Changes in Proteins in Pistils after Self- and Cross-Pollination in Japanese Pear

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Summary

The present study was performed to get a clue to a solution for the obfuscating mechanism with reference to self-incompatibility in Pyrus serotina.

1. The growth of pollen tubes showed the slow rate from approximately 36 hours after pollination and stopped completely in 48 hours at the portion near the ovary in a selfed style, but the tubes in a crossed one continued their growth and fertilization occurred in about 96 hours after pollination.

2. In the selfed style, the tips of pollen tubes began to swell in 36 hours after pollination and callose deposited abnormally on the surface of the tubes. However, in a crossed style, sharp tips of the tubes and no abnormality of callose deposition were observed at above time.

3. Soluble proteins and proteins dissociated by Tween 20 in selfed pistils exhibited unchanged level till 48 hours after pollination and increased thereafter gradually. On the other hand, these in crossed pistils decreased leisurely up to 24 hours and displayed steep increase after that time.

4. In the fraction of proteins dissociated by Tween 20, both electrophoretic patterns of proteins from selfed and crossed pistils exhibited little difference till 72 hours after pollination. However, in the fraction of soluble proteins, band 7 peak from the selfed pistils increased the height in 24 hours after pollination and then decreased. The band 7 from the crossed pistils displayed reverse patterns compared with that from the selfed pistils, that is to say, the band 7 decreased in 24 hours after pollination and then continued to increase gradually. Band 7 from the non-pollinated pistils showed intermediate patterns between above bands from selfed and crossed pistils.

Introduction

Most of the varieties of the Japanese pear have a strong gametophytic self-incompatibility. Since early years of this century, many workers have reported the morphological, histological and physiological phenomena relating to the self-incompatibility (1, 2, 14, 24, 25). Terami et al. determined the S-alleles of many varieties of the Japanese pear as the genetical interpretations (26), but an information from physiological standpoint in order to explain the incompatibility is still insufficient.

On the other hand, conventionally, the hybridizations by artificial pollination are still occupied as the main means for the breeding. In order to facilitate interspecific and intergeneric hybridization, in the first place the problems of the incompatibility and other obscure barriers for hybridization have to be solved, because it is essential to understand about the regulation of pollen germination and the growth of pollen tube in vivo.

Recently, some investigators have described about the relations between the contents of proteins (12, 15), enzyme activities (5, 6, 23) or the synthesis of protein through poly-somes (8, 9, 10, 11) and self-incompatibility systems in other plants. Actually, in the sporophytic self-incompatibility system, S-specific proteins are detected in stigmata of Brassica (18, 19, 20, 21, 22). These reports
obfuscate the possibility which proteins play some important roles in the metabolic system of self-incompatibility. However, above obfuscated possibility is not still reported about the relationship between the proteins and self-incompatibility system in Japanese pear.

This article describes about the qualitative and quantitative changes of proteins in the pistils after self-or cross-pollination.

Materials and methods

Branches (70－80 cm) of Pyrus serotina Rehd. var. Chojuro (as female) and var. Nijisseiki (as a source of cross pollens) which were provided by the courtesy of Torii orchard in Anjo city were cut off before flower opening (February). Then the cut end of them was put in water (10－20 cm in depth) and stored at 4°C. In the end of March, they were placed in a glasshouse (natural conditions) and were sometimes cut to renew each cut end. After castration in one day before anthesis, the castrated flowers were covered with paraffin paper bags. On the following day, moreover, they were pollinated and then covered with the bags again. Pollens used for the pollination had been stored in a desiccator at 4°C for one through two weeks. All experiments were achieved under natural conditions in the glasshouse.

The length of pollen tubes growing in the style was estimated with a fluorescent microscope (Olympus FLM) following a modification of the method described by Yano et al. (28). Self- or cross-pollinated pistils were got at intervals every 12 hours. After fixing in formalin-acetic acid-alcohol (FAA) for several weeks, they were washed in running tap water for one hour, soaked in 8 M NaOH for two hours, and washed again. Then they were stained with 0.1% solution of aniline-blue dye dissolved in 1/30 M K3PO4 for 24 hours. The stained styles were sealed with glycerin on a slide glass, crushed with a cover glass and prepared for the observation by the fluorescent microscopy. In order to estimate the length of pollen tubes, the most elongated 4 or 5 pollen tubes were measured.

