POLLEN TUBE INCOMPATIBILITY REACTION ON THE STIGMA IN SELF POLLINATED *SINAPIS ALBA* L.

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ABSTRACT

After selfpollination of *Sinapis alba* L. pollen tubes growth is inhibited on the stigma.

The pollen grains germinate 3-4 hours after pollination. The pollen give rise to one or more pollen tubes. They grow along the papillae. In the place of contact between the papilla and pollen tube the pellicula is digested. Then the direction of pollen tube growth changes completely. Pollen tubes grow back on the exine of their own pollen grain, or turn into the air. The pollen tubes growth was inhibited in 6-8 hours after selfpollination. After crosspollination usually there is no incompatibility reaction.

KEY WORDS: *Sinapis alba*, selfincompatibility, pollen-stigma interaction, pollen tube.

INTRODUCTION

Selfincompatibility is an important factor for seed-setting in many plants. *Brassicaceae* are as self-incompatible, and the mechanism of incompatibility is located on the stigma (Dickinson 1994; Dickinson and Lewis 1973; Nasrallah and Nasrallah 1984; Nettancourt 1977; Ockendon 1972; Roberts and Dickinson 1983). *Sinapis alba* is known as a crosspollinated plant. The aim of the present investigations was to localize the selfincompatibility barrier.

MATERIAL AND METHODS

Sinapis alba L. (*Brassicaceae*) plants were grown in a greenhouse in Lublin during the early spring 1994. They flowered abundantly, flowers in I-III inflorescence were emasculated and crosspollinated, or only isolated and selfpollinated. Pollen grain germination was checked in vitro.

Pistils from open flowers and some from buds were fixed in 2.5% glutaraldehyde or in a 1:3 acetic acid : ethyl alcohol mixture. Some pistils were macerated in 1N NaOH and stained with aniline blue for observations of the pollen tube growth under the fluorescence microscope (Nikon, UV 430 nm). Others were prepared for scanning electron microscopy (TESLA SEM). Pistils for scanning investigation were fixed and processed according to routine methods. Pistils were embedded in EPON resin. Semithin section (1 μ m) were stained with toluidine blue or PAS.

RESULTS

Several flowers are opening every day in the inflorescence of *Sinapis alba*. The anthers dehisce 2-4 hours after anthesis. The pistil has a solid style and dry type of stigma divided by a furrow (Figs 1, 2). On the stigma surface the epidermal cells develop as papillae coated with a thin pellicula. Before anthesis the cylindrical papillae are in a close proximity (Fig. 2). In open flowers their basal parts are swollen, the tips are loose (Fig. 4). During pollination the pollen grains are caught between the tips of the papillae (Figs 3, 5) many others papillae collapse (Fig. 3).

Self and crosspollinated pollen germinate after 2-3 hours. The pollen grains swell and pores are exposed. They are located in the furrow covered by a thin exine (Fig. 6). A pollen grain can give rise to 1, 2 or 3 pollen tubes. Especially after selfpollination many pollen grains form 2, 3 pollen tubes (Fig. 11). The pollen grains do not adhere strongly the stigmatic surface and can be easily washed away.

After crosspollination the growing pollen tube in 1 hour contacts the papillae. In the place of contact the pellicula is digested. The pollen tube grows along the papillae down to the base (Fig. 7). Then, it grows along the style and after 8 hours reach the ovules.

After selfpollination the pollen tubes grow normally until they touch the cell wall of the papilla. The direction of the growth is changed after 1 hour, many pollen tubes are turned out by 180° (Fig. 10, a thin arrow). After next two hours some growing pollen tubes turn round (Fig. 8), spread on the exine of their own pollen grains (Fig. 9) or stretch into the air (Fig. 11). The place of contact between the papilla and pollen tube often gives a bright callose fluorescence (Fig. 10, a thick arrow). In selfpollinated pistils no pollen tubes were found in the style or ovary 12 hours after pollination.

Fig. 1. Immature stigma of *Sinapis alba* from a bud 3-4 mm long, SEM x 300.
Fig. 2. The furrow of the immature stigma showing closely packed papillae, SEM x 270.
Fig. 3. Pollen grains retained between papillae, x 450.
Fig. 4. Immature stigma of *Sinapis alba* from a bud 3-4 mm long, SEM x 350.
Fig. 5. Papillae of mature stigma showing spaces between tips, SEM x 3500.

Fig. 6. Pollen grains on the stigma after anthesis, SEM x 3500

Fig. 7. Stigma 24 hours after crosspollination, SEM x 3500.

Figs 8, 9. A screwing pollen tube 24 hours after selfpollination, SEM x 4000.

Fig. 10. Callose fluoescence in the stigma 12 hours after self-pollination, x 450.

Fig. 11. Callose fluorescence of pollen tubes after self-pollination, x 800.

