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AUTHOR(S):

Miki-Hirosige, Hisako

CITATION:

Miki-Hirosige, Hisako. A Study of Tropism of Pollen Tubes to the Pistils (V) : Negative Tropism, Germination and Tube Growth of Pollen Grains in Primula obconica. Memoirs of the College of Science, University of Kyoto. Series B 1961, 28(3): 365-373

ISSUE DATE:

1961-12-20

URL:

http://hdl.handle.net/2433/258218

MEMOIRS OF THE COLLEGE OF SCIENCE, UNIVERSITY OF KYOTO, SERIES B,
Vol. XXVIII, No. 3, Article 8 (Biology), 1961

A Study of Tropism of Pollen Tubes to the Pistils
V. Negative Tropism, Germination and Tube Growth of Pollen Grains in *Primula obconica*

By

Hisako MIKI-HIROSIGE[1)]

Botanical Institute, College of Science, University of Kyoto

(Received July 21, 1961)

In 1955, the present author reported that the pollen tubes of *Camellia sinensis* showed a negative tropism to the steamed pistil slices of the same species, but this phenomenon was not observed in fresh pistils (MIKI, 1955). During the course of study of tropism reaction of the pollen tubes to the pistils of many higher plants (MIKI, 1961), it has been observed that pollen tubes of *Primula obconica* show negative tropism to the fresh style slices of the same species. It is the aim of the present study to see negative tropism, germination and tube growth of pollen grains in *Primula* in detail.

Material and Method

Pollen grains and pistils of *Primula obconica*, *P. japonica*, *P. sinensis* and *P. malacoides* were used as material. As the culture media of the pollen grains, 1.5% agar solution containing 25% sucrose was used. Methods of preparing pistil slices and of pollen grain culture were described in detail in the previous paper (MIKI, 1954).

Results

In the following five experiments are contained. In Exp. 1, tropism reaction of pollen tubes, pollen germination and pollen tube growth around the fresh pistil tissues (viz. stigma, style, ovary and ovule) are studied *in vitro*. Experiments 2 and 3 are concerned with existence of the active substances which inhibit the pollen germination, arrest the pollen tube growth and induce negative tropism, and in Exp. 4, stability of these active substances to heat is examined, and in Exp. 5, extraction of these substances from styles is attempted.

1) Formerly Hisako MIKI.

Exp. 1. *Tropism reaction, germination and tube growth of pollen grains spread around pistil slices on a culture medium.*

a. *Reaction of pollen grains around pistil tissues of* Primula obconica. Tropism reaction of pollen tubes, pollen germination and tube growth around stigma, style, ovary slices and ovules of *Primula obconica* were examined. Control tests were made without pistil slices.

Results obtained in this experiment are shown in Table 1.

Table 1. Pollen germination, tube growth and tropism reaction around the pistil slices in *Primula obconica.*

Objects	Control	Stigma	Style					Ovary and Ovule
Distance from a pistil tissue to pollen grains		Close to objects	Close to objects	Outside the germination inhibiting zone	Outside the intermediate zone	Not close to the intermediate zone	Outside the negative tropism zone	Close to objects
% of germinated pollen grains	14	16	0	2 – 10	14	52 – 78	14	14
Tube length (mm)	0.2	0.2	0	0.02 – 0.1	0.2	0.3 – 0.4	0.2	0.2
Tropism reaction	±	±	——	*	—	—	±	±
Name of zone			Germination inhibiting zone	Intermediate zone	Negative tropism zone			

* Tropism reaction is not clear since the pollen tubes are very short.

In this table it is seen that around the ovules, ovary and stigma slices the pollen germination and tube growth are not promoted nor arrested as compared with the control, and the pollen tubes show random tropism. On the contrary, around the style slice, pollen grains close to the style slice do not germinate ("germination inhibiting zone"). In most cases, the width of the "germination inhibiting zone" is about 1 mm. Some of the pollen grains outside of the "germination inhibiting zone" germinate and the germination percentage increases gradually as their distance from the slice increases, but their tropism reaction is not clear because the pollen tubes are very short ("intermediate zone"). Outside the "intermediate zone" pollen germination and tube growth are nearly equal to those of the control. Outside this region germination percentage of pollen grains and rate of the tube growth are higher than those of the control. In these two regions, pollen tubes show negative tropism to the style slice ("negative tropism zone", see Fig. 1). Outside the "negative tropism zone", pollen germination and tube growth are nearly equal to those of the control and the tubes show random tropism.

