THE GENETICS OF SELF- AND CROSS-INCOMPATIBILITY IN BRASSICA OLERACEA

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Received October 15, 1956

FOUR genetic schemes have been proposed to account for previous observations on self- and cross-incompatibility in *Brassica oleracea*: (1) oppositional *S* alleles at one locus, with the pollen behavior determined gametophytically by the genotype of the pollen itself (ODLAND and NOLL 1950); (2) system (1) modified by sympathetic *T* alleles at a second locus to account for instances of self-compatibility (KAKIZAKI 1930); (3) system (1) modified by what we interpret to be a polygenic system (ATTIA and MUNGER 1950); and (4) a system with oppositional alleles at two loci, with gametophytic control of pollen behavior (SEARS 1937; MIZUSHIMA and KATSUO 1953).

BATEMAN (1954, 1955) showed that two members of the Cruciferae, *Iberis amara* and *Brassica campestris*, have a sporophytic incompatibility system whereby pollen behavior is determined by the genotype of the pollen parent. BATEMAN suggested a similar system for *Brassica oleracea* and the present study using 'Calabrese Green Sprouting' broccoli shows this view to be correct.

PARENT PLANTS

Five flowering broccoli plants were moved from the field to an insect free greenhouse maintained at temperatures above 65°F. Compatibility relationships were determined during the winter of 1954–55 when the plants were six to nine months from seed. At first, at least 30 fresh flowers of each plant were selfed with fresh pollen. After about three weeks it was evident from the presence or absence of silique development that plant 1 was self-compatible and the others were self-incompatible. The five plants were then crossed to each other in all combinations. Emasculation was used only when plant 1 was the female, and for each cross except one over 20 flowers were pollinated.

The results varied considerably from plant to plant but there was no difficulty in deciding whether or not a cross was compatible. Incompatible pollinations resulted in the development of fewer siliques and fewer seeds per silique than compatible pollinations. Thus the average number of seeds per flower pollinated provided a good index of compatibility. This value is referred to as the "compatibility index".

Self-compatible plant 1 had a compatibility index of 4.0 when selfed, compared to 4.4 when crossed. Self-incompatible plants 2, 3, 4 and 6 had compatibility indexes of 0.0, 0.03, 0.28 and 0.01 respectively when selfed, compared to 6.7, 11.9, 13.8 and 9.2 in compatible crosses.

In crosses, plants 1 and 2 were compatible with each other and with the three other plants in both directions. The relationships among plants 3, 4 and 6 were the most

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interesting. Plants 3 and 4 were mutually compatible in both directions with a compatibility index of 12.2 when 3 was female, and 15.0 when 4 was female. Plants 4 and 6 were mutually incompatible with an index of 0.38 when 4 was female and 0.5 when 6 was female. However, the cross 6 × 3 was compatible, with an index of 10.7, while the reciprocal was incompatible with an index of 0.22.

For all crosses and selfings, samples of stigmas were collected 24 hours after pollination. These were fixed in alcohol-acetic, squashed in a drop of aceto-carmine and examined microscopically (SEARS, 1937). After compatible pollinations unstained pollen grains with tubes leading from them were abundant. After incompatible pollinations emptied grains were rare; most grains either did not germinate or produced very short tubes and consequently stained deeply. The results of the stigma squash method agreed exactly with the seed set data. This proved the reliability of the method for determining compatibility.

PROGENY FAMILIES

The compatibility relationships among parent plants 3, 4 and 6 immediately ruled out a simple gametophytic incompatibility system with alleles at one locus because, since plants 4 and 6 were cross-incompatible, they should have the same genotype, but their reactions toward plant 3 showed this was not so. Neither could KAKIZAKI'S *T* alleles explain the discrepancy, for plants 3, 4 and 6 were self-incompatible. On the other hand, similar situations have been found in plants with a sporophytic incompatibility system.

With a sporophytic system and alleles at one locus, the maximum number of *S* alleles present in plants 3, 4 and 6 should be four, because the allele common to 3 and 6 is not the allele common to 4 and 6. It was further predicted that the ten genotypes possible with these four alleles should be recovered from the progeny families 3 × 3, 4 × 4 and 3 × 4, while the genotype of 6 should be found in family 3 × 4. Accordingly, these three families were analyzed. In addition, families 4 × 1 and 1 × 1 were analyzed to study the mode of inheritance of self-compatibility from parent 1. Self-compatibility is widespread in broccoli (ANSTEY 1954).

