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Pollen Germination and Pollen Tube Growth in the Presence of Pistil Slices *in vitro*

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Several investigators reported that the pollen germination and the pollen tube growth were promoted in the presence of a stigma tissue of the same species on a culture medium (EAST and PARK, 1918; SASAKI, 1919; KNOWLTON, 1922; BRINK, 1924; GOTOH, 1931; VASIL'EV, 1937; MIKI, 1954). KÜHLWEIN (1948) reported promoting effects of the extracted substances from the pistils upon the pollen germination and pollen tube growth in angiosperms and gymnosperms.

On the other hand, MARTIN (1913) and SASAKI (1919) pointed out that a similar promotion was not observed in the case of *Trifolium*, and in the cases of *Zea* and *Brassica*, respectively.

JOST (1907) reported that the pollen germination was inhibited by stigma tissue of the same plant in *Hippeastrum* on the culture medium.

In the previous paper (MIKI, 1954), the present author reported the same effects of pistil slices on the pollen germination in *Lilium*. In this paper, it is intended to report how the pollen germination and the pollen tube growth of a species are affected by its own pistil slices or those from different species, and it is also attempted to extract active substances which control the germination and the tube growth.

Material and Method

Pollen grains were obtained from anthers which were just at anthesis, and pistils were taken from castrated flowers just bloomed. Names of plants used in the present study would be mentioned in the following descriptions of the results.

As the culture media of pollen grains, 1.5% agar solutions containing sucrose were used. The concentration of the sucrose was always 10%, except for *Vicia*, for which 25% sucrose was used. The hydrogen ion concentration of these media was between pH 6.2 and 6.4.

The hot sugar-agar solution stated above was put on a slide glass to form a layer of about 2 mm in thickness and after cooling slices of pistils were placed

on this agar plate. The methods for preparing pistil slices and a collodion membrane were described in detail in the previous paper (MIKI, 1954). Pollen grains were then spread around these slices with a slender brush. A slide glass, carrying the pistil slices and the pollen grains, was placed in a moist Petri-dish and incubated at 30°C. The germination percentage and the tube length were measured after 1, 2 and 3 hours in the pollen grains which were located within about 1 mm from the pistil slices. But the data after 3 hours were used for comparisons of the results in the following description. In each test the counting was made about 500–600 in number. The percentages in the following tables showed arithmetical mean values of several tests.

In extraction procedures, pistils were ground in a mortor with distilled water at about 5°C; the amount of water being 10 ml for one gram of pistils. The suspension was centrifugated at ca. 1,500 g for 10 minutes at room temperature. A clear supernatant was obtained (water extract 1). An equivalent amount of distilled chloroform was added to the supernatant and the mixture shaken in a separating funnel. The chloroform soluble fraction (chloroform extract) and the water soluble fraction (water extract 2) were obtained.

Strips of filter papers were steeped in the water extracts 1, 2, or the chloroform extract respectively and dried at room temperature. Pollen grains were spread around these filter papers on the culture medium, and percentages of germinated pollen grains and the pollen tube lengths were measured. For control, strips of filter papers, which had been dried after being steeped in distilled water or chloroform, were put on the culture medium.

Results

In the following description five experiments are contained. In Exp. 1, germination and tube growth around the fresh pistil slices (viz. stigma, style and ovary) are studied *in vitro*. Experiments 2 and 3 are concerned with existence of the substances which control the germination and the growth. In Exp. 4, stability of these active substances to heat is examined, and in Exp. 5, extraction of the substances is attempted.

Exp. 1. *Germination and tube growth of pollen grains spread around pistil slices on the culture medium.*

a. *In the same species.* Percentages of the germinated pollen grains and lengths of the pollen tubes around the stigma, the style and the ovary slices of the same species were measured. The plants used were *Lilium longiflorum*, *Narcissus tazetta* and *Hippeastrum hybridum*. Control measurements were made without pistil slices.

