INCOMPATIBILITY STUDIES IN COSMOS BIPINNATUS

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INTRODUCTION

STERILITY phenomena have been studied extensively in many genera—in Antirrhinum by BAUR (1919), FILZER (1926), and SIRKS (1927); in Capsella by SHULL (1929) and by RILEY (1932); in Linaria by CORRENS (1916) and SIRKS (1927); in Nicotiana by EAST (1915a, 1915b, 1918, 1919a, 1919b, 1919c, 1923, 1927), by EAST and PARK (1917, 1918), EAST and MANGELSDORF (1925, 1926) ANDERSON (1924), SIRKS (1927), EAST and YARNELL (1929); in Petunia by TERAO (1923) and by TERAO and U (1929); in Prunus by DORSEY (1919), SUTTON (1918), and CRANE (1925); in Verbascum by SIRKS (1917, 1926, 1927); in Veronica by LEHMAN (1919, 1922) and by FILZER (1926) and others.

But a thorough search of the available literature fails to reveal any reports on sterility in *Cosmos bipinnatus*. In fact, there seems to have been almost no study made on the sterility in any compositae. In the only Cosmos investigations recorded, MIYAKE, IMAI, and TABUCHI (1926, 1927) studied inheritance of flower color, pollen color, and floral characters but made no mention of sterility.

MATERIAL AND METHODS

In the course of breeding new varieties of Cosmos, sterility was first noted when attempts to self F_1 plants repeatedly failed to produce seed.

In 1936 a cross was made between large-flowered pink (from the variety "Sensation") and small-flowered crimson to develop a large-flowered crimson strain. The F_1 was grown in 1937 and consisted entirely of large-flowered crimson-flowered plants, indicating that crimson was dominant over pink, as had been previously recorded by MIYAKE, IMAI, and TABUCHI, and also indicated that large-flowered was dominant over small-flowered. Attempts to self individual plants in the F_1 population showed that they were all self-sterile. Therefore, four plants (well isolated from other Cosmos) were allowed to intercross at random. This produced a normal set of seed.

In 1938 a large F_2 population was grown and this population segregated for flower color and flower size. Crimson, pink, and white-flowered plants were noted, indicating that the original pink-flowered parent was heterozygous for flower color, and suggesting the existence of two factors for color, one a basic color factor and the other a diluting factor. The segregation in flower size was approximately three large-flowered to one small-flowered. (Of 108 plants, 74 were Crimson, 17 Pink, and 17 White.)

Twenty-five large-flowered crimson plants were selected and numbered and each was individually enclosed in a cheesecloth "cage" to prevent insect pollination (all open flower-heads and seed pods were removed prior to caging). On each plant several heads were left unpollinated as checks, and it was found that none of these unpollinated flower heads produced any seed. This indicated both that all plants were self-sterile and that the cages were effective in preventing any uncontrolled pollination.

After the sterility of all the plants was established, crosses were made between all the 300 possible combinations of the 25 plants.

In Cosmos the ray florets are neutral and all of the disc florets are hermaphroditic. Anthesis of the disc florets occurs centripetally and requires about a week for completion. Emasculation was unnecessary because of their established self-sterility. Pollen was shaken from each of the male parents into an individual sterile glassine bag and in each case it was applied by camel's-hair brush to caged flower heads in which the center disc florets had just opened. At this stage, practically all of the pistils in the disc were receptive.

Within a week or ten days following pollination, its effectiveness could be determined. It was ascertained at once that some combinations were completely fertile while others were more or less completely sterile. The sterile combinations were repeated in order to ascertain whether failure to set seed was the result of inter-sterility or faulty technique in crossing. It is significant that in all cases repetition gave approximately the same negative results.

When the seed ripened, each cross was picked separately and the number of seeds recorded.

(Inasmuch as the work was done primarily to develop a large crimson strain, the 25 selections were backcrossed with a multiple recessive (small-flowered white) to determine the genetic constitution of each. No detailed sterility studies were made in this connection, however.)

RESULTS

As noted above, all 25 selections made in the F_2, as well as the F_1 population, showed complete self-incompatibility.

It was further noted that some combinations were completely cross-fertile, producing nearly a full head of seed, while others were cross-incompatible, producing no seed at all, or, at best, very few seeds.

Moreover, when a plant, A, was incompatible with each of two other plants, B and C, then B and C were also cross-incompatible with each other. By further studies of the results, it was noted that the 25 selections fell into four intra-incompatible but inter-compatible classes as shown in table 1.