The progeny plants were studied during the fall and winter of 1955 in an insect free greenhouse maintained at temperatures above 50°F. Cuttings of the parent plants 1, 2, 3, 4 and 6 grown under these conditions had compatibility indexes of 8.16, 0.35, 0.73, 0.83 and 0.00 respectively when selfed. The cross 6 × 3 had an index of 16.1. At least twenty flowers were used in each of these pollinations. Thus the cooler conditions seemed to favor higher seed set than was obtained the previous season.

These compatibility indexes were calculated from ripe seeds, two to three months after pollination. Counts can be obtained on immature seeds one month after pollinations but they usually give higher indexes because of ovule abortion as the silique matures. However, analysis of the progeny families required a rapid means for determining compatibility relationships, and this was provided by the stigma squash method. Four flowers were used in each pollination, and the four stigmas were squashed on one slide. If ten or fewer compatible grains were found on a slide, the pollination was scored (−); if from 11 to 50, the results were noted (±); if over

50, (+). A compatible pollination could usually be recognized at a glance. All plants were first selfed. Emasculation was practised in subsequent crosses only if the female was self-compatible.

The results of the compatibility determinations are recorded on the accompanying checkerboard diagrams. Females are entered at the top of a diagram, males to the side. The second column and row indicates the plant number. 437, for example, refers to plant 7 from the cross 4 × 3. Parental plants are designated as, for instance, No. 4 instead of 4, except in figure 2B. Except in figure 3, the first column and row of a diagram contain the allele subscripts of the *S* genotype proposed for an individual or class. Active alleles are denoted by a dot superscript, recessive alleles by parenthesis, and incomplete recessiveness by a dot and parenthesis.

Family 3 × 3

This family of ten plants was produced by selfing parent 3 by bud-pollination (PEARSON 1929; SEARS 1937). All plants proved to be self-incompatible by the stigma squash method. The compatibility indexes, based on counts of immature seeds one month after selfing at least ten flowers on each plant, were 0.6, 0.4, 0.0, 2.6, 0.5, 0.4, 0.1, 0.9, 3.8 and 0.7 for plants 331 to 3310 respectively. In inter-sib crosses plants 331 and 332 were mutually compatible and their pollen conveniently divided the family into three groups (fig. 1A). In all, 90 of the possible 100 sib matings were completed with no further division of the three groups.

The compatibility pattern of this family is exactly as expected from selfing a heterozygote with a single locus, sporophytic, incompatibility system. Both alleles of the heterozygote were active in pollen and stigma. Accordingly parent 3 was assigned the genotype S_2S_3. The genotypes S_2S_2, S_2S_3 and S_3S_3 occurred in family 3 × 3 in the ratio 2:4:4. This interpretation was confirmed by the identical behavior of 335 and parent 3 in interfamily combinations (fig. 2B).

The results of family 3 × 3 not only rule out a simple gametophytic incompatibility system with alleles at one locus but also such a system with two loci (the hypothesis of SEARS 1937; and of MIZUSHIMA and KATSUO 1953) is denied by the following considerations. A plant with the genotype $A_1A_2B_1B_2$ will produce the four sorts of pollen, (A_1B_1), (A_1B_2), (A_2B_1) and (A_2B_2), so that its progeny by selfing will contain nine genotypes. Can any three of these genotypes behave like the classes of 3 × 3? First, the four genotypes $A_1A_1B_1B_1$, $A_1A_1B_2B_2$, $A_2A_2B_1B_1$ and $A_2A_2B_2B_2$ are ruled out because, unlike the classes of 3 × 3, they can accept pollen from the parent. The next task is to find two mutually compatible classes among the remaining five genotypes and this of course is impossible since all the pollen genotypes from these plants were also produced by the parent, and proved to be incompatible. Simpler parental genotypes homozygous at one or the other locus are more readily eliminated.

