

Nicotiana alataとN.langsdorffi間の種間不和合性の遺伝学的,生理学的分析

誌名	The Japanese journal of genetics
ISSN	0021504X
著者	高橋, 秀二郎
巻/号	49巻4号
掲載ページ	p. 247-256
発行年月	1974年8月

GENETICAL AND PHYSIOLOGICAL ANALYSIS OF INTERSPECIFIC
INCOMPATIBILITY BETWEEN *NICOTIANA ALATA* AND
N. LANGSDORFFII

HIDEJIRO TAKAHASHI

Laboratory of Applied Botany, Faculty of Agriculture, Kyoto University,
Kyoto 606

Received May 8, 1974

Interspecific incompatibility is found in many combinations of interspecific crossing, but our knowledge about its mechanism is still very few. Most of the study in interspecific incompatibility has been carried out in connection with self-incompatibility because of the wide existence of unilateral incompatibility: crosses between self-incompatible and self-compatible species are successful only when self-compatible ones are used as the female parents. This unilateral incompatibility was found first by Anderson and de Winton (1931) between self-incompatible *Nicotiana alata* and self-compatible *N. langsdorffii*. Lewis and Crowe (1958) showed the widespread occurrence of unilateral incompatibility between self-incompatible and self-compatible species, and supposed that the self-incompatibility allele itself has a dual role; i.e., 1) each self-incompatibility *S* allele in pistil inhibit growth of pollen tube of its own kind, 2) all self-incompatibility alleles share in inhibition of growth of pollen tubes which bear the allele for self-compatibility. Pandey (1964) studied the interspecific incompatibility between *N. alata* and *N. langsdorffii*, and assumed that in the style *S* gene produces two kinds of specificity; primary specificity responsible for the rejection of pollen with self-compatible *S_f* gene and secondary specificity responsible for the rejection of pollen with normal *S* gene. Though most of the workers claimed that close relationship existed between self-incompatibility and interspecific incompatibility, there are a few workers who thought that interspecific incompatibility was independent of self-incompatibility. Hogenboom (1972) stated that the inhibition of *Lycopersicon esculentum* pollen-tube growth in the style of *L. peruvianum* was governed by a number of independent dominant genes, and that self-incompatibility and interspecific pollen-tube-growth inhibition were separate phenomena.

In the present experiments, genes participating in interspecific incompatibility between *Nicotiana alata* and *N. langsdorffii* were analyzed, and it was tried to elucidate the relationship between self-incompatibility and interspecific incompatibility.

MATERIALS AND METHODS

Seeds of *N. alata* and *N. langsdorffii* supplied by Japan Monopoly Corporation were sown and grown up in the pots containing soil. Five plants were picked out from each

species and their self-incompatibility *S* alleles were identified. These plants and their *S* alleles were named as clones $L_1(S_1S_2)$, $L_2(S_1S_2)$, $L_3(S_3S_4)$, $L_4(S_5S_7)$ and $L_5(S_6S_7)$ in *N. langsdorffii* and clones $A_1(S_8S_9)$, $A_2(S_8S_9)$, $A_3(S_8S_{10})$, $A_4(S_{11}S_{12})$ and $A_5(S_{13}S_{14})$ in *N. alata*. Clones L_1 and L_2 were pseudo-self-compatible and they had same self-incompatibility *S* allele pair. Clones L_3 and L_5 were completely self-incompatible. Clone L_4 was completely self-compatible, and F_1 plants obtained by crossing clone $L_4 \times$ clone A_2 showed either complete self-compatibility or complete self-incompatibility. So, clone L_4 was truly self-compatible, and its *S* allele pair consisted of a self-compatible S_f gene and a normal *S* gene. All clones of *N. alata* were completely self-incompatible, and clones A_1 and A_2 had the same *S* allele pair.