TABLE 1

*Number of seeds set per cross—arranged according to sterility grouping (Failures (under 10) repeated).**

Sel. No.	2	3	6	8	9	10	13	14	18	19	22	1	4	5	7	17	20	21	25	11	15	23	24	12	16
2		1	0	0	0	1	11	0	1	0	1	40	66	80	72	30	73	67	66	67	28	70	78	83	60
3			0	0	1	1	0	0	0	0	3	43	38	62	48	43	41	50	74	43	50	81	76	35	60
6				0	0	0	3	1	2	0	0	78	58	89	64	40	54	72	39	15	59	7	54	54	58
8					0	0	0	0	0	2	0	81	30	64	54	23	32	50	36	61	15	113	40	53	49
9						2	0	8	2	0	3	68	49	67	70	62	51	39	47	61	49	53	55	73	75
10							0	3	2	5	0	21	51	73	70	53	60	39	46	79	38	59	30	52	58
13								1	1	0	0	71	71	52	82	59	75	82	67	88	6	27	00	86	51
14									1	3	0	86	58	37	26	53	101	60	37	23	76	53	63	89	16
18										0	0	30	10	45	30	22	3	5	6	38	56	17	69	58	49
19											0	77	57	50	38	65	64	67	62	69	52	40	40	79	82
22												89	21	70	36	46	27	66	48	89	2	66	47	75	74
1													0	2	5	0	0	1	3	78	36	61	56	45	66
4														0	2	0	9	0	4	60	49	33	55	45	40
5															2	0	0	2	39	106	90	64	70	88	77
7																3	0	5	0	49	49	44	29	39	51
17																	3	0	1	106	7	51	54	81	61
20																		0	1	58	9	67	71	71	42
21																			5	82	62	50	39	72	50
25																				87	50	68	63	65	43
11																					2	4	5	75	81
15																						0	2	90	0
23																							2	92	55
24																								70	66
12																									0
16																									

* In each case where a set of less than 10 seeds was obtained, the cross was repeated. The figures represent combined set in such cases.

Average number of seeds set in compatible crosses— 55.37.

Average number of seeds set in incompatible crosses— 1.79.

Any combination which produced five seeds or less was considered as an incompatible combination. The use of the term "incompatibility" is in no way to be taken to mean the complete failure to set any seed at all, for under certain conditions, such as the end of the flowering period, a few seeds may occasionally be obtained, a manifestation of what EAST (1923) has called "pseudo-fertility." Combinations producing 15 seeds or more were considered as compatible combinations. Those producing 6 to 14 seeds were considered as questionable. On this basis, the results are tabulated as in table 2, incompatibility being represented by a minus sign (–), and compatibility by a plus sign (+), with the questionable combinations symbolized by a question mark (?). According to this classification, the average number of seeds set by the incompatible combinations is 1.79, while the average number set by the compatible combinations is 55.37. This is more significant when it is taken into consideration that many of the incompatible combinations were repeated and the figure 1.79 thus represents, in many cases, more than one pollinated head of some of the combinations.

TABLE 2

Showing inter-compatibility and intra-incompatibility in Cosmos.

Sel. No.	2	3	6	8	9	10	13	14	18	19	22	1	4	5	7	17	20	21	25	11	15	23	24	12	16
2		–	–	–	–	–	?	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3			–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6				–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	?	+	+	+
8					–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9						–	–	?	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10							–	–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13								–	–	–	–	+	+	+	+	+	+	+	+	+	?	+	–	+	+
14									–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18										–	–	+	+	+	+	+	–	–	?	+	+	+	+	+	+
19											–	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22												+	+	+	+	+	+	+	+	+	–	+	+	+	+
1													–	–	–	–	–	–	–	+	+	+	+	+	+
4														–	–	–	?	–	–	+	+	+	+	+	+
5															–	–	–	–	+	+	+	+	+	+	+
7																–	–	–	–	+	+	+	+	+	+
17																	–	–	–	+	?	+	+	+	+
20																		–	–	+	?	+	+	+	+
21																			–	+	+	+	+	+	+
25																				+	+	+	+	+	+
11																					–	–	–	+	+
15																						–	–	+	–
23																							–	+	+
24																								+	+
12																									–
16																									

(–) = from none to 5 seeds per cross.
(?) = from 6 to 14 seeds per cross.
(+) = from 15 and up.

