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Nature and Inheritance of Self-incompatibility in *Capsicum pubescens* and *C. cardenasii*

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The genetic basis of self-incompatibilitiy in *Capsicum pubescens* and *C. cardenasii* was studied. Self-incompatibility was discovered for the first time in the normally self-compatible, cultivated species *C. pubescens*. All strains of *C. cardenasii* studied were self-incompatible.

Diallel matings of F_1 plants from intraspecific (*Capsicum cardenasii*) and interspecific (*C. pubescens* × *C. cardenasii*) crosses indicated a gametophytic system of incompatibility of the *Nicotiana* type in both species. Each F_1 progeny, regardless of the direction of the cross, constituted four intrasterile, interfertile groups. When self-incompatible *C. pubescens* or *C. cardenasii* plants were crossed with self-compatible *C. pubescens*, all F_1 and F_2 plants were self-compatible. F_1 plants accepted pollen readily from either parent. In contrast to *Nicotiana*, however, the self-incompatible strains accepted pollen readily from self-compatible plants.

The number of pollen-grain nuclei and the site of inhibition of pollen-tube growth substantiated the gametophytic interpretation of incompatibility in *Capsicum*.

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Nature and Inheritance of Self-incompatibility in *Capsicum pubescens* and *C. cardenasii*[1,2]

INTRODUCTION

THE GENUS *Capsicum* is a member of the family Solanaceae. Although extensive studies have been made on self-incompatibility in different genera of this family, *Capsicum* has been neglected, largely because of its relative unavailability.

The present study was initiated to determine the inheritance of self-incompatibility in *Capsicum cardenasii* and to explain observations that the F_1 and F_2 generations from crosses between *C. cardenasii* and self-compatible *C. pubescens* are self-compatible. These two species cross readily, and the hybrids are highly fertile. Shortly after this work was begun, a self-incompatible strain of *C. pubescens* was found and was included in the study.

The literature on self-incompatibility has been reviewed thoroughly by Sears (1937), Stout (1938), and Lewis (1949).

MATERIALS AND METHODS

The studies were confined to *Capsicum pubescens* R. & P. and *C. cardenasii* Heiser & Smith. Morphological features of both are given below.

Capsicum pubescens is known only as a cultivated species (Heiser and Smith, 1948; Rick, 1950; Eshbaugh, 1964). It is restricted to the higher elevations of South and Central America, from northern Argentina to central Mexico. Morphologically it is characterized by pubescent, rugose leaves, a rotate corolla with purple lobes and a white base, purple anthers, a calyx with five distinct teeth, and an irregularly wrinkled, black seed.

Capsicum cardenasii is a wild species known at present only from a restricted region near La Paz, Bolivia. The plant is essentially glabrous; the leaves are slender and nonrugose, and the branches slender and weak. The corolla is markedly campanulate, lavender to light purple, with yellow spots on the inner corolla lobes. The anthers are lavender; the calyx teeth are small. The smooth seeds are light brown.

Seeds were germinated in Petri dishes at a constant temperature of 70° F. The germinated seeds were transferred to 1-gallon cans and grown to maturity in an insectproof greenhouse. Studies of

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pollination and pollen-tube growth were made in a growth chamber maintained at 70° F.

Results of pollination were measured (1) by recording the number of pollinated flowers that set fruit and (2) by microscopic observation of pollen-tube growth in the styles 48 hours after pollination. The former method permits only a partial classification, because its accuracy is conditioned by female fertility and uncontrolled environmental factors. Studies of pollen-tube growth were therefore considered essential and were carried out by the fluorescent-dye technique described by Martin (1959).

For testing self-incompatibility, 20 to 25 flowers were self-pollinated and scored for fruit and seed set. In addition, at least three pollinated styles prepared by the fluorescent-dye technique were examined for pollen-tube growth.

Since the incompatibility response by any hybrid depends on the S genotype of its parents, diallel matings between F_1 plants were made. Eight plants were used, and the number of different intra-sterile, interfertile classes was determined. The eight plants were then used as testers and crossed with the remaining F_1 plants to verify the number of classes obtained in the diallel mating. This is an efficient, short-cut method, since large numbers of an F_1 population can be classified for *S* alleles with a minimum number of crosses.

