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AUTHOR(S):

Kato, Kazuo

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A Phenomenon in the Early Stage of Pollination in *Secale cereale* ; the Stigma Reaction

By

Kazuo KATO

Botanical Institute, Kyoto University

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Despite its importance, our knowledge of the changes and activities in both of the stigma cells and the pollen grains, especially at a very early stage after pollination, is surprisingly meagre, except for the works of STRASBURGER (1886) and FITTING (1909, 1910), and a further investigation is needed.

On the effect of pollen grains on stigma cells, it was found by STRASBURGER (1886) in crosses between species of *Orchis* (*O. masculata* × *O. Morio*) that the short pollen tube once emerged on the stigma is disorganized and the papillae of the stigma are turned brown in colour at the contact point with the pollen tube. According to him the colour change of the papillae may also be caused by ungerminated pollinia of *Orchis fusca* on *O. Morio*. From these facts it is seen that the pollinia give a certain change on the nature of the stigma cells by their contact at the pollination. Along a similar line remarkable investigations, both in observational and experimental, were made by FITTING (1909, 1910) on *Orchis*.

In *Secale cereale*, it has been found by the present author that the stigma cells differ in stainability of their nucleus, according whether they are pollinated or not. This subject has been taken up again and an investigation is in progress, a part of which will be reported here under as preliminaries.

The style of *Secale cereale* is bi-forked into two stigmas at its base, and numerous stigma filamsnts are born on these two stigmas for their entire length. The stigma cells composing the filament are arranged in four rows and their upper end is bent outwards, forming a shelf on which a pollen grain may easily be placed (Figs. 1 and 2). In the stigma cells their nucleus occupies the central position, and at both ends of the cells a large vacuole is seen. Along the cell wall and around the nucleus there is scanty cytoplasm in a thin layer, in which a few leucoplasts are contained.

When the pollinated style placed on a slide glass is stained with ordinary aceto-carmine, it is recognizable, in a short while, that in the stigma filament into which the pollen tube has been entered, the nulceus is stained deeply,

whereas other stigma filaments free from pollen grains, remain unstained (Fig. 1). As shown in Fig. 1, the difference in the stainability of the nuclei is distinct at a certain time after application of the aceto-carmine. This difference, however, is lost in about 20–30 minutes after the preparation, the nuclei being stained equally in the two kinds of filaments, pollinated and non-pollinated (Fig. 3).

In order to determine the shortest time necessary for stigma cells staining by pollination, the action time of the pollen, namely, the interval between the pollination and the application of aceto-carmine, was gradually shortened. By thus shortening the action time, one observes that in cases where the pollen grain has not put out a pollen tube, the stigma cell to which this ungerminated pollen attaches is stained deeper in colour than other non-attaching cells.

In *Secale cereale*, the pollen tube, in favourable condition, emerges from the germ pore, in one minute or more but in less than two, after the fall of pollen grains on the stigma. So the action time of the pollen was shortened from the grade of minutes to that of seconds, namely, from 50, 40, 30, 20 to 10 seconds*. In all these cases an easy stainability of the pollinated cell is recognizable. Accordingly, in order to manifest the easy stainability it is not necessary to enter the pollen tube into the stigmatic tissue but sufficient only to contact the pollen grain on the surface of the stigma cell for a short while (Fig. 2). However, as the action time of the pollen is shortened, the time that requires for being stained of the nucleus is prolonged.

In consequence of the pollination, various changes, other than the easy stainability, take place in the stigma cells. Among the changes, those in permeability to various stains and other substances; in shape and structure of the nucleus; and withering of the stigma cell, may be enumerated. To all physico-chemical changes which occur in the stigma cells by pollination, the term 'stigma reaction' is proposed, and for convenience of description this term will be used in the following.

It is usual that the stained cells are found more numerous in number in the apical direction of the filament from the pollinated cell than in the basal direction. It seems that when a stigma reaction takes place in a stigma cell this reaction is not only restricted within this cell but it may pass to neighbouring cells of the same filament, as seen below.

