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# Repressing the expression of self-incompatibility in crucifers by short-term high temperature treatment

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**Summary.** The effect of short-term high temperature on the expression of self-incompatibility was studied in detached flowers of *Brassica oleracea, B. campestris* and *Raphanus sativus.* The expression of self-incompatibility was repressed by treatment of pistils at 40 °C for 15 minutes. Treatment at 50 °C repressed self-incompatibility but it also disturbed pollen tube elongation into stylar tissue. S-glycoproteins did not show any quantitative changes during the intact pistil treatment under 50 °C. Callose was occasionally found in the treated papilla where the self pollen tube penetrated. The repressing effect of the 40 °C treatment was found to be reversible, and this reversibility depended upon the environmental temperature of plant. Plants grown at 15/5 °C (day/night temperature) completely recovered self-incompatibility 2 h after treatment, while those grown at 20/10°, 25/15 °C did not. The reversibility of the expression of self-incompatibility correlated with the distortion of plasma membrane in the papilla. It is considered that high temperature affects the pollen tube penetration system in pistils rather than the recognition system between pistils and pollen. The treatment of dehiscing anthers at 40 °C killed the pollen.

**Key words:** Self-incompatibility – Short-term high temperature – Crucifers

## Introduction

The effect of high temperature on the repression of self-incompatibility has been investigated in various plants (de Nettancourt 1977). In cruciferous plants whose self-incompatibility is controlled by a sporophytic system, it has been reported that a high temperature of about 30 °C weakened the degree of self-incompatibility (Johnson 1971; Gonai and Hinata 1972). The effect of a short-term high temperature has been reported only by Matsubara (1980), who did a phytotron treatment at 50 °C for 25 min in order to overcome self-incompatibility in *Raphanus sativus.* More data may be needed about such short-term treatments not only for the purpose of developing methods to obtain selfed seeds but also for studying the mechanism of self-incompatibility.

In the present experiment, the effect of a short-term high temperature was investigated paying special care to the following points: the flower was treated without any contact with hot water, since crucifers have dry stigma to which self-incompatibility is localized; instantaneous high temperature treatment was applied; the effect was observed for pollen-tube penetration, ultrastructure of pistils and S-glycoproteins.

## Materials and methods

The materials used were the homozygotes of $S^2$ and $S^{22}$ in *Brassica oleracea*, $S^1$, $S^7$, and $S^8$ in *B. campestris*, and $S^9$ and $S^{29}$ in *Raphanus sativus.* The genetic strains of *B. oleracea* were provided by N. P. A. van Marrewijk, Institute of Horticultural Plant Breeding, The Netherlands. The others were isolated from selfed progenies of a naturalized population in *B. campestris* and cultivars in *R. sativus* in our laboratory (Okazaki and Hinata 1984).

Newly opened flowers were detached at the receptacle. Each flower was set into a 1% agar gel which filled a test-tube (1.4 × 10 cm) to within about 1 cm from the lip. The test-tube with the flower was then inserted upside-down into another test-tube of a larger diameter. The upside-down flower was

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**Table 1.** Effect of high temperature treatment on pistils

| Treatment | | *B. campestris* | | | | *R. sativus* | | | |
|---|---|---|---|---|---|---|---|---|---|
| Temperature | Time | Self | | Cross | | Self | | Cross | |
| | | Stigma | Style | Stigma | Style | Stigma | Style | Stigma | Style |
| Control | | – | – | ++ | ++ | – | – | ++ | ++ |
| 35 °C | 15 min | – | – | ++ | ++ | – | – | ++ | ++ |
| 40 | 5 | – | – | | | | | | |
| | 10 | ± | ± | ++ | ++ | | | | |
| | 15 | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 50 | 5 | ± | ± | + | ± | | | | |
| | 10 | ++ | – | ++ | – | | | | |
| | 15 | ++ | – | ++ | – | ++ | – | ++ | – |

Pollen tube penetration into stigma and growth in style are shown by ++, +, ± and –, according to number of pollen tubes, abundant, 10–50, 5–10, and less than 5, respectively

placed as close as possible to the bottom of the larger test-tube, and the test-tube was put into hot water of the specified temperature. Thus, flowers were treated without any contact with water. Emasculated flowers were used for the pistil treatment and dehiscing anthers of flowers were used for the pollen treatment. The treated pistils were pollinated with untreated pollen and the untreated pistils were pollinated with treated pollen in the combination of similar and different S-genotypes, which are termed self- and cross-pollination, respectively.

