

# INHERITANCE OF SELF-INCOMPATIBILITY IN WILD SUNFLOWER<sup>1</sup>

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## Abstract

Crosses and reciprocals were made between wild sunflower (Helianthus annuus L.) and the cultivated line P-21, which is self-compatible and consists of equal numbers of the genotypes msms (male-sterile) and Msms (male-fertile). The wild sunflower set no seed when selfed.

F<sub>1</sub> plants were self-incompatible and male-fertile. Pairs of F<sub>1</sub> plants were crossed inter se to give F<sub>2</sub>, and were backcrossed to both parents. F<sub>2</sub> were classified for self-incompatibility and male-sterility. Cross-compatibility of F<sub>1</sub> plants and F<sub>2</sub> segregations indicated a single locus, S, for self-incompatibility, independent of ms.

Seven wild plants were crossed in all combinations. Results indicated that the self-incompatibility is determined sporophytically, and that at least five different S alleles were involved. Dominance of alleles was expressed in the pollen, and independent action in the style.

It is well known that a system of self-incompatibility contributes to the high level of cross-pollination in open-pollinated cultivars of sunflower (Helianthus annuus L.). Habura (1957) reported that a sporophytic incompatibility system was present in cultivated sunflower. He concluded that at least two multiallelic loci governed self-incompatibility, and expression was influenced by physiological factors. Ivanov (1975) found that reactions in both the pollen and stigma were involved, with the zone of incompatibility being at the surface of the stigma. The self-incompatibility was not always complete, allowing some selfing. Vranceanu et al (1974) concluded that self-incompatibility in cultivated sunflower is complex in both expression and inheritance. Luciano et al (1965) reported that the percentage of outcrossing varied in different genotypes over a range of 0 to 100%. Their data showed dominance, or possibly overdominance, for self-incompatibility in one cross, and overdominance for self-compatibility in another cross, with environment strongly influencing levels of self-incompatibility. Working with pure lines, Russell (1953) found that the average seed set under selfing bags varied from 4.2 to 155.3% of the average number of seeds set in heads without selfing bags. Lofgren and Nelson (1977) crossed a self-incompatible line that produced some seed with one that was fully self-compatible, and found that most of the selfed F<sub>2</sub> plants produced more than 50 seeds. They assumed a dihybrid inheritance with a ration of 12:3:1 (70% had fewer than 200 seeds per head, 23% had 200 to 350 seeds, and 7% had over 350 seeds per plant).

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In Israel, Pinthus (1959) found that the production of selfed seed in sunflower heads was much greater when temperatures were low than when they were high. When flowering occurred in November (average daily maximum temperature 16.5°C) 86% of the heads produced selfed seed with an average of 59.5 seeds per head, whereas when flowering occurred in August (average daily maximum temperature 27.5°C) the values were respectively 4% and 0.42 seeds per head.

Heiser (1954) reported that many of the wild species were fully self-incompatible. Our studies of the inheritance of fatty acid composition in selected materials of the wild version of H. annuus showed that those materials were fully self-incompatible. It was then decided to determine the inheritance of that incompatibility.

#### Materials and Methods

Male-sterile and male-fertile plants of a self-compatible line, P-21, derived from the cultivar Pereodivk, were crossed to nine wild plants in a greenhouse at Davis in 1973. Wild plants of H. annuus were grown from one collection obtained from Ontario, Idaho, in 1971, and accessioned UC71-34. In Spain that collection was identified as A0-6, for "alto oleic" collection No. 6. P-21 carries the allele ms for male-sterility, found by Leclercq (1966). If plants of genotype msms are pollinated by Msms plants, the progeny consists of equal numbers of the genotypes Msms (male-fertile) and msms (male-sterile).

