Theor. Appl. Genet. 54, 249-265 (1979)

THEORETICAL AND APPLIED GENETICS

Procedures for Identifying *S*-Allele Genotypes of *Brassica**

D.H. Wallace

Departments of Plant Breeding and Biometry, and of Vegetable Crops, Cornell University, Ithaca, N.Y. (USA)

Summary. Procedures are described for efficient selection of: (1) homozygous and heterozygous *S*-allele genotypes; (2) homozygous inbreds with the strong self- and sib-incompatibility required for effective seed production of single-cross F_1 hybrids; (3) heterozygous genotypes with the high self- and sib-incompatibility required for effective seed production of 3- and 4-way hybrids.

From reciprocal crosses between two first generation inbred (I_1) plants there are three potential results: both crosses are incompatible; one is incompatible and the other compatible; and both are compatible. Incompatibility of both crosses is useful information only when combined with data from other reciprocal crosses. Each compatible cross, depending on whether its reciprocal is incompatible or compatible, dictates alternative reasoning and additional reciprocal crosses for efficiently and simultaneously identifying: (A) the *S*-allele genotype of all individual I_1 plants, and (B) the expressions of dominance or codominance in pollen and stigma (sexual organs) of an *S*-allele heterozygous genotype. Reciprocal crosses provide the only efficient means of identifying *S*-allele genotypes and also the sexual-organ × *S*-allele-interaction types.

Fluorescent microscope assay of pollen tube penetration into the style facilitates quantitation within 24-48 hours of incompatibility and compatibility of the reciprocal crosses. A procedure for quantitating the reciprocal difference is described that maximizes informational content of the data about interactions between *S* alleles in pollen and stigma of the *S*-allele-heterozygous genotype.

Use of the non-inbred I_0 generation parent as a 'known' heterozygous *S*-allele genotype in crosses with its first generation selfed (I_1) progeny usually reduces at least 7 fold the effort required for achieving objectives 1, 2, and 3, compared to the method of making reciprocal crosses only among I_1 plants.

Identifying the heterozygous and both homozygous *S*-allele genotypes during the I_1 generation facilitates, during subsequent inbred generations, strong selection for or against modifier genes that influence the intensity of self- and sib-incompatibility. Selection for strong self and sib incompatibility can be effective for both homozygous inbreds and also for the *S*-allele heterozygote, thus facilitating production of single-cross F_1 hybrids and also of 3- and 4-way hybrids.

Key words: *Brassica* – *S* alleles – Incompatibility – Hybrid – Crucifer – Dominance

Introduction

Pearson (1932) first suggested that self incompatibility of *Brassica* be used to facilitate the cross fertilization required for hybrid seed production. Commercial hybrid production was achieved in Japan in 1950, in the United States in 1954, and in Europe in 1966 (Haruta 1962; Wallace and Nasrallah 1968; Johnson 1972). Since about 1960 Japanese companies have produced 3- and 4-way hybrids by crossing inbreds, and the resultant single-cross *S*-allele heterozygotes for commercial seed production. The first U.S. developed 3-way cross was released in 1971. Most U.S. seedsmen use a single self-incompatible inbred that is pollinated (topcrossed) with an open-pollinated cultivar. Seed production is only on the inbred, so neither plants nor land is used efficiently.

The knowledge and procedures essential to hybrid seed production have been discussed fragmentarily in many research reports. The purpose of this paper is to consolidate such information and to describe efficient procedures for identifying the *S*-allele homozygous inbreds and *S*-allele heterozygotes which have sufficient self- and sib-incompatibility to enforce the cross fertilization required for

* Department of Plant Breeding and Biometry paper No. 690

0040-5752/79/0054/0249/$3.40

commercial production of hybrid seed of the *Brassica* crops. Some new terms for describing facets of sporophytic incompatibility are introduced.

Background Information for Methods and Procedures

General Knowledge of Incompatibility

Sporophytic and gametophytic systems of incompatibility are reviewed by de Nettancourt (1977, 1972), Townsend (1971) and Arasu (1968). Physiological and biochemical aspects are reviewed by Heslop-Harrison (1974, 1975, 1975a) and Ferrari and Wallace (1977). General knowledge of these systems will assist in application of the procedures outlined herein.

First Inbred Generation Siblings

First inbred generation (I_1) siblings are by far the easiest population in which to identify homozygous and heterozygous *S*-allele genotypes. Occasionally all I_1 plants will be homozygous for the same *S*-allele [i.e. the non-inbred (I_0 generation) parent was an *S*-allele homozygote]. However, self incompatibility enforces cross fertilization, so selected I_0 plants usually will be *S*-allele heterozygotes. The I_1 generation will then segregate for three *S*-allele genotypes, two homozygous and one heterozygous. Presence of but one or two *S*-alleles in a population is guaranteed by bud selfing a selected I_0 plant to bypass the self incompatibility. Presence of more than two *S* alleles in a plant population makes genotype identification very difficult.

When the I_0 plant carries two *S* alleles, a minimum of 13 I_1 plants is required to provide 95% probability that all three possible genotypes are present: S_1S_1, S_1S_2 and S_2S_2. A 99% probability requires 19 I_1 plants.

The I_0 Parent as a 'Known' S-Allele Genotype

Since an I_0 plant is usually an *S*-allele heterozygote, it is, for its I_1 progeny only, a 'known' heterozygous *S*-allele genotype. Reciprocal crosses between this 'known' heterozygote and its I_1 progeny usually facilitate the easiest possible identification of the *S*-allele genotypes. Cuttings of the original I_0 should, therefore, be grown vegetatively through the time that the I_1 progeny is being grown, and vernalized and brought to flower at the same time as the I_1 progeny. For some *Brassica* species environmental manipulation will extend the flowering duration of the I_0 until the I_1 is flowering (Richards and Thurling 1973).

Recessive and Dominant S Alleles

Alleles S_2, S_5 and S_{15} (Thompson 1968; Wallace 1979) and some other less well recognized alleles (Johnson and Blyton-Conway 1976; Ockendon 1975; Lawson and Williams 1976) are generally recessive to nearly all other *S* alleles, especially in the pollen. Thus, the *S*-allele is inactive or weakly active, i.e. largely unexpressed, in heterozygous combination with most *S* alleles. Two highly recessive *S* alleles in heterozygous combination are often both largely inactive, this being the *S*-allele interaction called mutual weakening (Wallace 1979). Also, homozygotes of these highly recessive alleles usually have a low intensity of self incompatibility (Johnson and Blyton-Conway 1976). Since almost all plants of open pollinated cultivars are heterozygotes, these highly recessive *S* alleles are seldom fully expressed. Therefore, these recessive *S* alleles have high transmission to progeny and occur in as many as 65% of the plants of some open-pollinated *Brassica* populations (Johnson and Blyton-Conway 1976). In contrast, highly dominant *S* alleles almost always have near full activity (Visser 1977), resulting in lowered transmission. Consequently, highly dominant alleles occur in a minor proportion of plants of most open-pollinated populations.

Inbreds with low self- and sib-incompatibility are poor parents for hybrid seed production, especially since their incompatibility is further weakened by environmental influence (Johnson and Blyton-Conway 1976; Johnson 1971; Richards and Thurling 1973a)

Flow Charts of Procedures

As an aid to understanding the organization and sequence of all steps required for isolating and determining the merit of an inbred as a female parent for hybrid seed production, a flow chart of procedures is presented (Chart 1). Additional flow charts are given to aid comprehension of the alternative reasoning and steps used to identify the *S*-allele genotypes from (Chart 2) reciprocal crosses among the I_1 plants, or (Chart 3) using the I_0 parent as a 'known' genotype that is reciprocally crossed with its I_1 plants. Charts 2 and 3 are both amplifications of the interpretative procedures (Chart 1: Step 8) by which the *S*-allele genotypes are determined.

Methods

Evaluating the Parents

The first step is selection for horticultural, disease resistance and quality characteristics, and for diversity of origin (Chart 1: Step 1). The next three steps all involve pollination procedures as described next.

Pollination Procedures

Self and reciprocal cross pollinations, except bud pollinations, are with open flowers. Emasculation is unnecessary for preventing unwanted self pollination but anther removal during pollination is recommended if it facilitates transfer of pollen to the stigma. Pollen is transferred by rubbing dehisced anthers against stigmas.

Pollinated flowers should be marked below the lowest flower with a label held with a string looped around the raceme. Flowers or buds immediately above the highest pollinated flower on the raceme can be removed to separate pollinated from non pollinated flowers. A pressure-applied label which is readily removable can be lightly pressed onto the back of the string-held label. Information recorded on both labels includes: (1) a consecutive number for the pollination; (2) specific identification of the female and male parents; (3) number of flowers pollinated if other than standard and (4) the date.

