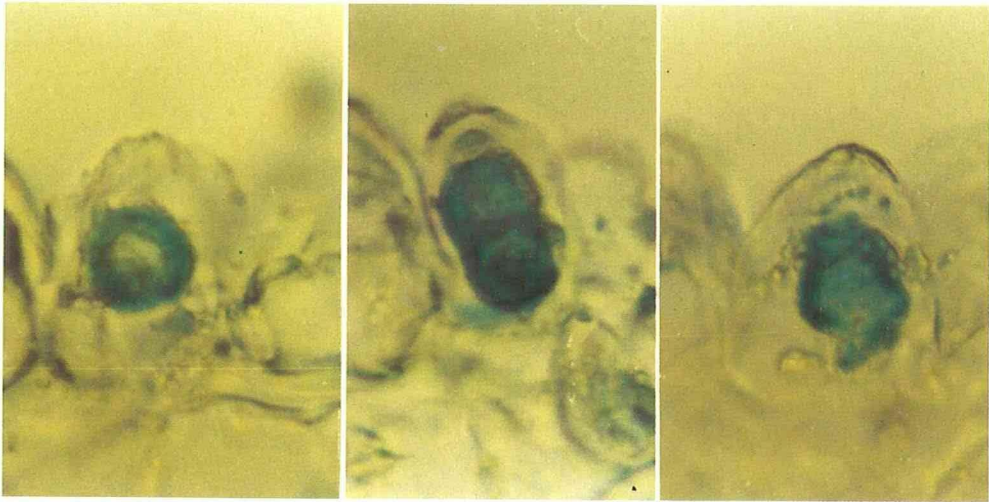


Fig. 1 Photomicrographs showing the upper epidermal cells in the bluing petals, after treatment of the Prussian blue reaction.