DISCUSSION AND CONCLUSION

EAST (1927) and EAST and MANGELSDORF (1925) studied the progeny of reciprocal crosses between two self-incompatible plants in Nicotiana (*N. forgetiana*×*N. alata*) and reported three incompatible classes all inter-compatible but intra-incompatible. The progeny of a cross between members of any two classes consisted of equal proportions of two of the three, the class to which the female parent belongs not being included. Accordingly, when class X was pollinated by class Y or class Z, class X did not appear among the progeny, it consisting only of classes Y and Z in equal numbers. Three alleles for incompatibility, s_1, s_2, s_3, were assumed to explain these results. The genotypes of the three classes were represented as follows:

class X—s_1s_3
class Y—s_1s_2
class Z—s_2s_3

A plant gives stimulus only to pollen carrying genes other than its own. Therefore, pollen carrying the s_2 factor is the only type which will fertilize s_1s_3 plants (class X), and from such a pollination, half of the progenies are s_1s_2 (class Y) and half are s_2s_3 (class Z).

Further studies by EAST and YARNELL (1929) resulted in the isolation of 15 such alleles.

BAUR (1919), FILZER (1926), and SIRKS (1927) studied sterility in *Antirrhinum hispanicum* and found that the mode of inheritance was similar to that of Nicotiana. Other species in which sterility has been studied and found to be inherited in the same manner as Nicotiana are: *Capsella grandiflora* by SHULL (1929); *Linaria vulgaris* by CORRENS (1916) and SIRKS (1927); *Petunia violacea* by TERAO (1923) and TERAO and U (1929); *Verbascum phoeniceum* by SIRKS (1917, 1926, 1927); *Veronica syriaca* by LEHMAN (1919, 1922) and FILZER (1926); and others.

Our data seem to indicate that incompatibility in Cosmos is inherited in a manner similar to that in Nicotiana. Since 23 of 25 plants fell into three groups, we assume three alleles, s_1, s_2, and s_3, to be present in our population, and that the constitution of the three groups were s_1s_2, s_1s_3, and s_2s_3; and that a plant gives stimulus only to pollen carrying genes other than its own, and further that probably a fourth gene, s_4, was present in the remaining two plants, since they were compatible with the other 23 plants but not with each other.

According to the Nicotiana scheme, when the original parents of a controlled cross (not reciprocal) have three alleles, one allele in common, the genotype of the female parent does not recur in the F_1, but if the F_1 plants are allowed to intercross at random, it occurs in half the plants in the F_2, with the other two combinations each making up one-fourth of the population. Consequently, we should expect in the F_2 50 percent of the s_1s_2 (female parent) and 25 percent each of s_1s_3 and s_2s_3 plants.

In our investigation of 23 plants, 11 fell in one group, 8 in another, and

4 in the third group. On the suggested scheme, the expectations would have been 11.5 in the one group and 5.75 in each of the other two.

However, since the F_1 population was small, it is highly possible that instead of two plants each (that is, equal numbers) of s_1s_3 and s_2s_3, there were instead three s_1s_3 plants and one s_2s_3 plant. Such proportions would give in the F_2 11.5 s_1s_2, 8.625 s_2s_3, and 2.875 s_1s_3. This expectancy is more in harmony with the observed data.

The possibilities of the original parents differing by two genes is precluded by the existence of only three certain groups in the F_2. Four sterility factors would give in the F_2 six classes, a condition which did not occur.

The fourth group of two plants was assumed to carry a factor, s_4, since it was compatible with all the plants in the other three groups. The s_4 factor probably was not present in the original parents of the cross. It could easily have been introduced through contamination in the F_1, since the F_1 population was open pollinated.

When the F_2 selections were back-crossed with small-flowered white plants in unrelated lines, plants were found which were compatible with all 25 selections. This indicates the existence of at least a fifth factor for sterility.

Further investigations are being conducted to study the behavior of the various combinations in the third generation.

SUMMARY

1. *Cosmos bipinnatus* is entirely self-sterile.

2. Some plants are inter-fertile while others are inter-sterile.

3. The existence of four allelomorphs for cross-sterility has been established; and the back-cross data indicate the presence of at least an additional fifth factor.

4. Any plant gives stimulus only to pollen carrying genes other than its own, and plants of the same genotype are cross-incompatible.

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