Measuring Self-Incompatibility of the Parent (Chart 1: Step 2)

Flowers of I_0 plants should be selfed before or at the time that buds are selfed to obtain I_1 seed. The I_0 might also be reciprocally crossed with an unrelated plant to check the I_0 male and female fertilities. If either the pollen tube penetration or seed set data

Flow Chart 1. Procedures for: Identifying *S*-allele genotypes, determining self- and sib-incompatibilities, and determining potential for use in hybrid seed production

Step 1: Select an I_0 plant for horticultural, disease resistance and other characteristics, and for diversity of origin

↓

Step 2: Cross reciprocally with testers of the highly recessive *S*-alleles

↓

Step 3: Assay for self incompatibility

↓

Step 4: Self by bud pollination to get I_1 generation seed

↓

Step 5: Grow, vernalize and bring the I_1 progeny to flowering

↓

Step 6: Make reciprocal crosses among I_1 sibling plants, or for improved efficiency, Make reciprocal crosses between the I_0 parent and the I_1 siblings

↓

Step 7: Score and record pollen tube penetration using fluorescent microscopy

↓

Step 8: Quantitate the reciprocal difference between the reciprocal crosses. Interpret the reciprocal difference and the pollen tube penetration scores according to the stepwise procedures of Flow Charts 2 or 3. Use Chart 2 if the data are from intercrosses among I_1 sibling plants. Use Chart 3 if the data are from reciprocal crosses between the I_0 parent and the I_1 sibling plants

↓

Step 9: Within each *S*-allele genotype, including heterozygotes if planning for 3- or 4-way crosses, select for strong self and sib incompatibility to stabilize any effects of modifying genes

↓

Step 10: Test for stability of self and sib incompatibility over stage of plant development, across variations of temperature and humidity, and through subsequent generations of inbreeding

↓

Step 11: Compare seed set data with the pollen tube penetration data

↓

Step 12: Consider 2-, 3- and 4-way compatibilities and incompatibilities among the alleles carried by the different inbreds and their heterozygotes, including potential for the *S*-allele heterozygote to fertilize or to be fertilized by possible selfs from a homozygous recessive genotype used as the female parent. If the commercial crop is the seed, as for oil seed, consider the potential for sufficient cross polination to effect adequate seed set

↓

Step 13: Test for nicking of pollination times and develop procedures for improving nicking

↓

Step 14: Produce single-cross hybrid seed

↓

Step 15: Test single-cross *S*-allele heterozygote for self and sib incompatibility

↓

Step 16: Produce 3- and 4-way hybrid seed

indicate weak self incompatibility (or fertility), the I_0 plant may be discarded. If the I_0 has superior horticultural merit only, its I_1 may be tested, but identifying *S*-allele genotypes and finding strong self- and sib-incompatibility will usually be difficult.

Testing for Highly Recessive and Dominant Alleles (Chart 1: Step 3)

It is desirable (Background Information: recessive and dominant alleles) that the highly recessive *S* alleles be identified in either the I_0 plant or I_1 progeny. This requires a homozygous tester for each such allele. If moderately active in either pollen or stigma, these alleles can be identified using reciprocal crosses between an I_0 plant and a homozygous tester. Any incompatibility for either reciprocal cross identifies the recessive allele. Identification may be easier with I_1 plants already shown to be the recessive genotype for their I_1 progeny. Being recessive does not indicate that the allele is a highly recessive *S*-allele.

Testers for S_2, S_5, S_{15} and other highly recessive, or dominant, alleles can be obtained from anyone who has matched *S* alleles against the respective alleles of the international *S*-allele collection (Ockendon 1975), or directly from that collection at the National Vegetable Research Station, Wellesbourne, Warwick, England.

Although advocated by others (Ockendon 1977; 1977a; Johnson and Blyton-Conway 1976), this author does not consider it necessary that the international-*S*-allele identity of dominant *S* alleles be specifically determined. The generalization that inbreds homozygous for dominant alleles have strong self- and sib-incompatibility does not always hold (Johnson and Blyton-Conway 1976). Also, recessive alleles can be highly incompatible (Smith et al. 1977). Therefore, selection of inbreds should be based primarily on strength of self and sib incompatibilities, and secondarily on dominance vs. recessiveness.

Obtaining Flowering I_1 *Plants* (Chart 1: Steps 4 and 5)

Bud pollinations (Chart 1: Step 4) should be done simultaneously with assaying the self-incompatibility of the I_0 parent (Step 2) and

testing for highly recessive-*S*-alleles (Step 3). The I_1 plants should then be grown, vernalized as required, and brought to flower (Step 5).

Pollen Tube Penetration Assay (Chart 1: Steps 6 and 7)

About 24 hours after pollination, two or more pollinated flowers should be removed. They may be wrapped together with the removable pressure-applied label. If seed set data will be collected, every other flower along the raceme can be taken. In the laboratory the pistils should be removed and placed in a test-tube onto which the removable label is transferred. Sufficient 1 N NaOH should then be added to cover the pistils which are then heated at 60°C for 1 hour, after which the NaOH is siphoned off. Aniline blue stain (2 gm/liter H_2O + 20 gm K_3PO_4) is then added and the pistils left overnight. In pairs, the pistils are then transferred to a microscope slide, with the removable label, and squashed in glycerol under a cover slide. Using filters that pass only wavelengths of 350 to 400 mμ, placed between a fluorescent light source and a light microscope, each pistil should be visually scored for pollen tube penetration into the upper style as described by Kho and Baer (1968). The removable pressure-applied label should accompany the pistils through all steps. It provides all identification required for recording the pollen tube penetration data. The pistils can be put into cytological fixatives, before heating in the NaOH (Kho and Baer 1968; Johnson 1971); this may reduce background fluorescence by tissues other than the pollen tubes.

The following scores are recommended: 0 = 0 tubes, 1 = 1-2, 2 = 3-5, 3 = 6-9, 4 = 10-14, 5 = 15-20, 6 = 12-50, 7 = 51-100, and 8 = 100 + tubes. The low and increasingly larger number of penetrated pollen tubes within each successively higher score maximizes ability to differentiate among pollinations with the strongest incompatibility. Recording scores rather than absolute numbers of tubes is preferred, because approximation of tube number requires less time. More importantly, scores better facilitate quantification and interpretation of the difference in incompatibility between reciprocal crosses.

Procedures for Identifying Genotypes

Reciprocal Crosses, and Recording and Interpreting Data

Beginning the Reciprocal Crosses (Chart 1: Step 6)

The most efficient first step is to begin to intercross the I_o reciprocally with each I_1 plant as it comes into flower. If the I_o is not available or is not flowering, the second most effective beginning procedure is to begin reciprocal crosses between the first flowering I_1 plants. As each I_1 plant comes into flower it should be reciprocally crossed to an I_1 that has already been reciprocally crossed to one I_1 sibling, and also reciprocally crossed to another I_1 that has not been crossed to an I_1 sib. This forms a chain of reciprocal crosses.

Recording Pollen Tube Penetration Data (Chart 1: Step 7)

As pollen tube penetrations into the style are scored, the pressure-applied removable label should be lifted from the microscope slide and transferred to a first notebook for cross pollinations at the position of its consecutive pollination number. Use a separate consecutive listing for selfs. The score for each pistil is then recorded next to the removable label. The consecutive pollination-specific number and the pollen-tube penetration scores are next recorded in a 'first-interpretative diallel table' (Table 1). Transferral of the pollination number with the data facilitates future correcting of inadvertent placement of data in an improper female × male cell, which sometimes occurs and is revealed while quantifying the reciprocal difference. Maximum benefit from pollen tube penetration data requires that it be transferred and interpreted on the day the scores are made, or on the following day.

In a first-interpretative diallel table (Table 1 is an example), designations of individual I_1 plants at the side and top of the table are in numerical order, which is random with respect to the *S*-allele genotypes that will be identified (Table 2). Initial interpretations (Step 8 of Charts 1-3) should be made directly after the data are recorded in the first-interpretative diallel table. Final summarization after interpretation, especially for formal presentation, usually requires transferral of the data from the first- to a second-interpretative diallel table (Table 2 is an example). Here, the data of each individual I_1 (or I_o) plant are positioned adjacent to the data of other I_1 (or I_o) plants that behave alike, i.e. that have the same *S*-allele genotype. For example, in Table 2, all plants of genotype S_aS_a that were used as a male parent are adjacent to each other at the left, plants of heterozygous genotypes S_aS_b used as male are in the center and all plants of genotype S_bS_b are at the right. Usages of the plants as female are positioned, in this same order, from top to bottom. Minimal effort is required for transferral of data from a first- to a second-interpretative table if it is done as soon as all genotypes can be derived from the pollen tube penetration data. All seed set data and subsequent pollen tube data can then be entered directly into the second-interpretative table.