RESULTS

Nature of Self-incompatibility

In self-compatible strains of *Capsicum pubescens,* no differences were observed in setting of fruits or in number of seeds per fruit when flowers were pollinated either in the bud stage or when fully open. Fruit set ranged between 50 and 60 per cent, and the seed number within each self-fertile strain remained fairly constant for each of the self-pollination methods (table 1).

TABLE 1

EFFECT OF SELF-POLLINATION AT DIFFERENT STAGES OF FLOWER DEVELOPMENT ON FRUIT SET AND NUMBER OF SEEDS PER FRUIT IN *CAPSICUM PUBESCENS*

Collection no. and flower stage	Effects of treatments		
	No. pollinations	No. fruit set	No. seeds per fruit
SA 265			
Unopened:			
Bud.....	10	5	30
Mature.....	10	6	31
Opened, mature..	10	6	28
SA 359			
Unopened:			
Bud.....	30	0	0
Mature.....	40	0	0
Opened, mature..	30	0	0

The barrier to self-compatibility appeared very strong in the self-incompatible *Capsicum pubescens* strain SA 359, since it failed to set any fruits even when the flowers were pollinated in the bud stage. The self-incompatibility reaction was very sharp when measured by pollen-tube growth, the tube seldom penetrating more than one fifth of the style length. Quantitative measurements of pollen-tube length within the style were not made, since no intermediate stage between the compatible and incompatible tubes was found.

Two different collections of *Capsicum cardenasii* were observed: SA 267 and Ac. 1793. Both were strongly self-incompatible, regardless of the age of the flower. Bud pollination, used to overcome self-incompatibility in some plants (e.g., *Brassica*), was ineffective with both species of *Capsicum.* As in cross-compatible stocks, pollen germination on self-pollinated flowers took place within six hours. Since pollen tubes did not penetrate more than one-fifth the length of the style, inhibition is therefore a function of the growth rate of the pollen tubes.

The type of self-incompatibility can be established from the number of self-incompatible classes in a segregating F_1 family. A class is considered to be a group of plants with the same incompatibility reaction. Thus, they are mutually cross-incompatible, but cross-compatible with individuals of other classes. Confirmation of classes was obtained from parental backcrosses.

F_1 families segregating for *S* alleles were obtained from intra- and interspecific hybridization between the self-incompatible *Capsicum pubescens* SA 359 and *C. cardenasii* SA 267 and Ac. 1793.

Capsicum pubescens SA 359 × *C. cardenasii* SA 267

A population of 15 plants was grown from this cross. Diallel matings were made between all F_1 plants. Four distinct intra-incompatible, intercompatible classes consisting of six, five, three, and one individuals, respectively, were distinguished on the basis of success or failure of the attempted crosses (table 2). A chi-square test indicated a good fit to the expected 1:1:1:1 ratio. All the F_1 plants were crossed reciprocally with both parents and set fruit freely. Reactions were precise, and it was possible to score results within two weeks.

Capsicum pubescens SA 359 × *C. cardenasii* Ac. 1793

A total of 27 F_1 plants was grown. Eight plants were intercrossed initially, and four classes of individuals were distinguished in numbers of three, three, one, and one, respectively (table 3). Individuals were cross-incompatible within each group, but compatible with members of other groups. The eight plants were then crossed as males to the remaining F_1 plants. Pollination results are summarized in table 4. Since the four groups varied in size from five to

TABLE 2

INTRA-INCOMPATIBLE AND INTERCOMPATIBLE GROUPS IN F_1 PLANTS FROM THE CROSS *CAPSICUM PUBESCENS* SA 359 × *C. CARDENASII* SA 267

Group and plant no.	Group and plant no.*															Parents	
	A-1						A-2					A-3			A-4		
	1	4	8	13	15	16	2	3	7	11	12	6	10	14	9	267	359
A-1:																	
1...	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
4...	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
8...	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
13...	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
15...	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
16...	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+
A-2:																	
2...	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+
3...	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+
7...	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+
11...	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+
12...	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+
A-3:																	
6...	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+
10...	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+
14...	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+
A-4:																	
9...	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+