Results obtained in this experiment are shown in Table 1.

From this table it is seen that in *Lilium longiflorum* the germination is more markedly promoted in the presence of the stigma and style slices than the presence of the ovary slices. In *Narcissus tazetta*, however, the germination is more

Table 1. Germination and tube growth of pollen grains around pistil slices in the same species.

Plant name	Hours after	Control		Kinds of pistil slices					
				Stigma		Style		Ovary	
		Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
Lilium longiflorum ×*L. longiflorum*	1	0	0	68	0.3	65	0.3	25	0.2
	2	15	0.1	80	0.4	68	0.9	50	0.6
	3	24	0.3	90	1.0	83	1.1	60	1.0
Narcissus tazetta ×*N. tazetta*	1	15	0.05	31	0.1	30	0.1	27	0.1
	2	18	0.05	48	0.2	34	0.3	40	0.3
	3	23	0.15	45	0.3	34	0.3	42	0.4
Hippeastrum hybridum ×*H. hybridum*	1	33	0.1	25	0.03	30	0.05	20	0.03
	2	37	0.3	31	0.2	46	0.3	54	0.3
	3	47	0.5	43	0.6	59	0.6	62	0.7

strongly promoted around the stigma and the ovary slices than around the style slices. In these species, tube growths are strongly promoted and their grades do not show any marked difference in spite of different kinds of sliced tissues. In *Hippeastrum hybridum*, however, the germination around the style and ovary slices is promoted than the germination of the control, but the germination around the stigma slices is nearly equal to that of the control. Tube growths are slightly promoted around these pistil slices than the growth of the control. Among the grades of these tube growths, any marked difference is not seen, irrespective of whatever part of the pistils is used.

In summarizing, the promoting tendency of the germination and the tube growth is conspicuous around the most parts of the pistil slices of the same species.

b. *In the same genus.* In this experiment, the pollen grains of *Lilium longiflorum* were spread around three parts of pistils of *L. elegans* (*L. elegans* ×*L. longiflorum*), and the pollen grains of *Vicia faba* were spread around the stigma and the ovary slices of *V. sativa* (*V. sativa*×*V. faba*).

Results obtained in this experiment are shown in Table 2.

From this table it is seen that both the germination and tube growth of *Lilium longiflorum* are markedly promoted around the pistil slices of *L. elegans*. In the case of *Vicia faba*, however, no discernible promotion is observed in the germination as well as in the tube growth.

c. *In the same family.* In each combination of *Hippeastrum hybridum*× *Narcissus tazetta*, *N. tazetta*×*H. hybridum* and *Clivia nobilis*×*H. hybridum*, the pollen grains were spread around the stigma, style and ovary slices.

Results obtained in this experiment are shown in Table 3.

Table 2. Germination and tube growth of pollen grains around pistil slices in the same genus.

Plant name	Hours after	Control		Kinds of pistil slices					
				Stigma		Style		Ovary	
		Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
Lilium elegans ×*L. longiflorum**	1	0	0	30	0.04	62	0.2	61	0.07
	2	15	0.2	70	1.1	69	1.1	61	1.1
	3	24	0.3	92	2.1	86	2.4	72	2.4
Vicia sativa ×*V. faba*	1	90	0.2	90	0.2			90	0.2
	2	91	0.4	90	0.4			92	0.4
	3	90	0.4	90	0.4			90	0.4

* In the following, A×B means pistils of A species being spread by pollen grains of B species.

Table 3. Germination and tube growth of pollen grains around pistil slices in the same family.