Calculating and Interpreting Reciprocal Differences (Chart 1: Step 8)

The first step toward interpretation of pollen tube penetration data is quantification of the reciprocal difference of each pair of reciprocal crosses. Numerical magnitude of the reciprocal difference (RD) is calculated by subtracting the smallest from the largest summation of the two pollen tube penetration scores of the reciprocal crosses, as follows. RD = (Scl + Sc2) − (Sc3 + Sc4), where Sc1 and Sc2 are pollen tube penetration scores for two pistils of a cross, and Sc3 and Sc4 are scores for the reciprocal cross. The arithmetic difference between these sums is the numerical magnitude of RD. This magnitude applies to both

Table 1. A first interpretative diallel table, illustrating the recording[a] of the consecutive pollination and pollen tube penetration data, followed by calculation and recording of the reciprocal difference

Stigma (♀) parent	Line plant	Pollen (♂) parent: I_o or I_o Plant number											
		I_o	I_1-1	I_1-2	I_1-3	I_1-4	I_1-5	I_1-6	I_1-7	I_1-8	I_1-9	I_1-10	I_1-11
	1					1			3	2			
	2 I_o					0			+16	0			
	3					0,0			8,8	0,0			
	4												
	1								8				
	2 I_1-1								0				
	3								0,0				
	4												
	1								24				
	2 I_1-2								+13				
	3								7,7				
	4												
	1								10				
	2 I_1-3								0				
	3								8,8				
	4												
	1	4							26				
	2 I_1-4	0							−1				
	3	0,0							8,7				
	4												
	1								12				
	2 I_1-5								+12				
	3								6,6				
	4												
	1								14				
	2 I_1-6								+16				
	3								8,8				
	4												
	1	6	7	17	19	25	15	13		11	9	23	21
	2 I_1-7	−16	0	−13	0	+1	−12	−16	Self	−15	−7	−12	−16
	3	0,0	0,0	0,1	8,8	8,8	0,0	0,0	0,0 0,0	1,0	1,2	2,2	0,0
	4												
	1	5							20				
	2 I_1-8	0							+15				
	3	0,0							8,8				
	4												
	1								18				
	2 I_1-9								+7				
	3								2,8				
	4												
	1								16				
	2 I_1-10								+12				
	3								8,8				
	4												
	1								22				
	2 I_1-11								+17				
	3								8,8				
	4												

[a] Within each cell of the diallel table, the pollination number is recorderd on line 1, the reciprocal difference on line 2, the pollen tube penetration scores on line 3, and total seeds for 3 pods on line 4

[b] These are actual data collected for an I_1 progeny in 1977. Identification of the I_1 genotypes was begun by making reciprocal crosses between the I_o parent and individual I_1 plants. With the good luck of finding a reciprocal difference with the third reciprocal cross, which has 7/16 probability, a homozygous recessive I_1 plant of genotype S_aS_a was successfully identified, using only the reciprocal crosses made on the first day of reciprocal pollinations. Thereafter, this homozygous recessive (S_aS_a) plant (I_1-7) was reciprocally intercrossed once with each of the other I_1 plants. The genotype of each of the 11 I_1 plants was identified with only 13 pairs of reciprocal crosses, i.e. with a total of 26 crosses

Table 2. A second interpretative diallel table, illustrating the reorganized and fully interpreted data[a] as compared to the same data in a first interpretative table[b]; the data herein are organized so that data of all plants that behave alike, i.e. that have the same *S*-allele genotype, are positioned adjacently

Stigma (♀) parent			Pollen (♂) parent: Assigned *S*-allele genotype											
			S_aS_a		S_aS_b								S_bS_b	
	Line plant no.	no.	I_1-1	I_1-7	I_o	I_1-2	I_1-5	I_1-6	I_1-8	II_1-9	I_1-10	I_1-11	I_1-3	I_1-4
S_aS_a		1		8										
	I_1-1	2	Self	0										+1
		3	0.0 0.0	0,0										8,8
		4	0 0	1										47
		1	7		6	17	15	13	11	9	23	21	19	25
	I_1-7	2	0	Self	−16	−13	−12	−16	−15	−7	−12	−16	0	+1
		3	0,0	0,0 0,0	0,0	0.1	0.0	0.0	1,0	1,2	2,2	0,0	8,8	8,8
		4	0	0 0	0	0	0	0	0	0	0	0	0	51
S_aS_b		1		3					2					1
	I_o	2	+16	+16	Self	0	0	0	0	0	0	0	−2	0
		3	8,8	8,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
		4	1	0	0	0	0	0	0	0	0	0	0	0
		1		24										
	I_1-2	2		+13		Self								+2
		3		7,7		1,2 0,0								
		4		0		0 1								0
		1		12										
	I_1-5	2		+12			Self							−1
		3		6,6			1,0 0,0							0,0
		4		0			0 0							0
		1		14										
	I_1-6	2		+16				Self						
		3		8,8				0,0 0,0						0,0
		4		1				0 11						0
		1		20	5									
	I_1-8	2		+15	0				Self					0
		3		8,8	0,0				0,0 0,2					0,0
		4		58	1				5 4					0
		1		18										
	I_1-9	2		+7						Self				0
		3		2,8						0,0 0,0				0,0
		4		0						0 0				0
		1		16										
	I_1-10	2		+12							Self			+3
		3		8,8							0,0 1,0			0,0
		4		23							1 13			0
		1		22										
	I_1-11	2		+16								Self		0
		3		8,8								0,0 5,3		0,0
		4		10								2 2		0
S_bS_b		1		10										
	I_1-3	2		0									Self	−2
		3		8,8									0,3 0,0	0,0
		4		20									0 0	0
		1		26	4									
	I_1-4	2	−1	−1	0	+2	+1		0	0	0	0	0	Self
		3	7,8	8,7	0,0	0,2	1,0		0,0	0,0	0,0	0,0	0,0	0,0 0,0
		4	34	0	0	0	0	0	3	0	0	0	0	0 0

[a] Within each cell of the diallel table, the pollination number is recorded on line 1, the reciprocal difference on line 2, the pollen tube penetration scores on line 3, and total seeds for 3 pods on line 4

[b] Pollination numbers are entered in the table for only those crosses that were required to identify the genotype of all 11 I_1 plants, as given in Table 1. The remaining crosses with the I_o parent and the I_1-4 plant, respectively, serve to illustrate the reduced information that is derivable from reciprocal crosses of a heterozygote S_aS_b and of a dominant homozygote S_bS_b with each I_1 plant, as compared with the genotype differentiation that is achieved for every reciprocal cross with a known homozygous recessive S_aS_a (I_1-7). The self pollinations test the intensity of self-incompatibility of each individual plant. The female × male cells of first- and second-interpretative tables should be large enough to permit recording the data of at least five pollinations

Table 3. Sexual-organ × *S*-allele-interaction Types I, II, III, and IV as derived from the two *S*-allele interactions (dominance[a] and codominance[a]) in factorial combination with the two sexual tissues (pollen and stigma)

	Pollen	Stigma		Pollen	Stigma
I	$S_a < S_b$	$S_a < S_b$	III	$S_a = S_b$	$S_a < S_b$
II	$S_a < S_b$	$S_a = S_b$	IV	$S_a = S_b$	$S_a = S_b$

[a] The allele to the open side of < is dominant while that to the closed side is recessive, and = indicates codominance, i.e. strong and near equal activity of both alleles

crosses of the reciprocal pair, but a negative (–) sign is arbitrarily prefixed for the cross with the smaller sum of pollen tube penetration scores (Sc1 + Sc2, or Sc3 + Sc4), and a plus (+) is prefixed for the cross with the larger sum. Thus, the – and + prefixes identify the most and least incompatible crosses respectively of the reciprocal pair of crosses and the magnitude of RD quantifies this difference. With the recommended pollen tube penetration scores of 0 to 8, and determining RD from scores for two pistils, the smallest and largest possible magnitudes of RD are 0 and 16, with all intermediate numbers of 2-15 being possible. A magnitude of 0 indicates no difference in incompatibility (or of the inverse, i.e. compatibility) of the reciprocal crosses, 1-4 indicates minimal difference, and magnitudes approaching 16 indicate a large difference. Reciprocal differences with intermediate magnitudes, near 8, often have a random distribution of + and – prefixes and are also numerically inconsistent, indicating weakened expression of incompatibility by one or both parents, in either pollen or stigma, or both. It might also indicate a degree of female or male sterility, or possibly strong sensitivity to environmental influence.

The RD permits a person looking at the data of one cross to approximate data of the reciprocal. The alternative is to view simultaneously the data of both crosses (Tables 1 and 2), which is cumbersome and must be repeated each time data of the two crosses are compared, or are compared with data of other reciprocal crosses.

Classification of the *S*-allele Interactions (Chart 1: Step 8)

The *S*-allele interactions often approach one of two extremes (Table 3, and Wallace 1979): (1) codominance, i.e. simultaneous full activity of both *S*-alleles of the heterozygote, (2) dominance, i.e. full activity of one *S*-allele of the heterozygote with complete inactivity of the other. The term dominance and its inverse, recessiveness, are used herein even when the dominance is only partial, that is the more active allele has only 60 to near 100% activity, and/or when the recessive allele retains some activity, perhaps 1 to 30% (Wallace 1979). The *S*-allele interaction is called codominance when both alleles have near equal activities of about 75 to near 100% (Wallace 1979). Dominance is symbolized by $S_a < S_b$, and codominance by $S_a = S_b$ (Tables 3 and 4).

Classification of the Sexual-Organ × *S*-Allele-Interaction Types (Chart 1: Step 8)

Four classes of incompatibility in *Brassica* were described and designated as mode of inheritance for *S*-allele interaction Types I, II, III and IV by Thompson and Howard (1959) and Haruta (1962). They have been further discussed by Wallace and Nasrallah (1968), MacKay (1977), De Nettancourt (1977) and Frankel and Galün (1977). However, the interactions that occur between *S*-alleles are codominance and dominance (Table 3), and intermediate activities of one or both alleles as briefly described in the preceding paragraph and documented by Wallace (1979). Types I, II, III and IV are derived from the two *S*-allele interactions (codominance and dominance) in factorial combination with the two sexual organs (pollen[1] and stigma) (Table 3). To recognize these factorial components, in this paper and Wallace (1979) the types are redesignated as sexual-organ × *S*-allele-interaction Types I, II, III and IV.