F<sub>1</sub> plants from the cross, msms P-21 x wild, and its reciprocal, wild x Msms P-21, were grown in a greenhouse in the spring of 1974 at Davis. Most were branched, but a few had only a single head. Incompatibility was established by permitting selfing of one head per plant (or half of the head where there was only one head). Incompatibility was evident at an early stage because the styles remained erect after pollination whereas the styles shriveled shortly after pollination when selfs or crosses were compatible. Incompatibility was verified by determining seed production at maturity.

Data for this study were obtained from crosses involving P-21 and one wild plant, A0-6-8. Because all of the above F<sub>1</sub> plants were self-incompatible, ten with several heads were intercrossed in pairs to give F<sub>2</sub> seed. Selected F<sub>1</sub> plants were crossed reciprocally to both parents to give BC<sub>1</sub> seed.

Where female parents were male-fertile and self-compatible, they were emasculated. Emasculations were made daily on all opening flowers by removing anthers with tweezers and rinsing the flowers with water.

The following populations were studied in the field at Cordoba, Spain, in 1976:

F<sub>2</sub>: (msms P-21 x wild F<sub>1</sub>) x (msms P-21 x wild F<sub>1</sub>)

F<sub>2</sub>: (wild x Msms P-21 F<sub>1</sub>) x (wild x Msms P-21 F<sub>1</sub>)

F<sub>2</sub>: (msms P-21 x wild F<sub>1</sub>) x (wild x Msms P-21 F<sub>1</sub>)

BC<sub>1</sub>F<sub>1</sub>: (msms P-21 x wild F<sub>1</sub>) x Msms P-21

BC<sub>1</sub>F<sub>1</sub>: (msms P-21 x (msms P-21 x wild F<sub>1</sub>))

BC<sub>1</sub>F<sub>1</sub>: (msms P-21 x wild F<sub>1</sub>) x wild

Heads were bagged before they flowered. Plants were identified as male-sterile or male-fertile during flowering. After harvest of achenes and removal of those that were empty, plants were classified as compatible (one or more achenes with seeds) or self-incompatible (no achenes with seeds).

A total of seven wild plants, each from a different location, were used in diallel crosses in a greenhouse at Cordoba, Spain, in the spring of 1976. The sources and accessions of the wild plants were: Woodland, CA, UC73-1; Phoenix, AZ, UC73-28; Ontario, ID, UC71-34; Davis, CA UC73-12; Houston, TX, Collection No. 073; Mulford, UT, HSP07-167; and Boron, CA, HSP07-404. The last three samples were provided by B.H. Beard, USDA. Crosses were made by rubbing heads together or by transferring pollen with a cotton swab on each of several days after the first whorl of flowers opened. All heads used in crosses were examined for levels of seed production to establish cross-incompatibility relationships.

Compatibility appeared to be higher in a greenhouse if populations were grown during the fall, spring and winter when temperatures were reduced. Consequently plants with 5 or more seeds per head were considered to be self- or cross-compatible. In the field during the summer when temperatures were high seed set was reduced, and plants which produced one or more seeds were considered to be self- or cross-compatible.

### Results

Table 1 gives the results from paired crosses of 10 self-compatible  $F_1$  plants obtained from crosses of male-sterile P-21 to the wild plant A0-6-8. Where five seeds or more were obtained from a cross, it was considered to be compatible.

TABLE 1. Compatibility of  $F_1$  sunflower plants obtained from a cross of male-sterile P-21 with one wild plant.<sup>1</sup>

Number of $F_1$ plant used as female	Number of $F_1$ plant used as a male									
	1 $\underline{Sx}^2$	2 $\underline{Sx}$	3 $\underline{Sy}$	4 $\underline{Sy}$	5 $\underline{Sx}$	6 $\underline{Sy}$	7 $\underline{Sy}$	8 $\underline{Sx}$	9 $\underline{Sy}$	10 $\underline{Sy}$
1 $\underline{Sx}^2$	-	-	+	+	-					
2 $\underline{Sx}$	-	-	+	(4)		+	+		+	+
3 $\underline{Sy}$		+	-	-	+	-	-		(3)	
4 $\underline{Sy}$	+		-	-	+		-	+	-	-
5 $\underline{Sx}$	-		+	+	-	+	+		+	+
6 $\underline{Sy}$		+	-			-	-			-
7 $\underline{Sy}$	+	+	-		+	(3)	-	+	-	-
8 $\underline{Sx}$	(3)	-	+	+		+	+	-		
9 $\underline{Sy}$	+	+	-	-		-			-	-
10 $\underline{Sy}$	+	(4)	-	-	+					-