Most of the experiments were carried out in spring and autumn in the greenhouse though a part of them were also performed in summer and winter in the phytotron. Generally, buds a day before anthesis were emasculated, bagged, and pollinated next day. Data scored in each table were average number of seed set which was calculated from more than 3 times repetition. In figures of this paper, more than 10 flowers were examined for each plot.

RESULTS AND DISCUSSION

I. Analysis of interspecific incompatibility

Table 1 shows the fertilities obtained by reciprocal interspecific and intraspecific crossing among 10 clones of *N. alata* and *N. langsdorffii*. When the plants were crossed with the pollen of another species they showed much lower fertility than when they were crossed with the pollen of their own species. The degree of decrease in fertility due to interspecific combination varied with the clones in both species, especially remarkable variation in fertility was observed among each clone of *N. langsdorffii*. Clone L_4 showed the lowest and clone L_5 the highest compatibility when they were reciprocally crossed with *N. alata*.

In order to examine whether these decreases in fertility in interspecific crossing depend on sterility after fertilization or interspecific incompatibility, next experiment was carried out. Clone L_1 was reciprocally crossed with clone A_1 , and F_1 plants were grown up. From the F_1 plants of both combinations (clone $L_1 \times$ clone A_1 and clone $A_1 \times$ clone L_1), each ten plants were picked out. These 20 F_1 plants were reciprocally crossed with their both parents to examine fertility. The result obtained is shown in Table 2. When the F_1 plants were back crossed reciprocally with both parental species, each combination showed as nearly high fertility as that obtained by intraspecific crossing among both parental species (ref. Table 1). This fact means that, in the F_1 plants, there is little disharmony between both parental genomes. So, it may be said that low fertility of interspecific crossing does not depend on sterility which is caused after fertilization, but is due to interspecific incompatibility which occurs in pistil before fertilization.

From the results shown in Tables 1 and 2, it may be concluded that there is bilateral interspecific incompatibility between *N. alata* and *N. langsdorffii*, though combination with *N. langsdorffii* as female is more compatible than its reciprocal one. It is

Table 1. Average number of seed per pod and fertility (%) obtained after reciprocal interspecific and intraspecific crossing among ten clones of *N. alata* and *N. langsdorffii*

♂ ♀										
	L ₁	L ₂	L ₃	L ₄	L ₅	A ₁	A ₂	A ₃	A ₄	A ₅
L ₁	—	—	456.2 (93.3)	510.8 (104.5)	347.4 (71.0)	181.3 (37.1)	169.5 (34.7)	185.0 (37.8)	213.7 (43.7)	157.3 (32.2)
L ₂	—	—	533.3 (109.1)	520.8 (106.5)	539.8 (110.4)	278.7 (57.0)	197.3 (40.3)	274.0 (56.0)	347.3 (59.0)	334.0 (68.3)
L ₃	466.8 (95.5)	448.8 (91.8)	—	459.7 (94.0)	364.0 (74.4)	202.1 (41.3)	200.5 (41.0)	205.3 (42.0)	189.3 (38.7)	185.3 (37.9)
L ₄	506.7 (103.6)	477.8 (97.7)	496.0 (101.4)	—	412.0 (84.3)	107.4 (22.0)	167.7 (34.3)	91.7 (18.8)	160.5 (32.8)	102.3 (20.9)
L ₅	593.6 (121.4)	573.3 (117.2)	556.3 (113.8)	538.0 (110.0)	—	416.3 (85.1)	430.3 (88.0)	402.7 (82.4)	435.0 (89.0)	415.8 (85.0)
A ₁	68.4 (6.3)	315.7 (28.9)	584.5 (53.5)	0.0 (0.0)	980.0 (89.7)	—	—	1249.5 (114.4)	1181.0 (108.1)	1132.7 (103.7)
A ₂	112.8 (10.3)	302.3 (27.7)	864.0 (79.1)	111.5 (10.2)	983.7 (90.1)	—	—	1157.0 (105.9)	1284.0 (117.6)	1123.7 (102.9)
A ₃	47.8 (4.4)	358.8 (32.9)	436.0 (39.9)	8.0 (0.7)	924.7 (84.7)	1224.5 (112.1)	1269.0 (116.2)	—	1200.3 (109.9)	1295.0 (118.6)
A ₄	0.0 (0.0)	248.0 (22.7)	182.7 (16.7)	0.0 (0.0)	760.7 (69.6)	988.0 (90.5)	907.5 (83.1)	933.5 (85.5)	—	892.3 (81.7)
A ₅	0.0 (0.0)	148.7 (13.6)	0.0 (0.0)	0.0 (0.0)	638.0 (58.4)	924.0 (84.6)	1102.0 (100.9)	825.0 (75.5)	969.7 (88.8)	—