General Instructions for Determining the *S*-Allele Interactions and Sexual-Organ × *S*-Allele-Interaction Types

Charts 2 and 3 present alternative procedures for completing Step 8 of Chart 1. Determining the *S*-allele interaction(s) and the sexual-organ × *S*-allele-interaction type of the *S*-allele heterozygous genotype of an I_1 progeny

1 A pollen grain is a single cell. It is not multicellular and differentiated, the usual criteria for designation as an organ. A pollen grain is an unusual cell; its two or three nuclei and cytoplasm migrate into a differentiated pollen tube. Sexual-organ was incorporated into the larger term 'sexual-organ × *S*-allele-interaction' because sexual-organ presents a comprehensible concept to a first-time reader; male-female × *S*-allele interaction failed to do so, and pollen-stigma × *S*-allele-interaction is too specific to include pollen-style × *S*-allele-interaction types that may be recognized.

Table 4. Expected incompatible and compatible interpretations and reciprocal difference interpretations[a] from reciprocal intercrosses among plants of the two homozygous and one heterozygous *S*-allele genotypes for sexual-organ × *S*-allele interaction Types I, II, III and IV

Sexual-organ × *S*-allele-interaction	*S*-allele interaction		Female genotype and phenotype[b] (♀)	Male genotype and phenotype[b] (♂)
	Stigma (♀)	Pollen (♂)		
I	dominance	dominance	$S_a\ S_a$ $S_a < S_b$ $S_b\ S_b$	$S_a\ S_a$ $S_a < S_b$ $S_b\ S_b$ Inc Com Com Com Inc Inc Com Inc Inc
II	codominance	dominance	$S_a\ S_a$ $S_a = S_b$ $S_b\ S_b$	$S_a\ S_a$ $S_a < S_b$ $S_b\ S_b$ Inc Com Com Inc Inc Inc Com Inc Inc
III	dominance	codominance	$S_a\ S_a$ $S_a < S_b$ $S_b\ S_b$	$S_a\ S_a$ $S_a = S_b$ $S_b\ S_b$ Inc Inc Com Com Inc Inc Com Inc Inc
IV	codominance	codominance	$S_a\ S_a$ $S_a = S_b$ $S_b\ S_b$	$S_a\ S_a$ $S_a = S_b$ $S_b\ S_b$ Inc Inc Com Inc Inc Inc Com Inc Inc

[a] Reciprocal crosses with a reciprocal difference are indicated by ◄- - -► and reciprocal crosses without a reciprocal difference by ◄——►

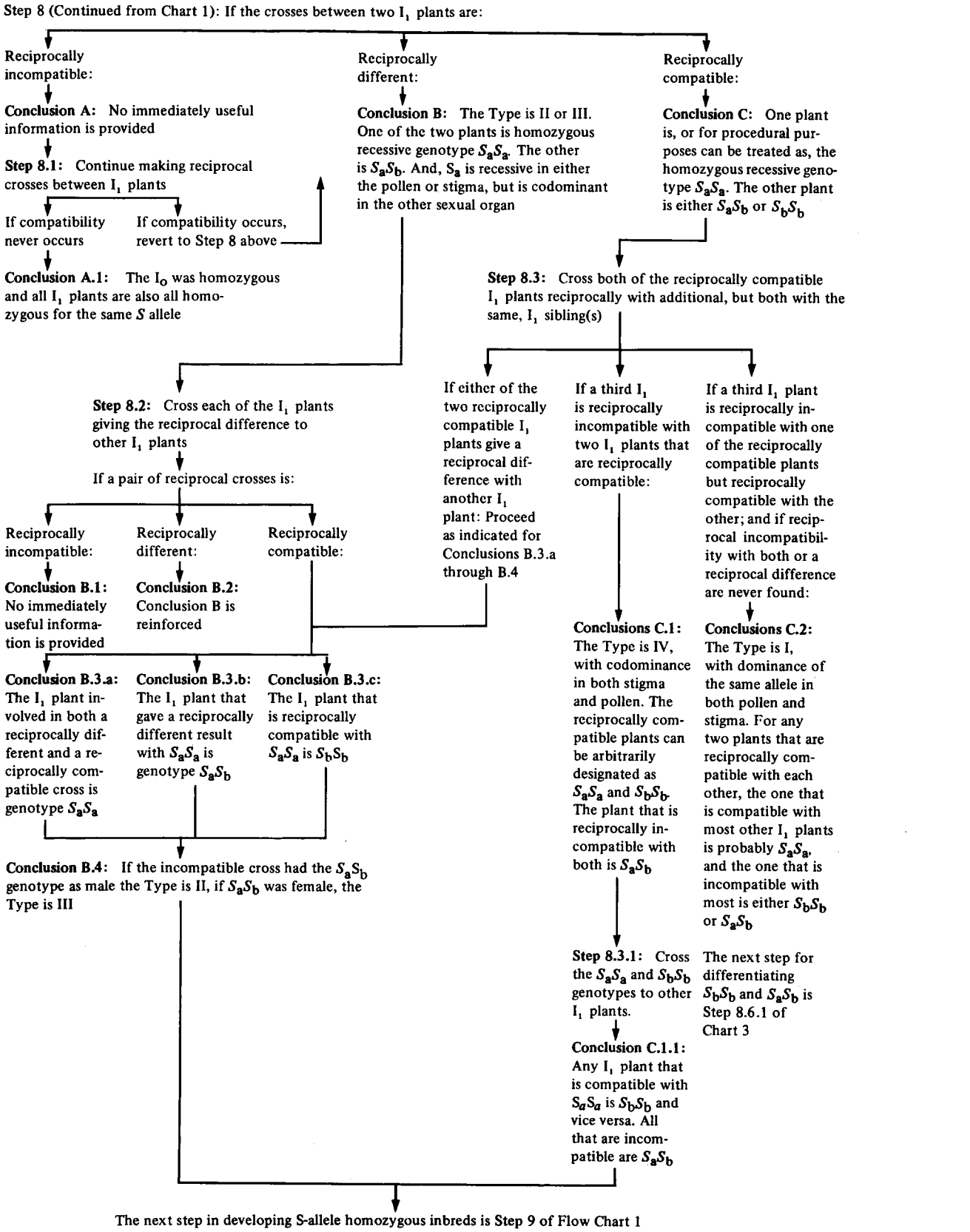
[b] The *S*-allele phenotype of heterozygotes is indicated by the symbols < and = where < specifies recessive vs. dominant and = indicates codominance. These phenotypes correspond with the described *S*-allele interactions in stigma and pollen. The phenotype for homozygotes always corresponds with the genotype

requires that the heterozygote be identified and reciprocally crossed with each of the two corresponding and identified *S*-allele homozygotes (Wallace 1979). Interpretation requires understanding of the sporophytic control over *S*-allele action, i.e. that the *S*-allele phenotype is the same for every pollen grain from an *S*-allele heterozygote, even though events after meiosis distributed one *S*-allele to half of the grains and the other *S*-allele to the other half[2]. This sameness of phenotype also applies for the *S*-allele activity (with dominance) or activities (with codominance) in the stigma (Wallace 1979).

The possibility of either codominant or dominant *S*-allele interaction occurring in the pollen and, independently of the same alternative codominant or dominant *S*-allele interactions occurring in the stigma, have been used to determine the incompatibility, compatibility and reciprocal-difference expectations for reciprocal crosses among the three I_1 genotypes. These expectations are given for each of the sexual-organ × *S*-allele-interaction Types I, II, III and IV (Table 4). The expectations, in turn, are used to interpret results from reciprocal crosses among the unknown genotypes of the I_1 sibling populations and/or to interpret results for reciprocal crosses between the I_0 parent and the I_1 plants. This facilitates essentially simultaneous determinations of: (A) the codominance or dominance that occurs in the pollen and in the stigma of the *S*-allele heterozygotes, (B) the sexual-organ × *S*-allele-interaction types, and (C) the *S*-allele genotypes of the I_1 plants.

2 This identical *S*-allele activity for every grain from an *S*-allele heterozygous plant, in spite of the *S*-allele genotype of the grain, is the basis for classifying the incompatibility of *Brassica* as sporophytically controlled. The identical activity for all grains is in sharp contrast to gametophytic control, where the *S*-allele activity of each pollen grain (male gamete) is fully dependent on the *S*-allele carried by that grain, i.e. upon its genotype (Wallace 1979).

Flow Chart 2. Procedures for identifying the *S*-allele genotypes and the sexual organ X *S*-allele-interaction types using reciprocal crosses among I_1 plants are:



[a] These procedures and conclusions are derivable from Table 4

Genotype and Type Identification Using Reciprocal Crosses Among I_1 Siblings

When two I_1 plants are reciprocally crossed, three combinations of incompatible and compatible results can occur: (A) both crosses may be incompatible (reciprocally incompatible), (B) one may be incompatible while the other is compatible (reciprocally different), or (C) both crosses may be compatible (reciprocally compatible). These results respectively give rise to conclusions A, B and C (Chart 2: Step 8). Each conclusion dictates a different series of reciprocal crosses and expected results for efficiently determining *S*-allele genotypes of the I_1 plants, and the sexual-organ × *S*-allele-interaction type of the heterozygote.

