SELF-INCOMPATIBILITY IN MARROW-STEM KALE, *BRASSICA OLERACEA* VAR. *ACEPHALA*

II. METHODS FOR THE RECOGNITION IN INBRED LINES OF PLANTS HOMOZYGOUS FOR S ALLELES

By K. F. THOMPSON and H. W. HOWARD
(*Plant Breeding Institute, Trumpington, Cambridge*)

(Received 24 April 1958)

I. Introduction

It has been shown by Thompson (1957) that self-incompatibility in marrow-stem kale, *Brassica oleracea* var. *acephala*, is determined by a multiple series of S alleles and that the self-incompatibility system is of the sporophytic type. In the present paper methods for the recognition in inbred lines of plants homozygous for S alleles are described and discussed. Such plants would be needed if the present mass-selection methods used in breeding the crop were replaced by a method involving the use of inbred lines to produce double-cross seed.

II. Methods

1. *Possible parental types*

As is summarised in Table 1, there are four possible types of parent plants which are heterozygous for S alleles. In the first type allele *a* is dominant to allele *b* in both pollen and style; in the second type *a* is dominant to *b* in the pollen but both alleles are active in the style; in the third type both *a* and *b* are active in the pollen but *a* is dominant to *b* in the style; and finally in the fourth type both alleles are active in both pollen and style. The possibility of *a* being dominant to *b* in the pollen but of *b* being dominant to *a* in the style (or the reciprocal possibility) is not considered because plants possessing two such alleles would be self-compatible and not self-incompatible.

2. *Recognising S allele homozygotes by the use of the parent plant*

It is easy with marrow-stem kale to maintain by vegetative propagation (stem cuttings) any selected parent from which an inbred family has been obtained by bud pollination. Hence one method of searching for S allele homozygotes would be to pollinate eleven members of the inbred family by their parent and also to pollinate the parent with the eleven inbred plants (eleven plants are used because this is the number needed at a probability level of $P=0{\cdot}95$ for there to be present at least one plant of each homozygote). Any plants giving compatible pollinations with the parent (i.e. the heterozygote *a b* of Table 1) would be homozygotes for an S allele which was recessive in either pollen or style or both (see Table 1). No compatible pollinations

Table 1. *Possible parent plant types and the percentages of compatible pollinations to be expected from pollinations between plants obtained by inbreeding* (ab, *selfed by bud pollination giving* 2 ab : 1aa : 1bb).

Type	Allele Relations*		Expected percentages of compatible pollinations with sister inbreds					
			used as female			used as male		
	Pollen	Style	*ab*	*aa*	*bb*	*ab*	*aa*	*bb*
1	$a>b$	$a>b$	25 ($\times bb$)	25 ($\times bb$)	75 ($\times ab$ and aa)	25 (onto *bb*)	25 (onto *bb*)	75 (onto *ab* and *aa*)
2	$a>b$	$a=b$	0	25 ($\times bb$)	75 ($\times ab$ and aa)	25 (onto *bb*)	25 (onto *bb*)	25 (onto *aa*)
3	$a=b$	$a>b$	25 ($\times bb$)	25 ($\times bb$)	25 ($\times aa$)	0	25 (onto *bb*)	75 (onto *ab* and *aa*)
4	$a=b$	$a=b$	0	25 ($\times bb$)	25 ($\times aa$)	0	25 (onto *bb*)	25 (onto *aa*)

*$a>b$ means a dominant (active), b recessive (inactive); $a=b$ means both a and b active.

would, however, occur if the two alleles were active in both pollen and style (i.e. type 4 of Table 1), and, since it is compatible pollinations which make possible the recognition of S allele homozygotes, the use of the parent plant is no good for such families. It is therefore suggested that it is better to use one inbred plant as a female 'tester' plant in pollinations with eleven others and to use another inbred plant as a male 'tester' plant in pollinations with eleven others. Even if both the 'tester' plants picked for use in the pollinations are heterozygotes, the situation will be no worse than if the parent plant (itself a heterozygote) had been used.

On the other hand the use of the parent has some advantages over using random plants in an inbred progeny. First it will be known that the parent plant is not self-compatible, and secondly the parent plant will have been chosen so as not to be either male or female sterile. The two 'tester' plants, chosen in an inbred family, can, of course, be tested to make sure that they are not self-compatible and that they are male and female fertile. Such tests, however, take time, and the results may not be available before the test pollinations for determining incompatibility groups have to be made.

3. *Suggested method for recognising S allele homozygotes*

The first part of the suggested method for recognising S allele homozygotes is to use one plant of the inbred family as a female parent and another plant as a male parent in pollinations with eleven plants (see previous section). Unless the parent plant was either a homozygote or a heterozygote with the two alleles having a type 4 relationship of Table 1, some compatible pollinations should be obtained (compatible pollinations may be found in some cases for type 4). The compatible pollinations will make it possible to split the family into two groups (with a very fortunate choice of the two 'tester' plants it may be possible to differentiate three groups for types 2, 3 and 4 of Table 1). The smaller group, if only two have been recognised, is likely to be the homozygotes (with the small number of plants involved care must, of course, be taken in using this assumption).