The average numbers of seed obtained after intraspecific crossing in *N. alata* and *N. langsdorffii* were 1092.2 and 498.0, respectively. These numbers were used to calculate the fertility (%) for each combination as denominator.

Table 2. Frequency of the F₁ plants showing various grades of fertility when they were reciprocally crossed with both parental species

Combination ♀ ♂	Total No. of plants examined	No. of plants showing various grades of seed set per pod					
		201~300	301~400	401~500	501~800	801~1100	1101~1400
clone L ₁ × F ₁ plants	20	2	13	4	1	0	0
clone A ₁ × F ₁ plants	20	0	0	0	0	17	3
F ₁ plants × clone L ₁	20	0	0	1	5	13	1
F ₁ plants × clone A ₁	20	0	0	3	13	4	0

supposed that workers who stated about unilateral incompatibility between self-incompatible *N. alata* and self-compatible *N. langsdorffii* overlooked following fact: there is also partial interspecific incompatibility in the combination of *N. langsdorffii* × *N. alata* as well as in its reciprocal combination.

In order to analyze the gene(s) participating in interspecific incompatibility, following experiments were performed. Among the F₁ plants obtained after crossing clone A₁ × clone L₁, a plant was picked out and named clone AL. Clone AL was selfed by bud pollination and F₂ plants were grown up. Table 3 shows the frequency of the F₂

Table 3. Frequency of the F₂ plants showing various grades of compatibility with the pollen of both parental species

Combination ♀ ♂	Total No. of plants examined	No. of plants showing various grades of seed set per pod					
		1~200	201~400	401~600	601~800	801~1000	1001~1200
F ₂ plants × { clone L ₁ clone A ₁	67	0	8	25	26	8	0
	66	3	17	26	14	5	1

Table 4. Frequency of the back-crossed progenies showing various grades of compatibility with the pollen of their parental species

Combination ♀ ♂	Total No. of plants examined	No. of plants showing various grades of seed set per pod					
		0~ 200	201~ 400	401~ 600	601~ 800	801~ 1000	1001~ 1200
(clone L ₁ × clone AL) × { clone L ₁ clone A ₁	50	0	2	11	28	9	0
	50	3	30	12	4	1	0
(clone A ₁ × clone AL) × { clone L ₁ clone A ₁	47	3	7	16	10	7	4
	47	0	0	6	12	19	10
(clone L ₁ × clone L-AL) × { clone L ₁ clone A ₁	41	0	7	19	11	4	0
	41	12	24	5	0	0	0
(clone A ₁ × clone A-AL) × { clone L ₁ clone A ₁	43	3	23	12	5	0	0
	43	0	0	0	11	19	13

Table 5. Number of seed in clones AL, L-AL and A-AL with the pollen of both parental species

