trichlorfon were very effective in killing Diabroticite beetles in a field of sweet corn (*Zea mays* L.) even after 3 heavy rains totalling 5 cm. Five cut fruit halves placed 5 m apart killed the following mean numbers of beetles after 5 days: methomyl (SCR 227 ± 95, WCR 19 ± 13) and trichlorfon (SCR 287 ± 159, WCR 40 ± 39).

It is evident from these and many other experiments performed with bitter fruits as poison baits, that these are effective in killing large numbers of Diabroticite beetles. Such traps have possible utility in protecting vegetable gardens from beetle attack and in monitoring beetle populations in corn fields. Various traps have been employed using homogenates of bitter fruits poured on vermiculite, sponges, or paper; and cut fruits placed in buckets and other containers.

Literature Cited

1. Audier, H. E. and B. C. Dass. 1966. Mass spectrometry of tetracyclic triterpenes. Part I – the cucurbitacin group. *Tetrahedron Letters* 20:2205-2210.
2. Chambliss, O. L., H. T. Erickson, and C. M. Jones. 1968. Genetic control of bitterness in watermelon fruits. *Proc. Amer. Soc. Hort. Sci.* 93:539-546.
3. _____ and C. M. Jones. 1966. Chemical and genetic basis for insect resistance in cucurbits. *Proc. Amer. Soc. Hort. Sci.* 89: 394-405.
4. _____ and _____ . 1966. Cucurbitacins: specific insect attractants in cucurbitaceae. *Science* 153:1392-1393.
5. Contardi, H. G. 1939. Estudios geneticos en *Cucurbita* y consideraciones agronomicas. *Physis* (Buenos Aires) 18:331-347.
6. DaCosta, C. P. and C. M. Jones. 1971. Resistance in cucumber, *Cucumis sativus* L. to three species of cucumber beetles. *HortScience* 6:340-342.
7. David, A. and D. K. Vallance. 1955. Bitter principles of cucurbitaceae. *J. Pharmacy Pharmacol.* 7:295-296.
8. Grebenscikov, I. 1955. Notulae cucurbitological II: Uber *Cucurbita texana* A. Gray und ihre Kreuzing mit einer hochgeguchteten *C. pepo* form. *Kulturpfl.* 3:50-59.
9. Howe, W. L., J. R. Sanborn, and A. M. Rhodes. 1976. Western corn rootworm and spotted cucumber beetle associations with *Cucurbita* and cucurbitacins. *Environ. Entomol.* 5:1043-1048.
10. Lavie, D. and E. Glotter. 1971. The cucurbitacins, a group of tetra cyclic triterpenes. *Forts. Chemie Organ. Naturstoffe* 29:307-362.
11. Metcalf, R. L. 1979. Plants, chemicals, and insects: some aspects of coevolution. *Bul. Entomol. Soc. Amer.* 25(1):30-35.
12. _____, R. A. Metcalf, and A. M. Rhodes. 1980. Cucurbitacins as kairomones for Diabroticite beetles. *Proc. Nat. Acad. Sci.* (USA). 77(7):3769-3772.
13. _____, A. M. Rhodes, Jane E. Ferguson, and Esther R. Metcalf. 1979. Bitter Cucurbita spp. as attractants for Diabroticite beetles. *Cucurbita Genetics Coop. Rpt.* 2, 38-39, June.
14. Rehm, S. 1960. Die Bitterstoffe der Cucurbitaceen. *Ergeb. Biol.* 22:108-136.
15. _____ and J. H. Wessels. 1957. Bitter principles of the Cucurbitaceae VIII. Cucurbitacins in seedlings, occurrence, biochemistry, and genetical aspects. *J. Sci. Food Agr.* 8:687-691.
16. Sharma, C. C. and C. V. Hall. 1973. Relative attractance of spotted cucumber beetle to fruits of fifteen species of Cucurbitaceae. *Environ. Entomol.* 2:154-156.
17. Watt, J. M. and M. G. Breyer-Brandwijk. 1967. The medicinal and poisonous plants of southern and eastern Africa, 2nd ed. E&S Livingston, Edinburgh.
18. Whittaker T. W. 1951. A species cross in *Cucurbita. J. Hered.* 43: 65-69.

