Genotype, protein, phenotype relationships in self-incompatibility of *Brassica*

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SUMMARY

The inheritance of self-incompatibility proteins was studied in three homozygous self-incompatible genotypes of *Brassica oleracea* var. *capitata* and their F_1 and F_2 progenies. The presence or absence in the stigma of incompatibility proteins was determined by immunodiffusion and independently by disk electrophoresis. Certain proteins (antigens) were present in F_1 and F_2 plants in exact correlation with segregation of the S alleles as determined by phenotypic expression of incompatibility. An S allele–protein–phenotype relationship was thus verified.

In *Brassica*, self- or cross-pollinations exhibit an incompatibility phenotype, i.e. incompatibility, compatibility or intermediate, as expressed through pollen germination and seed set. This paper reports genetic analyses from seed set data of the genotype–phenotype relationships, including S allele interactions in heterozygotes, among (1) three homozygous S allele genotypes, (2) the three heterozygous genotypes derivable from crosses among these homozygotes, (3) two of the three F_2 populations. The identified genotype–phenotype relationships are compared against stigmatic protein patterns determined by both serology and electrophoresis. The derived genotype–protein–phenotype relationships extend and support previous work (Nasrallah & Wallace, 1967a, b; Nasrallah, Barber & Wallace, 1970).

1. MATERIALS AND METHODS

(i) *Materials*

The three inbreds of *Brassica oleracea* var. *capitata* used were previously described and used in studies of self-incompatibility proteins (Nasrallah & Wallace, 1967; Nasrallah *et al.* 1970). The arbitrary designations S_1S_1, S_2S_2 and S_3S_3 are maintained; they indicate homozygosity for the three different S alleles. Hybrids S_1S_2, S_1S_3 and S_2S_3 from crosses among the three homozygous genotypes and F_2 populations derived from bud selfing S_1S_2 and S_2S_3 were also used. Data of Thompson (1968) and the authors (unpublished) indicate that cabbage allele S_1 corre-

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Table 1. *Average seed set per pollination from selfs, and crosses among three homozygous S-allele genotypes and their* F_1 *hybrids*

Female parent	Male parent					
	S_1S_1	S_2S_2	S_3S_3	S_1S_2	S_1S_3	S_2S_3
S_1S_1	0·4 (73)*	19·4 (18)	16·3 (20)	15·6 (17)	18·0 (7)	—
S_2S_2	28·6 (15)	0·12 (91)	22 (14)	2·5 (23)	—	1·9 (32)
S_3S_3	19·3 (20)	16·0 (17)	1·0 (56)	—	1·5 (20)	3·0 (47)
S_1S_2	1·8 (37)	1·1 (38)	—	4·4 (35)	—	—
S_1S_3	4·0 (16)	—	5·0 (15)	—	1·25 (12)	—
S_2S_3	—	0·9 (50)	3·4 (12)	—	—	0·5 (53)

* Number of flowers pollinated.

sponds to kale allele S_2 (Thompson & Taylor, 1966). Cabbage allele S_2 corresponds to kale allele S_{14} (Thompson & Taylor, 1966; Thompson, personal communication, June 1971) rather than kale allele S_{21} as stated by Thompson (1968).

(ii) *Methods*

Self- or cross-pollinations were performed by mechanical transfer of pollen to stigmas of flowers. Pollinated flowers were tagged and seed counts were obtained for individual mature pods. Seed counts of 15–25 seeds per pollination indicated a compatible phenotype while few or no seeds indicated an incompatible phenotype. Some pollinations gave intermediate seed sets.

Immunodiffusion methods were identical to those reported by Nasrallah & Wallace (1967a); the batches of antisera (AHS$_1$ and AHS$_2$) against S_1 and S_2 stigmatic homogenates were used. Heterologous absorption of sera was performed by absorbing AHS$_1$ with S_2 homogenates and AHS$_2$ with S_1 homogenates.

Acrylamide gel electrophoresis procedures were generally similar to those reported by Nasrallah *et al.* (1970) with the following modification. Freshly collected stigmas numbering 75–100 were homogenized in 0·2 ml of stacking gel, centrifuged at 5000g for $\frac{1}{2}$ h and the supernatant fluid then subjected to electrophoresis.