The second part of the suggested method consists of using the parent both as male and female in pollinations with any two inbreds which gave compatible pollinations. Since it is known that the parent is the heterozygote, the results of these pollinations should show which group, or groups, of plants are homozygotes and what is the relationship between the two alleles in the parent.

The third part of the suggested method consists of using the plant, or group of plants, now known to be homozygotes for one allele, to find the homozygotes for the other allele. This will not be necessary if the parent is of type 4 (see Table 1) since the only compatible pollinations are between homozygotes; and it will not be possible, except by progeny testing, if the parent is of type 1 because the heterozygotes and the homozygotes for the dominant allele behave similarly (see Table 1).

If no compatible pollinations are obtained from the first part of the suggested method, further pollinations can be tried or an alternative method used. This alternative method, which would take another year, involves crossing the parent with an S allele

homozygote known to be low in the dominance series. This would give plants with only one of the dominant alleles of the original parent, and these plants could be used to find plants of the inbred family which are homozygous for the other allele.

The experimental work on the recognition in inbred families of plants homozygous for S alleles described later in this paper has been carried out more or less as described in the suggested method above, but it was not, for example, always necessary or possible to use the parent plant.

4. *Experimental methods*

The inbred families were all grown from seed obtained by bud-pollinating the parent plants. As in Thompson (1957) all pollinations were made in the glasshouse. Plants were tested for being self-compatible or self-incompatible by pollinating about ten flowers of one inflorescence at the open-flower stage. Female fertility was checked from pollinations by an unrelated plant, not by bud-pollination as in Thompson (1957). Ten flowers on one inflorescence were used also in the pollinations to determine the cross-compatibility of two plants. The compatibility of crosses is expressed in the tables as the average number of seeds per fruit, the number of seeds being usually determined by examining green, immature fruits.

III. Results

1. *Family 6 (one allele dominant to the other in both pollen and style)*

The pollination results for family 6, obtained from plant B 33 selfed by bud pollination, are given in Table 2. Pollinations were made in both 1956 and 1957. Twelve of the sixteen plants were crossed as female parents in 1957 by 31/35 which was known to be compatible with B 33. With the exception of plant 8 all these pollinations gave a high set of seeds per fruit. Plant 8 also gave a low set of seeds per fruit when pollinated by plant 7 in 1957, although it gave a high set in 1956. All plants of this family therefore have a high female fertility.

The results from selfing the plants in the two years suggest that three plants (6, 9 and 16) are more or less self-compatible. If these three plants really are self-compatible, then it would appear that their self-compatibility is due to them being homozygous for a recessive gene which is independent of the S alleles.

In the search for S allele homozygotes fifteen plants of the family were all crossed onto two plants, 13 and 4, in 1956 and were crossed by a single plant, number 7. As can be seen from the results given in Table 2a, the crosses onto plant 13 as female parent split the twelve self-incompatible plants into two groups, nine plants (group A) being cross-incompatible with plant 13 and three (group B) being cross-compatible. Plant 7 as male parent also distinguishes the same two groups. Using plant 4 as a female parent in 1956, however, did not show any such division of the family, but a repeat of the 1956 pollinations in 1957 showed that plant 4 and plant 13 behaved similarly as female parents. The difference between the 1956 and 1957 results from using plant 4 as a female parent is probably to be explained by the fact that the 1956 pollinations

Table 2. *Family 6 from plant B33 selfed. (a) intra-family pollinations*

Group	Plant No.	Average number of seeds/fruit from pollinations							
		31/35 as male, 1957	Selfs 1956	Selfs 1957	Plant 13 as female, 1956	Plant 4 as female 1956	Plant 4 as female 1957	Plant 7 as male 1956	Plant 7 as male 1957
	1	23·7	0·7	0·2	0·7	13·6	1·2	23·0	25·7
	2	26·7	8·5	7·4	1·6	17·4	2·0	35·3	26·1
	3	20·6	0·1	1·0	0·6	2·5	0·0	24·3	20·0
	4	—	3·6	1·8	1·4	—	—	24·1	21·7
A	5	34·1	18·4	11·7	1·3	22·6	2·0	41·1	32·1
	8	5·9	4·0	0·1	0·4	29·4	8·9	16·3	3·4
	10	16·9	0·5	0·7	0·0	19·1	0·4	34·6	19·0
	11	20·3	10·0	7·1	0·8	23·9	5·2	24·2	21·1
	12	28·2	2·8	4·5	0·1	21·5	0·0	30·8	29·0
	13	—	0·3	—	—	17·6	—	18·0	—
	7	—	2·8	—	18·0	24·1	21·7	—	—
B	14	24·7	1·3	7·6	18·5	20·6	23·8	2·5	6·1
	15	—	12·1	—	11·6	31·7	—	2·6	—
	6	—	28·1	17·2	20·3	30·3	29·4	30·8	—
?	9	28·2	25·7	24·5	16·5	33·3	27·0	7·7	25·8
	16	31·6	20·2	24·5	16·1	33·7	23·5	6·7	30·2