Family 4 × 3

All ten plants of this family were self-incompatible when tested soon after they began to flower. The pollen of parent 4 divided the family into two groups; the pollen of 431 split both of these and the result was four groups. All plants were then tested

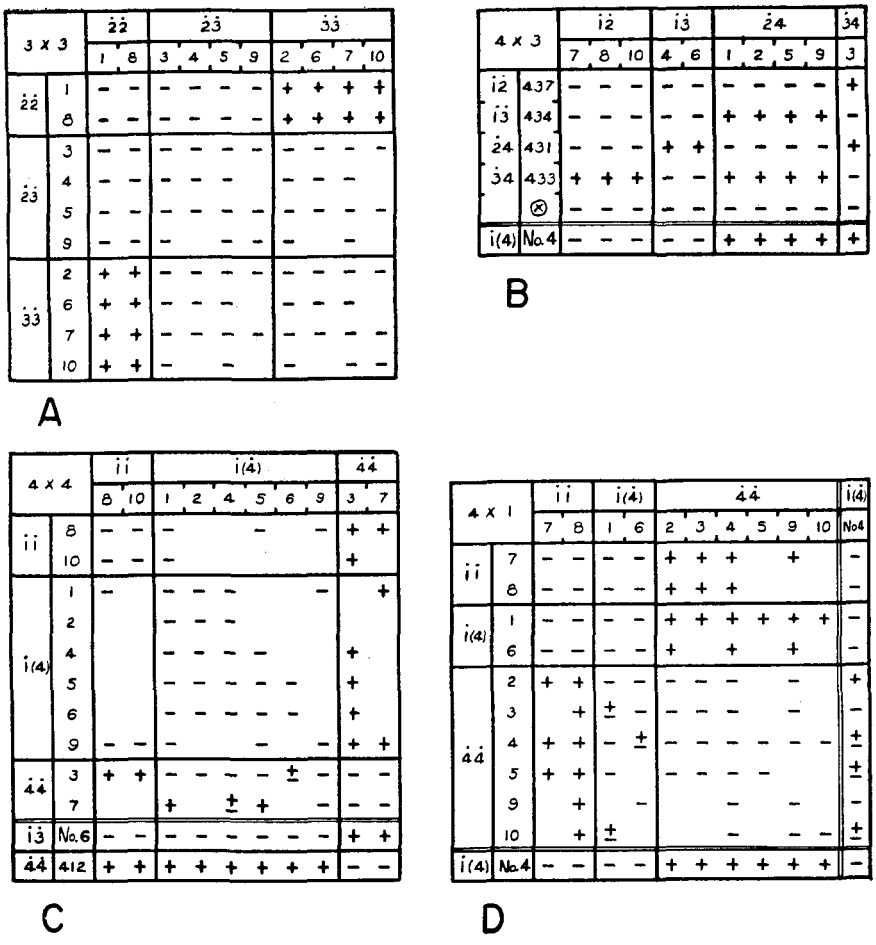

FIGURE 1.—A. Sib compatibility relationships in family 3 × 3. B. Sib compatibility relationships in family 4 × 3 and the reaction of pollen of parent 4 toward this family. C. Sib compatibility relationships in family 4 × 4 and the reaction of pollen of 412 and plant 6 toward this family. D. Sib compatibility relationships in family 4 × 1 and the reaction of pollen and stigmas of parent 4 toward this family.

by pollen of a member from each of the other three groups. No new divisions appeared (fig. 1B).

The reactions of the four groups were arranged to form a compatible diagonal (BATEMAN 1954) from which it was deduced that the classes of 437 and 434, which rejected the pollen of parent 4, had a common allele (S_1) from parent 4. It followed that classes of 431 and 433 must have the second allele (S_4) from parent 4. Similarly, when the pollen of 437 was used on the classes of family 3 × 3, (fig. 2B) it was found to have S_2. It followed that class 431 must also have S_2 while the classes of 434 and 433 must have S_3. Having established the class genotypes, it was evident from the