* + = compatible
− = incompatible

1:1:1:1 ratio of class sizes
P = 0.20–0.50

TABLE 3

RESULTS OF DIALLEL MATINGS OF EIGHT F_1 PLANTS FROM THE CROSS *CAPSICUM PUBESCENS* SA 359 × *C. CARDENASII* AC. 1793

Group and plant no.	Group and plant no.*								Parents	
	B-1			B-2			B-3	B-4		
	1	2	4	6	10	11	3	7	359	1793
B-1:										
1.....	−	−	−	+	+	+	+	+	+	+
2.....	−	−	−	+	+	+	+	+	+	+
4.....	−	−	−	+	+	+	+	+	+	+
B-2:										
6.....	+	+	+	−	−	−	+	+	+	+
10.....	+	+	+	−	−	−	+	+	+	+
11.....	+	+	+	−	−	−	+	+	+	+
B-3:										
3.....	+	+	+	+	+	+	−	+	+	+
B-4:										
7.....	+	+	+	+	+	+	+	−	+	+

* + = compatible
− = incompatible

1:1:1:1 ratio of class sizes
P = 0.20–0.50

TABLE 4

COMPATIBILITY CLASSIFICATION OF ENTIRE F_1 FAMILY FROM THE CROSS *CAPSICUM PUBESCENS* SA 359 × *C. CARDENASII* AC. 1793, BY MEANS OF TESTER PLANTS

Compatibility group (female parents)*	Compatibility group† and tester plant no. (male parents)				Parents	
	B-1 1, 2, 4	B-2 6, 10, 11	B-3 3	B-4 7	359	1793
B-1.....	−	+	+	+	+	+
B-2.....	+	−	+	+	+	+
B-3.....	+	+	−	+	+	+
B-4.....	+	+	+	−	+	+

* Groups and plant numbers:
B-1: 1, 2, 4, 5, 12, 23, 24, 25, 27 = 9
B-2: 6, 8, 9, 10, 11, 15 = 6
B-3: 3, 13, 14, 20, 21 = 5
B-4: 7, 16, 17, 18, 19, 22, 26 = 7

1:1:1:1 ratio of class sizes
P = 0.5–0.8

† + = compatible
− = incompatible

nine plants, a chi-square test was applied. Deviation from expected values was not significant. Crosses within groups were not successful, but plants of different groups crossed freely. All F_1 plants were reciprocally compatible with both parents.

Capsicum cardenasii SA 267 × *C. cardenasii* Ac. 1793

The self-incompatibility reaction of these two strains of *Capsicum cardenasii* has been noted individually with *C. pubescens* SA 359, which served as a stock common to both strains. Data on eight plants reciprocally intercrossed from a population of 18 F_1 plants from the cross between the two *C. cardenasii* strains are given in table 5. Four intra-incompatible, intercompatible classes were again obtained, with four, two, one, and one plants, respectively. The remaining 10 plants were placed in the four groups by the method indicated

TABLE 5

RESULTS OF DIALLEL MATINGS OF EIGHT PLANTS FROM THE CROSS *CAPSICUM CARDENASII* SA 267 × AC. 1793

Group and plant no.	Compatibility group* and plant number							
	C-1				C-2		C-3	C-4
	1	7	8	9	5	19	2	6
C-1:								
1	−	−	−	−	+	+	+	+
7	−	−	−	−	+	+	+	+
8	−	−	−	−	+	+	+	+
9	−	−	−	−	+	+	+	+
C-2:								
5	+	+	+	+	−	−	+	+
19	+	+	+	+	−	−	+	+
C-3:								
2	+	+	+	+	+	+	−	+
C-4:								
6	+	+	+	+	+	+	+	−