Table 2. (*b*) *pollinations with parent* (B 33). *B 33 Selfed—1956, 1·3; 1957; 7·6 seeds/fruit*

Group	Plant No.	Av. no. seeds/fruit from pollinations			
		B 33 as male		B 33 as female	
		1956	1957	1956	1957
A	1	2·0	0·0	0·0	—
	3	7·4	—	0·0	0·2
	8	0·8	—	—	—
	11	—	5·6	—	4·9
B	7	—	—	27·2	—
	14	26·7	24·0	—	24·9
	15	5·4	—	30·1	—
?	6	—	29·0	—	24·7
	16	26·0	—	27·2	—

were made on the first flowers of the first inflorescences to come into flower, while in 1957 they were made at a later stage.

The results in Table 2a suggest very strongly that the three plants of group B are homozygotes for an S allele which is recessive in both pollen and style to the other allele present in the parent plant. This was checked by reciprocal pollinations with the parent (Table 2b), and it was found that group B plants gave compatible pollinations both ways with the parent. Plants of group A, which presumably includes both the homozygotes for the other allele and the heterozygotes, are also as expected cross-incompatible with the parent. The homozygotes for the other allele can only be found by progeny testing as is illustrated for family 3 in the next section.

2. *Family* 3 (*one allele dominant to the other in both pollen and style*).

Family 3 of Thompson (1957) was obtained from selfing plant 31. There were a few self-compatible plants, and intra-family pollinations of the self-incompatible plants were all incompatible. A family from plant 31 crossed with another selection, known to be heterozygous for S alleles, contained four incompatibility groups. Thus plant 31 was not an S allele homozygote, and it is probable, though not proved, that the inactive S allele in plant 31 is a self-fertility allele, low in the dominance series. The recognition of homozygotes for the dominant (active) allele in family 3 thus requires the same method as would be needed for recognising similar homozygotes in family 6 and in other families where one allele is dominant to the other in both pollen and style (i.e. type 1 of Table 1).

The method for recognising the homozygotes is to cross eight plants of those known to be either homozygous or heterozygous for the dominant allele with a plant homozygous for the recessive (inactive) allele (this is again for a probability level of P=0·95).

Five plants from each of the crosses are then pollinated with plants homozygous for the recessive allele. If all of these pollinations are compatible, then the parent of this cross is a homozygote for the dominant allele (see Table 3). Alternatively the five plants from each cross can be pollinated with the original parent. If all of these pollinations are incompatible, then the parent of this cross is a homozygote for the dominant allele (see Table 3).

Table 3. *Recognition of plants homozygous for an S allele which is dominant in both pollen and style.*

(a) It is required to recognise $\dot{a}\dot{a}$ plants in a mixture of $\dot{a}\dot{a}$ and $\dot{a}(b)$ produced on selfing an $\dot{a}(b)$ parent ($\dot{a}$=dominant, active allele and (b)=recessive, inactive allele in the *ab* heterozygote).

(b) Normal method.

(i) Cross with *bb*

$\dot{a}\dot{a} \times bb \rightarrow$ all $\dot{a}(b)$

$\dot{a}(b) \times bb \rightarrow 1\ \dot{a}(b)\ :\ 1\ bb$

(ii) $\dot{a}(b)$ and *bb* seedlings from above crosses can be recognised from pollinations with either $\dot{a}(b)$ or *bb*.

(c) Modified method used for family 3 of this paper.

(i) Cross with *cc*.

$\dot{a}\dot{a} \times cc \rightarrow$ all $\dot{a}(c)$

$\dot{a}(b) \times cc \rightarrow 1\ \dot{a}(c)\ :\ 1\ b\dot{c}$

(ii) $\dot{a}(c)$ and $b\dot{c}$ seedlings from above crosses can be recognised from pollinations with either $\dot{a}(b)$ or *cc*.

For the recognition of plants homozygous for the dominant S allele of family 3 it was not possible to use the recessive S allele homozygotes of this family because such plants are probably self-compatible. In their place there was used plant 300/14 (or in one case 300/12), an S allele homozygote from family 2 of Thompson (1957). It was known that the allele in plant 300/14 was recessive to the dominant S allele in plant 31, but also active in the presence of the inactive S allele of plant 31 (Table 3). The crosses were made using plant 300/14 as female parent and the family 3 seedlings as males. Because 300/14 is non-hairy and because plant 31 is homozygous for a dominant gene which gives hairiness of the margin of seedling true leaves (Thompson, 1956), it was possible to check that the progenies did not include any accidental selfs.