Pollen-tube behavior was observed on the day following pollination using a fluorescent microscope after aniline blue staining. Score of ++ (abundant), + (about 10–50), ± (5–10) and – (less than 5) were given according to the number of pollen tubes penetrating into the stigma or growing into the style tissues. Three to five replicate observations were made.

For electron microscopy, stigmas were pre-fixed with 2% glutaraldehyde in a 0.05 M phosphate buffer pH 7.2 at 4 °C for 30 min and refixed with 1% $OsO_4$ in a 0.05 M phosphate buffer. The fixed stigma were dehydrated by an alcohol-acetone series and embedded in Spur resin. Sections (60–100 μm thick) were stained with 1% uranyl acetate in 50% ethanol and 0.1% lead citrate successively for 5 min each.

For quantitative analysis of S-glycoproteins, thirty stigmas were homogenized in 0.15 ml of phosphate buffer with a mortar and pestle. After centrifugation (10,000×g), a definite volume of supernatant was subjected to isoelectrophoretic analysis (Nishio and Hinata 1977). After staining with Coomassie Brilliant Blue (G250), quantities of S-glycoprotein bands were estimated by densitometry at 580 nm.

## Results

### *Self-compatibility immediately after treatment*

Pistils treated at 20 °C and 35 °C for 15 min expressed normal self-incompatibility, i.e. the pistils were penetrated by pollen tubes in cross- but not in self-pollination. The treatment at 40 °C for 5 min also did not affect self-incompatibility. However, penetrating pollen tubes were occasionally observed in self-pollination after the 40 °C – 10 min treatment. When the 40 °C – 15 min treatment was given to the pistils, an abundant number of pollen tubes penetrated up to the stylar tissue through the papilla cell wall in both self- and cross-pollinations (Table 1). It was occasionally observed in the treated self-pollinated stigmas that the penetrated papilla cells were strongly stained with fluorescence, suggesting callose deposition at the attached part (Fig. 1). In the 50 °C – 10 and 15 min treatments, the selfed pollen tubes penetrated the stigma tissue but they did not grow to the stylar tissue. The same was observed for cross-pollination. The pollen tubes stopped growing and the tips swelled (Fig. 1c). When the duration of the 50 °C treatment was shortened to 5 min, a few pollen tubes were observed in the stylar tissue in self- as well as in cross-pollinations. The response of pistils to these temperatures was similar in all three species, *B. oleracea, B. campestris* and *R. sativus.*

The treatment of dehiscing anthers at 20 °C for 15 min and 35 °C for 15 min did not have any effect on the behavior of self-incompatibility. When treatments at 40 °C for 15 min and 50 °C for 15 min were given, pollen germinated in neither self- nor cross-pollination on non-treated stigmas, ie. the treatments killed the pollen. In the 40 °C – 10 min treatment, no pollen was viable in *B. campestris* and very few pollen tubes penetrated papilla cells in the cross-pollination of *R. sativus* (Table 2).

In order to estimate the effect of temperature, the tip of a thermoelectric couple was placed upright in the test tube as if the tip were the pistil of a flower. When 40 °C was applied, 39 °C was reached within 30 s; in the case of 50 °C, 49 °C was reached within 1 min.