J. Amer. Soc. Hort. Sci. 105(6):842–846. 1980.

Overcoming Self-incompatibility in *Raphanus sativus* L. with High Temperature[1]

Sachiko Matsubara

Faculty of Agriculture, Okayama University, Okayama 700, Japan

Additional index words. Raphanus sativus

Abstract. **Self-incompatibility of *Raphanus sativus* L. was partially overcome by exposing plants to temperature of 30 to 50°C. The most effective treatment was 50°C for 25 minutes. Scanning electron microscopical (SEM) observation of pollen tubes on the papillae surface have shown that exposure of the gynoecium to 50°C for 25 minutes resulted in pollen tube growth following self-pollination that resembled that of cross-pollination. Openings of papillae and detached pollen grains and tubes were found as the result of successful pollen tube penetration of papillae. Fluorescence microscopical (FM) observation served to confirm these observations made by SEM. However, incompatible pollen failed to germinate although pollen grains were attached to the papillae by aid of their waxy surface substances.**

Most of *Raphanus sativus* cultivars are self-incompatible, and the response is generally known to be a sporophytic type (5, 30, 31). In order to breed pure lines or to retain parent lines for F_1 seed production, overcoming self-incompatibility is an important problem. The methods of overcoming self-incompatibility such as hormone application (8, 9, 18), temperature treatments (1, 3, 11, 12, 15, 25, 32), mentor pollen application (7, 14, 26, 28, 29), bud or old flower pollination (2, 27), placental, ovarian, or test tube pollination (4, 13, 20) and a mechanical or electric method (22, 23, 24) differ depending on plant genera. The self-incompatibility of *R. sativus* may be partially overcome by either bud pollination or CO_2 gas treatment (19), bud pollination has been used on a commercial basis. Recently, the effect of various high temperature treatments or mentor pollen application to promote pollen germination on incompatible stigma has been reported (30, 31).

This paper presents the result of studies conducted with high temperature treatments for overcoming the self-incompatibility with observations made during the course of these investigations with SEM and FM on the interaction between self-incompatible pollen and stigma.

Materials and Methods

Selections of self-incompatible plants from 'Honbashi-taibyo-Minowase' (H-Mino) and 'Minowase' (Mino) radish were used.

[1]Received for publication April 2, 1980.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

They were found to be cross-compatible. Seedlings were vernalized at 1°C for 2 weeks, and placed in a plastic greenhouse. Experiments were repeated several times from April to October, 1979. Flowering shoots which had been covered with paper bags a day before flowering were removed, and before treatment anthers were removed and kept in Petri dishes at room condition. The flowering shoots were treated as selected temperatures and durations in growth chambers as follows: 30, 35, 40 and 50°C for either 15, 20, 30, 45, 60, 90, 120 and 150 min. Stigmas were self-pollinated just after treatment, and the flowering shoots were dipped in an aqueous solution of 8-oxyquinoline and 3% sucrose at 25°, and observed for embryo development 2 weeks later.

In order to determine the type of stigma-pollen interaction by SEM, stigmas 0.5, 1 and 4 hr after self, cross, or self-pollination on stigma treated 50°C for 25 min (HT), as well as immature and mature stigmas before pollination were fixed in 40% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 at 5°C for 24 hr. After changing buffer 3 times during a 16 hr period, stigmas were then post-fixed with 1.6% osmium solution in the same buffer at 5° for 3 hr. The materials were dehydrated by the method of Gogue et al. (10). The tissue was critical point dried and mounted on a SEM stub with a drop of Bond (G. Konishi, Co.), coated with Au Pd, and viewed in the SEM (Akashi MSM-4S) at 21 KV accelerating potential. Observation of pollen tube growth in the gynoecium was made by FM. Stigmas were fixed after 1, 4, 10 or 24 hr of self- and cross-pollination and HT; dehydrated, embedded in paraffin, sectioned at 15 μm and stained in 0.1% anilin blue in 0.1 N K_3PO_4 for 3 hr, then mounted in 50% glycerin, and observed by FM within 2 to 3 days.