In this experiment, it is observed that the pollen tube growth is inhibited

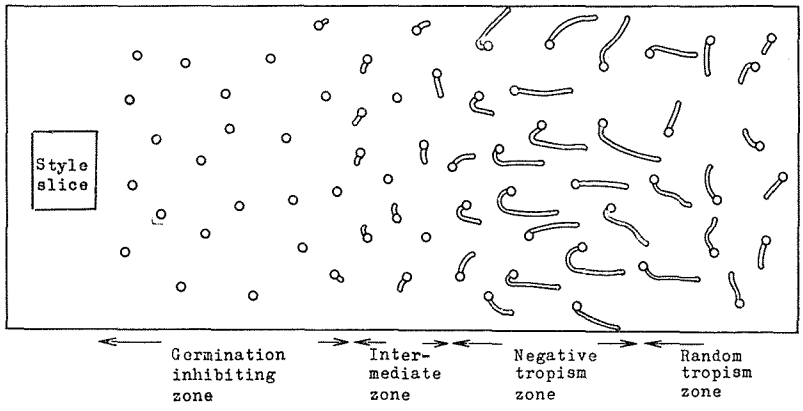

Fig. 1. Behaviours of pollen grains around a fresh style slice in *Primula obconica*.

near the style slice. In the next experiment, the effect of the style slice to the tube growth is tested.

b. *Effect of the style slice to germinated pollen tubes.* Pollen grains were spread on agar medium, and after 2 hours style slices were placed near these pollen grains which had germinated by that time. After 2 additional hours they were observed under microscope. For the control, the tube growth of the pollen grains spread on the medium without style slices was tested.

The results of this experiment show that growth of pollen tubes which are found close to the style slice is arrested, but the pollen tubes which are found remote from the slice go on their elongation as same as those of the control, and show negative tropism to the style slices.

c. *Reactions of pollen grains of various combinations in heterostyle-heteroanther flowers. Primula obconica* has heterostyle-heteroanther flowers, so that the following six combinations among the long-styled flowers and the short-styled flowers are possible.

Long-styled flower (pistil)×Long-styled flower (pollen), in the same flower.

Long-styled flower (pistil)×Long-styled flower (pollen), between different flowers.

Short-styled flower (pistil)×Short-styled flower (pollen), in the same flower.

Short-styled flower (pistil)×Short-styled flower (pollen), between different flowers.

Long-styled flower (pistil)×Short-styled flower (pollen), between different flowers.

Short-styled flower (pistil)×Long-styled flower (pollen), between different flowers.

For each of the above combinations, tropism reaction of pollen tubes, pollen germination and tube growth were tested.

The results obtained in this experiment are quite similar to those obtained in Exp. 1, a. That is, around the ovules, ovary and stigma slices, pollen germination and tube growth of pollen grains are nearly equal to those of the control and pollen tubes show random tropism. Around the style slice, however, pollen grains close to the style slice do not germinate ("germination inhibiting zone"). Outside the germination inhibiting zone, the germination percentage of pollen grains increases gradually as a distance from the pollen grains to the slice increases, but their tropism reaction is not clear because the pollen tubes are very short ("intermediate zone"). Away from the intermediate zone, the pollen germination and the tube growth are more markedly promoted than those of the control, and the pollen tubes show negative tropism to the style slice ("negative tropism zone").

It must be added here that, in *P. obconica*, the percentages of the germinated pollen grains and the tube growth of the two types of pollen grains (large pollen grains obtained from long-styled flowers, and small pollen grains obtained from short-styled flowers) do not show any difference. CORRENS (1889) and DAHLGREN (1918) reported that no difference in germination percentage was found between the two types of pollen grains obtained from the two types of flowers of *Primula*. Our results are quite in agreement with their reports.

d. *Reactions in other species of* Primula. Using stigma slices, style slices, ovary slices and ovules of *P. japonica*, *P. sinensis* and *P. malacoides*, the same tests as in Exp. 1, a have been carried out.

In this experiment, it is observed that, around the pistil tissues of these plants, the germination percentage and the rate of tube growth of the pollen grains from the same species are nearly equal to those of the control. These pollen tubes do not show negative tropism to the pistil slices.

From the results obtained in Exp. 1, it is presumed that an active substance or substances, inducing negative tropism and controlling the pollen germination and the tube growth, is contained in the style tissues. Experiments 2 and 3 are undertaken to confirm the existence of such substances.

Exp. 2. *Diffusion of active substances from styles to agar media.*

Style slices from long-styled flowers placed on the surface of an agar film on a slide glass were kept for various lengths of time in a Petri-dish with a moist filter paper. Then, the style slices were removed immediatly after spreading the pollen grains from long-styled flowers around the slices. Tropism reaction of pollen tubes, pollen germination and tube growth around the places on the culture medium from where the style slices had been removed were examined (the empty place). For comparison, culture media carrying the pollen grains only, or both the pollen grains and the style slices, were used.