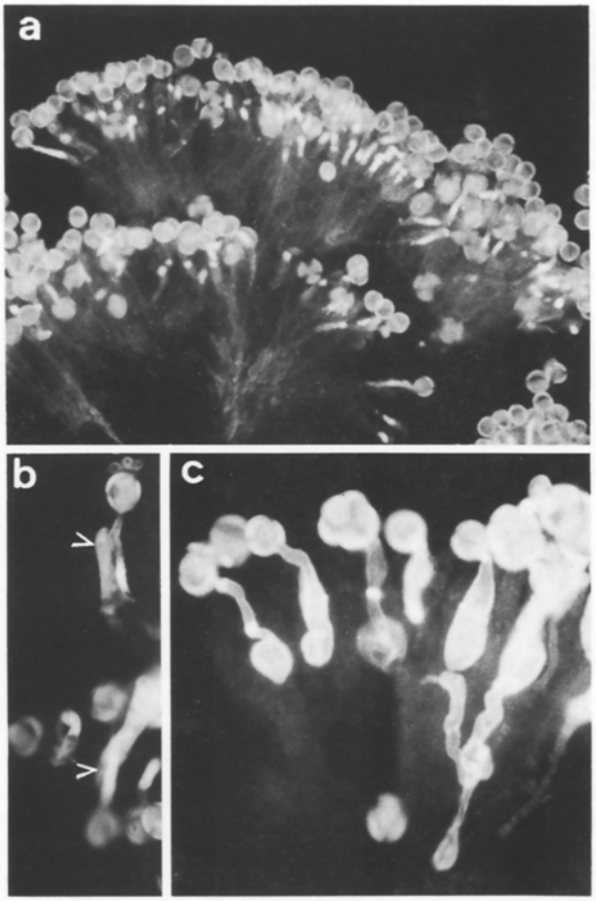


**Fig. 1a–c.** Pollen tube penetration during temperature treatments of pistils, when pollinated with untreated pollen, in *B. campestris*. **a** Many pollen tubes penetrated the pistil in self-pollination after the 40 °C – 15 min treatment (×100). **b** Callose formed in papillae (arrows) in which selfed pollen tubes had penetrated after treatment (×200). **c** Abnormal pollen tubes growing in pistil treated at 50 °C for 15 min (×200)

**Table 2.** Effect of high temperature treatment on dehiscing anthers

| Treatment | | *B. campestris* | | *R. sativus* | |
|---|---|---|---|---|---|
| Temperature | Time | Self | Cross | Self | Cross |
| Control | | – | ++ | – | ++ |
| 35 °C | 10 min | – | ++ | – | ++ |
| 40 | 10 | – | ++ | – | + |
| | 15 | – | – | – | +&– |
| 50 | 15 | – | – | – | – |

Pollen of the treated anther was pollinated onto un-treated stigmas, and germination of abundant, about half, and no pollen grains are shown by ++, + and –, respectively. In the case of +&–, variable results were obtained in more than 7 replications

**Table 3.** Recovery of self-incompatibility after high temperature treatment on pistils and anthers

| Pollination time after treatment (40 °C, 15 min) | *R. sativus* | | | *B. campestris* |
|---|---|---|---|---|
| | Pistil | Anther | | Pistil |
| | Self | Self | Cross | Self |
| Control | – | – | ++ | – |
| 0.h | ++ | – | – | ++ |
| 0.5 | ++ | – | – | ++ |
| 1.0 | ++ | – | – | +&– |
| 2.0 | +&– | – | – | +&– |
| 4.0 | – | – | – | +&– |

Presentation of data is the same as in Tables 1 and 2

**Table 4.** Effects of the environmental temperature of material plants on the recovery of self-incompatibility after the 40 °C – 15 min treatment in *Brassica compestris*

| Time duration in growth chambers | Pollination time after treatment | Temperature of growth chamber[a] | | |
|---|---|---|---|---|
| | | 15/5 °C | 20/10 °C | 25/15 °C |
| 2 days | 0 h | ++ | ++ | ++ |
| | 2 | – | ++ | ++ |
| | 4 | – | ++ | ++ |
| 4 | 0 | ++ | ++ | ++ |
| | 2 | – | ++ | ++ |
| | 4 | – | ++ | ++ |
| 7 | 0 | ++ | ++ | ++ |
| | 2 | – | ++ | ++ |
| | 4 | – | ++ | ++ |
| | | Field condition | | |
| 0 (Control) | 0 | | ++ | |
| | 2 | | – | |
| | 4 | | – | |

Pollen tube penetration is presented with the same symbols used in Table 1

[a] Day/night temperature

*Recovery of self-incompatibility*

The pistils treated at 40 °C for 15 min were kept at 25 °C in the dark for a specified period and then pollinated. When the intervening period was more than 2 h, the selfed pollen tubes could not penetrate the stigmas in many cases, ie. the treated papilla recovered their expression of self-incompatibility. Such recovery was not observed on those pistils treated at 50 °C for 15 min, however. In the dehiscing anther treatment, the pollen did not show any recovery of viability (Table 3).