Results and Discussion

The percentage of fruit set is indicated in Table 1. The highest percent fruit set obtained for both cultivars was by treating with 50°C for 20 to 25 min followed by 40° for 30 min (Mino) and 45 min (H-Mino). The treatments with 50° and 40° were effective from 5 to 30 min and 20 to 30 min, respectively, while shorter periods of treatment with 35° and 30° were ineffective. The number of seeds per fruit tended to increase with effective treatments which induced high fruit set. The most effective treatment was found to be at 50° for 25 min in both cultivars.

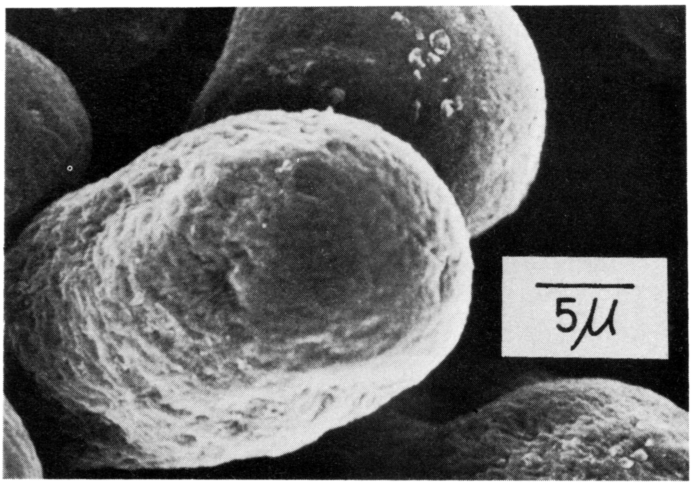

Fig. 1. Immature papillae covered with soft waxy substance. × 3000.

Immature and mature papillae of *Raphanus sativus* are presumably covered with a waxy substance as observed in *Brassica oleracea* L. (Gemmifera group) by Roggen (21), and are similar in size (Fig. 1 and 2), although they seemed to be softer in immature than in mature papillae. Overcoming self-incompatibility by bud-pollination may be related to the softness of immature papillae. Pollen grains failed to germinate after 30 min following selfing, but were attached to the papillae by aid of their waxy surface substances. The papillae did not change in shape after pollination (Fig. 3). They were still in the same shape 1 to 4 hr after self-pollination, however, on its surface, fragment materials which are apparently a part

Table 1. Effect of high temperature treatment of the gynoecium of *Raphanus sativus* L. to overcome self-incompatibility.

Treatment		H-Mino radish				Mino radish			
Temperature (°C)	Time (min)	No. of pollinations	No. of fruit	Fruit set per pollination (%)	No. of seeds per fruit	No. of pollinations	No. of fruit	Fruit set per pollinations (%)	No. of seeds per fruit
50	5	58	8	13.8	2.3	80	23	28.8	1.9
	10	41	11	26.8	2.0	90	20	22.2	2.4
	15	40	16	40.0	3.3	76	18	23.7	3.8
	20	45	30	66.7	3.5	59	29	49.2	3.3
	25	59	39	66.1	4.5	60	39	65.0	3.2
	30	79	12	15.2	2.9	76	10	13.2	2.2
40	20	39	8	20.5	3.2	50	13	26.0	2.5
	30	38	8	21.1	3.6	62	37	59.7	2.5
	45	27	13	48.1	4.3	64	20	31.3	2.3
	60	35	5	14.3	2.6	55	2	3.6	2.0
35	15	51	0	0	0	50	0	0	0
	30	42	2	4.8	1.0	53	16	30.2	3.6
	45	58	10	17.2	2.0	34	11	32.4	2.6
	60	41	22	53.7	5.3	41	10	24.4	1.5
	90	46	8	17.4	2.3	35	8	22.9	1.6
30	60	48	0	0	0	45	0	0	0
	90	45	0	0	0	59	7	11.9	2.3
	120	50	5	10.0	3.5	47	19	40.4	2.8
	150	31	1	3.1	3.0	39	0	0	0
Control		55	0	0	0	55	0	0	0