In this experiment, it is observed that around the empty place from style slices there are the inhibition and promotion of pollen germination and tube growth as well as the negative tropism reaction of pollen tubes, as in the case of Exp. 1, a.

Distances from a style slice to germinated pollen grains within width of "germination inhibiting zone" at various time intervals are shown in the following table.

Table 2. Distances from the place formerly occupied by style slices, within whose distances pollen germination is inhibited.

Styles are placed on agar film for (min.)	30	60	90	120	180
Distance from a style slice (mm)	0.4	0.4	0.5	1.0	1.1

From this table the following conclusion may be obtained: the longer the style slices are kept on the agar culture medium, the wider becomes the "germination inhibiting zone".

From the results obtained in Exps. 1 and 2, it is concluded that the negative tropism reaction of pollen tubes, the inhibition and promotion of the pollen germination and the tube growth are caused by some active substances which diffuse from the style slices on the agar medium.

Exp. 3. *Diffusion of the active substances through a cellulose*[2] *or collodion membrane.*

In this experiment it was examined if the active substances stated above would diffuse through cellulose or collodion membranes. To solve this question, style slices wrapped in cellulose and collodion membranes were placed on the agar media. Then, the pollen grains were spread around these wrapped slices. Some control tests were made with unwrapped style slices, and cellulose and collodion membranes without the style slices.

Results obtained in this experiment are shown in the following table.

Table 3. Pollen germination, tube growth and tropism reaction around the style slices wrapped in a collodion or cellulose membrane.

Objects	Wrapped style	Unwrapped style	Collodion or cellulose membrane only
% of germinated pollen grains	14	0	16
Tube length (mm)	0.2	0	0.2
Tropism reaction	±	—	±

In this table, it is seen that around the wrapped style slices tropism reaction of pollen tubes, pollen germination and tube growth do not show any appreciable difference from those obtained in the control test. Hence it may be assumed that the substances, which induce the negative tropism and control

2) Cellulose membrane made by Visking Co. (U.S.A.) was used. Pore diameter of the membrane is reported to be 24 Å.

the pollen germination and the tube growth, do not diffuse through the cellulose and collodion membranes.

Exp. 4. *Tropism reaction, pollen germination and tube growth around steamed style slices.*

The author has already reported that the substances which induce the positive tropism of pollen tubes in *Lilium longiflorum* and *Camellia sinensis* are metastable to heat (MIKI, 1954, 1955). In Exp. 4, the heat stability of the active substances, existence of which was proved in Exp. 3, was tested.

A test tube containing styles and a moist filter paper was kept in boiling water for 10 minutes. These steamed styles were then cut longitudinally and placed on the culture media. Tropism reaction, percentage of germinated pollen grains and the tube length around the style slices were studied.

In this experiment similar results to those obtained in Exp. 1 are obtained: The pollen grains close to the steamed slice do not germinate ("germination inhibiting zone"). Away from this zone, the pollen tubes are very short while the pollen grains germinate ("intermediate zone"). Outside this zone, both germination and tube growth are promoted and the pollen tubes show negative tropism to the slice ("negative tropism zone"). From these results it is concluded that the agents, which control the pollen germination and the tube growth and induce the regative tropism, are heat stable.

Exp. 5. *Extraction of active substances.*

Styles (0.1 g) were ground in a mortor with distilled water (1 ml) at about 5°C, and the suspension was centrifugated at ca. 1,500 g for 10 minutes at room temperature. A clear supernatant was obtained. Part of the supernatant was diluted 10, 100 and 10,000 times with distilled water. Strips of filter paper steeped in these four water solutions in different concentrations were dried. Pollen grains were spread on these filter papers on the culture medium. For control, strips of filter paper dried after being steeped in distilled water were placed on the culture medium.

Results obtained in this experiment are shown in Table 4.

Table 4. Tropism reaction, pollen germination and tube growth around the strips of filter paper steeped in water extracts from the styles*.

Concentration	Control	0.1 g/1 ml	0.1 g/10 ml	0.1 g/100 ml	0.1 g/10,000 ml
% of germinated pollen grains	14	0	5	12	12
Tube length (mm)	0.2	0	0.08	0.1	0.2
Tropism reaction	±	——	±	±	±

* Pollen grains within about 0.7 mm from the filter papers are counted.

In this table it is seen that in the concentration of 0.1 g/1 ml pollen grains do not germinate within about 0.7 mm from the filter paper (the "germination

inhibiting zone"), but in diluted extracts pollen grains germinate and they show random tropism. The growth of the pollen tubes is arrested in the cases of 0.1 g/10 ml and 0.1 g/100 ml. It is also seen that in the concentration of 0.1 g/1 ml, some of the pollen grains germinate outside the germination inhibiting zone, and the germination percentage increases gradually as distances from pollen grains to the filter paper increase, but the pollen tubes are very short ("intermediate zone"). Outside this zone, germination and tube growth are more markedly promoted than those of the control and the pollen tubes show negative tropism to the filter paper ("negative tropism zone"). Hence, it is assumed that these active substances are water soluble.

