Changes in the Amount and Composition of Stylar Canal Exudate after Self- or Cross-pollination in Self-incompatible *Lilium longiflorum* Thunb.

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**Summary**

Pollen tubes of self-incompatible *Lilium longiflorum* Thunb. grow in stylar canal exudates. Changes in the amount and composition of the exudate after self- and cross-pollination were investigated.

Stylar canal exudate injected into stylar canal promoted the growth of self-incompatible 'Hinomoto' pollen tubes. The promotion was more enhanced as the amount or concentration (expressed by carbohydrate concentration) of stylar canal exudate was increased. No significant difference was found in length between incompatible pollen tubes after injection at 80 mg•ml\(^{-1}\) carbohydrate concentration and cross-compatible 'Georgia' pollen tubes. Carbohydrate and protein contents of stylar canal exudate from self- or cross-pollinated pistils decreased as pollen tubes grew longer. Little difference between the contents of the canal exudates from self- and cross-pollinated pistils was observed. Sugar composition and molecular weight distribution of arabinogalactans which are main components of the canal exudate showed little differences by self- or cross-pollination. When pistils were administered \(^{14}\)C-glucose, the rates of incorporation of radioactivity into the canal exudates were not different between self- and cross-pollinated pistils. However, the amount of \(^{14}\)C incorporated into pollen tubes after cross-pollination was twice that fixed by pollen tubes after self-pollination. An interpretation of this phenomenon is that cross-pollination stimulated the secretion of the canal exudate more than did self-pollination.

Based on our findings, we attribute self-incompatibility in *Lilium* to the lack of stimulation of stylar exudate by a self pollen. This leads to an insufficient supply of canal exudate required for pollen tube growth.

**Introduction**

The expression of self-incompatibility is controlled by multiple alleles, S-genes (Nettancourt, 1977). Although S-gene specific proteins have been isolated in several plants, their functions in the expression of self-incompatibility are little known (Anderson et al., 1986; Harris et al., 1989; Jahnen et al., 1989; Mau et al., 1982, 1986). *Lilium longiflorum* Thunb. shows self-incompatibility, and incompatible pollen tubes are arrested in the style (Ascher and Peloquin, 1966). Although self-incompatibility of *Lilium* is not known to be related to the S-gene, *Lilium* is one of the most suitable materials for investigating the mechanism of self-incompatibility because of the following properties which most self-incompatible plants do not have: a) the flower of *Lilium* has a long, hollow stylar canal; b) exudate secreted into stylar canal is close related to the expression of self-incompatibility because pollen tubes grow in this medium; c) stylar tissues, pollen tubes and the canal exudate can be easily separated and subjected to the respective chemical analyses; and d) the anatomy of the style allows the direct effects of a substance on pollen tube growth to be observed.

The canal exudate is found to serve as nutrients for pollen tube growth (Labarca and Loewus, 1973) and to be mainly composed of arabinogalactans, whose average molecular weight is 50,000...
We previously reported that the canal exudate injected into stylar canal promoted the growth of incompatible pollen tubes (Ichimura and Yamamoto, 1991). We further reported that the diminution of self-incompatibility in the aged flower is caused by the accumulation of the canal exudate (Ichimura and Yamamoto, 1992). These findings suggest that self-incompatibility in Lilium longiflorum is closely related to the amount of the canal exudate.

In the present study, we investigated effects of the canal exudate on the growth of incompatible pollen tubes and changes in secretory amount and composition of the canal exudate after self- or cross-pollination.

Materials and Methods

1. Plant materials

The bulbs of Lilium longiflorum cvs. Hinomoto and Georgia were planted from September to December and grown in a greenhouse controlled at 10°-25°C. Flowers of ‘Georgia’ were used only as a source of cross-compatible pollen. From December to April, ‘Hinomoto’ flower buds, ca. 14.5 cm in length, were cut from the plant, and kept at 23°C under fluorescent light in water which was changed daily. Under these conditions, the buds opened 2 days after harvest. One day before anthesis, anthers were excised from the buds and allowed to dehisce on a silica gel. The pollen were stored at —20°C until used within a week.

2. Flower incubation after pollination

One day after anthesis, tepals and filaments were removed from the flower, then the flower was pollinated with about 5 mg of self-incompatible ‘Hinomoto’ or cross-compatible ‘Georgia’ pollen grains. The pollinated flowers were incubated in a jar with tap water at 25°C in the dark.