2. RESULTS

(i) *Incompatibility phenotypes of self and cross pollinations*

Each inbred was found to be self-incompatible, averaging 1·0 or fewer seeds per self-pollination (Table 1). Each was cross-compatible with the other two inbreds as indicated by 15–25 seeds per cross-pollination. Each of the F_1 hybrids S_1S_2, S_1S_3 and S_2S_3 was also self-incompatible. The self-incompatibility exhibited differed from that of the homozygous parents in that (1) two of the three hybrids averaged more than 1·9 seeds per self-pollination, (2) the range in seed set for individual pollinations was larger for each hybrid than for any homozygous parent, (3) there was higher plant-to-plant variability in seed set, (4) there was much increased seed set as the flowering season progressed.

Table 2. *Average seed set per pollination for reciprocal crosses among nine* F_2 *plants and their homozygous parents*

Phenotypic F_2 group	Plant no.	A			B					C	Parents	
		1	5	9	2	3	4	6	7	8	S_1S_1	S_2S_2
A	1	1·7 (9)*	0·0	0·7 (3)	17·0	18·5	—	22·5	15·5	20·7 (4)	0·6 (10)	21·2 (10)
	5	1·0	0·5	—	—	—	—	—	—	—	0·0	19·5
	9	1·5	0·5	0·0	—	—	—	—	—	—	0·5	22·5
B	2	9·0	3·0	16·5	—	12·3 (3)	1·0	10·0 (3)	3·5	0·0	1·0 (3)	1·0 (4)
	3	8·5	6·5	5·5	6·5	—	0·0	7·0	4·0	0·0	0·3 (3)	0·0 (3)
	4	0·0 (1)	—	0·0 (1)	—	—	—	—	—	0·0 (1)	0·0	0·0
	6	12·0 (3)	10·3 (3)	6·5	3·7 (3)	13·0 (3)	3·0	—	6·6 (3)	1·0	11·7 (3)	0·5
	7	4·5	10·5	13·0	—	3·0 (3)	1·0	3·0	—	0·0 (5)	4·3 (6)	0·4 (5)
C	8	24·5 (4)	24·6 (3)	24·3 (3)	4·3 (3)	4·5 (3)	0·0 (3)	1·6 (3)	0·0 (3)	0·1 (6)	22·5 (4)	0·0 (5)
Parents	S_1S_1	0·2 (8)	3·0 (4)	0·7 (3)	21·0 (7)	21·3 (3)	19·0	18·6 (3)	24·6 (6)	16·1 (9)	—	—
	S_2S_2	22·2 (5)	21·0	20·0 (3)	1·5 (4)	9·0	0·0 (1)	10·5	2·2 (4)	0·0 (4)	—	—

* Number of flowers pollinated if other than two.

Three of the four possible pollinations, including reciprocals, between hybrid S_1S_2 and its parents ($S_1S_2 ♀ \times S_1S_1 ♂$, $S_1S_2 ♀ \times S_2S_2 ♂$ and $S_2S_2 ♀ \times S_1S_2 ♂$) were incompatible, while the fourth ($S_1S_1 ♀ \times S_1S_2 ♂$) was compatible (Table 1). Reciprocal pollinations between S_1S_3 and its parents gave essentially identical results; the corresponding three pollinations ($S_1S_3 ♀ \times S_1S_1 ♂$; $S_1S_3 ♀ \times S_3S_3 ♂$; $S_3S_3 ♀ \times S_1S_3 ♂$) were incompatible, and the corresponding cross of S_1S_1 with the heterozygote ($S_1S_1 ♀ \times S_1S_3 ♂$) was compatible. All four of the reciprocal pollinations between hybrid S_2S_3 and its parents were incompatible. In general, using a heterozygote as either the male or female parent gave a higher seed set, i.e. a less incompatible phenotype, than self-pollinations of the homozygotes.

Families of nine and eleven F_2 plants were respectively derived from bud selfing S_1S_2 and S_2S_3. An attempt was made to self-pollinate each F_2 plant and to cross it reciprocally with each of its two homozygous parents and with each of its F_2 sibs. Some pollinations were missed because of asynchrony of flowering or insufficient flowers.