When Reciprocal Incompatibility Occurs Between Two I_1 Siblings (Chart 2: Conclusion A)

Reciprocal incompatibility provides no information that is immediately effective in identifying either the *S*-allele genotype or the sexual-organ × *S*-allele-interaction type. However, the incompatible results will assist with identifying and/or verifying the genotype of these plants and also the type, after the genotypes of several I_1 plants have been determined. The next step is reciprocal crosses between new pairings among I_1 plants (Chart 2: Step 8.1). If compatibility occurs between any two I_1 plants, revert to instructions for determining genotypes and type when compatibility occurs (Chart 2: Conclusion B or C). Until compatibility is found, as each I_1 plant comes into flower, it is most efficient to cross it: (A) with an I_1 previously crossed reciprocally with one I_1 sibling, and (B) with an I_1 not yet crossed with any sibling. The resultant two reciprocal-cross pairs for each I_1 plant test the *S*-allele activity in both its pollen and stigma, and form a chain in which these activities are tested against the *S*-allele-activities of all other I_1 plants. This reciprocal-cross chain is both minimal and sufficient for demonstrating that all the I_1 plants have the same *S*-allele activity. Incompatibility of the full chain demonstrates that each I_1 is homozygous for the same *S*-allele, and that the I_o parent was an *S*-allele homozygote (Chart 2: Conclusion A.1).

When a Reciprocal Difference Occurs (Chart 2: Conclusion B)

From the *S*-allele genotypes S_aS_a, S_aS_b, and S_bS_b within a segregating I_1 progeny, three reciprocal crosses can be made between two genotypes (Table 4). There are four sexual-organ × *S*-allele-interaction types, so there are twelve combinations of reciprocal-cross results (Table 4). A reciprocal difference occurs for only two of the twelve results, one for Type II and one for Type III (Table 4). It appears when: codominance occurs in the stigma (Type II, Table 4) or in the pollen (Type III, Table 4) of genotype S_aS_b, while dominance occurs in the other (pollen or stigma), – and when this heterozygote is reciprocally crossed with the homozygous recessive genotype S_aS_a (Table 4). These two pairs of reciprocal crosses (one each for Types II and III) are the only reciprocal crosses between two I_1 plants which directly indicate, by the reciprocal difference, the exact genotypes of the two I_1 plants and which simultaneously give an indication of the sexual-organ × *S*-allele-interaction type. The reciprocal difference does not indicate which I_1 plant is S_aS_a and which is S_aS_b, or whether the Type is II or III (Table 4). However, it facilitates tentative assignment to both I_1 plants of S_aS_a or S_aS_b, and also of sexual-organ × *S*-allele-interaction Type II or III. This is a more complete step toward final genotype assignment (Chart 2: Conclusion B) than data from any other self, cross or pair of reciprocal-cross pollinations between two I_1 plants can provide.

To achieve positive identification of the genotypes, both I_1 plants giving the reciprocal difference are reciprocally crossed to additional I_1 plant(s) (Chart 2: Step 8.2). To conform to an essential procedure when reciprocal compatibility occurs (Step 8.3), both plants are crossed to the same additional I_1 plant(s). If either of the two I_1 plants with the reciprocal difference is reciprocally compatible with a third I_1 plant (plant C), the genotypes of all three I_1 plants can be assigned. Plant A, with a reciprocal difference with I_1 plant B and with reciprocal compatibility with I_1 plant C is the homozygous recessive genotype S_aS_a (Conclusion B.3.a). Plant B, the second parent of the cross with the reciprocal difference is S_aS_b (Conclusion B.3.a). And, C which was reciprocally compatible with A, i.e. with genotype S_aS_a, is the homozygous dominant genotype S_bS_b (Conclusion B.3.c). The next procedure (Step 8.5) for identifying genotypes of the remaining I_1 plants is presented in detail under: When a Reciprocal Difference Occurs Between the I_o and I_1 Plant (Chart 3: Conclusion E)

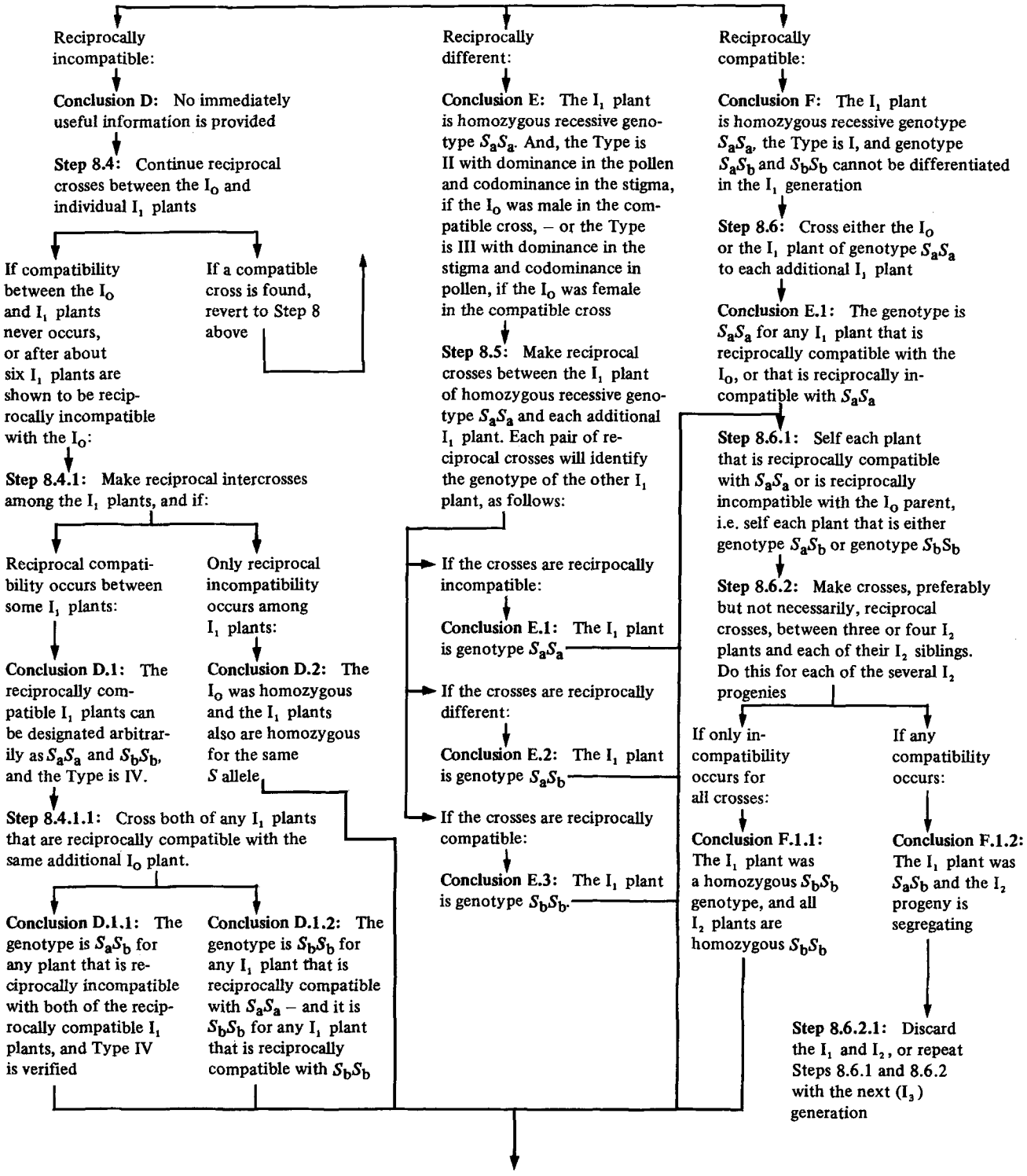
After the recessive and heterozygous genotypes have been specifically identified, the sexual-organ × *S*-allele-interaction type of the heterozygote can be determined. Its Type is II if S_aS_b was male in the compatible cross, and III if S_aS_b was female (Chart 2: Conclusion B.4).

When Reciprocal Compatibility Occurs Between I_1 Siblings (Chart 2: Conclusion C)

With reciprocal compatibility between two I_1 plants, one must be genotype S_aS_a which is one parent of all reciprocally compatible crosses (Table 4), and the other will be either S_aS_b or S_bS_b (Table 4, and Chart 2: Conclusion C). Reciprocal compatibility between I_1 plants occurs within all four types, so type is not indicated. Next, the two

Flow Chart 3. Procedures for identifying the *S*-allele genotypes and the sexual-organ × *S*-allele-interaction types using reciprocal crosses between the I_0 parent and individual I_1 plants.[a]

Step 8 (Continued from Chart 1): When the reciprocal crosses between the I_0 parent and an I_1 plant are:



The next step in developing *S*-allele homozygous inbreds is Step 9 of Flow Chart 1

[a] These procedures and conclusions are derivable from Table 4

reciprocally compatible I_1 plants are reciprocally crossed to the same additional I_1 plant(s) (Chart 2: Step 8.3). Crossing both to the *same* third (and if reciprocal compatibility again occurs, fourth, fifth, etc.) I_1 sibling is emphasized by the italics. Only this procedure, other than reciprocal crosses with the I_o parent, can indicate that the heterozygous genotype S_aS_b is of sexual-organ × *S*-allele-interaction Type IV. A further merit is that it always facilitates identification of all four types.

If either of two reciprocally compatible I_1 plants gives a reciprocal difference in crosses to a third I_1 plant C, plant A which is reciprocally different with C and reciprocally compatible with B is the homozygous recessive genotype S_aS_a (Chart 2: Step 8.3; Conclusion B.3.a); plant B is S_bS_b (Conclusion B.3.c); and plant C is S_aS_b (Conclusion B.3.b). The reciprocal difference indicates Type II or III. It is Type II if S_aS_b was male in the incompatible cross, and III if it was female (Conclusion B.4).