The results of pollinations of the seedlings in the progenies from the cross of 300/14 by family 3 plants are given in Table 4. Nearly all seedlings were highly self-incompatible, and nearly all showed a high female fertility when pollinated by the unrelated inbred 252/15. The pollinations by plant 31 and by plant 300/14 nearly all gave results which allowed a classification of the seedlings into the two expected incompatibility groups to be made. It was also found, as was expected, that seedlings incompatible with plant 31 were compatible with plant 300/14, and that seedlings compatible with plant 31 were incompatible with plant 300/14.

It was clear from the first results that only one inbred, plant 31/35, might be homozygous for the dominant S allele. That plant 31/35 is such a homozygote was shown by further tests on an extra four seedlings from the cross 300/14 x 31/35. The probability that 31/35 is not a homozygote but a heterozygote for the S alleles is less than 0·002.

3. *Family 7 (one allele dominant in the pollen, but both alleles active in the style).*

The results for family 7 from Plant A 168 selfed by bud-pollination are given in Table 5. All seedlings gave a high set of seeds per fruit when crossed with the unrelated plant A 162/2. One seedling, plant 2, appears to be at least partially self-compatible.

Table 4. *Further investigations of family 3 plants to find dominant S allele homozygotes.*

Cross	Seedling No.	Average number of seeds/fruit set by seedling: Self, open flowers	in crosses with plants: 252/15	31 = à(b)	300/14 = cc	Type of seedling H = à(c) P = bc
300/14 × 31/27	1	9·3	24·9	27·1	5·6	P
	2	2·6	28·7	1·0	13·9	H
	2[1]	—	35·6	2·5	35·3	H
	3	0·0	24·0	0·5	15·2	H
	4	—	5·6	12·1	14·2	? P
	4[1]	—	24·1	25·1	16·0	? P
	5	14·7	18·1	17·7	4·2	P
300/14 × 31/28	1	6·4	27·2	23·5	10·8	P
	2	2·6	27·3	1·0	15·9	H
	3	2·9	29·9	28·1	10·2	P
	4	3·3	24·0	0·1	9·5	H
	5	2·7	27·0	25·2	7·3	P
300/14 × 31/30	1	2·0	18·3	0·6	6·5	H
	1[1]	4·1	24·8	9·9	23·8	H
	2	2·3	15·5	2·8	9·6	H
	3	1·8	16·4	16·4	3·2	P
	4	—	24·1	21·9	0·2	P
	5	6·5	23·7	13·5	19·3	H
300/14 × 31/32	1	20·7	31·2	—	25·2	?
	2	10·6	23·6	25·6	6·5	P
	3	0·0	25·6	0·4	8·6	H
	4	—	28·4	28·1	2·7	P
	5	—	28·7	1·1	18·9	H
300/14 × 31/33	1	2·3	18·1	7·2	12·0	? H
	2	—	24·3	31·6	7·2	P
	3	16·4	24·6	23·2	0·4	P
	4	—	30·6	30·1	14·5	P
	5	7·2	18·4	30·0	7·5	P
300/14 × 31/35	1	0·4	32·5	4·9	24·6	H
	2	0·1	27·5	1·8	23·8	H
	3	0·1	26·8	0·5	13·2	H
	4	1·5	27·4	0·8	24·6	H
	5	—	22·7	1·4	14·2	H
	6[2]	1·7	28·0	0·3	21·9	H
	7[2]	1·9	25·8	1·0	20·1	H
	9[2]	3·3	31·2	1·2	21·5	H
	10[2]	3·1	25·6	0·5	19·6	H

Table 4. (*Continued*)

Cross	Seedling No.	Average number of seeds/fruit set by seedling			Type of seedling H=á(c) P=b.c
		Self, open flowers	in crosses with plants		
			252/15	31=á(b)	300/14=cc
300/14×31/36	1	—	33·5	34·3	0·5	P
	2	—	24·2	0·7	10·7	H
	3	—	25·2	3·1	16·7	H
	4	—	16·4	14·8	0·6	P
	5	0·1	21·7	29·6	0·0	P
300/14×31/37	1	2·5	25·0	1·8	20·4	H
	2	1·3	23·0	0·7	19·8	H
	3	—	27·2	28·5	2·4	P
	4	3·1	18·8	13·6	2·2	P
	5	—	28·0	28·9	5·7	P
300/14×31/38	1	0·2	20·3	18·5	0·2	P
	2	1·7	26·7	32·9	1·8	P
	3	0·8	23·7	—	4·1	? P
	4	3·3	26·1	22·3	0·6	P
	5	2·4	22·4	25·0	—	P
300/14×31/39	1	0·0	10·3	—	5·8	P
	1[1]	—	26·8	34·8	8·0	
	2	3·4	31·3	—	25·6	H
	3	0.5	25·8	5·1	16·2	H
	4	0·2	29·7	—	23·2	H
	5	0·4	32·6	2·9	27·2	H