Plant name	Hours after	Control		Kinds of pistil slices					
				Stigma		Style		Ovary	
		Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
Hippeastrum hybridum ×*Narcissus tazetta*	1	15	0.05	30	0.07	34	0.07	24	0.05
	2	18	0.05	34	0.2	42	0.3	26	0.1
	3	23	0.15	33	0.4	45	0.5	31	0.4
Narcissus tazetta ×*Hippeastrum hybridum*	1	33	0.1	26	0.05	31	0.05	40	0.05
	2	37	0.3	49	0.3	47	0.3	46	0.2
	3	47	0.5	49	0.7	53	0.8	42	0.7
Clivia nobilis ×*Hippeastrum hybridum*	3	47	0.5	9	0.4	5	0.6	9	0.3

It is seen in this table that the germination and tube growth of *N. tazetta* are promoted around all the three parts of pistils of *H. hybridum*. Around the stigma slices of *N. tazetta*, the germination of *H. hybridum* is nearly equal to that of the control, and around the style slices it is slightly promoted while around the ovary slices it is slightly arrested, but the tube growth is promoted around the three pistil slices. Around the three pistil slices of *C. nobilis*, however, the germination of *H. hybridum* is markedly arrested. The tube growth around the stigma and ovary slices is slightly arrested, while it is promoted slightly around the style slices.

d. *In the different families.* Combinations of plants used in this experiment are shown in table 4.

Table 4. Germination and tube growth of pollen grains around pistil slices in the different families.

Plant name	Hours after	Control		Kinds of pistil slices					
				Stigma		Style		Ovary	
		Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
Lilium elegans × *Hippeastrum hybridum*	1	33	0.1	0	0	0	0	0	0
	2	37	0.3	16	0.07	18	0.07	28	0.07
	3	47	0.5	25	0.1	32	0.1	38	0.1
Lilium longiflorum × *Hippeastrum hybridum*	1	33	0.1	10	0.05	1	0.02	2	0.02
	2	37	0.3	34	0.2	13	0.1	20	0.06
	3	47	0.5	49	0.2	27	0.2	27	0.2
Lilium longiflorum × *Narcissus tazetta*	1	15	0.05	35	0.07	35	0.07	34	0.07
	2	18	0.05	43	0.1	46	0.2	44	0.1
	3	23	0.15	42	0.2	45	0.3	44	0.2
Hippeastrum hybridum × *Lilium longiflorum*	1	0	0	0	0	0	0	0	0
	2	15	0.1	44	0.5	33	0.6	10	0.3
	3	24	0.3	84	0.8	49	1.0	53	0.4
Narcissus tazetta × *Lilium longiflorum*	1	0	0	0	0	0	0	0	0
	2	15	0.1	0	0	2	0.06	1	0.03
	3	24	0.3	23	0.1	30	0.1	1	0.03
Clivia nobilis × *Lilium longiflorum*	3	24	0.3	7	0.2	20	0.2	0	0
Gladiolus gandavensis × *Lilium longiflorum*	3	24	0.3	0	0	0	0	0	0
Gladiolus gandavensis × *Hippeastrum hybridum*	3	47	0.5	0	0	0	0	0	0
Gladiolus gandavensis × *Antirrhinum majus*	3	17	0.2	0	0	0	0	0	0

Results obtained in this experiment are given in Table 4.

Results of this experiment are classified into following five types:

i) *L. longiflorum* × *N. tazetta* and *H. hybridum* × *L. longiflorum*: In this type, the germination is markedly promoted in all cases, but the tube growth is markedly promoted in some cases and slightly in other cases.

ii) *L. elegans* × *H. hybridum* and *L. longiflorum* × *H. hybridum*: In this type, around the stigma slices of *L. longiflorum* the germination is equal to that of the

control, but it is arrested in the other cases. The tube growth is markedly arrested in all cases.

iii) *N. tazetta*×*L. longiflorum*: Around the style slices the germination is slightly promoted, and around the stigma slices it is equal to that of the control, but around the ovary slices the germination is arrested markedly. In general the tube growth is markedly arrested.

iv) *C. nobilis*×*L. longiflorum*: While around the style slices the germination is slightly arrested, around the stigma slices it is arrested markedly. There is no germination around the ovary slices. The tube growth is slightly arrested in the presence of the stigma and the style slices compared with that of the control.

v) *G. gandavensis*×*L. longiflorum*, *G. gandavensis*×*H. hybridum* and *G. gandavensis*×*A. majus*: In all cases of the combinations, no pollen grain germinates within a distance of about 1 mm from the pistil slices and only those pollen grains beyond this limit are capable of germination (Fig. 1).