Seed set data from reciprocal pollinations among the F_2 sibs from hybrid S_1S_2 permitted each plant to be placed into one of three F_2 phenotypic groups, arbitrarily designated A, B and C (Table 2). Placement into a phenotypic group was determined by the combination of incompatibility and compatibility phenotypes exhibited by the F_2 plant in the reciprocal crosses with its sibs. The combination of phenotypes exhibited by all F_2 plants within a group was similar and distinct from the combination of incompatibility phenotypes of F_2 plants in the other groups. These incompatibility phenotypes were as follows: all the F_2 plants that were selfed were self-incompatible; all F_2 plants within each group were cross-incompatible with each other or exhibited an intermediate incompatibility phenotype. In intergroup pollinations, plants of phenotypic groups A and C were reciprocally cross-compatible and plants of groups B and C were reciprocally cross-incompatible. These incompatibility and compatibility phenotypes for pollinations between and within groups of F_2 plants are summarized in Table 3. The F_2 plants 1, 5 and 9 were designated as phenotypic group A, plant 8 as group C, and plants 2, 3, 4, 6 and 7 as group B (Table 2).

Also shown in Table 2 for individual pollinations and summarized in Table 3 are the incompatibility phenotypes obtained for reciprocal pollinations of the F_2 plants with their homozygous parents S_1S_1 and S_2S_2. The plants of F_2 phenotypic group A were reciprocally incompatible with parent S_1S_1 and reciprocally compatible with parent S_2S_2. The reverse was true for the one plant of F_2 phenotypic group C; it was reciprocally compatible with parent S_1S_1 and reciprocally incompatible with parent S_2S_2. In contradistinction, the incompatibility phenotype of pollinations between plants of F_2 phenotypic group B and the homozygous parents was dependent upon whether the F_2 plant or the homozygous parent was used as female or as male. Group B ♀ × parent $S_1S_1 ♂$ was weakly incompatible while the reciprocal $S_1S_1 ♀ \times B ♂$ was fully compatible, and B ♀ × parent $S_2S_2 ♂$ was strongly incompatible while the reciprocal $S_2S_2 ♀ \times B ♂$ was weakly incompatible.

Selfing hybrid S_2S_3 also gave rise to three groups of F_2 plants, arbitrarily desig-

Table 3. *Summary of seed sets and incompatibility phenotypes* for reciprocal cross-pollinations within and among three phenotypically distinct* F_2 *incompatibility groups and for reciprocal pollinations with the parents* S_1S_1 *and* S_2S_2

Phenotypic F_2 group†	Genotype‡	A 1, 1	B (1), 2̇	C 2, 2	Parents 1, 1	Parents 2, 2
A	1, 1	1·0 (24)§ I	18·1 (18) C	21·0 (4) C	0·5 (13) I	21·8 (14) C
B	1̇, 2	9·0 (27) WI	5·4 (38) WI	0·17 (12) I	3·8 (17) WI	0·43 (16) I
C	2, 2	24·5 (10) C	2·1 (15) WI	0·2 (5) I	22·5 (4) C	0·0 (5) I
Parents	1, 1	1·0 (16) I	18·5 (21) C	16·1 (9) C	—	—
	2, 2	21·3 (10) C	4·1 (13) WI	0·0 (4) I	—	—

* I = incompatibility; WI = weak incompatibility; C = compatibility.
† See text for descriptions of F_2 phenotypic groups A, B and C.
‡ In heterozygotes, parentheses indicate an inactive allele and a dot indicates an active one.

nated M, N and O, as distinguished by the combination of compatible and incompatible phenotypes expressed in reciprocal crosses among the F_2 sibs (Table 4). The phenotypes were as follows: all the F_2 plants that were selfed were self-incompatible. All F_2 plants within each group were cross-incompatible with each other or exhibited intermediate cross-incompatibility. Plants of groups M and O were reciprocally cross-compatible. Plants of group N were reciprocally cross-incompatible with plants of both groups M and O. These phenotypic expressions are summarized in Table 5. Plants 6, 7 and 9 were designated as F_2 phenotypic group M; plants 2, 4 and 5 as group O; and plants 1, 3, 8, 10 and 11 as group N (Table 4).