DISCUSSION

Controlled pollination of *Sinapis alba* proved that it is a self-incompatible plant. The dry type of the stigma and relatively early incompatibility reaction are considered as evolutionary progressive sporophytic type of incompatibility (Nettancourt 1977). The dry stigma gives good conditions for hydration of self and foreign pollen grains. The self pollen grains are able to germinate on the stigmas but they cannot grow inside the pistil tissue. Very similar selfincompatibility reactions were described in *Brassica oleracea* and *Raphanus* (Dickinson and Lewis 1973; Ockendon 1972; Elleman et al. 1988). Dickinson found a very similar behaviour of self pollen tubes of *Brassica*. They tried to escape into the air from the contact with their own stigma. It is contrary to the most popular opinion that pollen tubes cannot grow without any stable support. Probably they "prefer" to grow along the supporting surface, but if it is not "suitable" the pollen tubes take the only possible opportunity of growing into the air. It means that they distinguish the character of the stigma after some time of close contact. In *S. alba* about 1 hour passed before the reaction is noticeable in the pollen tube growth.

Dickinson (1993 and 1994) and others (Roberts and Dickinson 1983; Elleman and Dickinson 1990) concentrated on the coating of pollen grains and enzymes which digest the pellicula and cuticle on the stigmatic cells in *Brassica*. The enzymes are probably esterases playing the role of signals for stigmatic cells. In *Brassica* the pollenkit reacts with pellicula and makes a kind of insoluble "glue" fixing the pollen grain to the stigma. In *Sinapis* such sticking reaction was not found. The pollen grains were only loosely connected with the stigma. Their shape and size adjusted to the distances between the tips of the papillae. We assume that, the role of esterases in *Sinapis* is not quite the same as it was suggested for *Brassica*. The beginning of germination and pollen tube growth were similar after self- and cross- pollination. Esterase digested the pellicula under the tip of the pollen tube, thus enabling the papilla and pollen tube to come into a direct contact and exchange chemical signals. It is not clear if the pollen surface

esterases or other substances excreted later from the pollen tube are signalling and triggering the incompatibility reaction. Callose spots described by Kerhoas et al. 1983 and by Roberts and Dickinson (1983) were also found in *Sinapis*. Thus appears not immediately after pollination but later when pollen tubes are in direct contact with papillae. This callose may to isolate two incompatible partners.

After several hours the incompatible pollen tubes drastically changed their growth direction in different ways, but always minimizing the contact with "unsuitable" stigma. It is very likely that after the stigmatic cells had been informed by signals from the pollen tube about their self-character the synthesis of some repellent substances began. The substances could be glycoproteins as it was found in *Brassica* (Nasrallah and Nasrallah 1984). Such substances can modify the pollen tube growth in vivo and in vitro (Roberts and Dickinson 1983; Dickinson 1994). The suggestions that the selfincompatibility barrier is located in hydrated cellulose-pectine layer of stigmatic cell wall appeared relatively a long time ago (Kanno and Hinata 1969). New considerations on selfincompatibility in *Brassicaceae* support that opinion (Dickinson 1993, 1994).

The mechanism of selfincompatibility in *Sinapis* can depend on the repulsion reaction (eg. excretion of S-glycoproteins) in the stigma blocking the pollen tube growth inside the pistil tissue.

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REAKCJA NIEZGODNOŚCI ŁĄGIEWKI PYŁKOWEJ NA ZNAMIENTU U SAMOZAPYLNEJ *SYNAPIS ALBA* L.

STRESZCZENIE

U *Sinapis alba* L. przeprowadzono samozapylenie i zapylenie krzyżowe aby stwierdzić, w jakim okresie fazy progamicznej działa bariera samoniezgodności. Po samozapyleniu i zapyleniu krzyżowym pyłek kiełkował z 1, 2 lub 3 porusów. Łągielki z pyłku pochodzącego z obcych roślin po wykiełkowaniu rosły ku papillom, a po wejściu w bezpośredni kontakt z ich ścianami rosły nadal wzdłuż tych ścian ku podstawie papilli. Samoniezgodne łągielki pyłkowe początkowo rosły ku papillom znamienia. W miejscu zetknięcia szczytu łągielki i papilli następowało wytrawienie pelikuli okrywającej papille. Po osiągnięciu bezpośredniego kontaktu między ścianą komórkową papilli i łągielki pyłkowej zmieniał się kierunek wzrostu łągielki. Większość samoniezgodnych łągielki zaczynała rosnąć w przeciwnym niż dotychczas kierunku. Ścieliły się one po egzynie własnego ziarna pyłku, a niekiedy rosły w powietrze, przy czym skręcały się jak sprężyny.

SŁOWA KLUCZOWE: *Sinapis alba*, samoniezgodność, interakcja pyłek – znamie, łągielka pyłkowa.