1 Repeat pollinations 2 Additional plants pollinated later.

3 Type of seedling—H=á(c), i.e. compatible with 300/14 (cc), but incompatible with plant 31 (á b)—see Table 3c.

Table 5. *Family 7 from plant A168 selfed.*

Group	Plant No.	A162/2 as male	Average number of seeds/fruit from pollinations						
			Selfs		Plant 2 as male	Plant 8 as female		Plant 20 as female	Plant 20 as male
			1956	1957		1956	1957		
C	6	30·0	—	1·1	—	2·2	0·0	34·8	24·4
	7	33·4	2·1	5·1	13·8	2·1	—	33·4	28·6
	10	22·1	—	0·7	—	0·4	—	33·7	24·6
	17	21·0	—	0·1	—	—	1·4	29·1	20·8
	19	28·1	—	2·3	—	—	0·3	27·0	30·0
D	1	28·4	—	1·7	—	2·9	1·3	30·6	6·0
	3	30·3	3·6	7·1	20·1	3·0	—	34·8	4·7
	4	24·2	—	1·5	—	4·2	1·1	34·2	4·9
	5	34·5	1·5	5·8	23·5	2·2	0·7	34·6	8·1
	8	33·7	1·2	4·0	13·4	—	—	34·4	2·5
	9	39·4	1·6	7·0	21·1	1·2	0·8	32·5	11·0
	11	34·3	—	2·7	—	0·7	—	30·2	14·9*
	18	30·0	—	8·3	—	—	1·8	32·3	3·7
E	20	21·5	—	0·8	—	—	2·5	—	—
?	2	—	13·7	—	—	13·4	—	—	—

*Pollination repeated=1·2 seeds/fruit.

The first pollinations made in the search for S allele homozygotes were with plant 2 as male parent in 1956. The five pollinations all appear to be partially compatible and are of no use for the analysis of the family. The second set of pollinations made in 1956 were with plant 8 as female parent. The nine pollinations other than that by plant 2 all appeared to be incompatible. Further pollinations with plant 8 as female parent were made in 1957 and all these were also incompatible. Since a total of thirteen plants gave incompatible pollinations with plant 8 as female parent, and since such a number of plants would be expected to include at least one plant homozygous for each of the two S alleles present in A 168, it is reasonable to suppose that plant 8 is heterozygous for two S alleles which have independent activity in the style (see Table 1).

A series of pollinations using another seedling, plant 20, as female parent were also made in 1957. It gave compatible pollinations with all the thirteen inbreds, including plant 8, crossed onto it. Assuming plant 8 to be heterozygous, this must mean that plant 20 is homozygous for an allele which is recessive in the pollen, i.e. we are dealing with a case of type 2 allele relationship of Table 1.

Recognition of the homozygotes for the other allele is now easy because only they will give compatible pollinations when plant 20 is used as a male parent (see Table 1). Of the thirteen inbreds tested, five appear to be homozygous for the S allele which is dominant in the pollen. These are the group C of Table 5, group D being the heterozygotes.

It was not possible to check the above analysis by pollinations with the parent, A 168, as this was dead. The segregation, found in the family, of 5 homozygous for one allele : 8 heterozygotes : 1 homozygous for the other allele with one possible self-compatible plant is a fair fit to a 1 : 2 : 1 ratio.

Family 7 is also of interest as showing a possible linkage of the S allele which is recessive in the pollen with one kind of female sterility. Thus plant 20, the recessive homozygote, produced 80 per cent of shrivelled seed when selfed by bud pollination or crossed as female parent with A 162/2; plant 11, one of the S allele heterozygotes, formed about 50 per cent of shrivelled seed when selfed by bud pollination; and plant 6, one of the dominant homozygotes, gave only 4 per cent of shrivelled seed when bud pollinated.

4. *Family* 8 (*both alleles active in the pollen, but one allele dominant in the style*).

All fourteen plants of family 8 from plant B 96 selfed by bud-pollination gave a good set of seeds when pollinated with the unrelated plant 300/10, and, with the exception of plant 7, they are all highly self-incompatible (Table 6).

The search for S allele homozygotes was begun by crossing thirteen plants onto plant 6 as female parent, and by using plant 14 as male parent in crosses with the other thirteen inbreds. As is shown in Table 6, plant 6 was compatible as female parent with four inbreds, but no compatible pollinations were found when plant 14 was used as male parent, although there was a rather high set with plant 4. The results for plant 14 suggest that this plant is a heterozygote and that the two alleles have independent

activities in the pollen (see Table 1). If this is so, plant 6 may be either of the two S allele homozygotes or the heterozygote, and the four inbreds giving compatible pollinations with plant 6 must be homozygotes for either the dominant or the recessive allele (see Table 1).

