

*Studies on the Expression of Color Tone
in Rose Petals. II*

*Changes of the Epidermal Structure in the Velvety
Dark Red Petals as Flower Develops.*

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It is well-known among many rose growers that the black tone of petals of velvety dark red roses becomes gradually lower in contrast with the red color coming out as the flower develops. On the view point of the author's previous paper that the expression of black tone of the velvety dark red petals was attributed to the shadow of epidermal cells, this phenomenon may be imputed to the morphological change of the petal surface as flower develops.

Various physiological and morphological changes may occur in opening flowers. In the case of rose flowers, there are found only a few physiological investigations, but so far as the author is aware we have none as to their morphological changes. For instance, SIEGELMAN et al. showed that the respiration of rose petals begun to increase just before flower development, became to maximum at fully-opened stage and thereafter depressed; WEINSTEIN et al. studied the fixation of carbon dioxide in the flower of various opening stages; WEINSTEIN reported that the contents of various organic compounds in petals varied diversely with flower development; and AHUJA et al. and YASUDA indicated that the quantity of the pigment in petals diminished slightly as flowering progressed.

In this paper the author studied the morphological change of epidermal structure in the petals of velvety dark red roses during the flower development, discussing the relation of this change to the gradual diminution of black tone.

Materials and Methods

The plants used in the present investigation were three velvety dark red rose cultivars, BONNE NUIT, CHARLES MALLERIN and JOSEPHINE BRUCE, grown outdoors. Their outermost insect- and disease-free petals were collected from the flowers in following stages respectively:

Unopened stage: the stage in which all calyxes developed, but petals did not yet.

Half-opened stage: the stage in which about half number of petals of a flower developed.

Mostly-opened stage: the stage in which about eighty per cent. of petals of a flower developed, pistils and stamens having not yet appeared.

Fully-opened stage: the stage in which all petals completely developed, and pistils and stamens appeared at the center.

Over-opened stage: the stage in which one or two outermost petals begun to fall.

About 5 cm squares were cut off from these petals, fixed in CRAF I^{O} , dehydrated with normal-butyl alcohol method, and sectioned at 15μ , after they were embedded in paraffin. The sections were stained by fast green. On the other hand, the sections cut by free hand without fixing and staining, were also prepared. Both paraffin section and free hand section were used for observation.

The surface views were photographed by lighting the petal surface at an angle of 30° .

Observation

The various types of epidermal structures observed in the petals during the flower development, are summarized in **Figs. 1-6** by means of schematic sketches.

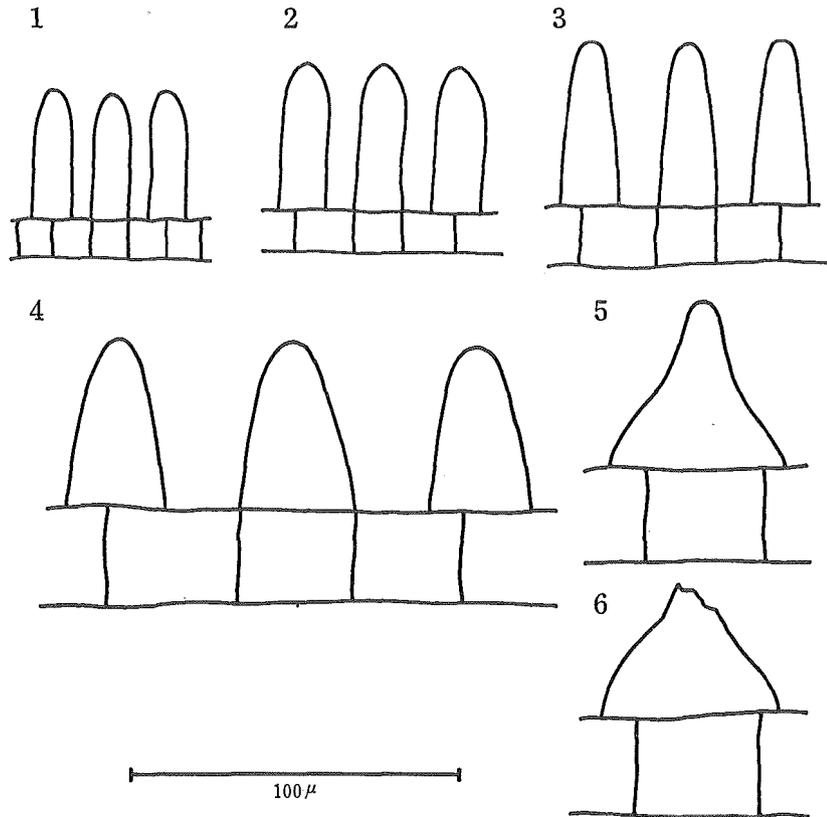
In the unopened and half-opened stages, the epidermal cells showed a obtuse, cudgel-like form (**Figs. 1 and 2**). The epidermal cells of both stages were essentially equal in form, but differed from each other in size, the cell of the latter stage being larger in some degree than that of the former.