In pollinations with the homozygous S_2S_2 and S_3S_3 parents (Tables 4, 5) F_2 plants of phenotypic group M were reciprocally incompatible with S_2S_2 or had intermediate incompatibility, and reciprocally compatible with S_3S_3. The F_2 plants of group O had exactly opposite phenotypes; they were reciprocally incompatible with parent S_3S_3 and reciprocally compatible with S_2S_2. The F_2 plants of group N were reciprocally incompatible with parent S_3S_3. They were also reciprocally incompatible with parent S_2S_2, but the incompatibility was intermediate or weak when the F_2 phenotypic group N plants supplied the pollen.

(ii) *S allele genotypes*

Three F_2 phenotypic groups, as identified above, representing three F_2 genotypes, are expected since self-incompatibility in *Brassica* is known to be controlled by

Table 4. *Average seed set per pollination for reciprocal crosses among eleven F_2 plants and their homozygous parents*

Phenotypic F_2 group	Plant no.	O			N					M			Parents	
		2	4	5	1	3	8	10	11	6	7	9	S_1S_1	S_2S_2
O	2	0·0 (2)*	0·0 (5)	0·0 (4)	0·5 (4)	1·3 (3)	2·0	2·0	0·5 (1)	20·2 (4)	21·2 (4)	18·3 (3)	16·4 (12)	0·0 (5)
	4	0·0	0·2 (5)	0·0 (5)	7·5	21·5	1·5	0·5	1·0	20·2 (4)	24·0 (3)	25·0 (3)	20·5 (8)	0·0 (6)
	5	0·0 (6)	1·4 (5)	0·0	0·0 (4)	14·0	9·5	14·0 (1)	7·0 (1)	22·5 (4)	18·0	19·5	23·0 (4)	0·0 (5)
N	1	0·4 (9)	0·0 (4)	0·0 (3)	0·0 (8)	1·0 (4)	—	—	—	1·2 (4)	—	2·0	0·2 (12)	0·0 (5)
	3	0·0 (4)	0·0 (7)	0·0 (3)	0·0 (5)	0·0 (3)	1·0	1·5	0·0	0·1 (7)	0·5	0·0	0·2 (6)	0·0 (5)
	8	0·1 (6)	0·0 (6)	0·1 (8)	1·8 (5)	1·0 (5)	—	0·5	0·5	0·0 (4)	1·4 (5)	0·0	0·0 (4)	0·0 (2)
	10	0·4 (5)	0·0	1·0	0·5 (4)	0·0 (1)	—	—	—	0·7 (4)	—	0·0	0·2 (4)	0·0 (3)
	11	0·0 (3)	0·0 (5)	0·0	1·5	0·0	—	0·2 (4)	5·5 (4)	0·0 (5)	—	0·0 (3)	0·1 (8)	0·0
M	6	20·4 (8)	22·5 (4)	21·0 (5)	1·3 (3)	0·3 (3)	—	12·0 (1)	23·0 (1)	—	0·0 (1)	0·0 (1)	0·4 (5)	24·0
	7	20·6 (3)	22·5 (4)	20·5 (4)	10·0	7·6 (3)	14·8 (5)	14·3 (3)	17·6 (3)	2·0 (7)	1·2 (5)	0·0 (6)	4·3 (8)	20·3 (3)
	9	25·0 (4)	25·5 (7)	23·5 (4)	3·0	7·5	14·0	3·3 (3)	12·0 (3)	0·2 (4)	0·0 (6)	0·0 (3)	0·7 (4)	23·5 (4)
Parents	S_2S_2	21·4 (5)	21·8 (5)	23·0 (3)	2·5	9·7 (3)	0·7 (3)	7·0 (7)	3·3 (6)	0·0 (3)	0·2 (5)	0·0 (5)	—	—
	S_3S_3	0·0	0·0 (1)	0·0 (1)	0·5	0·0 (1)	—	0·0 (1)	0·0 (1)	21·5	10·0 (1)	19·0 (1)	—	—

* Number of flowers pollinated if other than 2.