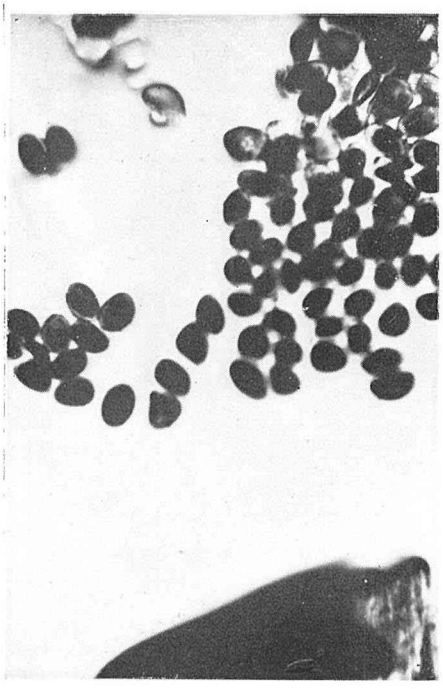

Fig. 1. Inhibition of pollen germination of *Hippeastrum hybridum* spread around the style slice (S) of *Gladiolus gandavensis*.

From Exp. 1, it is seen that the germination and the tube growth are promoted generally in the presence of pistil tissues of the same species. On

the other hand, in the presence of the pistil tissue of remote relationship, the germination is markedly promoted in some cases, and in other cases it is slightly or markedly arrested. In these cases the tube growth is also promoted or arrested independently of the germination.

Here, it may be assumed that the germination and the tube growth are controlled by an active substance or substances contained in the pistil tissue. Experiments 2 and 3 were undertaken to ascertain the existence of such substances.

Exp. 2. *Effects of active substances diffused from the pistil tissue on an agar medium.*

Pistil slices were placed on an agar film on a slide glass and kept 3 hours in a moist Petri-dish. Then the slices were removed immediately after the spreading of the pollen grains around the slices. For control, there were prepared some culture media carrying the pollen grains only, or both the pollen grains and the pistil slices. Names of plants used in this experiment were shown in Table 5.

Results obtained in this experiment are given in Table 5.

Table 5. Germination and tube growth of pollen grains to the active substances diffused from pistil tissues to agar.

Plant name	Control		Removed tissues						Fresh tissues					
			Stigma		Style		Ovary		Stigma		Style		Ovary	
	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
Lilium longiflorum ×*L. longiflorum*	24	0.3	65	1.0	60	0.9	50	0.9	90	1.0	83	1.1	60	1.0
Hippeastrum hybridum ×*H. hybridum*	47	0.5	45	1.1	57	1.1	59	1.0	43	0.6	59	0.6	62	0.7
Gladiolus gandavensis ×*Lilium longiflorum*	24	0.3	0	0	0	0	0	0	0	0	0	0	0	0
Gladiolus gandavensis ×*Hippeastrum hybridum*	47	0.5	0	0	0	0	0	0	0	0	0	0	0	0
Gladiolus gandavensis ×*Antirrhinum majus*	17	0.2	0	0	0	0	0	0	0	0	0	0	0	0

Results of this experiment show that in most cases of *Lilium* and *Hippeastrum* the germination and the tube growth are promoted around the places on the culture medium, from where the pistil slices of *Lilium* or *Hippeastrum* have been removed. Contrary to the above cases, the pollen grains of *L. longiflorum*, *H. hybridum* and *A. majus* do not germinate in the vicinity of the place from where the pistil slice of *Gladiolus gandavensis* has been removed. Hence, it is

stated that some substances, which control the germination and the tube growth (*Lilium* and *Hippeastrum*), or inhibit the germination (*Gladiolus*), are contained in the pistil tissues and that they diffuse from the tissue on the agar culture medium.