In the mostly-opened stage, the epidermal cells were lengthend to the horizontal direction to surface at the lower portion of cells, but at the upper portion the horizontal extension of cells was not so wide as in the lower portion, so they looked like a slender bell (**Fig. 3**).

In the fully-opened stage, two types of epidermal cells were recognized: one type was like a wide bell (**Fig. 4**), where the horizontal expansion was more considerable at the lower portion than in the case of preceding stage, but vertical elongation hardly happened; the other was a funnel-like type as a result of more extention at the base of cells than at the upper portion (**Fig. 5**).

In the epidermal cells of over-opened stage, three types of cells were generally discriminated: two of them were of the wide bell type and of the funnel-like type respectively, as being above mentioned; the rest was of the deformed-type, which was somewhat collapsed at the top (**Fig. 6**).

As a result of the sequence of enlargement in palisade parenchyma cells during the flower development, the spaces among the epidermal cells grew



Figs. 1~6 :—The schematic sketches of epidermal structures of the petals in various flowering stages.

wider and wider through all the stages of flower development.

The shadows of epidermal cells onto the petal surface were filled up among the spaces in the unopened stage and the half-opened stage (**Plates 1 and 2**). With the fully opened stage, however, they were not so abundant in all spaces (**Plate 3**).

Discussion

If a light of certain intensity is projected upon an object from a certain direction, producing its shadow on a plane, the visual form of the shadow is to depend upon the relative situation of the object and the plane. In the case of the surface structure of a petal, the object and the plane stand respectively for an epidermal cell and the space between the epidermal cell and a palisade parenchyma cell.

Though the direction of light was settled as described in the experimental procedure of this study, it may be said that there may fairly be irregular

reflection of light owing to the fact that the cell walls of petal surface are not perfectly flat.

On the early development of flowers including those of unopened and half-opened stages, it is expected that the irregular reflection of light takes place only at the top area of epidermal cells, since cudgel-like epidermal cells are arranged closely leaving very narrow spaces among them. Hence, it is no wonder that their shadows were filled up among the cells without gaps.

From plates 1 and 2, it can be easily noticed that the total red area in the petals at half-opened stage is larger than that at unopened stage, when the equal areas of them are compared. This seems to be brought about by the horizontal growth to petal surface of the cells in epidermis and palisade during the flower development from unopened to half-opened stage.

In the mostly-opened stage and fully-opened stage, not only the spaces among the epidermal cells were wider than that in preceding stages, but the side walls of epidermal cells were also slanting to petal surface. Such a surface structure is to be shined by both the direct ray and irregular reflection. The shadow of epidermal cell does not necessarily fill up the space continually. Accordingly, it can be quite natural that the total dimension occupied by red area in these stages was vaster than that in stages proceed.

In the last stage of flower development, namely over-opened stage, as the spaces among the epidermal cells were very wide, and their side walls were more oblique to surface than those of earlier stages, the epidermal structure seems to be not appropriate to yield shadow onto the surface. So that almost all petal surface can be occupied by the red area with the direct ray and irregular reflection of light.

It should be emphasized here that the red tone of petal surface becomes to be displayed by degree through the gradual elimination of shadow of epidermal cells with the progress of flower development.

⁽⁸⁾YASUDA stated that although anthocyanin quantity in the petals of BONNE NUIT was slightly reduced in the progress of flower development, the degree of its diminution was not enough to alter essentially the color tone from blackish to red. Hence, the decrease of black tone can not be attributed to the change of pigment contents in the petal.

From above discussion, the author in this paper intends to conclude as follows: the fact that the black tone becomes lower while red color slowly appears in the petals of velvety dark red roses during the flowering process, is attributed directly to the gradual elimination of shadow of epidermal cells on the petal surface.

