Pollen Tube Growth in the Pistils Grafted with Style of Different Cultivar or with Cross or Self Pre-pollinated Styles of *Lilium longiflorum*

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Summary

The self-incompatibility in *Lilium longiflorum* was investigated using the differently grafted pistils. Pistils of *Lilium longiflorum* cv. Georgia and Hinomoto were cut transversely into two parts at 15 or 25 mm from stigma tips. They were then grafted in different combinations with silicone rubber tube (2.5 mm in inner diameter). Pollen tube growth in the grafted pistils was somewhat reduced as compared with that in the intact style, but the incompatibility responses of the pollen tubes could be studied in the grafted styles.

In the pistils grafted with different cultivar, the appearance of self-incompatible responses was observed on the style from the same cultivar. The ovary end portion of the grafted styles seemed to be more influential.

In the pistils grafted with cross-pre-pollinated pistil segment, the incompatible pollen tube growth was faster than in the pistils grafted with non- or self-pre-pollinated pistil segments. The necessary condition for the rapid growth of pollen tube was generated in the styles after cross-pollination, with a probable velocity of 0.5 cm/h from the stigma to the ovary.

In self-pre-pollinated pistils, the necessary condition for the rapid growth of pollen tube seemed not to be generated in the style and further any inhibitor for the pollen tube growth was not produced.

Introduction

Lilium longiflorum shows the gametophytic self-incompatibility. Heslop-Harrison (3) suggested that the gametophytic self-incompatible response consists of two steps, such as recognition and rejection reactions. The recognition reaction occurred 3 hours after pollination on or in the stigma. However, Lawson and Dickinson(5) reported that in *Lilium henryi* the stigma played no role in the self-incompatibility response.

Spanjers(6) observed that the change of bioelectric potential in *Lilium longiflorum* styles following cross-pollination was different in pattern from that following self-pollination, and the transfer velocity of the poten-

tial toward the ovary varied between 1.2 and 1.9 cm/h. The principle and meaning of the bioelectric signals are not well understood, but the difference in signals generated after pollinations possibly may be correlated with different reactions in the *Lilium* styles. The different conditions in the styles between before and after pollination may result in the difference in the pollen tube growth. However, it is fairly difficult to completely remove pollen and pollen tubes from the stigma and stylar canal of pre-pollinated pistil. This difficulty can be avoided by using grafted pistils.

In this paper, we describe the effects of pollination on the pollen tube growth in the pre-pollinated styles using the grafted *Lilium* pistils, and discuss about the self-incompatibility responses including the roles of stigma and changes that occurred in pistils after pollination.

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Materials and Methods

Lilium longiflorum cv. Hinomoto and Georgia were grown in the greenhouse. Flowers were excised 3 days before anthesis, and they were kept in a jar containing tap water at 23°C. One day before anthesis, anthers were emasculated, and they were kept in desiccator with silica gel at -20°C.

One day after anthesis, some flowers were pollinated with incompatible (self) or compatible (cross) pollen. These pollinated flowers were incubated at 23°C for indicated period after pollination. For clarity these pistils are referred to as pre-pollinated pistils. After incubation, the tepals and filaments of flowers were removed, and the pistils were cut transversely into two parts at 15 mm or 25 mm from the stigma tip. Each segment from the ovary side parts was grafted to a 15 mm or 25 mm length of stigma segment end part of non-pollinated 'Georgia' pistil with a silicon rubber tube (2.5 mm in internal diameter and 5 mm long), respectively.

Fig. 1. The scheme of grafting method in the experiment. Pre-pollinated pistil segments were grafted at various times after pollination (the results are represented in Fig.2). G : Georgia, H : Hinomoto

The grafted pistils were again pollinated with 'Georgia' or 'Hinomoto' pollen, and then they were incubated at 25°C by the method of Hiratuka et al. (4). After incubation for 48 hours, the pistils were longitudinally bisected, and the length of pollen tube was measured after staining with 0.1% cotton blue solution. The scheme of a grafting experiment represents in Fig. 1.

