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Genetic Studies of Self Incompatibility in the Garden Chrysanthemum, *Chrysanthemum morifolium* Ramat[1]

L. W. Drewlow[2], P. D. Ascher and R. E. Widmer

Department of Horticultural Science, University of Minnesota, St. Paul, Minnesota (USA)

Summary. Self incompatibility was investigated in the hexaploid garden chrysanthemum, a member of Compositae. Nine sibling clones selected from a highly compatible cross were all self incompatible. 14.8% of the crosses between these sibs in diallel were compatible, but one sib, 67-111-42, accounted for 10 of the 12 compatible crosses. 67-111-42 was also more compatible than the remaining 8 sibs in crosses to other closely related plants. Crosses of the 9 sibs to 12 unrelated tester clones indicated that none were male or female sterile. Inbreeding via pseudocompatibility was successful in increasing homozygosity at the *S* loci. The percentage of compatible crosses obtained in 3 sib diallels of I_2 clones from crosses of 67-111-42 I_1 plants approached that of the original 9 × 9 diallel, but no one individual accounted for most of the compatible crosses. It was possible to separate the 9 sibs into 9 incompatibility patterns from the pollinations made in this study. The evidence suggests that the self-incompatibility reaction in the garden chrysanthemum is sporophytic and involves more than 1 locus.

Introduction

Self incompatibility, which is widespread in flowering plants, favors outbreeding by preventing the normal functioning of pollen tubes in styles of matched incompatibility specificity. Hughes and Babcock (1950) and Gerstel (1950) first described the sporophytic self-incompatibility reaction in *Crepis foetida* and *Parthenium argentatum* both members of Compositae. This incompatibility reaction is determined by a single locus with multiple alleles; the specificity of each pollen grain is determined by the diploid S genotype of the pollen producing plant. Sporophytic self incompatibility is characterized by allelic interactions ranging from independence to complete dominance and inhibition of pollen tubes occurs at the stigmatic surface. Other members of Compositae, *Cosmos bipinnatus* (Crowe, 1954), *Helianthus annuus* (Habura, 1957), *Chrysanthemum cinerariaefolium* (Brewer and Parlevliet, 1969), and *Carthamus flavescens* (Imrie and Knowles, 1971) have sporophytic self incompatibility. Fryxell (1957) and Mulford (1937) reported that *Chrysanthemum morifolium* was self incompatible but did not speculate as to the type of reaction.

Numerous crosses in the garden chrysanthemum breeding program at the University of Minnesota either failed to produce seed or produced only a few seeds per flower head, suggesting self incompatibility. Other members of Compositae having sporophytically determined self incompatibility are all diploids, while the garden chrysanthemum, *Chrysanthemum morifolium*, is a hexaploid. Thus we undertook a study with the garden chrysanthemum to determine the nature of the self incompatibility system in a hexaploid species.

Materials and Methods

Nine siblings were selected from progeny of the highly compatible cross Red Chief ♀ × 63-143-25 ♂ to study the self-incompatibility reaction of the garden chrysanthemum. The sibs were selected for similar bloom date, for numerous disk florets to produce pollen for crossing, and for red flower color to fulfill varietal breeding objectives.

Three flower heads were emasculated and pollinated for each cross made in the falls of 1968 and 1969. In the spring of 1969 and the fall of 1970 only 2 flower heads were pollinated. Crossing was conducted in a greenhouse that was screened to keep out insects. A night temperature of 60 °F was maintained. After the seeds ripened, seed number per flower head was recorded and compatibility of crosses was classified as follows: incompatible with under 10 seeds per head, possibly compatible with 10 to 20 seeds per head, and compatible with over 20 seeds per head.

In the fall of 1968 the 9 sibs were crossed in a diallel and backcrossed as the male to the male parent of the original cross. Lack of stock prevented backcrosses to the female parent. The 9 sibs were also crossed as males to 12 testers to determine whether any were male sterile. The above crosses were repeated in the spring and fall of 1969.