* + = compatible
− = incompatible

1:1:1:1 ratio of class sizes
P = 0.20–0.50

TABLE 6

COMPATIBILITY CLASSIFICATION OF ENTIRE F_1 FAMILY FROM THE CROSS *CAPSICUM CARDENASII* SA 267 × AC. 1793, BY MEANS OF TESTER PLANTS

Compatibility group (female parents)*	Compatibility group† and tester plant no. (male parents)				Parents	
	C-1 1, 7, 8, 9	C-2 5, 19	C-3 2	C-4 6	359	1793
C-1	−	+	+	+	+	+
C-2	+	−	+	+	+	+
C-3	+	+	−	+	+	+
C-4	+	+	+	−	+	+

* Groups and plant numbers:
C-1: 1, 7, 8, 9, 12, 15 = 6
C-2: 3, 5, 14, 19 = 4
C-3: 2, 11, 13, 16, 17 = 5
C-4: 6, 10, 18 = 3
+ = compatible
− = incompatible

1:1:1:1 ratio of class sizes
P = 0.5–0.8

above. Classification of the entire F_1 family on the basis of test pollinations is summarized in table 6. Deviation from the 1:1:1:1 ratio, as determined by chi-square test, was statistically non-significant.

Information on the number of self-incompatible classes was obtained from reciprocal crosses between only two strains of *Capsicum cardenasii*, SA 267 and Ac. 1793. A chi-square analysis for the reciprocal crosses (SA 267 × Ac. 1793, and Ac. 1793 × SA 267), assuming multiple-allelomorphic self-incompatibility, is given in table 7. The low-non-significant chi-squares are evidence that the two families were segregating similarly for an expected ratio of 1:1:1:1 for the four incompatibility classes.

Representatives from each of the classes of the three F_1 combinations were tested for compatibility. Reciprocal matings were attempted in as many combinations as possible. Seed set was obtained only from matings predicted to be compatible (table 8). From this it is clear that the *S* genes in both

TABLE 7

CHI-SQUARE ANALYSIS OF TWO RECIPROCAL F_1 FAMILIES OF *CAPSICUM CARDENASII* (267 AND 1793) FOR SEGREGATION INTO INCOMPATIBILITY GROUPS OF EQUAL SIZE

Family	SI groups	No. of plants	No. of plants expected	Chi-square	Probability
267	C-1	6	4.5	0.50	
×	C-2	4	4.5	0.05	
1793	C-3	5	4.5	0.05	
	C-4	3	4.5	0.50	
Totals		18	18.0	1.10	0.5–0.8
1793	C′-1	8	7.0	0.14	
×	C′-2	4	7.0	1.28	
267	C′-3	5	7.0	0.57	
	C′-4	11	7.0	2.28	
Totals		28	28.0	4.27	0.20–0.50
Pooled	1	14	11.5	0.54	
data	2	8	11.5	1.06	
	3	10	11.5	0.19	
	4	14	11.5	0.54	
Totals		46	46.0	2.33	0.5–0.8

TABLE 8

SEGREGATION OF BACKCROSS PROGENY OF THE F_1 HYBRIDS BETWEEN SELF-INCOMPATIBLE (SI) AND SELF-COMPATIBLE (SC) *CAPSICUM* SPP. TO THEIR RESPECTIVE SI PARENTS

Cross	*S* allele designation	No. of plants	No. of plants		*S* allele designation		Chi-square	Probability
			SI	SC	SI	SC		
(359 × 265) F_1	S^1S^f							
×		41	21	20	S^1S^2	S^2S^f	0.05	0.95–0.99
359	S^1S^2							
359	S^1S^2							
×		26	0	26	..	S^1S^f, S^2S^f	..	..
(359 × 265) F_1	S^1S^f							
(267 × 265) F_1	S^3S^f							
×		24	13	11	S^3S^4	S^4S^f	0.16	0.80–0.95
267	S^3S^4							

Capsicum pubescens and *C. cardenasii* are located at the same locus.

After it became evident that the self-incompatibility of SA 359 of *Capsicum pubescens* and of SA 267 and Ac. 1793 of *C. cardenasii* followed the *Nicotiana* type of self-incompatibility, we decided to test the inheritance in intraspecific and interspecific hybrids, using a self-fertile line of *C. pubescens*, SA 265, as a common male parent.