Conclusion and Discussion

In *Primula obconica* it is observed that around the style slice the pollen grains close to this slice do not germinate ("germination inhibiting zone"). Some of the pollen grains outside this zone germinate, and the germination percentage increases gradually as their distances from the slice increase, but the tropism reaction of the pollen tubes is not clear because the emerging tubes are very short ("intermediate zone"). Outside this zone germination and tube growth are nearly equal to those of the control. Outside this region germination and tube growth are markedly promoted compared with those of the control. In these two regions, pollen tubes show negative tropism to the style slice ("negative tropism zone"). Outside the "negative tropism zone", it is observed that germination and tube growth are nearly equal to that of the control and their tubes show random tropism.

It is assumed that an active substance or substances are contained in the style tissues of *P. obconica* judging from the results obtained in Exp. 1; they include a substance which controls the pollen germination, a substance which controls the pollen tube growth and a substance which induces the negative tropism. But it is not clear from this experiment whether the substances stated above are identical or not. From the results of Exp. 4, it is concluded that the substances stated above are heat stable. Moreover, it may be assumed that molecules of the substances are not small, because these substances do not diffuse through the collodion and cellulose membranes (Exp. 3). These active substances may be regarded as to be water soluble, because they are extracted from style tissues with distilled water (Exp. 5).

The present author has reported (1954, 1955) that some active substances which induce the postitive tropism to the pollen tubes of the same species are contained in the pistils of *Lilium longiflorum* and *Camellia sinensis*, and that the substances are metastable to heat, diffuse into the agar medium and pass the collodion membrane. She has also reported (1955, 1959) that some active substances which induce the negative tropism to the pollen tubes are contained in steamed pistil tissue of *Camellia sinensis*, and that they are heat stable,

diffuse from pistil tissues to agar medium, but do not diffuse through the membrane.

From the results obtained in Exp. 1, it is observed that, when the pollen grains are close to the style slice, both germination and tube growth are inhibited, while when the pollen grains are separated from the "germination inhibiting zone" the pollen germination and tube growth are promoted. These characteristics will be discussed in a next paper. The germination and growth inhibiting substances show an inhibiting effect in high concentration, while in low concentration they show promoting effect. BRANSCHEIDT (1930) and KUHN (1937) have reported that the existence of an active substance which promoted the pollen germination and that the percentage of pollen germination markedly decreased when the germination promoting substance was concentrated. The above interpretation supports these reports.

JOST (1907) reported that in *Hippeastrum aulicum* the pollen germination was inhibited on a culture medium around the stigma tissue of the same flower. YASUDA (1928, 1931) assumed in *Petunia violacea* existence of a germination inhibiting substance which was contained in the ovary and diffused from the ovary to the style. The present author has reported in the previous paper that pollen germination of *Lilium longiflorum*, *Hippeastrum hybridum* and *Antirrhinum majus* is inhibited around the pistil slice of *Gladiolus gandavensis* on a culture medium (MIKI, 1961). The results stated above are quite in accordance with the view derived from the present investigation that a pollen germination inhibiting substance is contained in the pistils of some plants.

Summary

1. In *Primula obconica* tropism reaction of pollen tubes, pollen germination and pollen tube growth around the ovules, ovary, style and stigma slices are studied.

2. Rates of pollen germination and pollen tube growth around the ovule, ovary and stigma slices are nearly equal to those of the control. The pollen tubes show random tropism. Contrary to the above cases, around the style slice, the pollen grains close to the style slice do not germinate ("germination inhibiting zone"). Some of the pollen grains germinate outside the germination inhibiting zone but the pollen tubes are very short. Here tropism reaction is not clear. The germination percentage, however, increases gradually as the distances from pollen grains to the slice increase ("intermediate zone"). Outside this zone, germination and tube growth are more markedly promoted than those of the control, and the pollen tubes show negative tropism to the style slice ("negative tropism zone"). Outside the "negative tropism zone" the germination and the tube growth are nearly equal to those of the control and the pollen tubes show random tropism.

3. In six combinations between long-styled flowers and short-styled flowers,

similar results are obtained to the above case.

4. The style tissue contains some active substances which induce the negative tropism of pollen tubes and control the germination and the tube growth of pollen. The substances are heat stable and water soluble, and diffuse from the style to an agar medium, while they do not diffuse through collodion and cellulose membranes.

The author takes pleasure in expressing her sincere appreciation for helpful suggestions throughout this work to Professor N. SHINKE and Dr. K. KATÔ of Kyoto University.

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