The recovery of self-incompatibility in treated pistils was variable depending upon the season of the experiment. The effect of environmental temperature on recovery was, therefore, investigated for plants grown

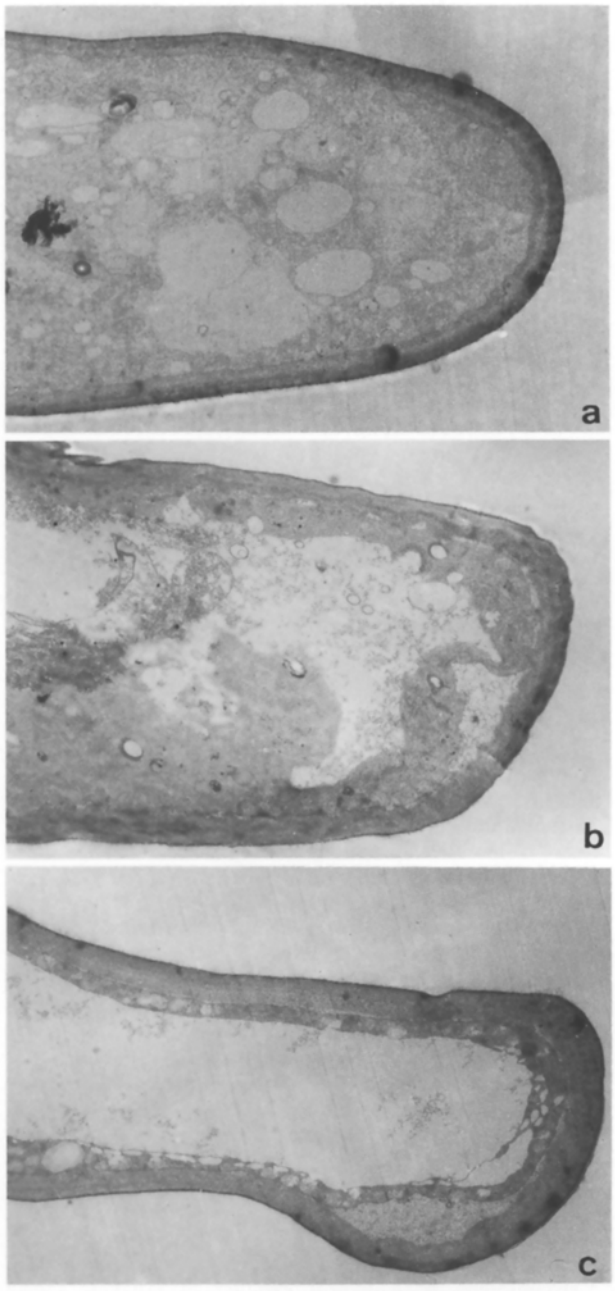


**Fig. 2a–c.** Ultrastructure of papilae of *B. campestris*. **a** A papilla before treatment. **b** A papilla immediately after treatment at 40 °C for 15 min, showing distortion of plasma membrane and vacuole. **c** A papilla 2 h after the end of the treatment showing recovery of the distortion of the plasma membrane

under controlled conditions. Plants grown at 15/5 °C (day/night temperatures) showed good recovery, while those at 20/10 °C and 25/15 °C did not (Table 4).

*Ultrastructure and S-glycoproteins*

The papillae treated at 40 °C for 15 min changed in ultrastructure after treatment; the plasma membrane

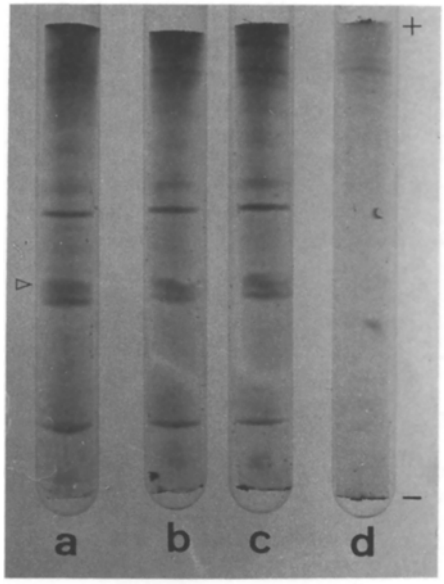


**Fig. 3.** Isoelectricfocusing profiles of stigma extracts after different temperature treatments for 15 min. *a* No treatment; *b* 40 °C; *c* 50 °C; *d* 60 °C. The band (pI 8.5, arrow) is specific to $S^8$ of *B. campestris*

and tonoplast were distorted and the vacuole expanded. Two hours after treatment, no distortion of plasma membrane in the pistils was observed though the expanded vacuole was still present (Fig. 2). The distortion of plasma membrane correlated with the expression of self-incompatibility.