An S_aS_b plant of Type IV has codominance in both pollen and stigma (Table 4). With codominance in both sexual organs, S_aS_b is reciprocally incompatible with both homozygotes, and only the two homozygotes are reciprocally compatible (Table 4). Therefore, when two reciprocally compatible I_1 plants A and B are both reciprocally incompatible with a third I_1 plant C: the Type is IV; plant C is S_aS_b; and plants A and B are homozygous genotypes S_aS_a and S_bS_b, with S_a and S_b designations being arbitrary because there is no recessive (or dominant) *S*-allele (Chart 2: Conclusion C.1). If the reciprocal difference and pollen tube penetration data in the interpretative diallel table (Table 1) suggest that one allele may be less active in the heterozygote than the other, then the homozygote with this less active allele should be designated S_aS_a.

The Type is I (Chart 2: Conclusion C.3) if a third I_1 plant is reciprocally compatible with one of the reciprocally compatible plants but is reciprocally incompatible with the other – if a reciprocal difference does not occur for any of the possible reciprocal crosses between two I_1 plants. Accepting no reciprocal difference for the population requires that each of the two reciprocally compatible I_1 plants be crossed reciprocally with four or five additional I_1 plants. If more than one pair of I_1 plants are reciprocally compatible, both of each pair can be reciprocally crossed with two or three additional I_1 plants. No reciprocal difference can be accepted for the population if it does not occur in these crosses.

Genotype and Type Identification Using Reciprocal Crosses Between the I_o Parent and I_1 Plants

When an I_o is reciprocally crossed with one of its I_1 progeny there are again three possible results: reciprocal incompatibility, a reciprocal difference, or reciprocal compatibility. Respectively, these give conclusions D, E and F (Chart 3: Step 8), which each dictates different procedures for identifying genotypes and type.

When Reciprocal Incompatibility Occurs Between the I_o and an I_1 Plant (Chart 3: Conclusion D)

If the I_o is reciprocally incompatible with an I_1 plant, no immediately useful conclusion can be drawn. The I_o is next reciprocally crossed with additional I_1 plants (Chart 3: Step 8.4), and if compatibility occurs instruction for compatible crosses (Conclusion E or F) should be followed. When compatibility has not occurred in reciprocal crosses between the I_o and five or six I_1 plants, it is probable that the Type is IV, or that the I_o was an *S*-allele homozygote. The I_o parent cannot distinguish between these alternatives, but a chain of reciprocal crosses among the I_1 siblings can. The chain (Step 8.4) is made by reciprocally crossing each plant with each of two I_1 sibs, as described in detail for Conclusion A. If two reciprocally compatible I_1 plants are now found, one can arbitrarily be designated S_aS_a, the other S_bS_b, and tentatively the Type is IV. These designations and Type IV will be verified if both reciprocally compatible I_1 plants are reciprocally incompatible with the I_o parent (Conclusion D.1), or if both are reciprocally incompatible with a third I_1 plant (Conclusion C.1). After verifying Type IV, the genotype is S_aS_a for any plant that is reciprocally compatible with S_bS_b, and vice versa (Conclusion D.1.2 or C.1.1), and all others are S_aS_b (Conclusion D.1.1 or C.1.1). Reciprocal compatibility is not essential for conclusion D.1.1, when Type IV has already been shown (Table 4), but reciprocal crossing is suggested for conformity with its general requirement.

If only reciprocal incompatibility occurs for the chain of reciprocal crosses among the I_1 plants (Step 8.4.1), the I_o was homozygous and each I_1 plant is homozygous for the same *S*-allele (Conclusion D.2).

When a Reciprocal Difference Occurs Between the I_o and an I_1 Plant (Chart 3: Conclusion E)

With a reciprocal difference between the I_o and an I_1 plant, the I_o is a 'known' heterozygote S_aS_b so the I_1 must be recessive S_aS_a, and the sexual-organ × *S*-allele-interaction Type is II, or III (Table 4, and Chart 3: Conclusion E). It is II if the I_o (known genotype S_aS_b) was male in the compatible and female in the incompatible cross, and it is III if it was female in the compatible and male in the incompatible cross (Conclusion E).

After indentifying the recessive S_aS_a genotype, subsequent reciprocal crosses should be between it and other I_1 plants (Step 8.5). For sexual-organ × *S*-allele-interaction

Types II and III, each reciprocal-cross pair between the recessive S_aS_a and another I_1 plant will always identify the genotype of the other I_1 plant. If the crosses are reciprocally incompatible, the other plant is also S_aS_a (Conclusion E.1). If one cross is compatible while its reciprocal is incompatible, the other I_1 is S_aS_b (Conclusion E.2). And, if the two crosses are reciprocally compatible, the other I_1 is dominant genotype S_bS_b (Conclusion E.3). Reciprocal crosses between a known genotype S_aS_b or S_bS_b and plants of unknown genotype are far less informative than crosses to a known S_aS_a (Tables 2 and 4). Reciprocal crosses between a known S_aS_b, including the I_o parent, and each I_1 plant will only identify more plants of recessive genotype S_aS_a. Dominant S_bS_b cannot be identified except by a previously identified recessive S_aS_a (Tables 2 and 4; Chart 3: Conclusion E.3).

When Reciprocal Compatibility Occurs Between the I_o and an I_1 Plant (Chart 3: Conclusion F)

If the I_o is reciprocally compatible with an I_1 plant: (A) the I_1 plant is homozygous recessive S_aS_a; (B) the Type is I; and (C) genotypes S_aS_b and S_bS_b cannot be differentiated in the I_1 generation (Conclusion F). Inability to differentiate is because Type I has dominance of allele S_b in both pollen and stigma, so the incompatibility phenotypes of S_aS_b and S_bS_b are identical (Table 4). Differentiation can be achieved in the I_2 generation, by segregation of S_aS_b (Conclusion F.1.2) vs. failure to segregate for S_bS_b (Conclusion F.1.1). Any I_1 plant that is incompatible with the I_1 of $S_a S_a$ genotype, or that is compatible with the I_o, is genotype S_aS_a (Conclusion F.1).

Summary of Merit of the I_o Parent as a 'Known' Genotype

No identified *S*-allele genotype exists within an I_1 population, but the I_o is S_aS_b if compatibility occurs between it and any I_1 plant, or between any two I_1 plants. A prerequisite for genotype identification is that an I_1 which is S_aS_a be one parent of the minimal required one pair, or of both of the minimal required two pairs, or of two of the minimal required three pairs of reciprocal crosses. That S_aS_a is a parent is evidenced whenever compatibility occurs for one or both of a pair of reciprocal crosses. The expected proportions among I_1 plants are 1/4:1/2:1/4 for S_aS_a, S_aS_b and S_bS_b. With the I_o as a known S_aS_b, the one required $I_o \times I_1$ reciprocal-cross pair is $S_aS_b \times S_aS_a$. It is directly recognized by a reciprocal difference for Types II and III and by reciprocal compatibility for Type I. Therefore, for Types I, II and III, each $I_o \times I_1$ reciprocal-cross pair has 1/4 chance of identifying the S_aS_a genotype, with 3/4 chance of failing. For reciprocal crosses between the I_o and two I_1 plants the chance of identifying S_aS_a is $7/16 = [1-(3/4)^2]$ and for three it is $37/64 = [1-(3/4)^3]$.

Identifying S_aS_a from reciprocal crosses among only I_1 plants requires for Types II and III, which have dominance in the pollen and codominance in the stigma, or vice virsa, a minimum of two reciprocal-cross pairs among three I_1 plants. The restrictions are that S_aS_a must be a parent of both pairs of reciprocal crosses and that the two pairs involve one plant of each of the three I_1 genotypes as follows: $S_aS_a \times S_aS_b$ and $S_aS_a \times S_bS_b$. Either reciprocal-cross pair may precede the other, so the chance of identifying S_aS_a with only two tandemly performed reciprocal-cross pairs is $[2 \times (1/4 \times 1/2) \times (1/4)] = 1/16$. This is seven times less efficient than two $I_o \times I_1$ reciprocal-cross pairs.

For Type I, with dominance in both pollen and stigma, identifying S_aS_a from reciprocal crosses among I_1 plants requires a minimum of three tandemly performed reciprocal-cross pairs among three I_1 plants as follows: $S_aS_a \times S_aS_b$, $S_aS_a \times S_bS_b$ and $S_aS_b \times S_bS_b$. The restrictions are that S_aS_a must be a parent of the first and also of either the second or third reciprocal-cross pair, and also that the three I_1 plants include one each of S_aS_a, S_aS_b and S_bS_b. The probability is again 1/16, being derived exactly as for Types II and III, since the required two crosses with S_aS_a are the same and when both are achieved the procedure makes the third required cross 100% available. This is 9.25 times less efficient than three pairs of $I_o \times I_1$ reciprocal crosses will identify an I_1 of S_aS_a genotype.

The three tandemly performed reciprocal-cross pairs required for Type I are also required for Type IV, with the same 1/16 probability of success. In contrast to Type I, however, this probability of 1/16 is infinitely more efficient than using reciprocal crosses of the $I_o \times I_1$, because the codominance in both pollen and stigma of the heterozygote causes all $I_o \times I_1$ crosses to be reciprocally incompatible, so no I_1 genotype can be identified from $I_o \times I_1$ crosses.