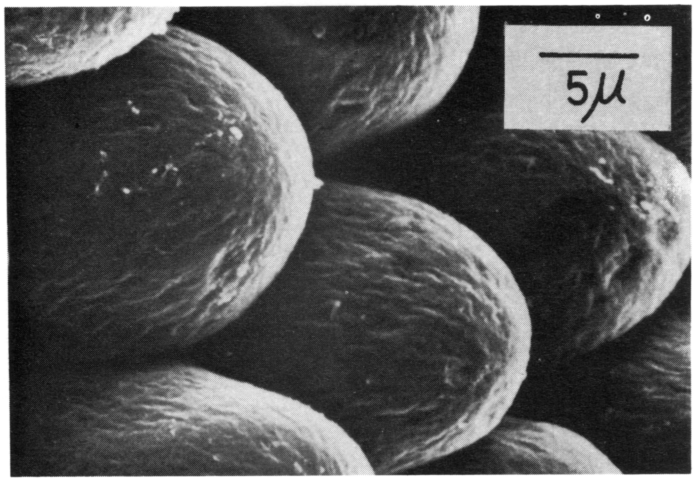

Fig. 2. Mature papillae covered with hard waxy substance. × 3000

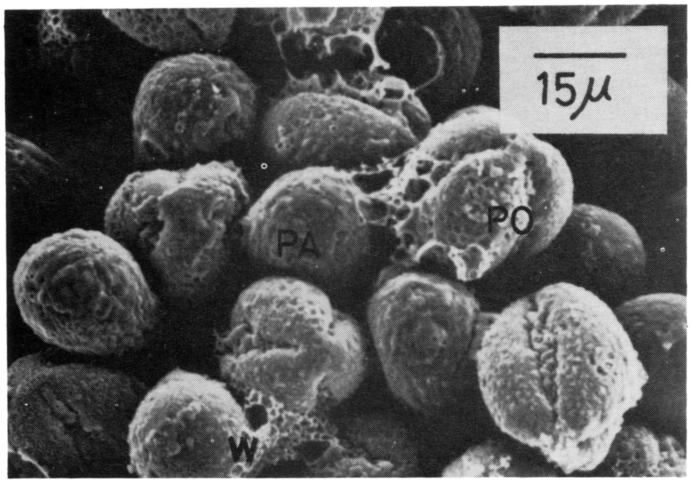

Fig. 3. Incompatible pollen behavior 30 min after self-pollination. PA= papilla; PO=pollen; W=waxy substance. × 1000