3. Measurements of pollen tube length

Pollen tube length of the pollinated flowers was measured by the method of Hiratsuka et al. (1983).

4. Collection of stylar canal exudate

A style with the stigma attached was excised from the top of the ovary. The stigma and 1 cm of the style were removed. Distilled water was injected into the stylar canal from the ovarian end with a syringe while the style was inverted. The eluate which dripped from the stigmatic end of the style was collected in an ice-cooled vessel. In a preliminary experiment in which cross-pollinated styles were flushed with 5 ml of water 48 hr after pollination, 93% of the canal exudate based on its carbohydrate content was recovered in the first 3 ml of eluate. Hence, the canal exudate was collected by flushing pollinated style with 3 ml of distilled water. From an unpollinated style, the canal exudate was collected with 1.5 ml of distilled water as described in the preceding paper (Ichimura and Yamamoto, 1991). The collected canal exudate was lyophilized and stored at —20°C.

5. Effects of the canal exudate on the in vivo growth of incompatible pollen tubes

One day after anthesis, tepals and filaments were removed from the incubated flower. The lyophilized canal exudate was dissolved and injected into the stylar canal through the center of the stigma with a microsyringe until overflow was observed on the stigmatic surface. The average injected volume was about 40 μl. The flower was then pollinated with either ‘Hinomoto’ or ‘Georgia’ pollen and incubated in a jar with tap water at 25°C for 48 hr in the dark. After incubation, the length of pollen tubes was measured. As the control, distilled water was injected into the stylar canal prior to pollination with incompatible and compatible pollen.

6. Collection of pollen tubes

After the collection of the canal exudate, the style was bisected with a razor blade. Pollen tube mass was pulled out with a pair of tweezers.

7. Assays for composition of the canal exudate

Carbohydrate concentration was determined by the method of Dubois et al. (1956) with glucose as standard, uronic acid by that of Galambos (1967) with glucuronic acid as standard, and protein by that of Bradford (1976) with bovine serum albumin as standard. Determination of the neutral sugar composition and gel filtration with Sephacryl S-300 were carried out as previously described (Ichimura and Yamamoto, 1991).

8. Incorporation of 14C into stylar canal exudate and pollen tubes
The seven incubated flowers at anthesis were administered 400 μl of 14C-U-glucose solution (14 μCi, 189 mCi·mmol⁻¹) by placing the cut pedicel in a small vial containing the labeled solution. Most of the labeled solution was absorbed by the pedicel within 4 hr. After absorption, distilled water was poured into the vial, and the flowers were incubated for 24 hr in the dark at 22°C. Then, the flowers were pollinated with about 5 mg of incompatible ‘Hinomoto’ or compatible ‘Georgia’ pollen grains, and incubated for 48 hr under the above conditions. After the incubation, the canal exudate and pollen tubes were collected as above. The collected pollen tubes were thoroughly homogenized in 1 ml of distilled water with a glass homogenizer. The homogenate was centrifuged at 3,000 × g for 20 min. The supernatant was decanted and the resulting pellet was resuspended in 1 ml of distilled water and recentrifuged at 3,000 × g for 20 min. This procedure was repeated twice and all supernatants were combined. The combined supernatant, final pellet and the canal exudate were transferred into separate vials and suspended in 5 ml of Scincisol (Wako Chem. Co.). Then, 14C in each fraction was measured with a liquid scintillation counter (Beckman).

### Results

1. **Pollen tube growth in the pistil**

Up to 12 hr after pollination, no difference in length was found between incompatible and compatible pollen tubes. However, 24 hr after pollination, the compatible pollen tubes grew longer than did those of incompatible ones. Subsequently, the difference in lengths between incompatible and compatible pollen tubes became greater with time after pollination. At 72 hr after pollination, compatible pollen tubes reached the ovary, whereas incompatible ones had grown only one-third of the distance (Fig. 1).

2. **In vivo effect of the canal exudate on the growth of incompatible pollen tubes**

The growth of incompatible pollen tubes in the ‘Hinomoto’ style was promoted by the injection of the canal exudate collected from unpollinated pistils. The tube growth was enhanced proportionately to the amount of exudate added as expressed by carbohydrate concentration. The injection of the canal exudate at a concentration of 80 mg carbohydrate·ml⁻¹ promoted the growth of incompatible pollen tubes comparable to the growth of compatible pollen tubes (Fig. 2).