Previously, 67-111-69 and 67-111-141 were crossed as the male parents to 5 clones unrelated to each other and to the 9 sibs. From the progeny of these crosses ten clones were selected as the female parent for pollination with the 9 sibs of the 67-111 series. A synopsis of crosses conducted with the 9 sibs is presented in Fig. 1.

To study the effect of inbreeding on decreasing the number of heterozygous S loci, a small number of selfed seeds of 67-111-42 and 67-111-141 were planted. From these seeds 6 inbred (I_1) clones of 67-111-42 and 4 inbred (I_1) clones of 67-111-141 grew and flowered. These clones

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Fig. 1. A synopsis of crosses made to study the genetic nature of self incompatibility in the hexaploid garden chrysanthemum

Red Chief (♀) × 63-143-25 (♂)
↓
9 seedlings selected from a progeny of 194.
67-111-23, -42, -63, -69, -105, -112, -141, -179, -184
The 9 siblings were:
1. Crossed in diallel.
2. Backcrossed as male to 63-143-25.
3. Crossed as males to 12 unrelated testers.

5 unrelated clones (♀) × 67-111-69 (♂)
× 67-111-141 (♂)
↓
10 selected clones (♀) × original 9 sibs (♂)

67-111-141 ⊗
↓
4 inbred (I_1) clones.
1. Crossed in diallel.
2. Crossed to 4 of the original 9 sibs.

67-111-42 ⊗
↓
6 inbred (I_1) clones
1. Crossed to 6 members of the original 9 sibs.
2. Crossed in diallel in 1969.
↓
6 I_2 populations (2–9 individuals/population).
1. Diallel sib mating within I_2 populations.
2. Intercrossed I_2 populations.
3. Selected progeny backcrossed to 67-111-42 I_1 clones.

67-111-42 × (heat treated)
↓
One I_1 clone – added to the 6 I_1 clones obtained and crossed in diallel.

were crossed in diallel in the fall of 1969 and 1970. Some of the crosses could not be made because of differences in bloom date, insufficient pollen production or male sterile plants. In the fall of 1970 one I_1 clone of 67-111-42 obtained by heat treatment was added to the diallel.

Six I_2 populations consisting of 2 to 9 individuals from crosses of I_1 clones of 67-111-42 were sib mated in a diallel to study the self-incompatibility reaction within I_2 sib families. Crosses were also made between the 6 I_2 populations but again not all crosses were possible. The selected progeny from the 67-111-42 I_2 populations were backcrossed to the 67-111-42 I_1 clones. I_1 clones from 67-111-42 and 67-111-141 were also crossed to members of the original 67-111 series (Fig. 1).

Results

The 9 siblings selected from the cross Red Chief ♀ × 63-143-25 ♂ were classified as being self incompatible when the three replications of the diallel were combined (Fig. 2). The only seeds resulting from self pollinations occurred in the fall of 1968, when clones 67-111-42 and 67-111-141 produced 11 and 13 seeds respectively. No seed was produced from 45 self pollinations made in the spring or fall of 1969. Of 81 possible crosses in the diallel only 12 were classified as being either compatible or possibly compatible (Fig. 2). Pollinations made during the spring of 1969 resulted in very poor seed production probably because of high night temperatures in the greenhouse. Crossing out of season was discontinued after this initial attempt. The 9 sibs could be divided into 3 incompatibility patterns on the basis of the diallel crossing block (Fig. 5A).

Fig. 2. Determination of incompatibility in the diallel crosses of the 9 sibs of the 67-111 series

♀ \ ♂	67-111-23	67-111-42	67-111-63	67-111-69	67-111-105	67-111-112	67-111-141	67-111-179	67-111-184
67-111-23	–	–	–	–	–	–	–	–	–
67-111-42	–	–	–	+	±	+	+	+	–
67-111-63	–	–	–	–	–	–	–	–	–
67-111-69	–	+	–	–	–	–	–	–	–
67-111-105	–	+	–	–	–	–	–	–	–
67-111-112	–	+	±	–	–	–	–	–	–
67-111-141	–	+	–	–	–	–	–	–	–
67-111-179	–	±	–	–	–	–	–	–	–
67-111-184	–	–	–	–	–	–	–	±	–

– Incompatible – less than 10 seeds/head.
± Possibly compatible – 10 to 20 seeds/head.
+ Compatible – More than 20 seeds/head.