Procedures for extraction of proteins from pear pistils are shown in Fig. 1. Pistils were homogenized in a glass homogenizer contain-
Soluble proteins (50 μl) and proteins dissociated by Tween 20 (100 μl) were analyzed by using 7.5% acrylamide gel for 6 hours at 7 mA per gel. Gels after electrophoresis were fixed with 10% of trichloroacetic acid (TCA) for 30 min and stained with 1% of amido black 10 B dye dissolved in 7% acetic acid overnight. Subsequently, they were destained electrophoretically and then were kept in the same acetic acid solution. In order to estimate molecular weight of proteins in the pistils by using the method of gel electrophoretic estimation (27), phosphorylase, bovine serum albumin, ovalbumin and lactate dehydrogenase were employed as marker proteins.

Results

The self-pollinated (selfed) pollen tubes stopped the growth at the lower portion of a style in 48 hours after pollination. The pollen tubes in the selfed style was almost the same growth rate compared with that in the cross-pollinated (crossed) ones till 36 hours after pollination. The difference of the length of pollen tubes between the selfed and crossed styles took place after 36 hours (Fig. 2). In 36 hours after pollination, many tips of the pollen tubes in selfed styles began to swell abnormally. The swelled tips were observed throughout the upper to the lower portions of the style (Fig. 3). In 96 hours after the cross-pollination, fertilization achieved completely in all pistils.

Protein contents in selfed or crossed pistils are shown in Fig. 4. The contents of soluble proteins in selfed pistils maintained unchanged level till 48 hours after pollination and thereafter increased gradually. On the other hand, the contents in the crossed pistils decreased a little in 24 hours after pollination and thereafter displayed a steep increase. The contents of soluble proteins in non-pollinated pistils were similar to those of selfed pistils. The contents of proteins dissociated by Tween 20 in selfed, crossed and non-pollinated pistils also showed almost the same tendency as these of soluble proteins, respectively.

Electrophoretic and densitometric patterns of soluble proteins and proteins dissociated by Tween 20 at zero time, just before pollination, are shown in Fig. 5. Each band of proteins was numbered from high molecular weight through low molecular one. Nine bands in the fraction of soluble proteins and fifteen bands (band 14th and 15th are not shown in the densitometric pattern) in the fraction of proteins dissociated by Tween 20 were detected, respectively. Four bands (band 1st, 2nd, 6th and 7th) in the fraction of soluble proteins corresponded to band 2nd, 4th, 10th and 12th in that of proteins dissociated by Tween 20 by estimation from the Rf value, respectively. In the fraction of proteins dissociated by Tween 20 in selfed and crossed pistils, their electrophoretic patterns exhibited no difference till 72 hours after pollination (data not shown), while in the fraction of soluble proteins, band 7 depicted interesting behavior. Namely, in selfed pistils, the height of band 7 peak increased in 24 hours after pollination and then decreased. On the other hand, the band 7 of soluble proteins in crossed pistils exhibited reverse patterns compared with the band 7 in selfed ones, that is, the height of band 7 peak decreased in 24 hours after pollination and then continued to increase gradually. However, band 7 in the non-pollinated pistils showed intermediate patterns between the band 7 patterns in selfed and crossed pistils (Fig. 6). Molecular weight of this protein, band 7 was 58,000.
Discussion

By means of homogenization by using polyclar AT, the resulting homogenate evaded browning by phenolic compounds (16) and KCN in the buffer also protected markedly proteins from browning in the homogenate, especially in the fraction of the proteins dissociated by Tween 20. In order to extract proteins from fruits which contain high contents of phenolic compounds and organic acids, some workers have homogenized tissues frozen in acetone (acetone powder), or in a buffer containing the reducing agents.

Fig. 3. The growth of pollen tubes in the style of P. serotina. A. A whole style in 6 hours after self-pollination (×45). pg: pollen grain, pt: pollen tube, st: stigma, sty: style. B. Pollen tubes in 24 hours after self-pollination (×800). C. A tube tip in 36 hours after self-pollination (×800). Note the sign of tip swelling. D. Pollen tubes in 48 hours after self-pollination (×400). Note the tips with abnormal swelling. E. Pollen tubes in 36 hours after cross-pollination (×200). Note the sharp tips.
It does appear that the self-incompatibility reaction may not localize in the style of Japanese pear, because many swelled tips of pollen tubes were observed throughout portions in selfed styles. It is also known that the self-incompatibility reaction in lilies has existed throughout all portions in the style and was not implicated in localization (3, 13).

Although the relation between the swelling of pollen tube tips in the selfed style and the self-incompatibility reaction is still not completely elucidated, our investigation may drop a hint about interesting problems between the swelling and the self-incompatibility reaction. On the other hand, we also observed some swelled tips of pollen tubes in the crossed style. Higuchi, who reported about swelled tips of pollen tubes in the selfed style of petunia, also has observed 15% abnormal (swelled) tips in crossed style (12). According to Higuchi's and our observations,
it may be possible that the self-incompatibility is caused as a refusible reaction against some substance(s) universally being in the style. Furthermore the different growth rate of each pollen tube in the selfed style may depend on the refusible intensity against the substance(s). An effort to substantiate this possibility has to be continued by the further investigations.