Inter-family		ii	i (4)		44		ii		i(4)		44		i(4)		44
		418	411	416	412	414	448	4410	441	449	443	447	112	115	1112
ii	418	−	−	−	+	+	−		−			+			
i(4)	411	−	−	−	+	+	−		−			+	−		
	416	−	−	−	+	+			−			+			
44	412	+	−	−	−	−	+	+	+	+	−	−	−		−
	414	+	−	±	−	−	+	+	+	+	±	−			
ii	448	−	−		+		−	−	−	−	+	+			
i(4)	441	−	−		+	+	−		−	−		+	−		
44	443		−	−	−	−	+	+	−	−	−	−			−
	447	+	−	±	−	−			+	−	−	−	−	−	−
i(4)	112	−	−	−	+				−				−	−	+
	115				+								−	−	+
44	1112				−						−	−	−	−	−

A

Inter-family		5?	23	i(4)	i3	22	23	33	i2	i3	2(4)	3(4)	ii	44	44
		2	3	4	6	331	335	332	437	434	431	433	418	412	447
5?	2	−				+		+					+	+	
23	3		−		−	−	−	−	−	−	−	−			
i(4)	4			−	−	+	+	+	−	−	+	+	−	+	+
i3	6		−	−	−	+	−	−	−	−	+	−	−		+
22	331	+	−	+	+	−	−	+	−	+	−	+		+	
23	335		−		−	−	−	−	−	−	−	−		+	
33	332	+	−	+	−	+	−	−	+	−	+	−		+	
i2	437		−	−	−	−	−	+	−	−	−	+	−	+	+
i3	434		−	−	−	+	−	−	−	−	+	−	−	+	+
2(4)	431		−	+	+	−	−	+	−	+	−	+	+	+	+
3(4)	433		−	+	−	+	−	−	+	−	+	−	+	+	+
ii	418	+		−	−						+	+	−	+	+
44	412	+		+		+		+	+	+	−	±	+	−	−
44	447			+	+				+	+	±	+	+	−	−

B

FIGURE 2.—A. Interfamily combinations involving representative plants of the genotypes S_1S_1, S_1S_4 and S_4S_4. B. Interfamily combinations involving cuttings of the original plants, and representative progeny plants. The highest activity of genotype S_1S_3 is shown.

	Class		A	B	E	D	C	F
		S	i4	i3	24	23	3?	2?
♂	A	i4	−	−	+	+	+	+
	B	i3	−	−	+	−	−	+
	E	24	+	+	−	−	+	+
	D	23	+	−	+	−	−	+
	C	3?	+	−	+	−	−	+
	F	2?	+	+	±	±	+	±

♀

FIGURE 3.—Summary and interpretation of the incompatibility relationships in SEARS' (1937) broccoli families 1-3 and 3-1.

compatibility pattern that S_1, S_2 and S_3 were active in the pollen and stigmas wherever present while S_4 was somewhere recessive.

An anomaly

From the compatibility relationships of parents 3, 4 and 6, it was predicted that family 4 × 3 should contain the genotype of parent 6. The interpretation of family 3 × 3 assigned the genotype S_2S_3 to parent 3 and, since 3 and 6 were incompatible, it followed that parent 6 had S_2 or S_3. Furthermore in family 3 × 3 both alleles of parent 3 were shown to be active in pollen and stigma so the reciprocal difference in compatibility between parents 3 and 6 must have been due to the common allele being active in the pollen of 6 but recessive in the stigma. It is evident that the postulated phenotype of parent 6 was not realized in family 4 × 3 where S_2 and S_3 were active wherever found.

This unexpected result required re-examination of the genotype of parent 6 by further experiment. Accordingly, male and female testers for S_1, S_2 and S_3 from families 3 × 3 and 4 × 3 were crossed with parent 6 (fig. 2B) and thus it was proved that parent 6 had the genotype S_1S_3. Since this genotype was represented in family 4 × 3 our prediction had indeed been realized! However, and much to our astonishment, plants 3 and 6, and also 335 (same genotype as 3) and 6, were now mutually incompatible in both directions. Other testers confirmed the activity of S_3 in both pollen and stigma of plant 6. Since, eight months previously, 3 and 6 were incompatible in one direction only, the only conclusion was that the compatibility relationship between the two plants had changed. The present and earlier pollinations were both extensive enough to rule out experimental error as the source of the discrepancy.