Combination ♀ ♂	No. of seed in each pod			Average No. of seed per pod
clone AL × { clone L ₁ clone A ₁	812,	845,	873	843.3
	641,	673,	674	662.7
clone L-AL × { clone L ₁ clone A ₁	873,	817,	767	819.0
	512,	521,	532	532.7
clone A-AL × { clone L ₁ clone A ₁	498,	568,	524	530.0
	956,	819,	931	902.0

plants which had various grades of compatibility with the pollen of both parental species. The frequency distribution of the progenies showing various grades of compatibility is continuous. This fact suggests that interspecific incompatibility does not depend on such definite gene as *S* in self-incompatibility, but is controlled by polygene. To ascertain this suggestion back crossed progenies were also examined. Clone AL

was back crossed as male with both parents (clones L_1 and A_1) and progenies obtained were grown up. These progenies were crossed as female with both parental species (clones L_1 and A_1) to examine compatibility. The result obtained is shown in Table 4. When an F_1 plant was back crossed with its one parental species, progenies obtained approached a little nearer to the character of the parental species in their character of interspecific incompatibility (ref. Tables 1 and 5). In further experiment, each one of the progenies obtained after crossing clone $L_1 \times$ clone AL and clone $A_1 \times$ clone AL was named clone L-AL and clone A-AL, respectively, back crossed again as male with its parental species (clones L_1 and A_1), and progenies obtained were grown up. These progenies were crossed with the pollen of clones L_1 and A_1 to examine compatibility. The result obtained is also shown in Table 4. When a back crossed progeny (clone L-AL or clone A-AL) was back crossed again with one and the same parental species, progenies obtained approached much nearer to that of this parental species in their character of interspecific incompatibility (ref. Tables 1 and 5). The frequency distribution of the back crossed progenies showing various grades of compatibility with both parental species was continuous. These facts also support the view that interspecific incompatibility is under polygenic control. Mather (1943) already suggested that polygene took part in interspecific incompatibility in *Petunia*, but he confused both kinds of polygenes participating in self-incompatibility and interspecific incompatibility. In *Lycopersicon*, Martin (1964, 1966 and 1968) suggested that both self- and interspecific incompatibility were controlled by two dominant genes, and polygene modified these incompatibilities. But in the present experiments, such dominant genes could not be detected. Moreover, as will be shown later, polygene which controls interspecific incompatibility may be distinguishable from that in pseudo-self-compatibility.

II. Analysis of pseudo-self-compatibility in *N. langsdorffii*

The frequency distribution in grades of self-compatibility of the progenies which were obtained by selfing the pseudo-self-compatible clone L_1 was continuous as shown in Table 6. This means that pseudo-self-compatibility in *N. langsdorffii* is probably controlled by polygene.

Table 6. Frequency in grades of self-compatibility of the progenies obtained by selfing of a pseudo-self-compatible plant (clone L_1)

No. of seed per pod	0	1~100	101~200	201~300	301~400	401~500
No. of plants	15	21	11	7	2	0

Fig. 1 shows the fertility when clones L_1 and L_2 were self-pollinated or reciprocally crossed. Though clones L_1 and L_2 have the same S allele pair (S_1S_2), the fertility obtained after selfing and reciprocal crossing differed by their combinations. Fig. 1 suggests that the pistil of clone L_1 has weaker incompatibility barrier than that of clone L_2 , whereas pollen growth-ability of clone L_2 is greater than that of clone L_1 .

In the previous report (Takahashi 1973), the author dealt with the pseudo-self-compatibility in *Petunia hybrida* and supposed as follows: The pistil of self-incompatible plant contains certain substance (S substance) which inhibits self-pollen-tube growth.

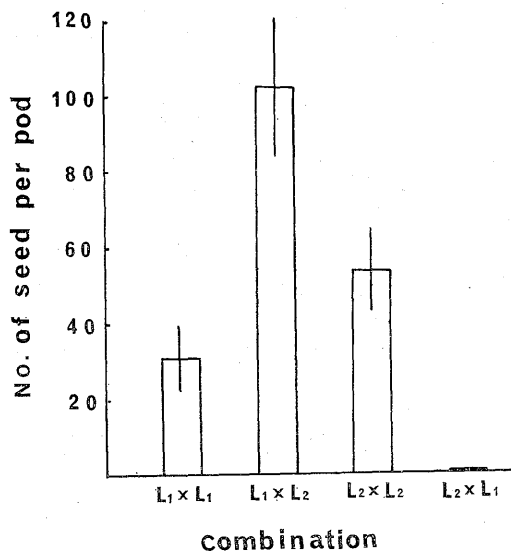


Fig. 1. Fertility obtained by selfing and reciprocal crossing of clones L₁ and L₂.