Hybrids between *Capsicum pubescens* and *C. cardenasii* were readily obtained by using the self-incompatible types as the female parent. Normally, the cross can be made in either direction between self-compatible strains of *C. pubescens* and self-incompatible strains of both species. In this instance, the self-compatible strain SA 265 of *C. pubescens* could be used as a male only. This revealed a unique type of unilateral

cross-incompatibility, which needs further investigation. All F_1 families from the three crosses were completely self-fertile. If the genotypes of SA 359, SA 267, and Ac. 1793 were annotated as S^1S^2, S^3S^4, and S^5S^6, respectively, and that of the self-fertile parent (SA 265) as S^fS^f, then the F_1 genotypes would be from S^fS^1 to S^fS^6.

All the F_2 plants derived from these three crosses were completely self-fertile. Apparently in the F_1 plants only the S^f pollen grains penetrated the styles, and the pollen grains containing incompatibility alleles were inhibited.

A single F_1 plant from the cross *Capsicum pubescens* SA 359 × SA 265 was backcrossed reciprocally to the SA 359 parent plant. F_1 plants of the constitution of S^fS^1 when backcrossed to the S^1S^2 parent gave backcross progenies that segregated in a ratio of 1 self-compatible:1 self-incompatible. All self-incompatible plants were incompatible with the original S^1S^2 parent. In the reciprocal cross, i.e., when the parent SA 359 was used as the female and the F_1 as the male plant, all the backcross progenies were self-compatible. Evidently, the S^1 pollen grains from the F_1 plant failed to reach the ovary, and only the S^f pollen grains effected fertilization. This is in complete agreement with the concept of independent action of S alleles in the style, one of the key characters of the gametophytically controlled system of self-incompatibility.

The other backcross, (*Capsicum cardenasii* SA 267 × *C. pubescens* SA 265) F_1 × SA 267, showed the same reactions; but in this case the reciprocal cross could not be attempted. Out of a total of 24 backcross plants, 13 were self-incompatible, which is close to the expected 1:1 ratio (table 8).

DISCUSSION

In crosses between different strains of the self-incompatible species *Capsicum cardenasii* or between *C. cardenasii* and a self-incompatible form of *C. pubescens,* the F_1 plants of each cross produced four groups of equal size. F_1 plants within a group were self- and cross-incompatible, but were fully compatible with all plants of the other three groups. All plants were compatible with either parent.

The explanation for this type of behavior, first suggested by Prell (1921), and developed and applied by later workers in *Nicotiana* and *Veronica* (East and Mangelsdorf, 1925; Filzer, 1926; Lehmann, 1926), is based on certain considerations: (1) the incompatibility reaction in some flowering plants is primarily a reaction between pollen tubes and secretions of the stylar tissues. (2) The individual pollen grains are haploid with respect to nuclear organization, and the tissue of the style is diploid. (3) Evidence indicates that partially selective fertilizations may occur that produce different classes in the reciprocal progenies of two plants. (4) Evidence also indicates that the progeny of two plants that are cross-compatible may segregate into no more than four groups of intra-incompatible individuals.

These recognized conditions are based on the following assumptions: (1) The diploid hermaphrodite, which is self-incompatible, possesses an allelic pair of special, oppositional incompatibility factors (as S^aS^b) which segregate during meiosis to give two classes of pollen, S^a and S^b. (2) All individuals are self-incompatible because both classes of pollen tubes encounter, in the pistil, the secretion of diploid cells, which contain the same S factors. (3) All members of the same genotype are cross-incompatible because of the same reactions as those that occur in self-pollination. (4) Two plants that have only one factor in common accomplish partial fertiliza-

tion; only the pollen without the common factor will function. This selective fertilization results in only two classes of progeny, one of which has a genotype different from that of either of the parents. (5) When there is no common *S* factor in the two parents, there is no partially selective fertilization. All classes of pollen function, and four classes appear in the progeny as $S^aS^b \times S^cS^d = S^aS^c$, S^aS^d, S^bS^c, and S^bS^d.