The cause of the change in compatibility relationships between plants 3 and 6 remains unknown. At first temperature was suspected since the present experiments were made under temperatures ten to twenty degrees lower than when the original crosses were made. Accordingly, two weeks after the change was discovered, the one plant of 6 and one of the two cuttings of 3 (designated 3B) were transferred to a greenhouse kept above 70°F. The other cutting of 3 (that is, 3A), the one used in first detecting the change, was left in the house kept above 50°F. Two days later the cross 6 × 3B was made at 88°F and proved to be compatible. The same cross was repeated next day at 81°F and was again compatible. In addition, 6 × 3A was also compatible, the pollen of 3A coming from 68°F just before pollination. Apparently the increased temperature returned the compatibility relationship to its first observed state. Plant 6 was then returned to the cool house. However, we decided to check the other plants with the same genotype as 6. Therefore on the following day, 6, 436 and 434 were pollinated by 3A at a temperature of 60°F. The first two were compatible while the last was incompatible. Thus the activity of S_3 in the pollen of 436 had changed while the plant remained in a cool house. Temperature as the immediate cause of the change was thus ruled out.

Deficiencies of soil nutrients were then suspected of causing the changes in compatibility relationships. The parent plants were first tested after having been grown in the same soil for several months. Also the cuttings showed chlorotic hunger symptoms when tested. Accordingly, cuttings of plants 3 and 6 were prepared. Plants of

each were grown in sand and fed with HEWITT's (1952, p. 189) standard complete nutrient solution to which sodium nitrate had been added. Control plants were raised on ordinary potting soil. Plant 6, in soil, had a compatibility index of 15.1 when pollinated by 3 grown in soil, and 18.8 when pollinated by 3 grown in sand. Similarly, plant 6, in sand with complete medium, had an index of 11.4 when pollinated by 3 grown in soil, and 12.9 when pollinated by 3 grown in sand. Thus, all crosses were compatible. Therefore the inactivity of S_3 in the stigmas of plant 6 cannot be attributed to deficiencies of the elements in the nutrient solution. The causal factor remains unknown.

Family 4 × 4

Since analysis of family 4 × 3 assigned the genotype S_1S_4 to parent 4, three genotypes were expected in family 4 × 4, the product of bud pollination. Since plants 4 and 6 had S_1 in common, the pollen of 6 was used to divide the ten plants of 4 × 4 into two groups, all plants being self-incompatible. Two plants, 443 and 447, were compatible with 6 and were given the genotype S_4S_4. The larger group was then tested with the pollen of 443, two crosses (S_1S_1) being compatible and six (S_1S_4) incompatible or partially incompatible. In all, 60 sib crosses were made, with several deviations from the clear-cut results hitherto encountered (fig. 1C).

The results indicate that S_1 was active in pollen and stigma of S_1S_4, but that S_4 was completely recessive in the pollen and partially recessive in the stigma. The potency of S_4 in the stigma S_1S_4 appears to be almost reduced to the threshold of activity. The variable results are perhaps due to minor modifying genes which swing the activity of S_4 from one side of the threshold to the other. The success of $S_1S_4 \times S_4S_4$ crosses appears to depend on modifiers in both pollen and stigma. Since the detection of S_4 in the heterozygote is uncertain, the assignment of genotype S_1S_1 may be questioned. Therefore, no important deductions have been based on genotype S_1S_1.

Family 4 × 1

The type of incompatibility system operating in broccoli was established by analyses of families 3 × 3, 4 × 3 and 4 × 4. Families 4 × 1 and 1 × 1, on the other hand, were investigated to learn how the absence of incompatibility in parent 1 was inherited.

Family 4 × 1 contained ten plants all of which were self-incompatible when they first came into bloom. Analysis of the family into its component classes proved difficult. The pollen of parent 4 was used to establish two groups (fig. 1D), but the reciprocal behavior of one group toward the stigmas of 4 was variable. Seventy-three sib combinations were made before it was realized that the compatibility pattern of family 4 × 1 was the same as 4 × 4, both having the genotypes S_1S_1, S_1S_4 and S_4S_4. This could only mean parents 4 and 1 had the same S genotype. It followed that the self-compatibility of parent 1 was not due to alleles at the S locus.