The specificity of the S substance depends on S allele and whose activity is probably controlled by polygene. The pseudo-self-compatibility may be caused by lowered activity of the S substance and increased vitality of the pollen. These views seem to be applicable to the pseudo-self-compatibility in *N. langsdorffii*, too.

III. Analysis of the relationship between self-incompatibility and interspecific incompatibility

Clones L₁ and A₁ were reciprocally cross-pollinated or clone L₁ was self-pollinated

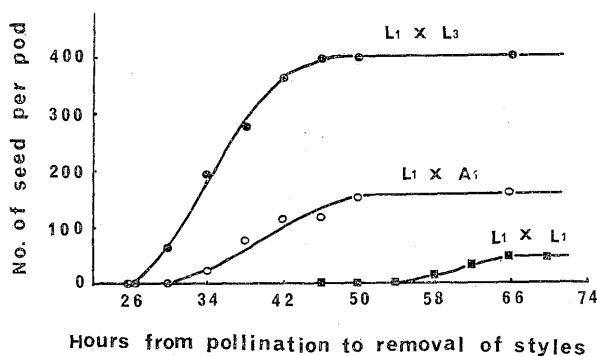


Fig. 2. Fertility of *N. langsdorffii* (clone L₁) whose styles and stigmas were removed at various hours after self-pollination and interspecific or intraspecific cross-pollination.

■: selfing of clone L₁, ○: interspecific crossing (clone L₁ × clone A₁), ●: intraspecific crossing (clone L₁ × clone L₃).

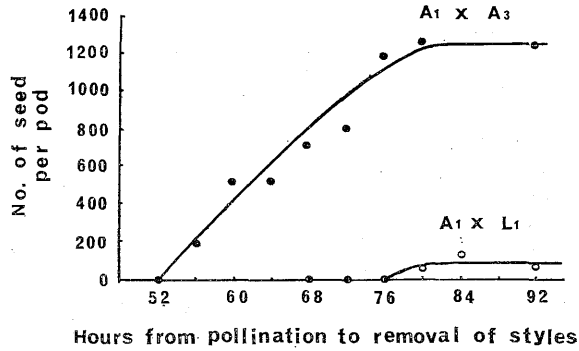


Fig. 3. Fertility of *N. alata* (clone A₁) whose styles and stigmas were removed at various hours after interspecific and intraspecific cross-pollination. ○: interspecific crossing (clone A₁×clone L₁), ●: intraspecific crossing (clone A₁×clone A₃).

at 25°C, and their styles and stigmas were removed at different times thereafter. The fertilities are shown in Figs. 2 and 3. These figures show that self- and interspecific incompatible pollen-tube growth is much slower than that of compatible one.

Fig. 4 shows the fertility of each clone in *N. langsdorffii* after selfing or interspecific