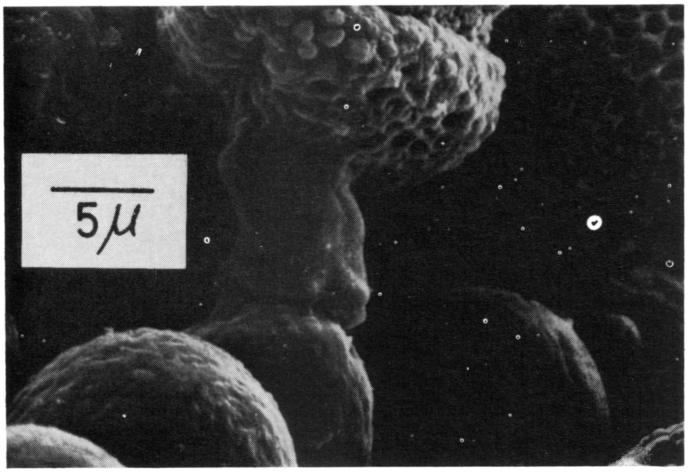

Fig. 4. Pollen germinated 30 min after cross-pollination. × 3000

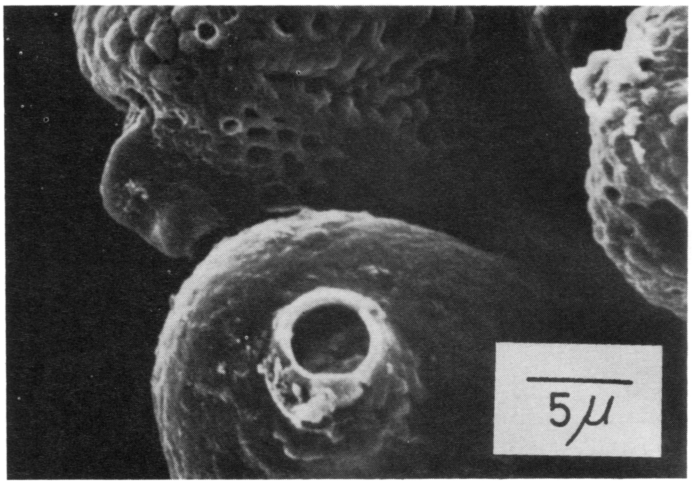

Fig. 5. Scar on a papilla and pollen which germinated 30 min after cross-pollination. × 3000

of the waxy substance which had been in between papillae and pollen grain were still found to stick to the papillae. In the case of cross-pollination, the germinated pollen adhered to the surface of papillae with pollen tubes emerging within 30 min (Fig. 4). Some pollen tubes that were not firmly adhered to the papillae may become detached during tube penetration (Fig. 5). Some papillae were shrunk, flatten in shape or appeared empty, and some empty pollen grains are found leaving the pollen tube on the papillae surface within 1 hr after cross-pollination. Papillae had 1 or 2 openings to which the pollen tubes had become attached and penetrated into, and the papillae were flattened within 4 hr after cross-pollination (Fig. 6). This behavior of pollen grain which is seen in stigma of self-incompatible combination in *R. sativus* resembles closely that in *B. oleracea* (Gemmifera group) (6, 21). Pollen walls became attached firmly and were connected with papillae by a waxy substance within 30 min after HT. Pollen tubes became embedded in the papillae; papillae had an opening by penetration of pollen tube and some were shrunk 4 hr after HT (Fig. 7). The behavior of pollen on papillae after HT resembled that of cross-pollination, but pollen germination and tube penetration began to start a few hours later than normal cross-pollination. The

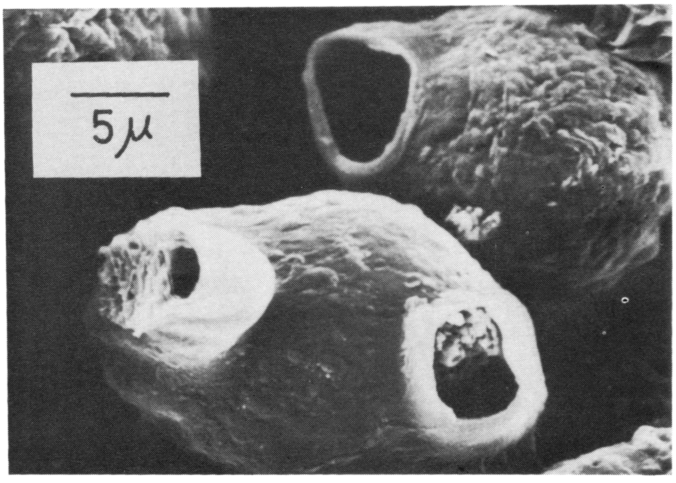

Fig. 6. Two openings in a papilla and a cast of pollen skin remaining on the papilla 4 hr after cross-pollination. × 3000

Fig. 7. Top view of papillae with pollen filling the interspaces. four hr after HT. x 1000