Family 1 × 1

This family, produced by ordinary selfing of self-compatible parent 1, contained 13 plants. The first, 111, developed many siliques upon isolation, so presumably was

self-compatible. The compatibility indexes after selfing at least ten flowers on each plant, for plants 112 to 1113 were 2.3, 16.1, 21.7, 0.7, 14.2, 9.8, 14.3, 12.6, 7.9, 13.4, 0.1 and 19.9 respectively. Seed counts were made four weeks after pollination. Thus only three plants, 112, 115, and 1112, can be considered self-incompatible. These pollinations were made about six weeks after the plants began to flower, a point which needs consideration.

STOUT (1922) reported that individuals of *B. chinensis* which at first were self-incompatible, became self-compatible during the middle part of the flowering season, but changed back to self-incompatibility at the end of the season. In most of our material the presence or absence of self-compatibility was determined only when the plants first began to flower. However, two plants, 433 and 414, which were self-incompatible at first, proved to be self-compatible when tested seven weeks later. (These two plants did not revert to self-incompatibility, and we suspect STOUT's end of season "self-incompatibility" was sterility due to the plants being exhausted by too many developing siliques.) Thus the strength of self-incompatibility waned in some of our plants and it is possible that the self-compatibility of parent 1 and some members of family 1 × 1 is of this nature.

Analysis of family 4 × 1 indicated that parent 1 had the genotype S_1S_4, the same as parent 4. Accordingly, the pollen of 4 was used to divide the self-incompatible members of 1 × 1 into two groups. The pollen of 4 was incompatible on 112 and 115 but highly compatible on 1112. Thus 1112 likely had genotype S_4S_4 as confirmed by six incompatible crosses between 1112 and other S_4S_4 plants (fig. 2A). The reciprocal crosses, using the pollen of 112, 115 and 1112 on 4, were incompatible. Intersib combinations (fig. 2A) showed the pollen of 1112 to be incompatible on 112 and 115 which indicated these two had the genotype S_1S_4. (The viability of 1112 pollen was checked on the stigmas of 331 (S_2S_2) where it was highly compatible.) These results amply confirm the conclusion that parent 1 possessed S_1 and S_4 and therefore the self-compatibility of parent 1 was not due to an allele at the S locus.

It is of interest that some S allele activity could be detected in self-compatible plants by the stigma squash method. Indeed, compatible selfings never resulted in so many emptied pollen grains as, for example, a cross between plants with no common S alleles. In addition, parent 1 and six self-compatible plants of 1 × 1 were tested for S_1 and S_4 in pollen and stigma. In plants 111, 117 and 119 no activity of either allele was detected. In plants 1, 114, 116 and 1110 there was no indication of S_1 activity but S_4 appeared to be slightly active in both pollen and stigma. It would seem that the factors responsible for self-compatibility had a greater effect upon the activity of S_1 than S_4, although the latter was sufficiently weakened to confer self-compatibility. It is noteworthy however, that parent 1 was the only plant of known genotype S_1S_4 to show any trace of S_4 activity in the pollen.

Interfamily combinations

Analysis of incompatibility in five families of broccoli revealed only four incompatibility alleles among the four parents 1, 3, 4 and 6. This represents a remarkably high concentration of S alleles but may be due to sampling rather than to any intrinsic feature of broccoli population structure.

Parents 1 and 4 had the same S genotype; therefore families 4×4, 4×1 and 1×1 segregated into the same classes. Results from interfamily crosses involving these three families are given in figure 2A. All crosses between plants with S_1 were incompatible as were all crosses between plants with genotype S_4S_4, except for one cross of the latter type which was slightly compatible. Pollen of S_1S_4 plants was always fully compatible on S_4S_4 stigmas which indicated S_4 to be completely recessive in S_1S_4 pollen, but in reciprocal crosses slight activity of S_4 in the stigmata of S_1S_4 was detected. However the stigmatic activity of S_4 in S_1S_4 was as erratic and unpredictable as it had been in intrafamily crosses.

Further interfamily combinations involving the ten genotypes possible with the four S alleles are given in figure 2B. The behavior of each genotype was exactly as expected thus proving the correctness of the genotype assignments. In addition, the uniqueness of each of the four alleles is obvious. It will be noticed that plant 2 was tested for each of the four alleles and found wanting. Thus five is the minimum number of S alleles studied.