3. **Changes in composition of stylar canal exudate after pollination**

Carbohydrate contents in the canal exudate from unpollinated styles gradually increased with time. Carbohydrate content in the canal exudate after self-pollination increased until 24 hr after pollination, thereafter it rapidly decreased; whereas that after cross-pollination increased for 12 hr after pollination and then gradually decreased. Protein contents in the canal exudates after respective pollinations followed a pattern similar as that of the carbohydrate content (Fig. 3).

Small variations were observed in sugar constituents of canal exudate collected from pollinated styles. Galactose and arabinose were detected in relatively large quantities compared with uronic acid and rhamnose. Change in each sugar content after respective pollinations showed almost the...
same pattern as those of carbohydrate- and protein-contents, indicating that the sugar composition of canal exudate differed little between self- and cross-pollinations (Fig. 4).

Elution profiles from Sephacryl S-300 gel column of canal exudates from pollinated pistils displayed two peaks of carbohydrates. The major peak yielded a polysaccharide, and minor peak, which was eluted at the same position with glucose, consisted of low molecular weight sugars. At 24 hr after pollination, respective peak heights of carbohydrate and protein after self-pollination were higher than were those after cross-pollination, but the molecular weight distributions of both carbohydrates and proteins in the canal exudates from self- and cross-pollinated pistils showed little difference. At 48 hr after pollination, major carbohydrate peaks from both self- and cross-pollinated pistils markedly decreased, whereas those from unpollinated pistils continued to increase. Two major peaks of protein were detected at 0 time. These peaks were much smaller 48 hr after pollination, but no marked difference in pattern was observed between canal exudates from self- and cross-pollinated pistils (Fig. 5).

The eluted portions containing the major carbohydrate peaks indicated by the bar in Fig. 5 were collected and their sugar compositions assayed separately. The hydrolyzate of each peak contained galactose, arabinose, uronic acid and rhamnose. No marked difference was found in sugar composition among peaks (Table 1).

4. Dry weights of the canal exudate and pollen tubes after pollination

Carbohydrate and protein contents of the canal exudate did not increase after self- or cross-pollination. However, to calculate the gross amount of the canal exudate secreted, the amount of canal exudate remaining in the stylar canal and the amount utilized by growing pollen tubes must be combined (Labarca and Loewus, 1973). The
amount of exudate we have expressed in this study based on carbohydrate and protein contents, represents only the amount remaining in the stylar canal. To examine the gross secretory amount of the canal exudate, we measured dry weights of the canal exudate and of pollen tubes after pollination. Although the dry weight of the canal exudate after cross-pollination was almost the same as that after self-pollination, the dry weight of compatible pollen tubes was far heavier than was that of incompatible ones (Table 2).

5. Incorporation of $^{14}$C into canal exudate and pollen tubes after pollination

The amount of $^{14}$C incorporated into the canal exudate from cross-pollinated pistils was nearly equal to that incorporated by self-pollinated ones; the specific activity per unit carbohydrate of the canal exudate from cross-pollinated pistils was slightly higher than that from self-pollinated ones (Table 3). On the other hand, incorporation of $^{14}$C into both supernatant and pellet made from compatible pollen tubes was almost twice that fixed by those fractions collected from incompatible pollen tubes (Table 4).

Discussion

Carbohydrate and protein contents in the canal exudate decreased as pollen tubes grew longer in the pistils (Figs. 1 and 3). The decrease is probably due to the uptake of the canal exudate by growing pollen tubes. This is supported by the findings that $^{14}$C was incorporated into pollen tubes growing in the canal exudate when the pistil was administered $^{14}$C-myo-inositol or $^{14}$C-glucose (Kroh et al., 1970; Labarca and Loewus, 1973), and that canal exudate injected into stylar canal promoted the growth of incompatible pollen tubes proportionate to the increase in carbohydrate concentration (Fig. 2).

The trends of carbohydrate and protein contents in the canal exudates from self- and cross-pollinated pistils were similar, changing little as time lapse after pollination increased (Fig. 3). Carbohydrate and protein contents of the canal exudate in Figs. 3, 4 and 5 represent only the amount remaining in the stylar canal. Thus, the values in these figures are not the gross amounts of the canal exudate in the self- and cross-pollinated styles.

