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A Study of Tropism of Pollen Tubes to the Pistils

VI. Behaviour of Pollen Grains to Stigma of Different Stages in Development

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In the preceding papers it has been reported that some pistils in florescence stage contain certain active substances which induce the positive or negative tropism of pollen tubes and control the pollen germination and pollen tube growth (Miki, 1954, 1955; Miki-Hirosige, 1961a and b). It is not clear, however, whether immature pistils contain these active substances or not. In *Lilium*, existence of these substances is confirmed more evidently in stigmas than in other parts of the pistils. It is aimed, therefore, in the present study to see effects of the active substances in different stages of stigma tissues in development on the tropism reaction of pollen tubes, pollen germination and the pollen tube growth.

Stigmas of *Lilium longiflorum* in the following four stages, in respect to the development of embryo sacs, were used.

Stage A. Embryo sac mother cell stage.
Stage B. Primary 2–4 nucleate stage (meiosis stage).
Stage C. Secondary four nucleate stage (florescence stage; early embryo sac development).
Stage D. Eight cell stage (four days after florescence; no pollination).

Tropism reaction of pollen tubes, germination and tube growth of pollen grains spread around stigma slices in the four stages stated above were examined. Control tests were made without the stigma slices. Methods of preparing the stigma slices and of culturing pollen grains were described in details in the previous paper (Miki, 1954).

Result obtained in the present study is shown in the following table.

In this table, it is seen that while in Stage A the percentage of the positive tropism reaction of pollen tubes to the stigmas is nearly equal to that of the control; in Stage B, the value is markedly higher than that in Stage A. The percentage reaches the maximum in Stages C and D. It must be emphasized

Table 1. Tropism reaction, germination and tube growth of pollen grains spread around the stigma slices in various growth stages of *Lilium longiflorum*.

Growth stages	Control	A	B	C	D
% of germinated pollen grains	24	66	68	90	84
Tube length (mm)	0.3	0.6	0.8	1.1	0.7
% of positive tropism	22	23	52	88	86

here that in Stage A the styles and the ovaries do not affect the tropism reaction of pollen tubes.

Both pollen germination and pollen tube growth are promoted in Stages A, B, C and D in the presence of the stigma tissues (Table 1). The promotion reach the maximum in Stage C.

From the results stated above, it is concluded that, while the substances which promote the pollen germination and the pollen tube growth are contained in the stigma tissues in all stages in pistil development (Stages A–D), the substance which induces the positive tropism appears in Stages B–D. It must be noted here that the substance which promotes the tube growth reaches the maximum in Stage C.

YASUDA (1930) has reported that in the style of a floral buds of *Petunia* the rate of the tube growth of the pollen grains from the same flower is rapid and the growth is normal in the bud stages as compared with those in the flower-opening stage. He has expressed the view that the special substance which inhibits the growth of the pollen tubes is not yet produced when the flowers are still in buds. KAKIZAKI (1930) and others have reported in *Brassica* that, while the self incompatibility exists in flower-opening stage, old flowers of three or four days after the florescence acquire the force of self fertilization, and have arrived at a conclusion that the secretion of the special substances will become weak as the physiological function is lost in the old flower.

Conclusion and Discussion

Results obtained in a series of the experiments carried out by the present investigator show that the pistils of many higher plants contain certain active substances which control the behaviour of the pollen grains. If the induction of positive tropism (MIKI, 1954, 1955), induction of negative tropism (MIKI, 1959; MIKI-HIROSIGE, 1961 a), promotion of pollen germination (MIKI-HIROSIGE, 1961 b), inhibition of pollen germination (MIKI-HIROSIGE, 1961 a and b), promotion of pollen tube growth (MIKI-HIROSIGE, 1961 b) and inhibition of pollen tube growth (MIKI-HIROSIGE, 1961 a) are caused from different substances respectively, six substances may be assumed. These substances are summarized in Table 2.

Table 2. Substances contained in pistil tissues which affect the behaviours of pollen grains.

Characters of substances	Plant names of pistils	Plant names of reacted pollen grains	Heat stability	Diffusion to agar film	Permeability of collodion or cellulose membrane	Extracted solvent	Literature
Induction of positive tropism	*Lilium longiflorum*	*Lilium longiflorum*	unstable	diffusible	permeable		MIKI, 1954
	Camellia sinensis	*Camellia sinensis*	unstable	diffusible	permeable		MIKI, 1955
Induction of negative tropism	*Primula obconica*	*Primula obconica*	stable	diffusible	impermeable	distil. water	MIKI-HIROSIGE, 1961 a
	*Camellia sinensis**	*Camellia sinensis*	stable	diffusible	impermeable	ethyl ether distil. water	MIKI, 1959
Promotion of pollen germination	*Lilium longiflorum*	*Lilium longiflorum*	stable	diffusible	permeable	distil. water	MIKI-HIROSIGE, 1961 b
	Hippeastrum hybridum	*Hippeastrum hybridum*	stable	diffusible	permeable	distil. water	
Inhibition of pollen germination	*Primula obconica*	*Primula obconica*	stable	diffusible	impermeable	distil. water	MIKI-HIROSIGE, 1961 a
	Gladiolus gandavensis	*Lilium longiflorum* *Hippeastrum hybridum* *Antirrhinum majus*	stable	diffusible	impermeable	distil. water chloroform	MIKI-HIROSIGE, 1961 b
Promotion of pollen tube growth	*Lilium longiflorum*	*Lilium longiflorum*	stable	diffusible	permeable	distil. water	MIKI-HIROSIGE, 1961 b
	Hippeastrum hybridum	*Hippeastrum hybridum*	stable	diffusible	permeable	distil. water	
Inhibition of pollen tube growth	*Primula obconica*	*Primula obconica*	stable	diffusible	impermeable	distil. water	MIKI-HIROSIGE, 1961 a

* Steamed pistil.