Results and discussion

Table 1 shows alterations in the pollen tube growth when pistils were grafted by silicon tube. In the grafted pistils, the growth of compatible pollen tube was somewhat reduced by the grafting operation, whereas the incompatible pollen tube growth was not significantly affected by the operation.

In the pistil grafted with pistil segment of different cultivar, the appearance of self-incompatible reaction was observed in the segment from the same cultivar, but the ovary end portion of the grafted styles seemed to be more influential (compared 49.7 mm with 33.7 mm in table 1). This result shows that at least the stigma part was not indispensable to induce self-incompatible reaction, and the result was similar to that of *Lilium henryi*(5).

Table 2 represents the growth of pollen tube in the pre-pollinated and then jointed pistils. The elongation of 'Georgia' and 'Hinomoto' pollen tubes in the 'Georgia'

Table 1. Pollen tube growth in the grafted pistils of *Lilium longiflorum*. The grafted pistils were incubated at 25°C for 48 hrs after pollination.

Graft combination*		Pollinated pollen	Pollen tube growth (mm±S. E.)
Stigma end	Ovary end		
non-Georgia	non-Georgia	Georgia (self)	27.8±1.5
		Hinomoto (cross)	61.6±4.4
non-Georgia	non-Hinomoto	Georgia	49.7±4.4
		Hinomoto	33.7±4.7
Control (intact Georgia pistil)		Georgia (self)	28.5±2.5
		Hinomoto (cross)	84.1±1.1

^a non-Georgia (Hinomoto) means non-pre-pollinated Georgia (Hinomoto) pistil segment.

pistils grafted with self-pre-pollinated 'Hinomoto' pistil showed no significant difference with that in the 'Georgia' pistils grafted with

Table 2. Pollen tube growth in the pistils grafted with self- or cross-pre-pollinated pistils of *Lilium longiflorum*. The grafted pistils were incubated at 25°C for 48 hrs after pollination.

Graft combination ^z		Pollinated pollen	Pollen tube growth (mm±S.E.)
Stigma end	Ovary end		
non-Georgia	non-Hinomoto	Georgia	49.7±4.4
		Hinomoto	33.7±4.7
non-Georgia	self-Hinomoto	Georgia	50.5±4.1
		Hinomoto	29.8±1.3
non-Georgia	cross-Hinomoto	Georgia	70.5±2.7
		Hinomoto	42.5±3.7

^z non-Georgia (Hinomoto) means non-pre-pollinated Georgia (Hinomoto) pistil segment, and self(cross)-Hinomoto means segment prepared from self(cross)-pre-pollinated pistil incubated at 23°C for 12 hrs after pollination.

Fig. 2. The incompatible pollen tube growth in the pistils of *Lilium longiflorum* 'Georgia' grafted with pre-pollinated 'Georgia' pistils in different periods after pollination.

A: Pre-pollinated pistils were cut at 15 mm from stigma tip just before grafting.

B: Pre-pollinated pistils were cut at 25 mm from stigma tip just before grafting.

The grafted pistils were incubated at 25°C for 48 hrs after self-pollination. Vertical lines indicate the standard errors.

non-pollinated 'Hinomoto' pistil. On the other hand, in the 'Georgia' pistils jointed with cross-pre-pollinated 'Hinomoto' pistil, the elongation of 'Georgia' and 'Hinomoto' pollen tubes was larger than that in the 'Georgia' pistils jointed with non- and self-pre-pollinated 'Hinomoto' pistils.

Brewbaker and Majumder(1) reported that an inhibitor for pollen tube growth exists in the pistil of *Petunia*. However, our results suggest that any inhibitor, which directly inhibits the pollen tube growth, is not produced in the pistils after self-pollination. Also we suggest that the necessary condition for the rapid growth of pollen tube is produced in the style after cross-pollination.

Fig. 2 shows the growth of incompatible pollen tube in the pistils grafted with pollinated pistils. When the pollinated pistils were cut at 15 mm from stigma tip at different times after pollination, the stimulative effect of cross-pollination on the incompatible pollen tube growth in the grafted pistils was evident 2 hours after pollination, and the maximum stimulation was attained at 4 hours after pollination (Fig. 2 A). When cut at 25 mm from stigma tip, the stimulative effect was evident at 4 hours after cross-pollination, and the maximum stimulation was attained after 6 hours (Fig. 2 B). This shows that another 2 hours is required for the longer style by 1 cm to receive its maximum stimulation.