- 1 Compatibility relations: + = compatible; - = incompatible.
- 2 Incompatibility allele assumed to have been obtained from the wild plant. The other allele in all cases is assumed to be  $\underline{Sf}$ .
- 3 Exceptional case, where 5 or fewer seeds were obtained when none were expected.
- 4 Exceptional case, where no seed was produced when some were expected. In all cases, pollinations were made on late heads with stored pollen.

Because all  $F_1$  plants from msms P-21 x A0-6-8 were self-incompatible, it is assumed that the wild plant carried no allele for self-compatibility. It is assumed that the genotypes of the wild plant and P-21 were respectively SxSy and SfSf. On that assumption there should be two groups of  $F_1$  plants, one with the genotype SxSf and the other with the genotype SySf. There should be equal numbers of each. Crosses of the same genotype should be incompatible, and crosses of different genotypes should be compatible. A cross and its reciprocal should behave the same.

With very few exceptions, the data of Table 1 support the assumption. Three of the exceptions can be explained as due to suspected transfer of a few pollen grains by insects. Another possibility is pseudocompatibility due to the action of modifier genes. The other two exceptions could have been due to our use of stored pollen, which might have been nonfunctional, thus resulting in no crossed seeds where they were expected.

There were about equal numbers of the two incompatibility genotypes among  $F_1$  plants, namely 4 SxSf and 6 SySf.

Where  $F_2$  generations were grown from the above  $F_1$  plants, all stemming from crosses of male-sterile P-21 and a single wild plant A0-6-8, they should result from intercrosses of  $F_1$  plants with the genotypes SxSf and SySf. The  $F_2$  should have equal numbers of the following genotypes: SxSy:SxSf:SySf:SfSf. The expected ratio would be 3 self-incompatible to 1 self-compatible. If the P-21 parent were male-sterile, the  $F_2$  would segregate three male-fertile (Ms) to one male-sterile (msms). With independent inheritance for the two loci, the expected frequency would be in the ratio of 9 self-incompatible male-fertile; three self-compatible, male-fertile; 4 male-sterile. The actual frequency was 456:122:181, not a very good fit to the 9:3:4 ratio (P in a chi-square test was 0.05 to 0.10, and P in a heterogeneity chi-square test was 0.25 to 0.50). Two additional  $F_2$  populations listed under Materials and Methods gave good fits to expected ratios.

Table 2 gives data from the  $BC_1F_1$  population obtained from (msms P-21 x A0-6-8  $F_1$ ) x msms P-21. There was a good fit of the data to the expected ratio. In two other backcross populations where was good agreement between actual data and expected ratios.

TABLE 2. Actual and expected numbers of  $BC_1F_1$  plants in male-fertility and compatible classes.  $BC_1F_1$  plants from (male-sterile P-21 x A0-6-8) x male-fertile P-21.

	Number of plants	
	Actual	Expected
Self-incompatible, male-fertile	71	66.38
Self-compatible, male-fertile	71	66.38
Male-sterile	35	44.24

P in chi-square goodness-of-fit test to a 3:3:2 ratio = 0.20 - 0.50.

Table 3 gives the results from crossing seven wild sunflower plants. All seven plants were self-incompatible. The interplant incompatibility relationships can be explained if alleles at a single locus were involved, and alleles show dominance in the pollen and independent action in the style. The dominance relationships are assumed to be as follows:  $\underline{S1} > \underline{S2} > \underline{S3} > \underline{S4} > \underline{S5}$ . One allele in each of plants 6 and 7 was not positively identified. It is assumed that the system is sporophytic in nature because of differences among reciprocal crosses.