Fig. 3. Determination of incompatibility of crosses between the 9 original sibs and 10 related tester clones

♀ \ ♂	67-111-23	67-111-42	67-111-63	67-111-69	67-111-105	67-111-112	67-111-141	67-111-179	67-111-184
68-37-19	–	+	–	–	–	–	–	–	–
68-39-99	–	+	–	±	+	+	–	+	+
68-39-178	–	+	–	–	–	+	–	–	–
68-50-13	–	+	–	–	–	–	–	–	–
68-50-57	0	0	–	–	–	+	–	0	0
68-50-65	–	+	–	+	–	±	–	–	±
68-52-10	–	+	–	–	–	–	–	–	–
68-59-91	–	–	–	–	–	–	–	–	–
68-60-48	–	+	–	–	–	+	–	–	±
68-60-107	–	+	–	–	–	–	–	–	–

– Incompatible – Less than 10 seeds/head.
± Possible compatible – 10 to 20 seeds/head.
+ Compatible – More than 20 seeds/head.
0 Cross was not made.

In crosses using the 67-111 series as pollen parent to 10 clones selected from crosses having either

Fig. 4. Determination of incompatibility of crosses between the 9 original sibs of the 67-111 series and 12 unrelated tester clones

♀	♂ 67-111-23	67-111-42	67-111-63	67-111-69	67-111-105	67-111-112	67-111-141	67-111-179	67-111-184
'Minn Autumn'	+	±	−	−	+	−	−	+	+
'Redheart'	−	−	−	+	±	+	−	−	−
'Royal Pomp'	+	+	±	+	+	+	+	+	+
'Apache'	−	+	−	−	−	+	−	−	+
'King Kushion Red'	−	−	−	−	−	+	−	−	±
66-183-1	±	±	−	−	+	+	−	−	+
66-160-3	−	−	−	−	−	+	−	−	+
'Lipstick'	−	−	−	+	−	+	−	+	−
63-111-2	+	+	−	+	+	+	+	+	+
'Ruby Mound'	−	+	−	−	−	−	−	−	+
66-58-18	+	+	±	+	+	+	−	±	+
'Superior'	+	−	−	+	+	+	+	+	+

− Incompatible — Less than 10 seeds/head.
± Possibly compatible — 10 to 20 seeds/head.
+ Compatible — More than 20 seeds/head.

67-111-69 or 67-111-141 as the male parent, 23% of the crosses were compatible (Fig. 3). Three of the sibs, 67-111-23, 67-111-63, and 67-111-141, did not produce any compatible crosses when crossed to the 10 clones. In contrast 67-111-42 was classified as being compatible with 8 of the 9 closely related clones. The sibs could be separated into 6 incompatibility patterns on the basis of these crosses (Fig. 5B). Crosses to 12 unrelated tester clones indicated that none of the 9 sibs were male sterile (Fig. 4). Two of the sibs, 67-111-112 and 67-111-184, unilaterally compatible with one sib in the diallel, were compatible with 10 of the 12 tester clones. Clone 67-111-42, the most compatible sib in the 9×9 diallel, was compatible with only 7 of the 12 testers. Only 52.8% of the crosses made to tester clones were classified as compatible. Each of the 9 sibs could be separated into a distinct incompatibility-compatibility pattern from the test cross data (Fig. 5C). Apparently, each of the 9 sibs is of a different genotype.