Fig. 4 will indicate that catabolisms (degradation) of proteins in the crossed pistil maintained predominance up to 24 hours after pollination, and subsequently, anabolisms (synthesis) of proteins gained an advantage. But no quantitative difference occurred in self- and non-pollinated pistils up to 48 hours after pollination. From the evidence which the growth of pollen tubes was inhibited in about 36 hours after self-pollination, above phenomena may be due to the growth inhibition of selfed pollen tubes or the promotion of crossed ones. Linskens and Tupy, who reported with regard to the difference of protein contents in the styles of petunia, have stated that protein contents maintained the lowest level during 12 and 24 hours after self-pollination and a little reduction was observed in the styles after cross-pollination (15). The difference between their results and ours can be explained on the assumption that the biological reactions are subject to variations of the plant materials and environmental factors. In petunia ovaries, Deurenberg found that the contents of ribosomal protein decreased till 6 hours after pollination and increased from 6 to 42 hours in self- and cross-fractions, but that in non-pollinated fraction increased up to 24 hours (9). It is noteworthy, however, that the metabolism of the protein in the pistil (style and ovary) before fertilization bring about a difference induced by types of pollination.

Concerning the behavior of electrophoretic patterns of soluble proteins, we pay attention to band 7th which increased in 24 hours after pollination in selfed pistils and then decreased (Fig. 6). On the other hand, this band 7th from crossed pistils depicted reverse patterns compared with that from selfed pistils, and that in non-pollinated pistils mediated between both above patterns. These results seem to conduct that different metabolisms of certain protein(s) are forced by cross- or self-pollination, respectively. In view of the behavior of band 7th among selfed, crossed and non-pollinated pistils, a very attractive hypothesis may be adopted in the following way. There is certain protein(s) which has something to do with the growth of pollen tubes in the pistil of Japanese pear. This protein participates in inhibition of the growth of pollen tubes at the initial phase (at around 24 hours), but will be essential for the latter half of tube growth. Furthermore, the synthesis and decay of this protein depends upon types of pollination. Self-incompatibility reaction is caused by the protein synthesized in the selfed pistil in 24 hours after pollination and also by that degraded after that time. Although our present data do not reveal about the biological meaning and characterization of this protein(s), there is a report which pushes forward our data. That is to say, an explanation with reference to self-incompatibility reaction and enzyme has been reported by Bredemeijer (5, 6). He supposed a hypothesis that some peroxidase isozyme takes part in inhibition of the growth of pollen tubes accompanying the incompatibility reaction in Nicotiana alata.

It is very difficult to shed light on the relationship between this protein in the metabolic pathway and the recognition of self-incompatibility reaction. However, in order to establish that hypothesis, further experiments in regard to the reaction manner for the recognition have to be continued from the physiological and biochemical standpoints.

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Literature Cited

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ニホンナンの和合及び不和合受粉後の花粉管の
生長に伴うタンパク質の変動

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摘 要

ニホンナンの自家不和合性の、生理的機構を解明する
手がかりを得るために、「長十郎」の切枝を用い、和合「二
十世紀」，不和合（自花）受粉後の花粉管の生長及びその
形態，雌しつい中のタンパク質の質的，量的変化を調べた。

1. 自家受粉した花粉管は，およそ 36 時間後に生長
速度をおとし，48 時間後には花柱の基部で生長を停止
した。一方，他家受粉のものは，およそ 96 時間後に受
精に至った。

2. 自家受粉した花粉管の先端は，36 時間後には肥
大し，カロースが異常に付着し始めるものが見られた。
しかしこの時，他家受粉のものは，先端が と がってい
た。

3. 自家受粉した雌しつい中の水溶性及び膜バウンド性
のタンパク質量は，受粉後 48 時間まで変化はなく，そ
の後徐々に増加した。他家受粉した雌しついでは，両方の
タンパク質とも受粉後 24 時間ごとに一時的な減少があ
り，その後急激な増加が見られた。

4. 電気泳動で調べたタンパク質の泳動パターンは，
膜バウンド性の分画では，自家及び他家受粉の間に 72 時
間で差は見られなかった。しかし水溶性分画では，自
家受粉のものは，受粉後 24 時間後に No.7 バンドのピー
クが増加し，その後減少した。一方他家受粉でのこの
バウンドは，自家受粉のものと逆のパターンを示した。つ
まり，24 時間後に一時的に減少し，その後徐々に増加
した。無受粉でのこのバウンドは，自家受粉と他家受粉と
の中間的な動きを示した。