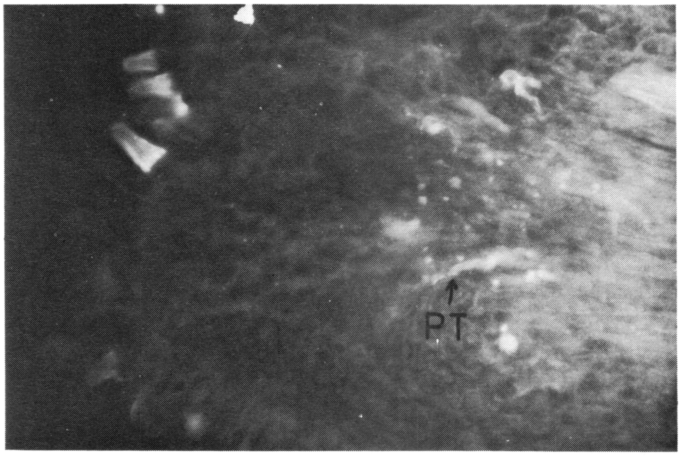

Fig. 9. Pollen-tube growth in the stigmatic tissue 4 hr after cross-pollination. PT=pollen tube. x 50

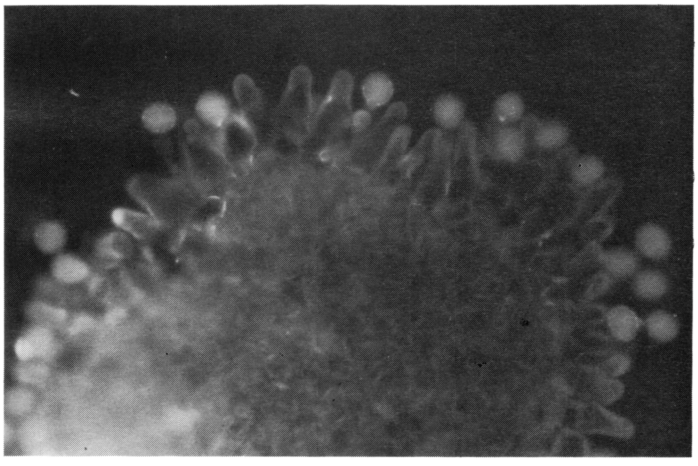

Fig. 8. Pollen on the stigmatic papillae surface 24 hr after self-pollination. x 50

fluorescent staining method is based on the selective absorption by callose of the dye, anilin blue (16). When stained, callose fluoresces UV light at 356 nm. Fluorescing pollen tubes did not appear in cross or longitudinal section of stigmas when observed within 24 hr after self-pollination (Fig. 8). Conversely, several pollen tubes per cross section at the center of the stigma were observed in cross-pollination after 1 hr (Table 2), in the middle style within 4 hr (Fig. 9), and at the upper part of the ovary within 10 hr. Although pollen germination was observed within 1 hr after HT, pollen-tube growth to the middle of stigma was first observed within 4 hr, at the middle of style within 10 hr (Fig. 10) and at the upper ovary within 24 hr. Pollen-tube growth of HT started later than cross-pollination. These results confirm the observation made by SEM.

High temperature treatments have been found to be effective for overcoming self-incompatibility in many plant genera, such as *Brassica oleracea* (32), *Chrysanthemum* (25), *Lilium* (1, 3, 12, 15, 18) and *Oenothera* (11). Temperatures used by these authors were, however, lower than 35°C or for only 5 min at 50°. The high range of temperatures used in the present experiment may be explained by the alteration of the waxy substance of papillar wall to a semisolid physical nature. This change is probably not enzymatic, although Roggen (21) and Carter et al. (6) stated that in cross-pollination the papillar wall is

Table 2. Pollen-tube growth in the gynoecium of 'H-Mino' radish as observed by fluorescence microscopy.