In summary, S_aS_a is the key I_1 genotype; it must always be recognized before another I_1 genotype can be identified (Thompson and Howard 1959). $I_o \times I_1$ reciprocal crosses are 9.25 times as efficient at identifying S_aS_a than $I_1 \times I_1$ reciprocal crosses for Type I, and 7.0 times more efficient for Types II and III, but infinitely less efficient for Type IV. The choice as to whether to use $I_o \times I_1$ or $I_1 \times I_1$ reciprocal crosses depends in part, therefore, on the relative proportions of Types I, II, and III vs. Type IV. Type IV commonly accounts for about 25% or less of the observed sexual-organ × *S*-allele interaction types, and reciprocal incompatibility for crosses between the I_o and six or seven I_1 plants clearly indicates a necessity to change to reciprocal crosses among the I_1 plants. Thus, with 7.0 to 9.25 fold higher efficiency for $I_o \times I_1$ reciprocal

crosses for about 75% of all I_1 populations, there is a large advantage for using the I_o if it can be brought into flower for a second time that is also simultaneous with flowering of its I_1 progeny.

Progenies for Which S-Allele Genotypes Cannot be Easily Identified

The sexual-organ × *S*-allele-interaction Types I, II, III and IV are extremes; the heterozygotes have highly active (codominant or dominant) or highly inactive (recessive) *S* alleles, and both alleles are highly active in the homozygous genotypes. The breeder must recognize that heterozygotes exist that are intermediate between these types, including extreme mutual weakening of both *S*-alleles (Wallace 1979). These intermediates can be common in populations with many highly recessive *S*-alleles (Webster 1973), and the alleles may not be fully active homozygotes. It is for this reason that I_o plants lacking high self incompatibility should preferably be discarded directly. I_1 progenies from heterozygotes of such intermediate types will have intermediate levels of reciprocalness and variable and poorly repeatable intensities of self- and sib-incompatibility. Such progenies will not have sufficiently high self- and sib-incompatibility of the homozygotes, or of the heterozygotes, to warrant the greater effort required for identifying the *S*-allele genotypes. For such progenies using the I_o as a known heterozygous *S*-allele genotype may assist identification of the I_1 genotypes by more than the expected 7.0 to 9.25 increases in efficiency.

Selecting Homozygous S-Allele Genotypes Without Determining the Type

A procedure previously used for selecting *S*-allele homozygotes is to self each I_1 plant, make diallel crosses among all plants within each I_2 progeny, and select the I_2 progenies that have reciprocal sib-incomaptibility among all I_2 plants. Such selection has usually been done with a full diallel, but a chain of reciprocal crosses among all I_2 plants as described herein could suffice. Thirteen I_2 progenies are required to give 95% probability of identifying at least one I_2 population that is homozygous for each of the two *S* alleles. On the average half of these I_2 progenies will have sib-incompatibility among all plants, and half of these will be genotype S_aS_a and half S_bS_b. That one homozygous I_2 population is S_aS_a and a second is S_bS_b is proven when several intercrosses between plants of two truebreeding I_2 progenies consistently show reciprocal compatibility. These procedures do not differentiate between the dominant and recessive alleles, so the S_aS_a and S_bS_b designations will be arbitrary, and sexual-organ × *S*-allele-interaction types will not be determined. Failure to identify recessive and dominant alleles and the sexual-organ × *S*-allele-interaction type prevents planning for production of 3- and 4-way hybrids, particularly since instances of mutual weakening between *S* alleles are not detected. Using the reciprocal-cross procedures outlined herein requires less total effort and gives more complete information.

Using the Seed Set Data

Of the flowers pollinated for each cross or self, some are left on the plant until maturity. About 60 days after pollination, when the pods are mature but before they shatter, the mature pods should be removed along with the string-held label that identifies the pollination. The pods for each pollination should be put in an envelope or bag, along with the label. The seeds should then be threshed and placed in a small envelope to which the label is attached with transparent tape. The number of pods and the total seed count should be recorded on the envelope. For comparisons with the pollen-tube penetration data, this information can be recorded on line 4 of the cell of the second-interpretative table that identifies the o+ and o− parents and the specific pollination (Table 2). Recording seed set data on the second- (Table 2) rather than first-interpretative table (Table 1) is preferred because subsequent transferral of data is never required, and because interpretation is easier when all data for all plants of the same genotype are adjacent and arranged for a diallel cross among known S_aS_a, S_aS_b and S_bS_b genotypes (as illustrated in Table 2).

The seed-set data will usually confirm the pollen tube data. When discrepancies occur and recording of data in the wrong cell has been eliminated, the seed set may be given the stronger consideration. It is lack of seed set from self- and sib-pollinations and abundance of seed set from outcrosses, not number of pollen tube penetrations, that are the primary considerations for hybrid seed production. However, a decision to rely on the seed set data requires full acceptance of these data. Table 1 of Wallace (1979) indicates general agreement between data for pollen tube scores and seed set data. Table 2 of this paper, however, indicates some major discrepancies; physiological or environmental perturbations obviously reduced the seed set of some well documented expressions of compatibility. Such reductions can result from water stress, insect damage or physiological and other environmental effects that occur after pollen tube germination. The breeder should only continue development of an inbred line when the seed set data and pollen tube penetration data are in reasonable agreement, or when a reasonable and acceptable explanation for discrepancies is available.

If desired, reciprocal differences can be calculated

from the reciprocal seed sets (see text and Table 1 of Wallace 1979).

Selecting for or Against Modifying Genes in Inbreds

Several studies indicate an effect of genes at other than the *S*-locus on the level of expressed incompatibility in *Brassica*. Thompson and Taylor (1971) and Nasrallah (1974) have each identified a gene for which the dominant allele weakens expression of incompatibility. It is not known if these were the same or different modifying genes. Thompson concluded that the dominant allele weakened expression of only S_{15} and other *S* alleles that are generally highly recessive. The dominant allele of the gene Nasrallah studied modified activity of his allele S_2 in the stigma; it did not alter S_2 activity in the pollen. The dosage effect of this allele was additive; one dominant allele conditioned quantities of an S_2-specific antigen in the stigma, and seed sets, that were intermediate to levels in plants homozygous for the recessive and dominant alleles. Thus, the allele is dominant only in that one dose overcomes expression of incompatibility vs. compatibility on an either-or basis; it is partially dominant when incompatibility vs. compatibility is considered on a continuous scale. Nasrallah and Wallace (1968), Thompson and Taylor (1971), Richards and Thurling (1973a), Nasrallah (1974) and Haruta (1962) have all demonstrated modification of the strength of incompatibility by an apparent polygenic complex.

The procedures described in this paper permit positive identification in the I_1 generation of the homozygous and also heterozygous *S*-allele genotypes. This facilitates selection among I_1 plants of the same genotype for strength of sib- and self-incompatibility, and further such selection among plants of each I_2 or later-generation progeny (Chart 1: Steps 9-10). Because the *S*-allele genotypes are known and fixed, any variation in strength within the I_2 generation and variation or change within successive generations must result from segregation of modifying genes (Johnson 1972). Thus, the described procedures will greatly improve efficiency of selecting *Brassica* inbreds for the strong incompatibility needed. The procedures described herein are newly available, so more experience is required before the best additional steps for selecting the appropriate modifying genes can be detailed. As suggested by Johnson (1971, 1972 a,b) the intensities of self- and sib-incompatibility of early, intermediate and late flowering of each inbred, and under different temperatures and humidities, should all be determined (Chart 1: Step 10).

Planning for 3- and 4-Way Hybrids

Primary requirements for producing 3- and 4-way hybrdids are a single-cross F_1 that is highly self- and sib-incompatible, and for some *Brassica* crops, similarity of the two inbred parents of each single cross hybrid for horticultural characteristics in order to maintain uniformity in the 3- and 4-way hybrids approaching that of single-cross hybrids. Both requirements are facilitated by identifying the *S*-allele genotypes and sexual-organ × *S*-allele-interaction types in the I_1 generation.

The strength of self- and sib-incompatibility among plants within each of the homozygous genotypes S_aS_a and S_bS_b, and also among plants within the heterozygous S_aS_b, will be largely determined simultaneous with identification of the *S*-allele genotypes. A chain of reciprocal crosses among the I_1 plants within each genotype may suffice for determining the intensities of sib-incompatibilities. A complete diallel among all I_1 plants within each genotype as previously advocated (MacKay 1977) will be more informative, but is not essential. The I_1 data about self- and sib-incompatibility of the S_aS_b genotype will greatly facilitate planning for production of 3- and 4-way hybrids, since this commercial seed will be produced on genotype S_aS_b (Chart 1: Step 12).

For maximizing uniformity and capitilizing on the incompatibility data acquired during *S*-allele identification, it is assumed that the *Brassica* breeder will choose to make the 3- and 4-way hybrids using inbreds derived from the same I_o parent. The acquired incompatibility data permits more effective selection within the S_aS_a and S_bS_b genotypes in I_1, and especially in I_2, for similarity of horticultural characteristics, optimum nicking of flowering times, for maximum seed set from crossing and for F_1 hybrid vigor. A definite advantage from use of such closely related inbreds may be better cross pollination, because bees may be unable to differentiate between flowers of the two closely related inbreds (Faulkner et al. 1977).