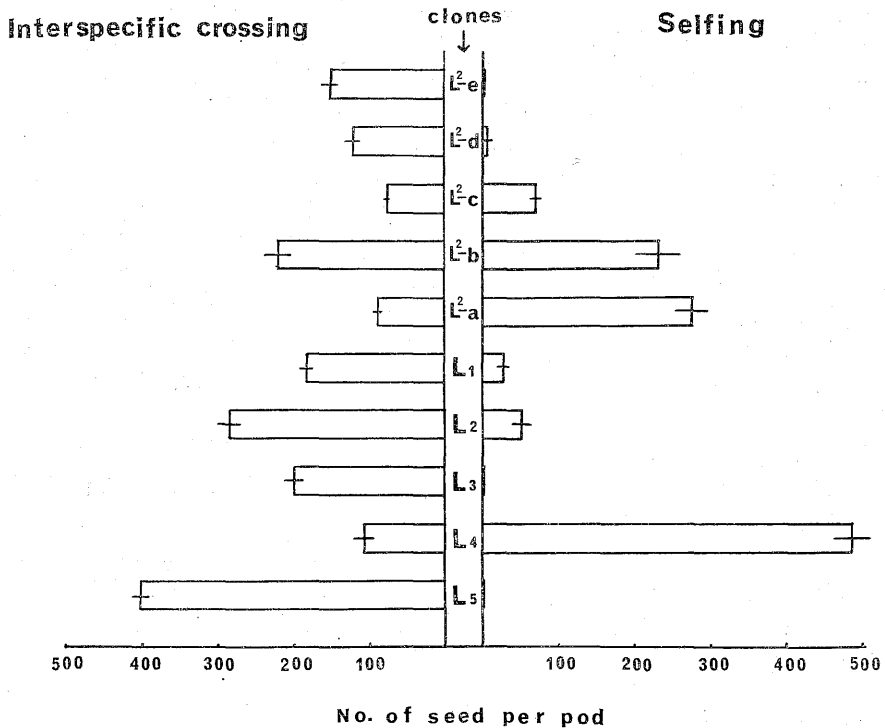


Fig. 4. Fertility of each clone of *N. langsdorffii* in selfing or interspecific crossing with the pollen of *N. alata* (clone A₁).

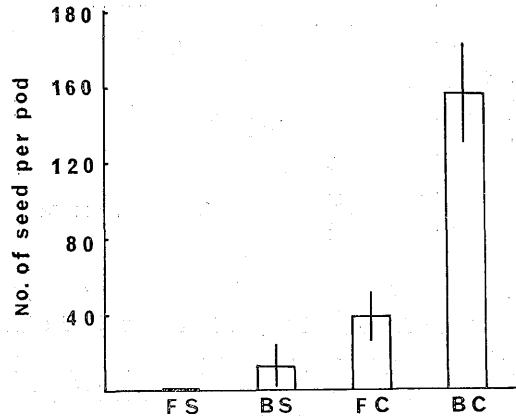


Fig. 5. Fertility of flowers and buds of *N. alata* (clone A_3) after selfing or interspecific crossing with the pollen of *N. langsdorffii* (clone L_1). FS: selfing of flower, BS: selfing of bud, FC: interspecific crossing of flower, BC: interspecific crossing of bud.

crossing with the pollen of *N. alata*. Clone L_4 which was truly self-compatible showed much stronger interspecific incompatibility with the pollen of *N. alata* than clone L_5 which was completely self-incompatible. So, it may be said that self-compatible S_f gene does not weaken the interspecific incompatibility barrier in pistil. As shown in Table 1, when clone $L_4(S_5S_f)$ was crossed as male with *N. alata*, not only the pollen with S_f gene but also the pollen with normal S gene was inhibited in their growth in the pistil of *N. alata* before fertilization. So, neither the dual function theory (Lewis and Crowe 1958) nor the two specificity theory (Pandey 1964) seem to be correct. Clones L^2 -a, L^2 -b, L^2 -c, L^2 -d and L^2 -e were progenies obtained by selfing of pseudo-self-compatible clone L_1 . These clones showed various strength of self-incompatibility and interspecific incompatibility, but strength of both incompatibilities did not correlate. As mentioned above (Fig. 1), clone L_2 had stronger self-incompatibility barrier in pistil than clone L_1 , but clone L_2 showed weaker interspecific incompatibility with *N. alata* than clone L_1 . From these facts it may be said that polygenes which control interspecific incompatibility and strength of self-incompatibility are different each other.