DISCUSSION

The present findings indicate that broccoli has a sporophytic incompatibility system controlled by S alleles at one locus. Gene interaction in heterozygotes involving the four fully analyzed alleles was such that S_1, S_2, and S_3 acted independently of each other in pollen and stigmas. On the other hand, S_4 was recessive in the pollen and greatly weakened in the stigma. These features are expected in sporophytic systems.

Several modifications of normal S allele activity were encountered. Some self-incompatible plants became self-compatible with age, a feature which has long been known but is inadequately understood. In other plants, the activity of one S allele fluctuated while the other allele remained active so that self-incompatibility was retained. This demonstrated the biochemical independence of the activities of S_1 and S_3. Similarly, the forces responsible for the self-compatibility of parent 1 reduced the activity of S_1 more than the recessive S_4. This indicated the independence of the events which conferred self-compatibility from those governing the dominance of S_1 over S_4. Probably other deviations from regularity remain to be discovered in Brassica incompatibility systems.

Against the evidence for a variously modified sporophytic incompatibility system in *B. oleracea*, we must weigh the proposals of other workers for gametophytic systems. First, the work of KAKIZAKI (1930), especially his Family 11 of 'Toyodawase' cabbage provides clear-cut, although limited, evidence for a gametophytic system. This is irreconcilable with our findings unless contamination be invoked. However, the data obtained by most workers (ODLAND and NOLL 1950; ATTIA and MUNGER 1950; MIZUSHIMA and KATSUO, 1953) can be equally well explained by a sporophytic system.

SEARS' (1937) work on broccoli merits special consideration on account of the complexity of the data. At least six compatibility classes can be recognized in SEARS' table 3. Designating these classes by letters, the first twelve plants, from left to right, comprise class A. Class B contains only the thirteenth plant; Class C the next three

plants; Class D the next two; Class E the next two, and the last three plants belong to the ambiguous Class F. Thus, family 1–3 and its reciprocal 3–1 contain five self-incompatible classes. SEARS proposed two loci of oppositional alleles to explain this. However, if the class reactions are summarized on a checkerboard diagram as in figure 3, classes A, B, E and D form a compatible diagonal. By using the method of BATEMAN (1954), the genotypes S_1S_4, S_1S_3, S_2S_4 and S_2S_3 may be assigned classes A, B, E and D respectively. The compatibility relationships in families 1–3 and 3–1 suggest a linear dominance sequence, $S_1 = S_3 > S_2 =$ or $> S_4$, for the pollen, and $S_1 = S_2 = S_3$ for the stigma. (However, if family 1–1 contains S_1S_2 plants, then $S_1 > S_2$ in the stigma.) The fifth class, C, can be interchanged for B or D without destroying the compatible diagonal (fig. 3). However, as Class C shows less cross-incompatibility than B or D, we regard it as exceptional and due to some modification which inactivated either S_1 or S_2 while S_3 remained active in the pollen and stigma. This change is perhaps similar to that observed in parent 6 of our material. Indeed, SEARS noted changes in the cross-compatibility relationships of some of his broccoli plants when they were moved from the greenhouse to the garden. These plants, however, belonged to a different series than those of his table 3. Nevertheless, the occurrence of such changes supports the above interpretation of SEARS' data. If this interpretation is correct, SEARS' data confirm the findings of the present study.

SUMMARY

Self- and cross-incompatibility in 'Calabrese Green Sprouting' broccoli (*Brassica oleracea* var. *italica*) was found to be controlled by multiple oppositional alleles at one locus. The pollen reaction is sporophytically determined. Four S alleles were analyzed in all combinations. Three alleles act independently of each other in heterozygotes; the fourth is recessive to the others in the pollen and incompletely recessive in the stigma. The incomplete recessiveness of S_4 in the stigma appeared to be conditioned by modifiers.

Inactivation of S allele activity occurred with time in some plants. Progeny studies showed the self-compatibility of another plant was not due to alleles at the S locus. A third type of inactivation involved only one S allele of a heterozygote so that the plant remained self-incompatible.

ACKNOWLEDGMENTS

The author wishes to acknowledge his indebtedness to PROFESSOR KARL SAX, under whose guidance this work was carried out. Financial assistance from the Bussey Institution of Harvard University is also gratefully acknowledged. Greenhouse facilities provided by the Canada Department of Agriculture enabled this work to be completed.

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