The knowledge acquired during genotype identification about the reciprocal compatibility, reciprocal incompatibility, or the reciprocal difference between the *S*-allele heterozygote and each of its counterpart homozygotes permits planning to produce most of the single-cross seed using the inbred with the dominant allele as the female parent. This is desirable because any unintended selfs in the single-cross will all be reciprocally incompatible with all actual single-crosses, thus permitting near-total elimination of inbreds in the 3- or 4-way hybrid seed. When a recessive homozygote is the female parent of the single-cross used as female parent of a 3- or 4-way hybrid, any unintended selfs within the single cross will be reciprocally compatible with the single cross for Type I (Table 4), and compatible in one direction but incompatible in the other for Types II and III (Table 4). Type IV is most desirable for use as female parent of the single-cross used in producing a 3- or 4-way hybrid, since the single cross is reciprocally incompatible with both of its homozygous inbred parents.

Other Aspects of Producing Hybrid *Brassica* Seed

It is procedures for identifying the *S*-allele genotypes and sexual-organ × *S*-allele-interaction types in the I_1 generation (Chart 1: Step 8 – expanded in Charts 2 and 3), and the advantages derivable therefrom, that are discussed herein. Most aspects of matching inbreds, selecting for or adjusting the nicking of flowering and numerous mechanical procedures for producing single-cross and 3- and 4-way hybrids have not been adequately discussed (Chart 1: Steps 11-16), and indeed have never been written about in detail. Cursory coverage has been given to some aspects (Thompson 1964; Johnson 1972 a, b; Nieuwhof and Garretsen 1975; Carter and McNeilly 1975; Faulkner et al. 1977; Roggen and van Dijk 1976; Gowers 1975; Ockendon 1978).

Acknowledgement

Appreciation is due: Susan Fast for pollinations; Bruce Rich for microscopical observations; Steffie David for typing many versions of the manuscript. The authors thanks J. Hawk, E. Kueneman, Georgia Voss, for concepts incorporated into the manuscript, and numerous colleagues and seedsmen for reviews. This work was partially supported by grants from J. Harris Company, Inc. and from Agway, Inc.

Literature

Arasu, N.T.: Self-incompatibility in angiosperms: A review. Genetica **39**, 1-24 (1968)

Carter, A.L.; McNeilly, T.: Increased atmospheric humidity post pollination: A possible aid to the production of inbred lines from mature flowers in the Brussels sprout (*Brassica oleracea* var. 'gemmifera'). Euphytica **25**, 531-536 (1975)

Faulkner, G.J.; Smith, B.M.; Daycott, A.: Matching inbred lines of Brussels sprouts for flowering characteristics as an aid to improving F_1 hybrid seed production. Ann. Appl. Genet. **51**, 21-28 (1977)

Ferrari, T.E.; Wallace, D.H.: A model for self-recognition and regulation of the incompatibility response of pollen. Theor. Appl. Genet. **50**, 211-225 (1977)

Frankel R.; Galun, E.: Pollination mechanisms, reproduction and plant breeding. Berlin Springer 1977

Gowers, S.: Methods of producing F_1 hybrid swedes (*Brassica napus* ssp. *rapifera*). Euphytica **24**, 537-541 (1975)

Haruta, T.: Studies on the genetics of self- and cross-incompatibility in cruciferous vegetables. (English transl. available from Minneapolis, Minn.: Northrup, King & Co. Takii Plant Breed. and Exp. Sta., Kyoto, Japan. Res. Bul. 2 (1962)

Heslop-Harrison, J.: The physiology of the incompatibility reaction in the *Cruciferae*. In: Proc. Eucarpia Meeting – Cruciferae 1974 (eds. Willis, A.B.; North, C.), Invergowrie, Dundee: Scottish Res. Inst., 14-18 (1974)

Heslop-Harrison, J.: Incompatibility and the pollen-stigma interaction. Ann. Rev. Plant Physiol. **26**, 403-425 (1975)

Heslop-Harrison J.: Male gametophyte selection and the pollen-stigma interaction. In: Gamete Competition in Plants and Animals (ed. Mulcahy, D.L.), pp. 177-189. North-Holland Publ. Co.: Amsterdam, The Netherlands 1975a

Johnson, A.G.: Factors affecting the degree of self-incompatibility in inbred lines of Brussels sprouts. Euphytica **20**, 561-573 (1971)

Johnson, A.G.: Problems in breeding and seed production of hybrid sprouts. The Commercial Grower. **24**, 749-750 (1972)

Johnson, A.G.: Overcoming problems of incompatibility. The Commercial Grower. **1**, 787-789 (1972a)

Johnson, A.G.: Alternative approaches which keep down seed costs. The Commercial Grower 1972b

Johnson, A.G.; Blyton-Conway, J.: Inbred lines with dominant incompatibility alleles for use as parents of F_1 hybrid Brussels sprouts. Euphytica **25**, 175-183 (1976)

Kho, Y.O.; Baer, J.: Observing pollen tubes by means of fluorescence. Euphytica **17**, 298-302 (1968)

Lawson, J.; Williams, W.: The effect of genotype on levels of pseudo-compatibility and the production of improved varieties in *Brassica oleracea*. J. Hort. Sci. **51**, 123-132 (1976)

MacKay, G.R.: A diallel cross method for the recognition of *S*-allele homozygotes in turnip, *Brassica campestris* L. *ssp rapifera*. Heredity **38**, 201-208 (1977)

Nasrallah, M.E.: Genetic control of quantitative variation in self-incompatibility proteins detected by immunodiffusion. Genetics **76**, 45-50 (1974)

Nasrallah, M.E.; Wallace, D.H.: The influence of modifier genes on the intensity and stability of self-incompatibility in cabbage. Euphytica **17**, 495-503 (1968)

Nettancourt, D. de: Self-incompatibility in basic and applied researches with higher plants. Genetica Agraria **26**, 163-216 (1972)

Nettancourt, D. de: Incompatibility in Angiosperms. Berlin-Heidelberg-New York: Springer 1977

Nieuwhof, M.; Garretsen, F.: Hybrid breeding in early spring cabbage (*Brassica oleracea* var. 'capitata' L.F. *alba* D.C.). II. Euphytica **24**, 87-97 (1975)

Ockendon, D.J.: Dominance relationships between *S* alleles in the stigmas of Brussels sprouts (*Brassica oleracea* var. 'gemmifera'). Euphytica **24**, 165-172 (1975)

Ockendon, D.J.: Rare self-incompatibility alleles in a purple cultivar of Brussels sprouts. Heredity **39**, 149-152 (1977)

Ockendon, D.J.: The production of inbred lines of Brussels sprouts with dominant *S* alleles. Euphytica **26**, 749-754 (1977a)

Ockendon, D.J.: Effect of hexane and humidity on self-incompatibility in *Brassica oleracea*. Theor. Appl. Genet. **52**, 113-117 (1978)

Pearson, O.H.: Breeding plants of the cabbage group. Calif. Agr. Exp. Sta. Bul. 532. (1932)

Richards, R.A.; Thurling, N.: The genetics of self-incompatibility in *Brassica campestris* L. ssp *oleifera* Metzg. I. Characteristics of *S*-locus. Control of self-incompatibility. Genetica **44**, 428-438 (1973)

Richards, R.A.; Thurling, N.: The genetics of self-incompatibility in *Brassica campestris* L. *ssp. oleifera* Metzg. II. Genotypic and environmental modification of *S* locus control. Genetica **44**, 439-453 (1973a)

Roggen, H.; van Dijk, A.J.: Thermally aided pollination: New method of breeding self-incompatibility in *Brassica oleracea* L. Euphytica **25**, 643-646 (1976)

Smith, B.M.; Johnson, A.G.; Conway, J.: Inbred lines with stronger self-incompatibility. National Vegetable Research Station,

Wellesbourne, Warwick, England. Ann. Rep. pp. 22-23 (1977)
Thompson, K.F.: Triple-cross hybrid kale. Euphytica **13**, 173-177 (1964)
Thompson, K.F.: Classified *S* alleles for *Brassica* breeders. In: Proceedings of *Brassica* Meeting of Eucarpia. (ed. Dixon, G.E.) pp. 25-28 (1968)
Thompson, K.F.; Howard, H.W.: Self-incompatibility in narrow-stem kale, *Brassica oleracea* var. 'acephala'. II. Methods for the recognition in inbred lines of plants homozygous for *S* alleles. J. Genet. **56**, 324-340 (1959)
Thompson, K.F.; Taylor, J.P.: Self-incompatibility in kale. Heredity **27**, 459-471 (1971)
Townsend, C.E.: Advances in the study of incompatibility. In: Pollen: Development and Physiology. (ed. Heslop-Harrison, J.) London Butterworths 1971
Visser, D.L.: The effect of alternating temperatures on the self-incompatibility of some clones of Brussels sprouts (*Brassica oleracea* L. var. 'gemmifera' (D.C.) Schulz). Euphytica **26**, 273-277 (1977)
Wallace, D.H.: Interaction of *S* alleles in sporophytically controlled self incompatibility of *Brassica*. Theor. Appl. Genet. **54**, 193-201 (1979)
Wallace, D.H.; Nasrallah, M.E.: Pollination and serological procedures for isolating incompatibility genotypes in the crucifers. Cornell Univ. Agr. Exp. Sta. Memoir 406, 23 pp. (1968)
Webster, J.C.: The use of fluorescence microscopy in studies of self-incompatibility of cabbage. M.S. Thesis, Cornell University, 138 pp. (1973)

Received October 21, 1978
Communicated by H.F. Linskens

Prof. Dr. D.H. Wallace
Department of Plant Breeding and Biometry
Cornell University
Ithaca, New York 14853 (USA)