Pollination	Time after pollination (hr)	No. of pollen-tube per section			
		Stimgatic surface	Stigma middle	Style middle	Upper ovary
Self	1	–	–	–	–
	4	–	–	–	–
	10	–	–	–	–
	24	–	–	–	–
Cross	1	5	2	–	–
	4	7	5	2	–
	10	4	1	2	1
	24	10	5	2	2
50°C for 25 min following selfing	1	4	–	–	–
	4	10	–	–	–
	10	7	3	2	–
	24	8	3	1	1

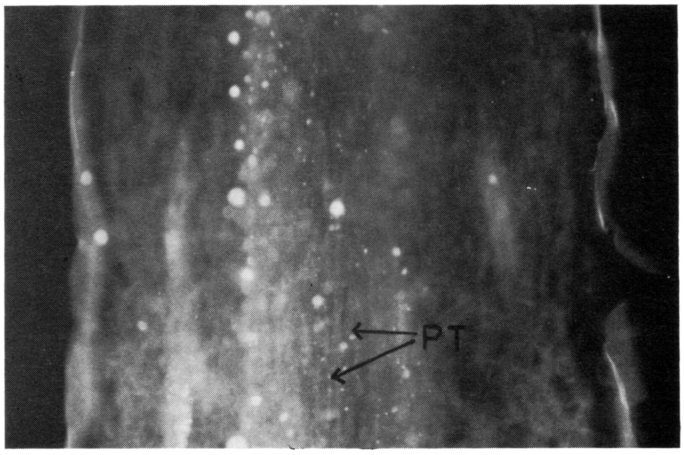

Fig. 10. Pollen tubes in mid-stylar tissue 10 hr after HT. x 50

digested by enzyme activity secreted from pollen tubes. Effective pollination methods used for overcoming self-incompatibility such as using a steel-brush (22) and electrically stimulated (23, 24) were also due to removal of waxy substances of the papillar wall.

Temperature may also overcome incompatibility, by denaturing proteins involved in the incompatibility reaction.