Test pollinations of two of the four inbreds (plants 10 and 12, see Table 6b), which gave compatible pollinations with plant 6, with the parent show clearly that they are homozygotes for the allele which is recessive in the style because it is only such homozygotes which give compatible pollinations with the heterozygote (see Table 1, type 3 parent). Test pollinations of one of the ten inbreds which did not give compatible pollinations with plant 6 (see Table 6 B, plant 16) and of plant 6 itself with the parent confirms that these two plants are either heterozygotes or homozygotes for the dominant allele.

Having recognised homozygotes for the allele which is independent in the pollen and recessive in the style, it is now easy to search for the homozygotes for the other allele. Plants homozygous for this allele, which is active in both pollen and style, will

Table 6. *Family 8 from plant B96 selfed.* (*a*) *Intra-family pollinations.*

Group	Plant No.	Average number of seeds/fruit							
		300/10 as male	Selfs	Plant 6 as female	Plant 14 as male	as female parents			
						5	10	12	13
F	4	28·8	0·4	0·6	16·6	24·4	—	—	—
G	3	28·7	0·7	0·2	0·5	2·8	—	—	—
	6	29·5	0·2	—	1·4	—	0·1	—	—
	7	29·3	10·2	1·1	12·7	—	—	1·2	—
	9	27·7	0·6	0·7	4·0	—	—	—	2·1
	11	26·2	0·0	0·1	0·6	—	—	2·1	—
	14	32·7	0·5	1·4	—	7·5	—	—	—
	15	24·1	0·2	0·2	3·4	9·3	—	—	—
	16	23·8	0·2	2·1	0·1	—	—	—	0·7
	17	21·5	0·0	1·2	2·9	—	—	—	2·9
H	5	30·0	1·4	29·9	7·5	—	—	—	—
	10	25·8	0·1	31·9	2·3	—	—	—	—
	12	27·4	0·1	35·0	1·1	—	—	—	—
	13	20·5	0·1	32·7	4·0	—	—	—	—

(*b*) *Pollinations with parent.* (*B 96*). *B 96 Selfed—9·4 seeds/fruit*

Group	Plant No.	Average seeds/fruit	
		B 96 as male	B 96 as female
G	6	4·4	5·8
	16	—	3·8
H	10	1·6	32·9
	12	2·5	32·8

give compatible pollinations when used as male parents in pollinations with the homozygotes for the other allele (see Table 1). All four plants which gave compatible pollinations with plant 6 as female parent were crossed with the ten plants which did not give compatible pollinations with plant 6. A single pollination, plant 5 x plant 4, was found to be compatible. This means that plant 4 is homozygous for the allele which is dominant in the style. The check pollinations using plant 4 as male parent with plants 10, 12 and 13, the other three plants in group H of Table 6, have not yet been made.

5. *Family* 9 (*both alleles active in both pollen and style*).

The pollination results for family 9 from plant B 120 selfed by bud-pollination are given in Table 7. The results are complicated by the fact that several of the inbreds show a high degree of female sterility, see especially the results for pollinations by the unrelated plant 31/35. All the plants with high seed sets by 31/35 are, however, highly self-incompatible.

The search for S allele homozygotes was commenced by using plant 8 as female parent and plant 19 as male parent in pollinations with the fifteen other inbreds. Plant 19 gave no compatible pollinations and is therefore probably heterozygous for two alleles which have independent activities in the pollen. Fortunately it was noticed for plant 8 that there was a pronounced colour change of the stigmatic surface from yellow to dark brown or black in the two days following pollination by plant 31/35. No such change in stigmatic surface colour occurred when plant 8 was selfed nor when it was crossed with plants 10 and 17 and with all the plants (including plant 19) of group K of Table 7. There were, however, colour changes with the four plants of group L. Since similar stigmatic surface colour changes had been noticed previously in some other plants for compatible and not for incompatible pollinations, it was suspected that the four plants of group L were S allele homozygotes; although, when figures for seeds per fruit were obtained later, there were no indications of any compatible pollinations because of the high female sterility of plant 8. It is also interesting to note that Bateman (1954) observed a flushing of the stigmatic surface colour in *Iberis amara* after compatible but not after incompatible pollinations.

The next step in the analysis of the family was to use plant 9, one of the suspected S allele homozygotes, as a female parent in crosses with plant 8 and the eleven inbreds which had given no colour changes when used as male parents with plant 8. Plant 9 was not the best plant of the four to have chosen as it was found later to have a certain degree of female sterility. Its use did, however, enable three plants homozygous for the other allele to be recognised (i.e. group J of Table 7).

The final pollinations were made using plants 17, 14 and 18 as female parents. They were chosen for their high seed fertility (see pollinations by 31/35) and represent one S allele homozygote (group J), the heterozygote (group K) and the other S allele homozygote (group L) respectively. The results for these pollinations show conclusively that the two alleles in this family have independent activities in both pollen and style (i.e. a type 4 relationship of Table 1).

Table 7. *Family 9 from plant B120 selfed.*
Intra-family pollinations.