Fig. 5 shows the fertility of flowers and buds of *N. alata* (clone A_3) after selfing or interspecific crossing with the pollen of *N. langsdorffii* (clone L_1). The buds used were 2 days before anthesis. Though the flowers of *N. alata* produced no seed by self-pollination, small number of seeds were obtained when the buds were self-pollinated. When these buds were cross-pollinated with the pollen of *N. langsdorffii*, they produced more seeds than when the flowers were used. Effect of bud pollination also exists in interspecific incompatibility.

IV. General discussion

The results obtained hitherto can be summarized as follows.

1. There was bilateral interspecific incompatibility between *N. alata* and *N. langsdorffii*.

dorffii, though combination of female *N. langsdorffii* and male *N. alata* showed higher compatibility than its reciprocal one.

2. The gene which controls interspecific incompatibility is not supposed to be a definite gene such as *S* in self-incompatibility, but is polygene. Though pseudo-self-compatibility in *N. langsdorffii* is also probably controlled by polygene, this polygene differs from that participating in interspecific incompatibility.

3. In both interspecific- and self-incompatible combinations, the growth rate of pollen-tube was much slower than that in compatible combination.

4. In both interspecific- and self-incompatible combinations, bud pollination increased the fertility.

To explain these facts the author wishes to propose following hypotheses. Enzyme(s) which is contained in pollen and is indispensable to pollen-tube growth shows the highest activity in the pistil of its own species. Activity of this enzyme(s) decreases in the pistil of other species, so that most of pollen tubes stop to grow before fertilization. Polygene controls factors which effect enzyme activity, for example, affinity between enzyme(s) and substrate(s), pH and other chemical conditions in the pistil. In self-incompatibility, inactivation of such enzyme(s) is caused by the *S* substance, and activity of the *S* substance is controlled by another polygene. When physical and chemical factors in the pistil are favourable for slowly growing incompatible pollen, for example, short style length, long life-time of the pistil or proper concentration of various chemical substances, a few vital pollen can continue to grow until fertilization even in the presence of such inactive enzyme(s). The pistil of *N. langsdorffii* is more favorable for incompatible pollen to reach to ovary than that of *N. alata*: much shorter style length of the former than the latter may be one of the factors. So, in *N. langsdorffii*, a little inactivation of the *S* substance causes pseudo-self-compatibility more easily than in *N. alata*, and in interspecific pollination, the pollen of *N. alata* in the pistil of *N. langsdorffii* can succeed in fertilization more easily than in its reciprocal combination. By this reason, there are far more pseudo-self-compatible plants in *N. langsdorffii* than in *N. alata*, and in interspecific crossing, the combination with female *N. langsdorffii* shows higher fertility than its reciprocal one. Furthermore the pistil of the bud is more favorable for incompatible pollen to reach to ovary than that of the flower: shorter length and longer life-time of bud's style may be important factors. So, bud pollination increases fertility in both incompatibilities.

Though the phenomena of both incompatibilities are very similar, the author supposes that the causes of both incompatibilities are quite different.

SUMMARY

Genetical and physiological analyses were undertaken in interspecific incompatibility between *Nicotiana alata* and *N. langsdorffii*. Experiments were also done to elucidate the relationship between self-incompatibility and interspecific incompatibility.

There is bilateral interspecific incompatibility between *N. alata* and *N. langsdorffii* though the combinations with *N. langsdorffii* as female show higher compatibility than its reciprocal one. It is supposed that the gene which controls interspecific incompati-

bility is not a definite gene such as *S* in self-incompatibility, but is polygene. Though pseudo-self-compatibility in *N. langsdorffii* is probably also controlled by polygene, polygenes participating in self- and interspecific-incompatibility are different each other. In both incompatibilities, the growth rate of incompatible pollen-tube is much slower than in compatible combination, and fertility increases by bud pollination. To understand these facts, hypotheses are proposed in the present paper.

ACKNOWLEDGMENT

The author wishes to express his gratitudes to Drs. Kotaro Watanabe and Atsushi Takimoto for their kind advices in preparation of this manuscript.

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