TABLE 3. Compatibility and assigned genotypes of seven plants of wild sunflower used in a diallel cross. Greenhouse, Cordoba, Spain, 1976.<sup>1</sup>

Female plant		Plant number and dominant allele of the male parent <sup>2</sup>						
Plant Number	Genotype	1( <u>S2</u> )	2( <u>S1</u> )	3( <u>S3</u> )	4( <u>S4</u> )	5( <u>S1</u> )	6( <u>S3</u> )	7( <u>S5</u> )
1	<u>S2S5</u>	-	+	+	+	+	+	-
2	<u>S1S2</u>	-	-	+	+	-	+	+
3	<u>S3S4</u>	+	+	-	-	+	-	+
4	<u>S4S5</u>	+	+	+	-	+	+	-
5	<u>S1S4</u>	+	-	+	-	-	+	+
6	<u>S3Sx</u> <sup>3</sup>	+	+	-	+	+	-	+
7	<u>S5Sx</u> <sup>4</sup>	+	+	+	+	+	+	-

1 Compatibility relations: + = compatible; - = incompatible.

2 Assumed dominance relations in the pollen:  $\underline{S1} > \underline{S2} > \underline{S3} > \underline{S4} > \underline{S5}$ .

3 Sx can be S3 or another allele recessive to S5.

4 Sx can be S5 or another allele recessive to S5.

Plant 6 was assigned the genotype S3Sx, where Sx could be S3 or any allele recessive to S5. Likewise, in plant 7, Sx could be S5 or any allele recessive to S5. Sx could be the same allele in plant 6 as in plant 7, e.g., S6, if it were recessive to both S3 and S5. In other words, if plants 6 and 7 are not homozygotes there must be a series of six or seven incompatibility alleles.

Assuming the genotypes assigned to plants 1 to 5, homozygotes should be obtained from crosses of the following plants: 1 x 2, giving S2S2; 1 x 4, giving S5S5; 3 x 5, giving S4S4; 4 x 1, giving S5S5; 4 x 3, giving S4S4; 4 x 5, giving S4S4; and 5 x 3, giving S4S4. This means that 7 of the 15 compatible crosses should give homozygotes, a rather high value.

### Discussion

The results show a pattern of inheritance for incompatibility very similar to that reported by Imrie and Knowles (1971) for a wild safflower species, *Carthamus flavescens* Spreng. *C. flavescens* is very similar to the cultivated species, *C. tinctorius*, and the two species intercross readily to produce an F<sub>1</sub> plant that has normal meiosis and normal fertility of outcrossed to related materials. *C. tinctorius* is a self-compatible species, although genotypes do vary in levels of outcrossing. In both sunflower and safflower the following similarities prevailed: the wild version was uniformly self-incompatible; the system of incompatibility was sporophytic; inheritance was governed by a series

of alleles at a single S locus; dominance was expressed in the pollen; and independent action of alleles was expressed in the style.

In safflower the allele for self-compatibility, from C. tinctorius, was intermediate in a dominance series of S alleles. This meant that some F<sub>1</sub> plants of crosses of C. flavescens and C. tinctorius were self-compatible and some were self-incompatible. In limited tests, the Sf allele of sunflower was always recessive to the S alleles for self-incompatibility in the wild type.

In safflower the site of the incompatibility reaction is the surface of the stigma (Imrie and Knowles, 1971), the same as in sunflower (Ivanov, 1975).

In some genotypes of safflower the allele Sf for self-compatibility was dominant to some incompatibility alleles in a greenhouse, but not in the field, presumably due to temperature effects. It is believed that temperature may have been involved in the production of a few seed on presumed self-incompatible sunflower plants under greenhouse conditions.

In the wild plants used in this study there were at least five, and possibly six or seven, incompatibility S alleles.

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