Summary

Three cultivars of velvety dark red roses, BONNE NUIT, CHARLES MALLERIN and JOSEPHINE BRUCE, were investigated concerning to the change of epidermal structure of petals during the flower development.

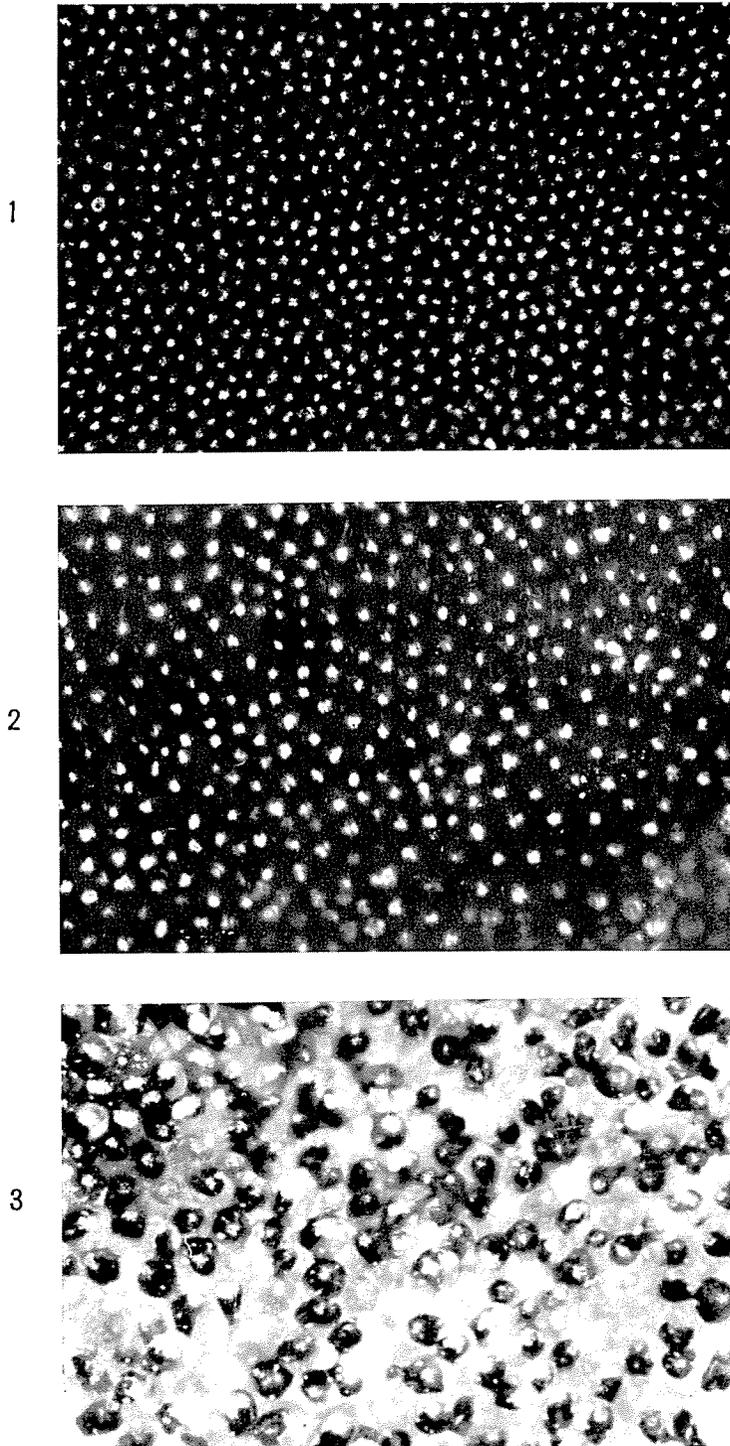
It was observed that the more developed flowers had the wider spaces among epidermal cells and the slant side wall to petal surface of epidermal cells, resulting in the condition that the epidermal structure grew less suitable to produce the unbroken shadow of epidermal cells.

The findings mentioned in this paper led the author to the following conclusion: the fact that the black tone becomes gradually lower and red color appears slowly with the progress of flowering in the petals of velvety dark red roses, is attributable mainly to the gradual elimination of the shadow of epidermal cells onto the petal surface.

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Plates 1~3 :—Surface views of petals of *Bonne Nuit* in various flowering stages, 1 : unopened stage; 2 : half-opened stage; 3 : fully-opened stage.