Exp. 3. *Germination and tube growth of pollen grains spread around the pistil slices wrapped in collodion and cellulose membranes*[1].

In this experiment, pistil slices were wrapped in a collodion or cellulose membrane, and put on the agar media. Then the pollen grains were spread around these wrapped pistil slices. Some control tests were made with unwrapped pistil slices, and collodion and cellulose membranes without the pistil slice. The names of plants used and their combinations and the results obtained in this experiment were given in Table 6.

Table 6. Germination and tube growth of pollen grains spread around pistil slices wrapped in collodion and cellulose membranes.

Plant name	Collodion or cellulose membrane only		Wrapped tissues in membrane						Unwrapped tissues					
			Stigma		Style		Ovary		Stigma		Style		Ovary	
	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
In same species														
Lilium longiflorum	25	0.4	90	1.0	80	1.0	58	1.0	90	1.0	83	1.1	60	1.0
Hippeastrum hybridum	35	0.6	40	0.6	55	0.6	60	0.6	43	0.6	59	0.6	62	0.7
Narcissus tazetta	25	0.2	43	0.3	50	0.3	40	0.4	45	0.3	34	0.3	42	0.4
In different species														
Hippeastrum hybridum × *Narcissus tazetta*	25	0.2	30	0.4	45	0.5	30	0.4	33	0.4	45	0.5	31	0.4
Hippeastrum hydridum × *Lilium longiflorum*	25	0.4	80	0.8	47	1.0	50	0.4	84	0.8	49	1.0	53	0.4
Lilium longiflorum × *Hippeastrum hydridum*	35	0.6	50	0.2	25	0.2	26	0.2	49	0.2	27	0.2	27	0.2
Lilium longiflorum × *Narcissus tazetta*	25	0.2	40	0.2	43	0.3	42	0.2	42	0.2	45	0.3	44	0.2
Gladiolus gandavensis × *Lilium longiflorum*	25	0.4	24	0.4	23	0.4	24	0.4	0	0	0	0	0	0
Gladiolus gandavensis × *Hippeastrum hydridum*	35	0.6	32	0.6	34	0.6	34	0.6	0	0	0	0	0	0
Gladiolus gandavensis × *Antirrhinum majus*	18	0.2	17	0.2	16	0.2	18	0.2	0	0	0	0	0	0

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

From this table it is seen generally that around the wrapped pistil slices both the germination percentage and the tube length are nearly equal to those around unwrapped pistil slices except the cases of *Gladiolus*. Hence, it may be assumed that the controlling substances diffuse through the membranes.

Around wrapped pistil slices of *Gladiolus gandavensis*, the germination and the tube growth are nearly equal to those of the case without pistil slices. Then, it may be assumed that the inhibiting substances do not diffuse through the membrane.

From the results of Exps. 2 and 3, it is concluded that the pistil tissues contain some active substances, i.e. the controlling substances of the germination and the tube growth.

Exp. 4. *Germination and tube growth of pollen grains spread around the steamed pistil slices.*

In this experiment, the heat stability of the active substances was examined. Test tubes containing a moist filter paper and pistils of *Lilium longiflorum*, *Hippeastrum hybridum* or *Gladiolus gandavensis* were kept in boiling water for 10 minutes. These steamed pistils were cut and then placed on the culture media. Percentage of the germinated pollen grains and tube length around three kinds of the steamed pistil slices were measured. Combinations of used pollen grains and pistils were shown in Table 7. For comparison, culture media carrying the pollen grains only, or both the pollen grains and fresh pistil slices, were used.

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

Table 7. Germination and tube growth of pollen grains around steamed pistil slices.