Literature Cited

1. Ascher, P. D. and S. J. Peloquin. 1966. Influence of temperature on incompatible and compatible pollen tube growth in *Lilium longiflorum*. *Can. J. Genet. Cytol.* 8:661-664.
2. _____ and _____. 1966. Effect of floral age on the growth of compatible and incompatible pollen tube in *Lilium longiflorum*. *Amer. J. Bot.* 53:99-102.
3. _____ and _____. 1970. Temperature and the self-incompatibility reaction in *Lilium longiflorum* Thunb. *J. Amer. Soc. Hort. Sci.* 95:586-588.
4. Belatokova, V. and J. Tupý. 1968. Test-tube fertilization in *Nicotiana tabacum* by means of an artificial pollen tube culture. *Biol. Plant.* 10:266-270.
5. Brewbaker, J. L. 1957. Pollen cytology and self-incompatibility system in plants. *J. Hered.* 48:271-277.
6. Carter, A. L., S. T. Williams, and T. McNeilly. 1975. Scanning electron microscope studies of pollen behaviour on immature and mature Brussels sprout (*Brassica oleracea* var. Gemmifera) stigmas. *Euphytica* 24:133-141.
7. Dayton, D. F. 1974. Overcoming self-incompatibility in apple with killed compatible pollen. *J. Amer. Soc. Hort. Sci.* 99:190-192.
8. Emsweller, S. L. and N. W. Stuart. 1948. Use of growth regulating substances to overcome incompatibilities in *Lilium*. *Proc. Amer. Soc. Hort. Sci.* 51:581-589.
9. _____, J. Uhring, and N. W. Stuart. 1960. The roles of naphthalene acetamide and potassium gibberellate in overcoming self-incompatibility in *Lilium longiflorum*. *Proc. Amer. Soc. Hort. Sci.* 75:720-725.
10. Gogue, G. L., H. P. Rasmussen, and G. R. Hopper. 1976. Structure of a single tissue prepared for analysis by light, scanning and transmission electron microscopy. *J. Amer. Soc. Hort. Sci.* 101:224-228.
11. Hecht, A. 1964. Partial inactivation of an incompatibility substance in the stigmas and styles of *Oenothera*. p. 237-243. *In* H. F. Linskens (ed.) Physiol. fertilization. North-Holland, Amsterdam.
12. Hopper, J. E., P. D. Ascher, and S. J. Peloquin. 1967. Inactivation of self-incompatibility following temperature pretreatments of styles in *Lilium longiflorum*. *Euphytica* 16:215-220.
13. Kanta, K. and P. Maheshwari. 1963. Intraovarian pollination in some *Papaveraceae*. *Phytomorphology* 13:215-229.
14. Knox, R. B., R. R. Willing, and A. E. Ashford. 1972. Role of pollen-wall proteins as recognition substances in interspecific incompatibility in poplars. *Nature* 237:381-383.
15. Kwack, B. H. 1965. Stylar culture of pollen and physiological studies of self-incompatibility in *Oenothera organensis*. *Physiol. Plant.* 18:297-305.
16. Majumder, S. K., K. R. Kerns, J. L. Brewbaker, and G. A. Johannessen. 1964. Assessing self-incompatibility in pineapple by a pollen fluorescence technique. *Proc. Amer. Soc. Hort. Sci.* 84:217-223.
17. Matsubara, S. 1973. Overcoming self-incompatibility by cytokinins treatment on *Lilium longiflorum*. *Bot. Mag. (Tokyo)* 86:43-46.
18. _____. 1977. Overcoming self-incompatibility of *Lilium longiflorum* by chemical and high temperature treatments and endogenous levels of plant growth regulators after pollination. *Adv. Plant Repr. Physiol.* 189-199.
19. Nakanishi, T. and K. Hinata. 1975. Self-seed production by CO_2 gas treatment in self-incompatible cabbage. *Euphytica* 24:117-120.
20. Rangswamy, N. S. and K. Shivanna. 1971. Overcombin self-incompatibility in *Petunia axillaris* (Lam.). B.S.P. II. Placental pollination *in vitro*. *Golden Jubile Vol. J. Indian Bot. Soc.* 50:286-296.
21. Roggen, H. P. J. R. 1972. Scanning electron microscopical observations on compatible and incompatible pollen-stigma interactions in brassica. *Euphytica* 21:1-10.
22. _____ and A. J. Van Dijk. 1972. Breaking incompatibility of *Brassica oleracea* L. by steel-brush pollination. *Euphytica* 21: 424-425.
23. _____ and _____. 1976. 'Thermally aided pollination': a new method of breaking self-incompatibility in *Brassica oleracea* L. *Euphytica* 25:643-646.
24. _____, _____, and C. Dorsman. 1972. 'Electric aided' pollination: a new method of breaking incompatibility in *Brassica oleracea* L. *Euphytica* 21:181-184.
25. Ronald, W. G. and P. D. Ascher. 1975. Effects of high temperature treatments on seed yield and self-incompatibility in Chrysanthemum. *Euphytica* 24:317-322.
26. Sastri, D. C. and K. R. Shivanna. 1976. Attempts to overcome interspecific incompatibility in *Sesamum* by using recognition pollen. *Ann. Bot.* 40:891-893.
27. Shivanna, K. R. and N. S. Rangswamy. 1969. Overcoming self-incompatibility in *Petunia axillaris*. I. Delayed pollination with stored pollen and bud pollination. *Phytomorphology* 19:372-380.
28. Stettler, R. F. 1968. Irradiated mentor pollen: its use in remote hybridization of black cottonwood. *Nature* 219:746-747.
29. Sree Ramula, K., G. M. M. Bredmeijer, and A. J. G. Van Gastel. 1977. Influence of mentor pollen on gametophytic intraspecific incompatibility in *Nicotiana*. *Incom. Newsl.* 8:87-90.
30. Tatebe, T. 1977. Studies on the physiological mechanism of self-incompatibility in Japanese radish. IV. Effect of high temperatures on self-incompatibility (in Japanese). *J. Japan. Soc. Hort. Sci.* 46: 48-51.
31. _____. 1979. Studies on the physiological mechanism of self-incompatibility in Japanese radish. VI. Effect of recognition pollen on self-incompatibility (in Japanese). *J. Japan. Soc. Hort. Sci.* 48:195-198.
32. Visser, D. L. 1977. The effect of alternating temperatures on the self-incompatibility of some clones of Brussels sprout (*Brassica oleracea* L. var. *Gemmifera* (DC.) Schlz). *Euphytica* 26:273-277.