Fig. 5. Diagram of incompatibility — compatibility patterns of the 9 sibs of the 67-111 series in the various crosses. All sibs underlined with a common line have the same pattern

A. Patterns obtained from the 9 × 9 diallel (Fig. 2)
$\underline{42}\quad\underline{23\quad 63\quad 184}\quad\underline{69\quad 105\quad 111\quad 141\quad 179}$

B. Patterns from crosses to 10 related testers (Fig. 3)
$\underline{42}\quad\underline{69}\quad\underline{112}\quad\underline{184}\quad\underline{105\quad 179}\quad\underline{23\quad 63\quad 141}$

C. Patterns from crosses to 12 unrelated testers (Fig. 4)
$\underline{42}\quad\underline{23}\quad\underline{63}\quad\underline{69}\quad\underline{105}\quad\underline{141}\quad\underline{112}\quad\underline{179}\quad\underline{184}$

The 9 clones of the 67-111 series were female fertile. Seven of the clones set 10 or more seeds per flower head pollinated (Fig. 2). Clones 67-111-23 and 67-111-63 set 2 seeds per flower head when crossed with 67-111-112 and 67-111-42.

Three (sibs 67-111-42, 67-111-112 and 67-111-184) of the 9 sibs of the 67-111 series were compatible when backcrossed as males to the male parent of the original cross. Reciprocal differences were obtained when the 67-111 series was used as the female parent in backcrossing. For example, the cross 63-143-25 × 67-111-42 produced 30 seeds per flower head while the reciprocal cross produced only 2 seeds per head.

None of the I_1 clones of 67-111-42 and 67-111-141 were self compatible. When the 6 I_1 clones of 67-111-42 were crossed in a diallel, 17.3% of the crosses were classified as being either compatible or possibly compatible. All crosses were made in the diallel of 67-111-141 inbreds with 28.1% either compatible or possibly compatible. Reciprocal differences were found in both of the diallel crossing blocks. For example, the pollination 69L-20-2 × 69L-20-1 set 16 seeds per head while the reciprocal set no seeds.

Six sib families were obtained from the crosses made with I_1 clones of 67-111-42. The sib families ranged in size from 2 to 9 individuals. Out of 26 clones only one clone (70D-113-1) was classified self-compatible. The percentage of compatible crosses ranged from 0 to 25% in diallel crosses made within the 6 sib families. The 3 largest sib families had 9.1%, 14.3%, and 15.3% compatible crosses. Again reciprocal differences were found.

Forty-five crosses were made among the 6 sib families. Only 6.5% of the crosses were compatible. A total of 22 crosses were made to I_2 clones using 4 of the 9 original sibs as the female parent 11 times and as the male parent 11 times. When the 4 sibs were used as the female parent only one cross was compatible, however, when they were used as the pollen parent 3 crosses were compatible and another possibly compatible. Sib 67-111-42 did not produce any compatible crosses when it was used as the pollen parent and only 1 possible compatible cross when it was used as the pistillate parent.

Twenty-five crosses were made between I_1 clones of 67-111-42 and 67-111-141, and 5 of the original 9 sibs. When the original sibs were used as the male parent, 6 out of 15 crosses were either compatible or possibly compatible. Ten crosses were made using the original sibs as female parent and 3 of these were classified as being compatible, all 3 involving 67-111-42.

Discussion

The garden chrysanthemum is a hexaploid (Dowrick, 1953), making possible 1, 2 or 3 loci for self incompatibility. If 3 loci are operative, each clone could have 6 S alleles and the progeny from a compatible cross as many as 12 different S alleles. Only 0.25% of the crosses would be expected to be compatible in a progeny from a compatible cross of individuals having 3 loci with independent action and a sporophytic incompatibility system. This compares with 25% compatible crosses expected with 1 locus and 2.8% compatible crosses with 2 loci operating. The 14.8% compatible crosses in the 9×9 diallel of the siblings was higher than expected from a 2 or 3 loci sporophytic system but lower than expected from one with only 1 self incompatibility locus.