A case was observed where between two closely placed filaments one pollen grain was lodged, and its pollen tube had penetrated into one of these filaments. In this case the stigma reaction was recognized in both these filaments. In other words, in order to cause a stigma reaction it is not

* With shortening of the action time difficulties are accompanied in making the preparation.

necessary to attach the pollen grain to a stigma cell at the germ pore but sufficient to attach it at any place of the pollen surface. This suggests that the pollen grain has a substance which acts on the stigma cells to change their nature, and that at the inception of germination this supposed substance is activated all over the surface of the pollen grain. The stigma cells between which the pollen tube has entered are more readilly stained with the aceto-carmine as compared with those to which the ungerminated pollen grain attaches. This indicates that the pollen tube also sets free a similar substance which causes the stigma reaction.

The stigma reaction occurs in immature as well as in mature stigmas. In their immature state the stigma filaments are of a form of smooth column and not feathery (Fig. 4). On the surface of such stigma filaments too, pollen grains may germinate and their pollen tube presents a winding form, since between the cells no gap has not been formed into which the pollen tube to enter. In the young stigma filaments the aceto-carmine penetrates so easily into the cells that the stigma reaction is recognizable only in a short duration after an application of aceto-carmine.

S. cereale is known to have a strong tendency to self sterility, but the stigma reaction is seen in both cases of neighbour pollination and strictly of self pollination.

In the stigma reaction, its first manifestation is the increase in permeability of the cell membrane in the pollinated stigma cells. To this change follow changes of the nucleus in shape and structure. In a natural and non-pollinated state, the nuclei in the stigma cells are spherical in shape and their inner structure is apparently granular. Within a short minutes after the pollen attachment no discernible change is seen in the nuclear structure, even where the pollen tube has entered enough in the stigmatic tissue. Twenty minutes or more after the pollination, withering of the stigma filaments is conspicuous. Due to the withering the stigma cells become more or less narrow in width and in which the nuclei change their shape from spherical into ellipsoidal. These nuclei are still granular and no discernible change is seen in the structure, but they take up readily the stain being coloured more deeply than the spherical nuclei. The ellipsoidal nucleus has a tendency to move towards the apical in the cell.

In cases when the pollen grain attaches anywhere midway of the stigma filement and the action time of the pollen is long enough, the ellipsoidal nuclei are fouud to be more numerous in number in the upper cells of the filament from the point of the pollen attachment than in the lower cells. This situation corresponds with the above-mentioned fact that the easier stainability of the stigma cells (nuclei) is presented in the apical part of the filament from the point of pollen attachment than in the basal.

When the action time of pollen grans is long in grade of hours, the

nuclei take other shapes than spherial or ellipsoidal, and there may be found those of fusiform and vermiform. In such nuclei, the nuclear threads coalesce in places into thicker ones and deep staining of these parts is conspicuous.

In a more advanced state of cell degeneration, due to the stigma reaction, the nucleus becomes obscure in contour and is melted into cytoplasm (karyolysis). In very withered stigma cells, pollinated naturally in the field, it is usual that the nucleus is broken into pieces as globules stained red with the carmine. In these globules no structure can be seen.

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Explanation of Plate

Figs. 1–4, are photomicrographs of stigma filaments in *Secale cereale*. They are taken from aceto-carmine preparations. Magnification in all figures is ca. 300×, except for Fig. 4, which is ca 600×.

Fig. 1. While nuclei are stained in two stigma filaments, into each of which a pollen tube has entered, those in other filaments with no pollen grain, remain unstained.

Fig. 2. Nuclei are stained in only one filament, to which a pollen grain attaches.

Fig. 3. Stigma filaments in prolonged staining. Nuclei are stained in all filaments, whether they are pollinated or not.

Fig. 4. Young stigma filaments showing no projection of their composing cells.