Group	Plant No.	Average number of seeds/fruit							
		31/35 as male	Selfs	Plant 19 as male	as female parents				
					Plant 8	Plant 9	Plant 17†	Plant 14†	Plant 18†
J	8	1·7*	0·0	0·8	—	7·8	—	0·9	19·3
	10	0·5	0·0	0·0	0·5	10·9	2·5	0·7	—
	17	15·8	0·4	3·6	0·3	9·0	—	—	20·4
K	1	4·1	0·0	0·0	0·5	0·5	—	0·1	0·6
	3	4·5	0·0	0·3	1·4	0·5	—	—	0·3
	4	8·8	0·0	0·0	1·7	1·0	—	—	1·3
	11	16·0	0·7	0·6	1·8	0·5	4·7	0·0	0·7
	12	12·3	0·0	0·8	0·6	0·0	—	—	0·1
	13	7·2	0·1	0·5	0·9	0·9	1·4	0·2	1·7
	14	22·9	0·1	0·2	0·7	1·0	3·4	—	0·2
	15	2·9	0·0	0·6	0·8	0·7	—	0·0	0·1
	19	2·1	0·3	—	0·8	2·1	3·6	0·2	1·6
L	2	19·9	0·0	0·2	2·8*	—	—	0·0	—
	9	8·7	0·2	2·1	1·6*	—	19·0	0·1	—
	16	0·2	0·0	0·0	1·4*	—	—	—	—
	18	14·3	0·3	1·6	1·8*	—	17·3	0·1	—

* These pollinations showed darkening of the stigma.
† These figures are based on averages of seed set from 5 to 7 flowers instead of 10.

Table 8. *Family 10 from plant A162 selfed.*
Intra-family pollinations and with plant 300/10.

Group	Plant No.	Selfs	Average number of seeds/fruit from					
			31/35 as male	Plant 4 as female	Plant 7 as male	300/10 as male	Plant 2 as female	Plant 5 as female
M	1	0·3	25·0	0·1	0·2	6·9	—	0·0
	3	0·1	25·3	0·0	0·3	7·9	—	0·9
	4	0·1	28·3	—	0·0	1·0	—	—
	6	0·0	5·1	0·0	0·0	0·1	0·3	0·2
	7	0·2	26·0	0·0	—	5·0	—	—
	8	0·0	4·5	0·0	0·0	2·0	—	0·2
	13	0·0	17·3	0·2	1·6	5·7	—	0·0
N	2	—	25·5	0·0	0·0	29·9	—	—
	5	0·0	2·6	0·0	0·0	5·6*	—	—
	12	0·0	9·5	—	0·0	13·9	—	0·2
	15	2·2	28·0†	0·0	0·0	24·3	0·6	0·0

* Repeated—18·4 at same time as pollinations made on plant 5 as female.
† From only 4 flowers. Only 25 per cent good seed, remainder shrivelled.

6. *Family* 10 (*both alleles active in both pollen and style*).

In family 9, the parent of which has two alleles with independent activities in both pollen and style, it was fortunate for the recognition of S allele homozygotes that one of the two plants chosen for the first set of pollinations was an S allele homozygote itself. Family 10, which will now be considered, can be used to illustrate a possible procedure when neither of the two plants chosen for the first set of pollinations is a homozygote (see also penultimate paragraph of part II section 3).

The results for family 10 from plant A 162 selfed by bud-pollination are given in Table 8. All plants are self-incompatible and all but three gave a high set of seeds per fruit when pollinated by the unrelated plant 31/35. In the search for S allele homozygotes plant 4 was used as female parent and plant 7 as male parent in pollinations with the other inbreds. No compatible pollinations were found. This suggested that the two alleles in the parent were both active in both pollen and style, plants 4 and 7 both probably being heterozygotes.

Fortunately a pollination had been made in the previous year between plant 4 of family 10 as female and plant 300/10 (see family 2 of Thompson, 1957) and found to be incompatible. Because plant 300/10 was known to be heterozygous for an allele which was dominant to the other in both pollen and style, it followed that the dominant allele in 300/10 was one of the alleles present in family 10, and that 300/10 could be used as a parent to recognise homozygotes for the other allele in this family. In other cases it would, of course, be necessary to produce plants of similar allele relationship type to plant 300/10 from crosses with a homozygote for an S allele low in the dominance series. Using 300/10 as a male parent, four compatible pollinations were obtained, and these suggest that the group N plants of Table 8 are homozygotes for the S allele not present in 300/10. An attempt to find the homozygotes for the other S allele by using group N plants as female parents was not, however, successful, and it would appear that all the plants in group M of Table 8 are heterozygotes. Since, however, group M plants are incompatible as female parents with 300/10 and as male parents with group N, this other allele must be active in both pollen and style. The allele present in group N plants is also active in both pollen and style, because group N plants give incompatible pollinations both as male and female parents with two of the heterozygotes, plants 4 and 7.

