

A Study of Tropism of Pollen Tubes to Pistils
III. Negative Tropism of Pollen Tubes in *Camellia sinensis*

By

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Several investigators (MOLISCH, 1894; MIYOSHI, 1894; LIDFORSS, 1909; BRINK, 1924; TSAO, 1949) reported that the pollen tubes showed positive tropism to pistils in some higher plants in vitro. In *Lilium longiflorum* and *Camellia sinensis*, pollen tubes show positive tropism to substances which diffuse from slices of the fresh pistils (MIKI, 1954, 1955). Experiments were carried out to clear the nature of these substances, but we were not able to determine them. During the course of these investigations, however, we have observed that pollen tubes show negative tropism to styles of *Camellia sinensis*, when the styles are killed by hot steam (MIKI, 1955). In the present investigation we have attempted to extract these active substances which are responsible for negative tropism in this plant, and tested the response of the pollen tubes to different concentrations of the extract from the styles.

Method of Extraction

Pistils of *Camellia sinensis* were killed by steam in a test tube at 99°C for 10 minutes followed by removal of the styles from the pistils. Then, the styles of 0.4 g were ground in an agate motor with some distilled water. The suspension was centrifugated at 4,000×g for 10 minutes at room temperature. Clear supernatant was obtained. It was then put in an evaporating dish and dried up on a boiling water bath. Yellow residue was left in the dish. 1 ml of distilled water was added to this residue. Small strips of filter paper¹⁾ were steeped in this solution and they were dried. These paper strips were put on an agar layer, about 2 mm thick, on a slide glass. Then, pollen grains were spread around the strips, leaving a clear zone with about 0.2 mm width from the strip. The slide glass was placed in a Petri dish and was kept in an incubator at 30°C. After 2 hours, grade of the tropism of pollen

1) The filter paper used in this test was No. 6 made by TOYO ROSHI Co., Ltd, and it was cut into 0.3 cm in width and 2 cm in length.

tubes to the strips was determined. In this test, it was observed that all pollen tubes show "negative tropism" to the strips.

In the previous paper, the present author reported that the tips of all pollen tubes first elongated toward the steamed pistil slices, and then they elongated toward the opposite direction of the slices when the tips arrived at a distance of 0.2~1 mm from the slices (MIKI, 1955). No pathological appearances were observed either in the germination of the pollen grains or in the elongation of the pollen tubes. Tropism of this type was designated as "negative tropism" in the previous paper (MIKI, 1955).

Similar result was obtained when absolute ethyl alcohol or ethyl ether was used for extraction. Therefore, it is concluded that the substances which are responsible for negative tropism in this plant, are extractable by water, ethyl alcohol or by ethyl ether.

Experiments

Exp. 1. *Relative Solubility of the Substances to Water and Ether.*

To 10 ml of ether extract (ether extract *a* in Fig. 1) from steamed styles, was added 10 ml of distilled water. This mixture was shaken in a separated funnel and separated into the ether extract *b* and the water extract *a*. Then, the water extract *a* was shaken with 10 ml of ethyl ether followed by separation of the mixture into two fractions, that is, the water extract *b* and the ether extract *c*. Strips of filter paper were steeped in these four fractions and dried on a water bath. The tropic response of pollen tubes to the strips obtained from each fraction, was tested. The results are given below.

Steamed styles: All pollen tubes showed negative tropism to the slices of the steamed styles.

Ether extract a: All pollen tubes showed negative tropism to the strips which had been steeped in this solution.

Ether extract b and c: Pollen tubes elongated quite at random²⁾ in respect to direction, when the strips were steeped in these extracts.

Water extract a and b: All pollen tubes showed negative tropism to the strips which had been steeped in these extracts.

In these cases all pollen grains germinate normally and pollen tubes elongate smoothly. The results of the tests stated above are shown in the following figure.

2) Tropism of this type is designated as "at random tropism" in this paper.

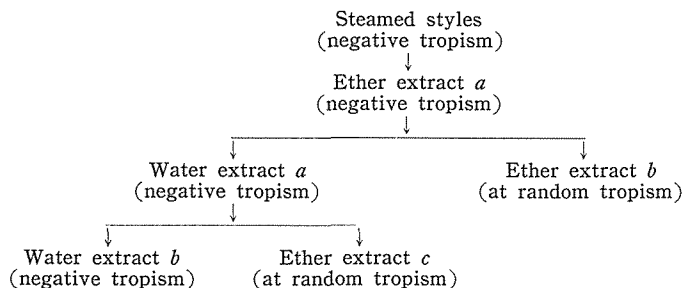


Fig. 1. The appearance of tropism of pollen tubes of *Camellia sinensis* to the ether extracts and the water extracts from the steamed styles.

From the above results, it is concluded that the substances in question are water soluble. Therefore, only the water extraction was used in the following tests.

Exp. 2. *Effect of Extract in Different Concentration.*

0.4 g of styles were ground with some distilled water as in the method of extraction. Then, the suspension was centrifugated. Clear supernatant was dried up on a boiling water bath. The residue which was obtained by this method was dissolved in 1 ml distilled water. This solution (0.4g/ml) was employed as the standard solution, but the solution probably contains many different substances. For the sake of convenience, however, this solution was used as standard in the germination experiment. This standard solution was successively diluted with distilled water. Strips of filter paper were steeped in these solutions and dried on a water bath. Then, these strips were used to determine the tropic response of pollen tubes as described in the preceding page of this paper. The results obtained in this experiment are given below.