Table 5. *Summary of seed sets and incompatibility phenotypes* for reciprocal cross-pollinations within and among three phenotypically distinct* F_2 *incompatibility groups and for reciprocal pollinations with the parents* S_2S_2 *and* S_3S_3

Pheno-typic F_2 group[†]	Geno-type[‡]	O 3, 3	N 2, 3	M 2, 2	Parents	
					2, 2	3, 3
O	3, 3	0·17 (46) I	4·6 (32) WI	21·5 (28) C	18·8 (24) C	0·0 (17) I
N	2, 3	0·14 (69) I	1·0 (56) I	0·24 (42) I	0·18 (34) I	0·0 (22) I
M	2, 2	22·4 (44) C	9·7 (36) WI	0·6 (34) I	2·3 (17) WI	22·4 (9) C
Parents	2, 2	21·7 (13) C	4·71 (21) WI	0·08 (13) I	—	—
	3, 3	0·0 (4) I	0·2 (5) I	20·5 (4) C	—	—

* I = incompatibility; WI = weak incompatibility; C = compatibility.

† See text for descriptions of F_2 phenotypic groups A, B and C.

‡ In heterozygotes parentheses indicate an inactive allele and a dot indicates an active one.

multiple alleles at the S locus (Bateman, 1955). That parents S_1S_1, S_2S_2 and S_3S_3 are homozygous for three different S alleles is shown by compatibility for cross-pollinations among them (Table 1) and by segregation of both hybrid S_1S_2 and hybrid S_2S_3 into the expected three F_2 phenotypic groups. The comparable cross pollinations (Tables 1–5) indicate that plants of F_2 phenotypic group A behave like parent S_1S_1, plants of group B behave like hybrid S_1S_2, and the single plant of group C behaves like parent S_2S_2. Likewise, in comparable cross-pollinations F_2 phenotypic groups M, N and O respectively have incompatibility phenotypes like parent S_2S_2, hybrid S_2S_3 and parent S_3S_3. These data indicate that F_2 phenotypic groups A, B, and C respectively are genotypes S_1S_1, S_1S_2 and S_2S_2 and groups M, N and O are genotypes S_2S_2, S_2S_3 and S_3S_3.

(iii) *S allele phenotypes of pollen and stigma*

The S allele phenotypes of pollen from heterozygotes S_1S_2 and S_2S_3 were determined by crossing the respective homozygotes with pollen from the heterozygote, and the S allele phenotype of the heterozygous stigmas were determined by pollinating separate stigmas with pollen from the respective homozygotes. The S allele phenotype for pollen of S_1S_2 plants was found to be that of allele S_2, as shown by mean seed sets from all (51) of the $S_2S_2♀ \times S_1S_2♂$ pollinations in Tables 1, 2 and 3 of 2·8 seeds per pollination, as contrasted with 17·3 seeds from 46 $S_1S_1♀ \times S_1S_2♂$ pollinations. This S_2 phenotype indicates that allele S_2 is active (dominant) in S_1S_2 pollen while S_1 is largely inactive (recessive). Stigmas of S_1S_2 simultaneously expressed both S_1 and S_2 allele phenotypes as shown by mean seed sets of 4·6 seeds

from 81 $S_1S_2 \female \times S_1S_1 \male$ pollinations and 0·8 from 66 $S_1S_2 \female \times S_2S_2 \male$ pollinations, indicating that both alleles are simultaneously active (co-dominant) in S_1S_2 stigmas. These means, 4·6 and 0·8, also indicate respectively that S_1 is less active than S_2 in S_1S_2 stigmas.

Both alleles of S_2S_3 plants are simultaneously active in both pollen and stigmas as indicated by mean seed sets of 5·9, 3·5, 0·5 and 0·5 respectively for 89 pollinations of $S_2S_2 \female \times S_2S_3 \male$; 82 pollinations of $S_3S_3 \female \times S_2S_3 \male$; 126 pollinations of $S_2S_3 \female \times S_2S_2 \male$ and 103 pollinations of $S_2S_3 \female \times S_3S_3 \male$ (Tables 1, 4, 5). Activity of both alleles is weakened in S_2S_3 pollen but not in S_2S_3 stigmas, as indicated by the 5·9 and 3·5 mean seed sets when S_2S_3 pollen was placed on homozygous stigmas in contrast with the two 0·5 means when S_2S_3 stigmas were pollinated with homozygous pollen.