According to morphological observations by Reiss et al. (1985), the diameters of pollen grain and pollen tube are 100 and 15 µm, respectively. Provided that the shapes of pollen grains and of pollen tubes are spherical and cylindrical, respectively, the volume of pollen tubes when they reach

![Fig. 4. Changes in sugar contents of the canal exudate from un-( ), self-( ) or cross-pollinated ( ) pistils.](image)
Fig. 5. Gel filtration profile of the canal exudate from self-, cross- or unpollinated pistils. Each fraction was assayed for carbohydrate (○) and protein (●). Eluate corresponding to polysaccharide peak indicated by the bar was collected, and its sugar composition is shown in Table 1. Vo: void volume. Vt: bed volume.

Table 1. Sugar composition in polysaccharide fraction of the canal exudate from self-, cross- or unpollinated pistils.

<table>
<thead>
<tr>
<th>Time after pollination (hr)</th>
<th>Rhamnose (%)</th>
<th>Arabinose (%)</th>
<th>Galactose (%)</th>
<th>Uronic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un 0</td>
<td>6.6 (0)</td>
<td>33.0 (0)</td>
<td>39.6 (0)</td>
<td>20.8 (0)</td>
</tr>
<tr>
<td>Self 24</td>
<td>7.1 (0)</td>
<td>38.0 (0)</td>
<td>40.5 (0)</td>
<td>14.4 (0)</td>
</tr>
<tr>
<td>Cross 24</td>
<td>6.8 (0)</td>
<td>38.0 (0)</td>
<td>40.8 (0)</td>
<td>14.4 (0)</td>
</tr>
<tr>
<td>Self 48</td>
<td>6.7 (0)</td>
<td>34.9 (0)</td>
<td>40.3 (0)</td>
<td>18.1 (0)</td>
</tr>
<tr>
<td>Cross 48</td>
<td>7.2 (0)</td>
<td>35.8 (0)</td>
<td>42.9 (0)</td>
<td>14.1 (0)</td>
</tr>
<tr>
<td>Un 48</td>
<td>5.6 (0)</td>
<td>38.0 (0)</td>
<td>41.6 (0)</td>
<td>14.8 (0)</td>
</tr>
</tbody>
</table>

* Polysaccharide fraction was obtained by collecting the eluate indicated by the bar in Fig. 5.
the ovary is estimated to be 30 times greater than that of pollen grains. Thus, most constituents of pollen tube are supplied from stylar tissues. The total dry weight of the canal exudate and pollen tubes from cross-pollinated pistils was much heavier than that from self-pollinated pistils (Table 2). This suggests that the gross secretory amount of the canal exudate after cross-pollination was greater than that after self-pollination.

To prove this postulation, we measured the incorporations of $^{14}$C into both the canal exudate and pollen tubes when the pistils were supplied with $^{14}$C-glucose. Although the amount of $^{14}$C released by respiration of pollen tubes was not detected, incorporation of $^{14}$C into pollen tubes after cross-pollination was almost twice that incorporated after self-pollination. These results indicate that

Table 2. Dry weights of canal exudate and pollen tubes from self-, cross- or unpollinated pistils.

<table>
<thead>
<tr>
<th>Time after pollination (hr)</th>
<th>Canal exudate DW (mg/10 styles)</th>
<th>Pollen tubes DW (mg/10 styles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self 48</td>
<td>2.1 ± 0.4*</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Cross 48</td>
<td>3.0 ± 0.1</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Un 48</td>
<td>9.4 ± 0.7</td>
<td>—</td>
</tr>
<tr>
<td>Self 72</td>
<td>1.9 ± 0.2</td>
<td>trace</td>
</tr>
<tr>
<td>Cross 72</td>
<td>3.1 ± 0.7</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Un 72</td>
<td>9.2 ± 0.3</td>
<td>—</td>
</tr>
</tbody>
</table>

* Each value represents mean ± standard error (n = 3).

Table 3. Incorporation of $^{14}$C into the canal exudate from self- or cross-pollinated pistils 48 hr after pollination.