In spite of the above assumption, it is not established definitely in this series of experiment whether all of these substances are different or not. Some considerations on this question are given as follows:

1) *Tropism inducing substances.* Two substances which induce the tropism reaction of pollen tubes are found in the pistil tissues; one induces the positive tropism and the other does the negative. The former substance is found in the fresh pistils of many phanerogams such as *Lilium*, *Heppiastrum* and *Narcissus* (MIKI, 1954, 1961), and the latter is found in the fresh styles of *Primula obconica* (MIKI-HIROSIGE, 1961). In *Camellia*, however, the fresh pistil tissues induce the positive tropism, while the tissues of pistils treated at 99°C induce the negative tropism (MIKI, 1955). Therefore, it is concluded that the substance which induces the positive tropism in *Camellia* and *Lilium* is metastable to heat. Moreover, this substance diffuses through both the collodion and cellulose membranes. Contrary to the above substance, the substance which induces the negative tropism is stable to heat and does not diffuse through these membranes.

Considering the facts stated above, it is quite safe to conclude that these two substances are quite different. In the pistils of *Lilium*, it is assumed that only the substance which induces the positive tropism in contained (MIKI, 1954), while the style of *Primula obconica* contains only the negative tropism inducing substance. In *Camellia sinensis*, however, it is assumed that both the positive and the negative tropism inducing substances are contained is the pistils, and that the former is more active in fresh tissues. When the pistils are heated, only the former substance is destroyed while the latter exhibits its activity (MIKI, 1955).

2) *Germination and tube growth controlling substances.* In many plants such as *Lilium*, *Narcissus*, *Camellia* and *Heppiastrum*, both the germination and the tube growth of pollen grains near the pistils of the same species are promoted, but this promoting effect is not recognizable in the pollen grains not close to the pistils (MIKI, 1954, 1961).

Then, it is questioned whether the tropism controlling substances are different from the germination and the tube growth controlling substances or not. The results of the experiment reported in this paper suggest a strong probability that these two substances are different, because the substances which promote the germination and tube growth are contained in stigma in all stages in the course of pistil development, while the substance which induces the positive tropism appears only in the later stages of the pistil development.

Then comes the question that whether the germination controlling substance differs from the tube growth controlling substance or not. Unfortunately, this question is not clearly solved in this series of experiments, but the result obtained in the experiment reported in 1961 probably supports a view that the germination and the tube growth are controlled by different active substances

(MIKI-HIROSIGE, 1961b). In this experiment it is confirmed that, when the pollen grains of *Hippeastrum* are spread around the style slices of *Clivia*, the pollen germination is markedly arrested while the tube growth is slightly promoted. Moreover, when the pollen grains of *Hippeastrum* are spread around stigma slices of *L. longiflorum*, the tube growth is arrested while the germination coefficient is almost equal to that of the control.

There is another question whether the pollen germination promoting substance and the pollen germination inhibiting substance are same or not. The results of the experiment carried out by the present investigator on *Primula obconica* are regarded sufficient to give a certain clue to solve this question (MIKI-HIROSIGE, 1961a). It is observed in this plant that in the pollen grains close to the style slices the germination of pollen grains are inhibited, while in the pollen grains away from the style slices the germination is promoted. It is fair, therefore, to assume that both the inhibition and the promotion are caused from different concentrations of an active substance. At least in *Primula*, however, there is no reason to infer that the germination promoting substance and the germination inhibiting substance are different. While in *Primula* the germination controlling substance does not diffuse through the cellulose and collodion membranes, in *Lilium* and *Hippeastrum* the germination promoting substance diffuses through these membranes. Therefore, it is assumed that the germination promoting substance in *Lilium* and *Hippeastrum* and the germination controlling substance in *Primula* are different. Then, it is inferable that the substances which promote the germination are not always the same.

Similar relation between the tube growth controlling substance in *Primula* and the tube growth promoting substance in *Lilium* and *Hippeaserum* is observed. Therefore, it is assumed that the tube growth promoting substance in *Lilium* and *Hippeastrum* and the tube growth controlling substance in *Primula* are different.

In the series of this experiment, it is not observed that the pollen grains or the pollen tubes show a special behaviour around a special part of the pistil. For example, in *Lilium* the tropism reaction around stigma slices is stronger than that around the style or the ovary slices, while in *Camellia sinensis* it is stronger around style slices than that around the other.

Generally speaking, it is concluded that the positive tropism reaction, the promotion of pollen germination and that of the pollen tube growth are seen when the relationship between the plant from which the pistil is taken and the plant from which the pollen grains are taken is near, but this tendency is hardly observed when the relationship between the two plants is remote. It must be noted here, however, that a few exceptional cases are observed in this series of the experiment. These facts may be explained as follows: Some active substances, which control the tropism reaction, the germination and the tube growth, are contained together in the pistils of higher plants, and the behaviours of the pollen grains to the pistils are caused from the difference in localization of the active substances, difference in the time in which the active substances are produced, difference in concentration of these substances and other factors.

Summary

1. Tropism reaction, germination and tube growth of pollen grains spread around the stigma slices are studied in different stages in pistil development of *Lilium longiflorum*.

2. Substances that promote the pollen germination and the pollen tube growth are detected in the stigma tissues in all stages of development, while the substance which induce the positive tropism is found only in the later stages.

3. Relation among the substances which control the tropism, the germination and the tube growth of pollen grains is discussed.

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