(iv) *Protein phenotypes of stigmas*

Electrophoretic separation of basic proteins of stigmatic homogenates of parents S_1S_1, S_2S_2, and their F_1 and F_2 progenies are shown in Fig. 1. The arrow identifies a protein band present in parent S_2S_2 but lacking in parent S_1S_1. Hybrid S_1S_2 has this band and the F_2 plants show segregation. Plants 1, 5 and 9, which were designated as F_2 phenotypic group A and identified as genotype S_1S_1, lack this protein band, while plants 2, 3, 4, 6 and 7, which were designated as group B and identified as genotype S_1S_2, and plant 8, which was designated as group C and identified as S_2S_2, all have it. Fig. 2 shows electrophoretic separations for S_2S_2, S_3S_3 and their F_1 and F_2 progenies. The arrow again identifies the distinct band of parent S_2S_2. This band also occurs in the S_2S_3 hybrid and in all the F_2 plants of phenotypic groups M (plants 2, 4 and 5) and N (1, 3, 8, 10 and 11), which were respectively identified as genotypes S_2S_2 and S_2S_3. The band is not present in plants 6, 7 and 9 which were designated as F_2 phenotypic group O and identified as genotype S_3S_3.

A second difference in band pattern between S_3S_3 and S_2S_2 was identified on the acrylamide gels and indicated by the symbol ● (Fig. 2). This band is present in parent S_3S_3 but lacking in S_2S_2. The F_1 (S_2S_3) and F_2 plants 1, 4, 5, 6, 7, 8, 9 and 10 have this band while plants 2, 3 and 11 do not. The segregation pattern is clearly independent of the incompatibility reaction and is controlled by a gene other than the S locus.

Homogenates of stigmas from each of the nine F_2 plants of family S_1S_2 were separately tested against heterologously absorbed AHS_1 and AHS_2 antisera. Homogenates from each of the three plants of F_2 phenotypic group A, i.e. genotypes S_1S_1, formed a precipitation band when tested against AHS_1 but failed to react with AHS_2. The single plant designated as phenotypic group C and identified as S_2S_2 reacted reversely, forming a precipitation band against AHS_2 but not against AHS_1. All five plants assigned to group B and identified as S_1S_2 reacted with both antisera. The 11 F_2 plants of family S_2S_3 were each tested against AHS_2. Tests against AHS_3 were not possible because this antiserum had been completely used. All three plants of F_2 phenotypic group M (genotype S_2S_2) and all five plants of group N reacted against AHS_2, while the three F_2 plants designated as group O failed to react.
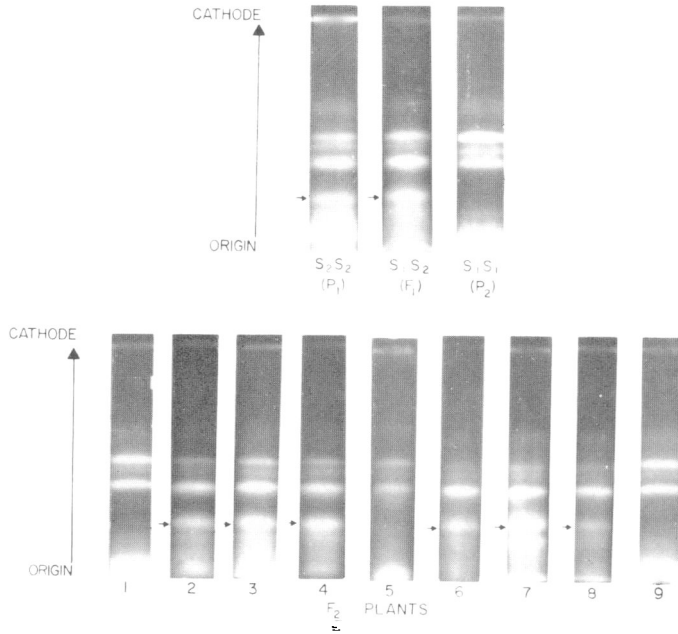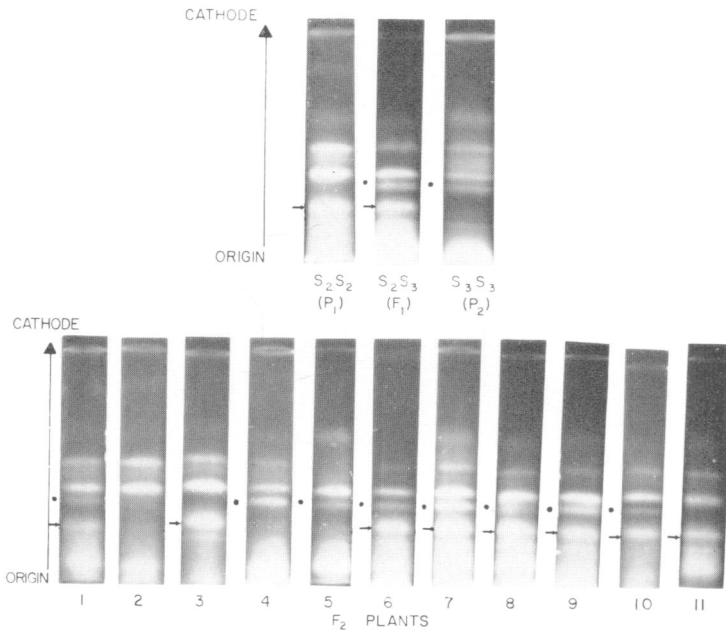
Fig. 1. Electrophoretic separations on acrylamide gels of the basic proteins from stigmatic homogenates of two parents S_1S_1 and S_2S_2 and their F_1 and F_2 generations. The S_2 band is indicated by the arrow.