Plant name	Control		Steamed tissues						Fresh tissues					
			Stigma		Style		Ovary		Stigma		Style		Ovary	
	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
Lilium longiflorum ×*L. longiflorum*	24	0.3	88	1.0	80	1.0	61	1.0	90	1.0	83	1.1	60	1.0
Lilium longiflorum ×*Hippeastrum hybridum*	47	0.5	47	0.2	25	0.2	26	0.2	49	0.2	27	0.2	27	0.2
Hippeastrum hybridum ×*H. hybridum*	47	0.5	42	0.6	59	0.6	60	0.6	43	0.6	59	0.6	62	0.7
Gladiolus gandavensis ×*L. longiflorum*	24	0.3	0	0	0	0	0	0	0	0	0	0	0	0
Gladiolus gandavensis ×*H. hybridum*	47	0.5	0	0	0	0	0	0	0	0	0	0	0	0
Gladiolus gandavensis ×*Antirrhinum majus*	17	0.2	0	0	0	0	0	0	0	0	0	0	0	0

From this table it is concluded that the active substances are heat stable, because the percentages of germinated pollen grains and tube lengths around the steamed pistil slices are nearly equal to those in the cases of fresh pistils. Extraction of the substances from the pistils was undertaken.

Exp. 5. *Extraction of the active substances.*

Pollen grains of *Hippeastrum hybridum, Narcissus tazetta* and *Lilium longiflorum* were spread around dried filter papers which had been steeped in the water extracts 1, 2 or the chloroform extract from the pistils of *H. hybridum* and *L. longiflorum.* Moreover, pollen grains of *L. longiflorum, H. hybridum* and *Antirrhinum majus* were spread around the filter papers which had been steeped in water and chloroform extracts from the pistils of *Gladiolus gandavensis.*

Results obtained in this experiment are shown in Table 8.

Table 8. Pollen germination and tube growth to the water and chloroform extracts from the pistils of *Hippeastrum hybridum, Lilium longiflorum* and *Gladiolus gandavensis.*

Pollen grains	Control				Extract					
	Distil. water		Chloroform		Water extract 1		Water extract 2		Chloroform extract	
	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
a. Extracted from *Hippeastrum.*										
Hippeastrum hybridum	36	0.7	41	0.2	46	0.9	63	0.7	37	0.1
Narcissus tazetta	23	0.1	23	0.2	26	0.1	21	0.2	22	0.2
Lilium longiflorum	35	0.4	20	0.2	44	0.7	30	0.5	15	0.2
b. Extracted from *Lilium.*										
Lilium longiflorum	35	0.4	20	0.2	49	0.9	31	0.7	14	0.3
Hippeastrum hybridum	36	0.7	41	0.2	30	0.1	38	0.7	39	0.7
Narcissus tazetta	23	0.1	23	0.2	34	0.3	18	0.3	25	0.2
c. Extracted from *Gladiolus.*										
Lilium longiflorum	35	0.4	20	0.2	0	0	0	0	0	0
Hippeastrum hybridum	36	0.7	41	0.2	0	0	0	0	0	0
Antirrhinum majus	17	0.3	13	0.2	0	0	0	0	0	0

From this table it is seen that in the presence of an extract from *Hippeastrum,* the promotion of the germination of *Hippeastrum* is conspicuous with both the water extracts 1 and 2 as compared with the chloroform extract. The pollen tube growth of *Lilium* is promoted in the presence of both water extracts, while the germination is promoted with the water extract 1 and is not affected

with the water extract 2, and with the chloroform extract it is slighty arrested. In *Narcissus* the germination is slightly promoted with the water extract 1, and the tube growth is slightly promoted with the water extract 2.

In the presence of *Lilium* extracts, the germination and the tube growth of *Lilium* and *Narcissus* are both promoted with the water extract 1, but with the water extract 2 tube growth is promoted but the germination is almost equal to that of the control. With the water extract 1 of *Lilium*, the germination of *Hippeastrum* is slightly arrested and the tube growth is markedly arrested. This fact is in accordance with the result of Exp. 1, d.