<table>
<thead>
<tr>
<th>Uptake (cpm/style)</th>
<th>(cpm/style/carbohydrate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self 12,649 ± 825*</td>
<td>29.4 ± 2.8</td>
</tr>
<tr>
<td>Cross 12,650 ± 292</td>
<td>33.5 ± 0.5</td>
</tr>
</tbody>
</table>

* Each value represents mean ± standard error (n = 3).

Table 4. Incorporations of $^{14}$C into pollen tubes 48 hr after self- or cross-pollination.

<table>
<thead>
<tr>
<th>Supernatant (cpm/style)</th>
<th>Pellet (cpm/style)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self 1,197.6 ± 21.6*</td>
<td>268.4 ± 17.2</td>
</tr>
<tr>
<td>Cross 2,383.6 ± 97.6</td>
<td>638.4 ± 15.4</td>
</tr>
</tbody>
</table>

* Each value represents mean ± standard error (n = 3).

the total amount of the canal exudate secreted after cross-pollination is much greater than that after self-pollination. There are two possible explanations. First, the secretion of the canal exudate is promoted by cross-pollination. Secondly, the secretion is inhibited by self-pollination. Yamada (1965) and Miki-Hirosige et al. (1987) showed histochemically that cross-pollination triggered the secretion of the canal exudate. In the present study, carbohydrate and protein contents of the canal exudate rather increased until 24 hr after self-pollination. Hence, the first explanation seems to be more plausible.

The dry weight of the canal exudate from unpollinated pistils was greater than that from cross-pollinated pistils. But because the amount of the consumption by respiration of pollen tubes can not be estimated, it seems that the amount of the canal exudate secreted from unpollinated pistils was not necessarily greater than that from cross-pollinated pistils.

The present study confirms that the main component of the canal exudate from unpollinated pistils was an arabinogalactan (Ichimura and Yamamoto, 1991). The trends in carbohydrate and protein contents with time after self- and cross-pollinations were similar (Figs. 3 and 4). Molecular weight distribution of carbohydrate in the canal exudate was not changed by self- or cross-pollination although absolute amounts were different (Fig. 5). Moreover, sugar composition of polysaccharide fractionated through Sephacryl S-300 gel column was not changed by the pollen source (Table 1). Therefore, it seems likely that the components of the canal exudate are neither changed by pollination nor selectively absorbed by pollen tubes growing in the canal exudate. Labarca and Loewus (1973) also reported that gel filtration profile on Sephadex G-100 was not changed by self- or cross-pollination.

Two theories have been proposed for the elucidation of the mechanism of self-incompatibility (Nettancourt, 1977). One assumes that the growth of incompatible pollen tubes is inhibited by a specific substance. The other supposes that the cessation of the growth of incompatible pollen tubes is caused by a lack of nutrients supplied from pistils. Many workers, using different plants have reported that the style extract including S-specific proteins inhibited in vitro growth of incom-
compatible pollen tubes (Clarke et al., 1985; Harris et al., 1989; Jahnen et al., 1989; Sharma and Shivanna, 1982; Shivanna et al., 1981; Williams et al., 1982). However, pollen tubes in vitro in these reports grew far less than those in situ, and thereby in situ growth of pollen tubes could not be reproduced in these experiments. Furthermore, whether these substances inhibit the growth of incompatible pollen tubes in situ still remains uncertain.

If specific inhibitor participates in the expression of self-incompatibility, the canal exudate can have inhibitor because the growth of incompatible pollen tubes ceased immersing in the canal exudate.

In the present study using Lilium, we showed that the canal exudate from unpollinated pistils markedly promoted the growth of incompatible pollen tubes as much as that from unpollinated pistils. Self-incompatibility in Lilium is overcome by pollinating aged flower (Ascher and Peloquin, 1966; Ichimura and Yamamoto, 1992). We previously showed that the diminution of self-incompatibility with aging of flowers was accompanied with an accumulation of canal exudate. In the aged flowers, the growth of incompatible pollen tubes was reduced when the stylar canal was flushed to remove the canal exudate (Ichimura and Yamamoto, 1992).

In the present study, we indicated that the secretory amount of the canal exudate after cross-pollination was greater than that after self-pollination.

From these findings together with the present results, we conclude that self-incompatibility in Lilium is attributed to insufficient secretion of the canal exudate which serves as nutrients for the growth of pollen tubes.

Acknowledgement

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Literature Cited