IV. Discussion

1. *Recognition of S allele homozygotes*

The methods necessary to recognise S allele homozygotes in inbred families from parents heterozygous for alleles with any of the four dominance relationships shown in Table 1 have been described in the previous part of this paper, two sections being given to cases of type 1 relationship, because progeny testing is necessary in such cases, and two sections to the type 4 relationship, because it may be advisable here also to raise progenies in order to obtain the compatible pollinations necessary to start the analysis of a family. It can be seen that the methods used are successful. They are, however,

somewhat laborious, and it is therefore worth considering an alternative method for producing S allele homozygotes. This can be done by doubling the chromosome number of haploids which can be obtained fairly easily in marrow-stem kale (Thompson, 1956 and unpublished). Further experiments are, however, needed with the doubled haploids before one can judge the relative merits of the two methods for obtaining S allele homozygotes.

2. *Self-compatibility*

It appears from the results for family 3 of Thompson (1957)—see part III section 2 of this paper—and from the results for family 6, given in part III section 1, that self-compatible plants are found in the selfed progeny of self-incompatible plants, and that they may be either homozygotes for an S allele low in the dominance series or homozygotes for a recessive allele which is not a member of the S allele system. These two types were also found by Bateman (1954) in *Iberis amara*.

3. *Pseudo-compatibility*

Pseudo-compatibility, i.e. the production of a relatively high set of seeds per fruit from either self- or cross- pollinations which at other times are incompatible, was frequent in some of the families investigated, see particularly the results from using plant 4 of family 6 as a female parent in 1956 as compared with 1957.

This pseudo-compatibility is not just a feature of marrow-stem kale but is found in other varieties of *Brassica oleracea*, in other species of *Brassica* and in other cruciferous genera. Thus pseudo-compatibility was found by Sampson (1957) in the variety "Calabrese Green Sprouting" broccoli of *B. oleracea*, and Bateman (1955, page 59) states that two forms of *B. campestris* and also the radish (*Raphanus sativus*) were unsuitable for critical work on incompatibility systems because of the large amount of pseudo-compatibility which occurred. Stout (1922) reported that plants of *B. chinensis* which were at first 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. Further investigations on pseudo-compatibility are needed, and in any such investigations it would be advisable to use cuttings so that results can be obtained from more than one plant of each genotype investigated.

4. *Female sterility*

Female sterility of two different types was found in families 7 and 9. In family 7 the sterility is not noticed if green fruits with immature seeds are examined—it thus has no effect on the counts of seeds per fruit used in deciding whether crosses are compatible or incompatible. In mature fruits, however, a high percentage of shrivelled seeds is found. The sterility is probably due to a recessive gene, or genes, in the same linkage group as the S alleles. In family 9, on the other hand, there is no apparent linkage of the factor or factors producing female sterility with the S alleles, and the sterility is due to ovule abortion at a very much earlier stage than occurs in family 7 so that the sterility does interfere with the counts of seeds per fruit at the green fruit stage.

V. Summary

1. Methods for recognising S allele homozygotes in inbred families from parents heterozygous for alleles which may have four possible dominance relations are discussed.

2. Examples are given of the recognition in inbred families of homozygotes for two S alleles present in the parents when one allele is dominant in both pollen and style, when one allele is dominant in the pollen only, when one allele is dominant in the style only, and finally when both alleles are active in both pollen and style.

3. Self-compatible plants in the inbred offspring of self-incompatible plants may be either homozygous for a self-fertility allele in the S series or for a recessive allele independent of the S allele series. Female sterility may also be due to genes linked with the S alleles or independent of them.

4. Pseudo-compatibility occurred in many families, but it was possible to ignore it in the analysis of the pollination results for the recognition of S allele homozygotes.

Acknowledgements

The skilful assistance given by Mr. J. P. Taylor during the whole of the work is gratefully acknowledged. The authors also wish to thank Messrs. D. R. Glendenning, W. M. Steele and T. Walker, who, while students at the Plant Breeding Institute, worked out the S allele relationships for two of the families discussed in this paper.

References

Bateman, A. J. (1954). Self-incompatibility systems in Angiosperms. II. *Iberis amara*. *Heredity*, **8**, 305–22.

Bateman, A. J. (1955). Self-incompatibility systems in Angiosperms. III. *Cruciferae*. *Heredity*, **9**, 53-68.

Sampson, D. R. (1957). The genetics of self-and cross-incompatibility in *Brassica oleracea*. *Genetics*, **42**, 253–63.

Stout, A. B. (1922). Cyclic manifestation of sterility in *Brassica pekinensis* and *B. chinensis*. *Bot. Gaz.* **73**, 110–32.

Thompson, K. F. (1956). Production of haploid plants of marrow-stem kale. *Nature*, Lond. **178**, 748.

Thompson, K. F. (1957). Self-incompatibility in marrow-stem kale, *Brassica oleracea* var. *acephala*. I. Demonstration of a sporophytic system. *J. Genet.*, **55**, 45–60.