In *Dactylis glomerata* L., a tetraploid grass with the 2 loci gametophytic self incompatibility system, cross compatibility was less than the 43.8% expected from a diploid with doubly heterozygous parental plants but greater than the 12.5% expected from the tetraploid (Lundqvist, 1969). Lundqvist suggested the higher than 12.5% cross compatibility could have resulted from the presence of self fertility alleles or the occurrence of double reduction in the population.

Sib 67-111-42 was responsible for 10 of the 12 compatible crosses in the diallel. When 67-111-42 was backcrossed as the male to the original male parent and crossed to closely related clones, a high percentage of compatible crosses were obtained, suggesting that 67-111-42 was different from the other 8 sibs. The presence of a self fertility allele in 67-111-42 is not probable. Plants of 67-111-42 were self incompatible as were the 6 I_1 plants obtained from selfing 67-111-42 and all except 1 of the I_2 progeny obtained from crosses among the I_1 plants.

The presence of homozygosity at one or more of the S loci operating in 67-111-42 could account for the high degree of compatibility with closely related clones. Brewbaker (1955) using tetraploid white clover from a colchicine doubled diploid found that crosses with single allele differences expected to be incompatible were compatible. He assumed that crossing over between the S locus and the centromere during quadrivalent association followed by double reduction resulted in homozygous S alleles. Dowrick (1953) reported that quadrivalent associations occur in the chrysanthemum. Double reduction may have reduced the number of different S alleles in 67-111-42 making it compatible with more of its sibs. Another mechanism that results in homozygosity of the S alleles is dominance among S alleles in the sporophytic system (Lewis, 1954). Reciprocal differences in cross compatibility were found indicating that dominance among S alleles was present.

An attempt to separate chrysanthemum clones into incompatibility classes by inbreeding 67-111-42 was not successful. Inbreeding was probably successful in increasing the homozygosity of the S loci as the percentage of compatible crosses obtained from 3 sib diallels of I_2 clones from crosses of 67-111-42 I_1 plants approached that of the original 9×9 sib diallel. The percentage of compatible crosses in the 9×9 diallel was inflated by the high compatibility of 67-111-42 (10 of the 12 compatible crosses), whereas, no individual clones in the I_2 sib families accounted for most of the compatible crosses.

Considering that most of the compatible crosses in the 9×9 diallel involved 67-111-42, which seemed to be different from the other 8 sibs, the self-incompatibility reaction in the garden chrysanthemum did not appear to be of the 1 locus multiple allelic sporophytic type found in other members of Compositae (Crowe, 1954) and Cruciferae (Bateman, 1955). The 14.8% compatible crosses obtained in the 9×9 diallel is probably an overestimation resulting from 67-111-42. Separation of the 9 sibs into 9 distinct incompatibility patterns by the diallel and by outcrossing, as well as the fact that only 52.8% cross-compatibility occurred in outcrossing support the theory that more than 1 locus is operating. The reaction appeared to be sporophytic in nature as it is in other members of Compositae. Inhibition of the pollen tubes occurred at the stigmatic surface, reciprocal differences were found in several of the diallel crossing blocks, and the self incompatibility reaction appears to be stable in a polyploid.

Information on the nature of self incompatibility in a hexaploid and the evolution of self incompatibility after changes in ploidy level can be obtained from the study of self incompatibility in the garden chrysanthemum. Sterility is not a problem with *C. morifolium* as it was with hexaploid *Ipomoea* where Martin (1968) resorted to diploid species to study self incompatibility. The best approach for continued study appears to be inbreeding. By increasing the homozygosity at some of the S loci, progeny should segregate into incompatibility classes allowing the determination of the functional relationship of loci to each other.

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L. W. Drewlow
Bodger Seeds Ltd.
Box 607
Lompoc, Calif. 93436 (USA)
P. D. Ascher
R. E. Widmer
Department of Horticultural Science
University of Minnesota
St. Paul, Minnesota 55101 (USA)