These results indicate that the necessary condition for the rapid growth of pollen tube is produced in the style from the stigma to ovary with the velocity of 0.5 cm/h after cross-pollination (Fig. 2). This velocity is much smaller than that of bioelectric potential suggested by Spanjers(6). Fig. 2 also indicates that there is about one hour of time lag from the cross-pollination to produce the necessary condition for rapid growth of pollen tube. Probably, the necessary condition will be generated when pollen tubes penetrated into stigma and entered stylar canal. Donk(2) reported that the respective peak of RNA synthesis was observed in *Petunia hybrida* style at 3 and 12 hours after self-pollination, and discussed that the recog-

nitition would occur at the very beginning of pollen tube-style interaction, in accordance with the first peak of RNA synthesis.

Our results strongly suggest that the necessary condition for the rapid growth of pollen tube is produced only in cross-pollinated style, and no inhibitor for the pollen tube growth is involved in the self-incompatibility response in *Lilium longiflorum*.

Literature Cited

1. BREWBAKER, J. L. and S. K. MAJUMDER. 1961. Cultural studies of pollen population effect and the self-incompatibility inhibition. Amer. J. Bot. 48: 457—464.
2. DONK, J. A. W. M. VAN DER. 1974. Synthesis of RNA and protein as a function of time and type of pollen tube-style interaction in *Petunia hybrida* L. Molec. gen. Genet. 134: 93—98.
3. HESLOP-HARRISON, J. 1978. Genetics and physiology of angiosperm incompatibility systems. Proc. R. Soc. Lond., B 202: 73—92.
4. HIRATUKA, S., T. TEZUKA and Y. YAMAMOTO. 1983. Use of longitudinally bisected pistils of *Lilium longiflorum* for studies on self-incompatibility. Plant & Cell Physiol. 24: 765—768.
5. LAWSON, J. and H. G. DICKINSON. 1975. The site of the incompatibility reaction in *Lilium henryi*. Incompatibility Newsletter 6: 18—21.
6. SPANJERS, A. W. 1981. Bioelectric potential changes in the style of *Lilium longiflorum* Thunb. after self- and cross-pollination of the stigma. Planta 153: 1—5.

テッポウユリの異品種の花柱および前受粉した雌ずいの 花柱を組合せて接合した花柱内における花粉管伸長

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摘 要

テッポウユリの自家不和合性の機構を明らかにするため、異品種またはあらかじめ受粉した雌ずいと未受粉雌ずいを接合して、新たに与えられた花粉の花粉管伸長を調べた。雌ずいは、柱頭側から 1.5 または 2.5 cm のところで切断して 2 分し、シリコンゴム管を用いて接合した。

接合雌ずいでは intact 雌ずいと比べて花粉管伸長は若干劣ったが、自家不和合反応が明らかに認められた。

異品種の雌ずいを接合して受粉した結果、自家不和合反応は自家の雌ずい切片部分で表われたが、子房側切片の影響がより強く表われた。

あらかじめ受粉した雌ずい（子房側）と未受粉雌ずい

（柱頭側）を接合し自家受粉した。前もって他家受粉した花柱内では、未受粉、あるいは前もって自家受粉した花柱内に比べ、自家花粉の花粉管伸長が顕著に促進された。前もって行った受粉と接合操作までの時間間隔を変えて調べた結果、前もって他家受粉した場合柱頭から子房に向かって、花粉管を伸長させるに必要な条件が 0.5 cm/h の速度で作られて行くことが示された。前もって自家受粉した花柱内では上述のような花粉管を伸長させるに必要な条件は作られなかった。また、花粉管伸長抑制物質も作られなかった。

以上の結果から、テッポウユリの自家不和合性は、自家受粉の場合花粉管伸長の条件が作られないことによるものと考えた。

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