In the case of standard solution, that is 0.4 g/ml solution, all pollen tubes show negative tropism. In the 0.4×10^{-1} g/ml solution, most pollen tubes show negative tropism, but some pollen tubes show positive tropism. In the solution, 0.4×10^{-4} g/ml solution $\sim 0.4 \times 10^{-10}$ g/ml solution, pollen tubes elongate at random and do not show any remarkable tropic response. In all these cases, pollen grains show good germination and their pollen tubes elongate smoothly. We may, therefore, conclude that the active substance to which pollen tubes show the negative tropism is soluble in water, and the substances do not give any injurious effects on the germination of pollen grains nor the elongation of pollen tubes. Isolation of these active substances stands in need of paper chromatography.

Exp. 3. *Hydrogen Ion Concentration.*

Alkaline or acid solution is frequently used for solvents in paper chromatography. Therefore, in this experiment, it was tested whether the alkaline or the acid solution gives some effects on the active substances or not. Water extract from steamed tissues shows pH 4.6. By adding dilute NH_4OH solution, pH of this extract was

adjusted to pH 9.0. In this solution, active substances were not destroyed. By adding dilute hydrochloric acid or dilute acetic acid to this alkaline solution, pH was reduced to pH 4.4. Strips of filter paper were steeped in these alkaline and acid solution and dried. The tropic response of pollen tubes to these strips was tested. Pollen tubes then showed negative tropism to each strip which was steeped in water extract, either alkaline or acid. These results are shown in the following figure.

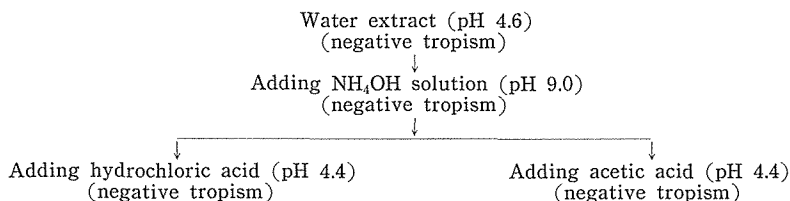


Fig. 2. The appearance of tropism of pollen tubes of *Camellia sinensis* to a different hydrogen ion concentration of the water extract of the steamed styles.

From above result, it is readily seen that, in every medium of different concentrations of hydrogen ions, all pollen tubes show clear negative tropism. From this fact, it is concluded that the activity of the water extract of steamed styles is retained both in alkaline and acid media.

Exp. 4. Paper Chromatography.

Separation of the active substances by paper chromatographic method was carried out. In this experiment, distilled water, ethyl alcohol (70% and 100%), *n*-butanol-acetic-water (4:1:2)³⁾ and *n*-butanol-ammonium-water (4:1:2)⁴⁾ were used as solvents. Development was given in these solvents in 2 hours at 30°C. The filter paper made by TOYO ROSHI Co., Ltd. No. 50 was used in this test. After the development, the filter paper was cut off into 10 equal parts. To each part of them the tropic response of pollen tubes was tested. In all these tests, pollen tubes show negative tropism to the whole part of Rf 9-10, and the middle part of Rf 8.

In this experiment, it is seen that the separation of the active substances is not easy, but this experiment is now under investigation.

Conclusion and Discussion

The active substances, which are responsible for negative tropism of pollen tubes in *Camellia sinensis*, are water soluble and are stable for heat, acid and alkali. Moreover, they are extracted with ethyl alcohol and ethyl ether.

3) pH of this solution was 5.0.

4) pH of this solution was 7.4.

In higher concentration of water extract pollen tubes show negative tropism, and in lower concentration, they elongate at random and show neither the positive nor the negative tropism. Around the strips of the filter paper which are steeped in dilute solution, pollen grains show good germination and the pollen tubes elongate smoothly. Therefore, it is concluded that pollen tubes show the negative tropism to the concentrated solution of water extract, while they do not show the negative tropism to the diluted solution.

TSAO (1949) has reported that the pollen tubes of *Hippeastrum Johnsoni* show positive tropism to the slices of its pistil. TSAO has tried to extract the active substance to which pollen tubes show positive response, but she was not able to separate this substance. In her paper, she has concluded that the active substance was water soluble, and stable for heat, acid and alkali. The substances which are reported in this paper are water soluble, and stable for heat, acid and alkali. In these respects, the substance reported by TSAO resembles the substances reported in this paper, but the latter substances have caused the negative tropism while the substance reported by TSAO caused positive tropism.

Summary

In *Camellia sinensis*, pollen tubes show negative tropism to the slices of the style which was killed by steam in a test tube at 99°C for 10 minutes. It has been shown that this response is caused by special substances (MIKI, 1955). In this paper, extraction of the active substances which are responsible for negative tropism in this plant, have been attempted. The active substances are soluble in water, ethyl alcohol and ethyl ether, and stable for heat, acid and alkali. The pollen tubes show the negative tropism to the concentrated solution of water extract of steamed styles, while they do not show negative tropism to the diluted solution.

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