From the above results it comes to a conclusion that the substances in question, which control the germination and the tube growth, are water soluble and that the substances are contained in the water extract 1.

Moreover, each of the three extracts from pistils of *Gladiolus* shows inhibitory effects. These results apparently indicate that the substances are both water and fat soluble.

Using the water extract 1 from *Gladiolus*, effects of diluted concentrations to germination were examined. The water extract 1 which inhibited the germination was diluted 100 and 10,000 times with distilled water. Strips of filter paper were steeped in these solutions and then dried. The pollen grains of *L. longiflorum*, *H. hybridum* and *A. majus* were spread around these filter papers on the culture medium.

Results obtained in this experiment are shown in Table 9.

Table 9. Pollen germinations and their tube growth to dilutions of the first water extract from the fresh pistils of *Gladiolus gandavensis*.

Pollen grains	Control (distil. water)		Concentration of extract			
			1 g/1,000 ml		1 g/100,000 ml	
	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)	Germinated pollen (%)	Tube length (mm)
Lilium longiflorum	35	0.4	23	0.3		
Hippeastrum hybridum	36	0.7	45	0.3	51	0.4
Antirrhinum majus	17	0.2	15	0.1	18	0.1

From this table it is seen that diluted solutions have less inhibitory effect, but rather promote the germination of *Hippeastrum*.

Conclusion and Discussion

In *Lilium longiflorum* and *Narcissus tazetta*, germinations and tube growths of pollen grains are promoted around the pistil slices of the same species; while in *Hippeastrum*, around the style and ovary slices they are promoted and the germination is nearly equal to that of the control around the stigma slices (Exp. 1, a). Jost (1907) reported that in *Hippeastrum aulicum* the germination

was inhibited with the stigma tissue, but in the present study of *Hippeastrum hybridum* pollen grains germinated around the stigma slices of the same species.

In the genus *Lilium*, both germinations and tube growths are promoted around the pistil slices obtained from different species of the same genus. In the genus *Vicia* there is no such promoting effects (Exp. 1, b).

In cases where the sources of pollen grains and pistils are of different genus in the same family (Amarillidaceae), the promoting effect of the germination is observable in most cases in the combinations of *Hippeastrum*×*Narcissus*, and *Narcissus*×*Hippeastrum*, whereas in the combination of *Clivia*×*Hippeastrum* the germination is markedly arrested. The tube growth in these combinations is promoted in most cases, and in some cases it is arrested slightly (Exp. 1, c).

In the cases where the sources of pollen grains and pistils are of different families, the promoting effect to both the germination and tube growth is conspicuous in some cases (e.g. *Hippeastrum hybridum*×*Lilium longiflorum*), and the arresting effect is recognizable in some cases (e.g. *Lilium elegans*×*H. hybridum*), and the inhibiting effect is seen in some other cases (e.g. *Gladiolus gandavensis*×*L. longiflorum*).

So far as the present investigation concerns, it is highly safe to conclude that the pollen germination and the tube growth of one species are generally promoted in the presence of a pistil of the same species, while in the presence of a pistil of remote relationship they are promoted only in exceptional cases, but in most cases they are rather not affected or inhibited.

From the above results, it is seen that the pistil tissue contains a substance or substances which control the germinations and tube growths. The substance is heat stable and diffuses from pistils on an agar culture medium. The substance which is contained in *Lilium* and *Hippeastrum* diffuses through cellulose and collodion membranes, but the substance which is contained in *Gladiolus* does not.

The water extract 1 from the pistils of *Lilium* and *Hippeastrum* promotes the germination and the tube growth. Therefore, it seems that the promoting substances are water soluble.