Results obtained in the present studies indicate inheritance of incompatibility that conforms to the *Nicotiana* scheme shown above. But inheritance similar to this may also be expected under other schemes.

According to the *Capsella* type of incompatibility, as reported by Riley (1932, 1936), an F_1 population could be composed of four incompatibility classes, but each class would not be

TABLE 9

COMPATIBILITY CLASSIFICATION OF DIALLEL CROSSES OF F_1 PLANTS BY MEANS OF TESTER PLANTS FROM EACH COMPATIBILITY GROUP OF *CAPSICUM PUBESCENS* SA 359 AND *C. CARDENASII* SA 267 AND AC. 1793

	Compatibility group*											
Group	A-1	A-2	A-3	A-4	B-1	B-2	B-3	B-4	C-1	C-2	C-3	C-4
A-1	–											
A-2		–										
A-3			–									
A-4				–								
B-1					–			ALL COMPATIBLE				
B-2						–						
B-3							–					
B-4								–				
C-1			ALL COMPATIBLE						–			
C-2										–		
C-3											–	
C-4												–

* – = incompatible

fertile with all others. The *Capsella* scheme obviously does not apply to our data.

Gerstel (1950), in presenting a type of genetic control for incompatibility in guayule (*Parthenium argentatum*), emphasized that the reaction takes place between two sporophytes. To account for the reciprocal difference, he assumed that certain alleles are dominant in the male but not in the female. Crosses between F_1 groups in guayule may show reciprocal differences. The guayule system, then, cannot explain the pattern observed in *Capsicum*.

Kakizaki (1930), working with cabbage, reported an exhaustive search for the basis of incompatibility reactions. His observations indicated that: (1) the progeny of two plants may contain more than four genotypes; (2) when a progeny segregates into two classes, both may be fertile with both parents; and (3) two sister plants that are cross-sterile may be of different genotypes. To explain these and other facts, he proposed a series of *S* factors which act in the manner of *Nicotiana,* but which are modified by a series of *T* factors that favor fertilization, yet are not alleles of the *S* factor. He called this the "associate type" of incompatibility. Our data cannot fit this scheme, because no more than four groups were found in the pro-

geny of any two plants. No progenies composed of two groups were found in which both groups were fertile with both parents. Nor does the behavior of *Capsicum cardenasii* resemble that of *Crepis* or *Parthenium* (Hughes and Babcock, 1950; Gerstel and Riner, 1950). Crosses between the compatible plants, which may produce four types of offspring, do not always produce the same type; and incompatibility may be found between groups that are not genetically identical. Also striking is the difference which often exists if the cross is made reciprocally. This occurs in the *Nicotiana* scheme only when homozygotes are produced by bud pollination or other methods that alter normal crossing behavior.

Representatives from each of the classes distinguished in several families were selected to test intercompatibility. The results from reciprocal matings (table 9) agree with those expected from a gametophytic system of incompatibility. Seed set was obtained only from matings considered to be compatible according to the *Nicotiana* scheme.

TABLE 10

COMPARISON OF SELF-INCOMPATIBLE SYSTEMS IN CERTAIN PLANTS, BASED ON THEORETICAL EXPECTATIONS FOR VARIOUS CHARACTERISTICS

System number	Representative type of incompatibility system	No. of classes in F_1 generation*	Reciprocal differences in F_1 generation*	Classes of F_1 hybrids in crosses to a compatible species	No. of nuclei in pollen grain*	Site of inhibition
I	Primulaceae	2-3	No	1 or 2	binucleate	..
II	Lythraceae	4	Yes	More than 2	binucleate	..
III	Compositae	4	Yes	2	trinucleate	stigma
IV	*Nicotiana*	4	No	2	binucleate	style
V	Gramineae	4-16	No	4	trinucleate	stigma
VI	*Physalis*	4-6	Yes	More than 2	..	..

* Underlined items are those expectations in agreement with the experimental results reported here.