Fig. 2. Electrophoretic separations on acrylamide gels of the basic proteins from stigmatic homogenates of two parents S_2S_2 and S_3S_3 and their F_1 and F_2 generations. The S_2 band is indicated by the arrow while the symbol (●) identifies another protein difference between S_3S_3 and S_2S_2.

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3. DISCUSSION

The serological tests of stigmatic homogenates of individual F_2 plants of hybrid S_1S_2 identified three protein patterns which correlated exactly with the S allele genotypes and expressed S allele activities. All parental, F_1 or F_2 plants identified as genotype S_1S_1 and shown to have only S_1 stigmatic activity possessed only an S_1 stigmatic antigen (protein). All plants identified as S_1S_2 and shown to have co-dominant activity for alleles S_1 and S_2 were shown to have both the S_1 and S_2 stigmatic antigens. All plants identified as S_2S_2 and having only S_2 stigmatic activity had only the S_2 antigen. For hybrid S_2S_3 no antiserum against allele S_3 was available but the S_2 stigmatic antigen was present in all parental, F_1 and F_2 plants identified as heterozygous or homozygous for S_2, i.e. in all plants showing S_2 activity. All parental, F_1 and F_2 plants of both hybrid S_1S_2 and S_2S_3 that were heterozygous or homozygous for allele S_2 and showed S_2 activity in the stigma had a protein band, as identified by electrophoretic separation of basic stigmatic proteins, that was absent in genotypes not carrying the S_2 allele. This protein band was shown in this and a previous study (Nasrallah *et al.* 1970) to be the S_2 antigen.

The data clearly demonstrate an S allele–protein–phenotype relationship for the cabbage stigmas. A similar S allele–protein–phenotype relationship seems logical for pollen but an S allele specific pollen substance has not been identified. The dominant or co-dominant S allele activities in pollen and stigmas of the heterozygous plants and the specific S allele activities in both pollen and stigma of the homozygous genotypes clearly interact to give the incompatibility phenotypes observed for the self and cross-pollinations among parental F_1, and F_2 genotypes. All the data are readily explained by multiple alleles at a single locus, with sporphytic control of S allele action in the pollen, and with interactions of dominance or co-dominance between the S alleles in heterozygous pollen and stigmas as previously reported for *Brassica* and related Cruciferae (Bateman, 1952, 1954, 1955; Thompson, 1957; Haruta, 1962; Odland, 1962). Compared with homozygotes, the heterozygotes frequently showed weakened activity, particularly in the pollen, i.e. when heterozygous pollen was placed on homozygous stigmas.

The molecular basis for these allelic interactions is not understood. The S allele proteins are absent from stigmas of cabbage flower buds (Nasrallah & Wallace, 1967*b*). They are synthesized during a period of about 2 days, just prior to anthesis, so that full incompatibility is expressed at anthesis. In addition to expressing co-dominance in the stigma as shown in this study, our unpublished data (see also Thompson & Taylor, 1966) indicates that S_2 is dominant in the stigma to some alleles and recessive to others, and that S_1 shows mutual weakening when paired with certain alleles. Quantitative and qualitative assays of the S allele proteins in these heterozygous genotypes would elucidate the biochemical basis of allelic interactions.

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