The next problem is whether the germination controlling substance and the growth controlling substance are the same or not. When pollen grains of *Lilium* and *Narcissus* are spread around the pistil slices of their own species, both the germination and the tube growth are promoted (Table 1). But in the case of *Clivia*×*Hippeastrum*, the germination is strongly arrested while the tube growth is not markedly affected (Table 3). Moreover, when the pollen grains of *Hippeastrum* and *Lilium* are spread around stigma slices of *L. longiflorum* and *Narcissus* respectively, the tube growth is arrested while the germination is almost equal to that of the control (Table 4). From the above results it is concluded that the germination and the tube growth controlling substances must be different. In Table 9, it is observed that, while the germination of *Hippeastrum hybridum* is promoted with the diluted water extract from the

pistils of *Gladiolus*, the tube growth is arrested. This result supports the above presumption.

KUHN (1937) has presumed that the same substance would be responsible for both the promotions of the pollen germination and the pollen tube growth in *Mattiola*. But KÜHLWEIN (1948) has reported that they would be different substances. The results obtained by ADDICOTT (1943) have supported the view that the germination of pollen grains and the growth of the pollen tube are at least in part physiologically independent.

A number of investigators have reported that several chemical substances, such as indole acetic acid, boron, colchicine and Vitamin B_1, promote the pollen germination or the tube growth *in vitro* (SMITH, 1942; ADDICOTT, 1943; O'KELLEY, 1957; FUKUI *et al.*, 1958; and others). But it is not clear whether these chemical substances are working in a pistil tissue or not.

In the previous paper (MIKI, 1961), it has been reported that pollen germinations of some species are inhibited around some pistil slices and this inhibition is of more frequent cases where the plants from which the pollen grains and pistils obtained are in a remote relationship each with the other. In the present work the active substance or substances which inhibit pollen germination are extracted from the pistil tissue of *Gladiolus gandavensis*, whose pistil slices inhibit pollen germination of many other different species. The substances are water and fat soluble, and diluted solutions of the water extract 1 become to be less inhibitory, and show rather promoting effects. Therefore the explanation is that one substance is contained in the pistil tissue of *Gladiolus* at least, and that substance shows inhibiting effects in high concentration, while it shows a promoting effect in low concentration. BRANSCHEIDT (1930) and KUHN (1937) reported that the percentage of pollen germination was markedly decreased when the germination promoting substance was concentrated. The substances stated above will be discussed in a next paper.

Summary

1. Germination percentage and tube length of pollen grains spread around pistil slices belonging to the same or other species are examined. Then, existence and heat stability of the active substances are examined, and extraction of these substances is attempted.

2. In *Lilium longiflorum*, *Narcissus tazetta* and *Hippeastrum hybridum*, it is seen that germination and tube growth are generally promoted around the three kinds of pistil slices (stigma, style and ovary) of the same species (Table 1).

3. In species in the same genus, it is seen that germination and tube growth are promoted in *Lilium*, but they are not affected in *Vicia* (Table 2).

4. In the same family of Amarillidaceae, it is observed that germination and tube growth of *Narcissus tazetta* are promoted around the pistil slices of *Hippeastrum hybridum*. Around the all pistil slices of *Clivia nobilis*, however,

germination of *H. hybridum* is markedly arrested, and tube growth is slightly arrested around the stigma and ovary slices, while it is promoted slightly in the presence of the style slices (Table 3).

5. In different families, several cases are seen. In some cases germination and tube growth are both promoted or both arrested, and in some cases the germination and tube growth are independently promoted or arrested from the other. Moreover, in other cases, germination is inhibited entirely (Table 4).

6. It is assumed that the pistil tissue contains a substance or substances which control the germination and a substance or substances which control the tube growth. These substances are heat stable and diffuse from pistils to an agar culture medium. The substances which are contained in *Lilium* and *Hippeastrum* diffuse through collodion and cellulose membranes but the substances which are contained in *Gladiolus* do not.

7. Extraction of the controlling substances of germination and tube growth is made from the pistils of *Hippeastrum hybridum* and *Lilium longiflorum* and that of the germination controlling substances from the pistils of *Gladiolus gandavensis*. It is concluded that the former substances are water soluble and the latter water and fat soluble.

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