Cipar, Peloguin, and Hougas (1964) have arrived at the same conclusions for *Solanum tuberosum.*

It was not possible to make tests of compatibilities in the backcross progeny that would have satisfied the criteria of the *Nicotiana* scheme (Stout, 1938). Nevertheless, the absence of reciprocal differences in crossing behavior among the four different classes of the F_1 precludes the presence of a complex system of self-incompatibility, such as those found in *Physalis* (Pandey, 1957), *Parthenium* (Gerstel, 1950), the Cruciferae (Bateman, 1955; Kakizaki, 1930), or *Secale cereale* (Lundquist, 1956).

As a quick review, a comparative sketch of self-incompatibility systems with respect to theoretical expectations is presented in table 10. It is evident that self-incompatibility as found in the *Capsicum* species is in close agreement with the multiple-allelomorphic hypothesis. That the *Nicotiana* scheme alone explains the present data is further substantiated by the results obtained from the site of pollen germination and the number of nuclei present in the pollen grain. Pollen germination in incompatible forms was observed to be normal; the tubes penetrated through the stigmatic layers, but inhibition typically occurred in the stylar tissue just beneath the stigma. Cytological studies indicated that mature pollen grains of both *Capsicum cardenasii* and *C. pubescens* are binucleate. These findings agree with the prediction based on the hypothesis that, among homomorphic angiosperms, species with binucleate pollen grains would have gametophytic systems of self-incompati-

bility, and species with trinucleate pollen grains, sporophytic systems (Brewbaker, 1957; Pandey, 1958, 1960).

The evidence obtained from reciprocal matings of F_1 progenies, backcrosses of F_1 individuals to their respective parents, diallel crosses involving *S* allele differentials, cytology of pollen grains, and site of inhibition lends ample support to the contention that *Capsicum* species follow the gametophytic pattern of inheritance of the *Nicotiana* type.

Although the self-incompatibility system follows the *Nicotiana* scheme, it differs from the types most commonly found. Whenever *Capsicum cardenasii* or the self-incompatible *C. pubescens* was crossed with self-compatible strains of *C. pubescens,* all F_1 and F_2 plants were self-compatible. Also, the F_1 plants were highly female-fertile when pollinated by either parent.

In all the intraspecific and interspecific crosses between self-fertile and self-sterile plants, self-compatibility was inherited as a dominant character in the F_1. Only self-fertile plants were observed in the F_2 populations obtained from either the intraspecific *Capsicum pubescens* cross (SA 359 × SA 265) or the interspecific *C. cardenasii* × *C. pubescens* cross (SA 267 × SA 265). However, a ratio of 1 self-compatible:1 self-incompatible plant was obtained in the backcross when the F_1 was used as the female parent. Such segregation was not observed in a reciprocal backcross, i.e., when the F_1 was used as the male parent. All the progeny of this reciprocal cross were self-compatible.

East and Yarnell (1929) found that the factor of self-fertility, present in *Nicotiana langsdorffii,* is allelic to the oppositional-factor series. When a plant bearing the self-fertility factors was crossed with a plant homozygous for one of the self-sterility factors, the F_1 was self-fertile; and if this was then selfed, a completely self-fertile F_2 resulted.

In selfed plants of the constitution S^1S^f or S^2S^f, such as would likely be obtained if *Capsicum pubescens* SA 265 (S^fS^f) were crossed with SA 359 (S^1S^2), probably only pollen tubes bearing the factor S^f would function. In that case, the F_2 generation from any single plant would consist of equal numbers of plants S^fS^f and S^1S^f or S^fS^f and S^2S^f. No self-sterile plants would be obtained.