Pistils treated at different temperatures for 15 min were subjected to the S-glycoprotein analysis. We could find neither quantitative differences nor alterations in the isoelectric band profiles between the stigmas of the control and those of the 40° and 50 °C treatments. When a 60 °C treatment was applied, however, S-glycoprotein bands were detected in neither *B. campestris* nor *B. oleracea* (Fig. 3).

When high temperature treatments (15 min) were applied to the stigma homogenate, the quantity of S-glycoproteins was equal in both the control and the 40 °C treatment, but it decreased in the 50 °C treatment to about half that of the control.

## Discussion

In the present experiment, instantaneous high temperature treatment was applied to pistils without any contact with hot water. According to a simulation by a thermocouple, it was estimated that the specified temperature was reached within 1 min. Treatment at 40 °C for 15 min was effective enough to repress the expression of self-incompatibility in pistils. Temperatures

higher than 50 °C also repressed self-incompatibility in pistils, but the pollen tube elongation into stylar tissue was concomitantly restricted.

A similar phenomenon was observed in the hot water treatment of stigma tips in *Ipomea fistulosa* by Prabha et al. (1982). It is assumed that some substances or stylar membrane systems necessary for pollen tube elongation were coincidentally affected by the 50 °C treatment. Matsubara (1980) reproted that treatment of whole plants in a growth cabinet at 50 °C for 25 min was effective for overcoming pistil self-incompatibility in *Raphanus*. The discrepancy in the effect of the 50 °C treatment is believed to be due to the differences in the methods of treatment – growth cabinet as opposed to test-tube; condition of materials – intact as opposed to detached pistils. We could not resolve this, because of the difficulty of shortening the lag time of treatment and of regulating temperature distribution in a growth cabinet. However, it is possible that self-incompatibility in pistils can be completely repressed by high temperature, and the optimum conditions are a function of temperature and duration. Stronger treatment may bring about deleterious effects for pollen tube growth.

According to Marcucci et al. (1982), dry pollen of Rosaceae and Liliaceae retained germinating ability even after 24 h at 40 °C and 50 °C. In contrast, treatments at 40 °C or more for 15 min killed pollen in dehiscing anthers in the present material. In rice, emasculation for hybridization is routinely done by dipping inflorescences in hot water at 42 °C for 7 min. The heat sensitivity of pollen is variable depending upon species and water content; however, anthers seem to be more sensitive to high temperature than pistils.

Genetic analysis of self-compatibility versus self-incompatibility revealed that the expression of self-incompatibility is controlled not only by *S* alleles but also by a complementary modifying gene. It was considered that the *S* alleles participate in a recognition reaction between pollen and stigma, and the complementary modifying gene participates in the metabolism of pollen tube penetration (Hinata et al. 1983; Hinata and Okazaki 1986). Regarding the treatment at 40 °C for 15 min the presence of S-glycoproteins in the stigma was recognized. Further, callose was occasionally found in the self-pollinated papilla. These results may suggest the occurrence of a recognition reaction in the treated stigmas. Distortion of the plasma membrane in treated papilla and its reconstruction along with the recovery of self-incompatibility may imply that normal metabolism in the papilla was disturbed by the heat treatment, though the production and role of heat shock proteins has yet to be determined. We considered that high temperature affects the pollen-tube penetrating system, which is controlled by the plasma membrane as well as the complenentary modifying gene. However, other considerations are also possible. For example, high temperature could block the information transfer system from the recognition reaction to pollen-tube penetrating system, etc. Relations between high temperature and the waxy substances on stigma remain to be investigated.

Self-incompatibility is widely utilized in the commercial production of $F_1$ hybrids in Brassica crops. In practice, self- or sister-brother fertilization occurs occasionally, producing so-called sib seed contamination depending upon environmental conditions. It is assumed that high temperature is one of the causes for the occurrence of the sib seed contamination, and that escape from high temperatures decreases such contamination. On the other hand, a short-term high temperature treatment may be utilized as one of techniques for producing selfed seeds, although untreated pollen should be provided.

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