According to the oppositional hypothesis, the behavior of S^f and S^1 alleles can be established in a number of ways: (1) by fertility of F_2 progenies; (2) by study of backcross progeny, using the self-incompatible parent as the female; (3) by examination of backcross progeny when the F_1 is used as the female; (4) by intercrossing behavior of self-incompatible individuals obtained from the backcross in procedure 3; (5) by crossing the self-incompatible plants obtained from procedure 3 to the original self-incompatible parent; and (6) by pollen-tube growth studies. In a cross $S^1S^2 \times S^fS^f$, the F_1 plants would be either S^1S^f or S^2S^f. To avoid the error of intercrossing F_1 plants, suspected to have been committed by early workers, the F_2 was obtained from a single, selfed F_1 plant only, designated tentatively as S^1S^f. This F_1 plant should have given equal numbers of S^fS^f *and* S^1S^f plants, all self-fertile, provided S^1 pollen-tube growth was inhibited in the S^1S^f style. If the pollen tubes containing the S^1 factor had grown normally, then the F_2 generation should have consisted of $S^1S^1 + S^1S^f + S^fS^f$. Since no self-sterile plants were observed in the F_2 progeny, the S^1 pollen tubes apparently failed to reach the ovules.

All individuals in the backcross progeny were self-fertile when the original self-incompatible parent was used as the female and the F_1 with the assumed S^1S^f genotype was used as the male. With the self-incompatible parent designated S^1S^2 and the F_1 as S^1S^f, the backcross progeny should have consisted of $S^1S^1 + S^1S^2 + S^1S^f + S^fS^f$ plants if the S^1 pollen tubes had normal growth in the S^1S^2

style. The absence of self-incompatible plants indicated that S^1 pollen tubes failed to grow in the parental style.

The reciprocal backcross (F_1 S^1S^t × self-incompatible parent S^1S^2) presented quite a different picture. Half of the individuals were self-incompatible and half were self-compatible. Assumptions about the inhibition of the S^1 pollen tube cannot be made from these data, since the 1:1 ratio can be expected under both conditions of S^1 pollen-tube growth. Assuming that the S^1 pollen tube is able to grow normally in the S^1S^t style, the progeny from this cross should consist of $S^1S^1 + S^1S^2 + S^1S^t + S^2S^t$ in equal numbers, which would give a 1:1 ratio. If it is assumed that the S^1 pollen tube has an incompatible reaction, then the progeny would constitute S^1S^2 and S^2S^t genotypes, i.e., the same 1:1 ratio. To determine if this were true, all self-incompatible plants from this backcross were again backcrossed as females to the original self-incompatible parent. If the self-incompatible backcross progeny consists of S^1S^1 and S^1S^2 plants, the situation could be expected when the S^1 pollen tube is not inhibited in the S^1S^t style. Half of the plants (S^1S^1) should be compatible with the S^1S^2 parent, because the S^2 pollen tubes would grow unobstructed, while the S^1S^2 plants would be cross-incompatible with the S^1S^2 parent. None of the self-incompatible plants from the backcross progeny was compatible with the self-incompatible parent. This behavior indicates that all the backcross progenies were of the same genotype, S^1S^2, and that the S^1 pollen tubes had failed to grow in the S^1S^t style.

Pollen-tube growth studies also lend support to the above conclusions. A microscopic examination of the styles from a self-pollinated F_1 (S^1S^t) plant showed that about half the pollen tubes had normal growth, while the rest had little. This indicates that the S^1 pollen tubes were inhibited in the S^1S^t styles.

Lewis and Crowe (1958) discussed the possible origin and evolutionary significance of self-compatibility genes in self-incompatible plant groups. They suggested that in self-incompatible (SI) species the first step in the evolutionary sequence was a mutation (*Sc*) to self-compatibility and that the *Sc* pollen would be functional on SI plants. Later evolutionary steps would result in the change of *Sc* to *SC*, the latter being non-functional on SI plants; they refer to this as the "(SI × SC) inhibition." This represents the end of the evolution from self-incompatibility to self-compatibility in a species. The *Sc* mutant, then, represents a recent evolutionary change and *SC*, an older mutant.

Our results, according to Lewis and Crowe's interpretation, would place the self-compatibility of *Capsicum pubescens* in the *Sc* class, representing a recent evolutionary change from the self-incompatibility still found in this species. Since the examples cited by Lewis and Crowe in support of their ideas were based on interspecific crosses, *C. pubescens* represents the second instance in which the *Sc* incompatibility has been found within a species. The first record is that by Hardon (1967) in *Solanum pennellii*.

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