GENETIC STUDY OF THE OCCURRENCE OF MALE FERTILE PLANTS IN CYTOPLASMIC MALE STERILE LINES OF SUNFLOWER

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INTRODUCTION

At present, the only cytoplasmic male sterility used in sunflower commercial hybrid seed production is that provided by the cytoplasm of *Helianthus petiolaris* Nutt. in the presence of the genome of *H. annuus* L. (LECLERCQ, 1969).

However, an important difficulty encountered in using this type of cytoplasmic male sterility is constituted by the frequent occurrence of male fertile plants in its progenies, when the male sterile line A os backcrossed to its recurrent partner B. (LECLERCQ, 1971; ENNS, 1972; VRANCEANU and STOENESCU, 1976).

The present study proposed itself to elucidate the genetic nature of this phenomenon, with the view of establishing the procedure of obtaining entirely sterile progenies.

MATERIALS AND METHODS

Five germplasm entries (CF I-CF V), the genealogy of which is presented in Table 1, Have been used as cytoplasmic male sterility sources. The number of Romanian male sterile lines investigated, in which pollen fertility occurred in BC_1—BC_9, amounted to 561. The normal lines that produced fertile or partial fertile F_1 generations when crossed to cytoplasmic male sterility sources, have been considered as pollen fertility restorers, and were not included in this study.

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The genetic control of the occurrence of male fertile plants in BC_1 was studied in six cytoplasmic male sterile lines of different origin (Table 2). The occurrence of pollen fertility in late backcross generations was studied in two cytoplasmic male sterile lines, S-1358 A (BC_4) and P-1380 A (BC_5), and four successive selfing generations (S_1—S_4) of the respective fertile plants were analysed. Ten fully fertile S_3 sublines originating from S-1358 A and P-1380 A (Table 3) were included in a complex system of crosses, together with the male sterile lines V-2612 A and EL-8455 A and their fertile counterparts V-2612 B and EL-8455 B.

The effect of the repeated selection of the individual crosses "sterile x fertile" that gave fully sterile progenies was examined in four generations of the lines S-1358 (A x B) and P-1380 (A x B).

The investigations were conducted at Fundulea in 1975-1979 both in field nursery and green houses. Crosses and self pollinations were performed under paper or cheese cloth bags. The fertile plants used as female parents were emasculated manually. Pollen fertility was estimated visually by direct observations or microscopically by staining with carmin acetic. Sunflower plants with 0-10% fertile pollen were classified as sterile, with 10-85% as partial fertile and with 85-100% as fertile.

RESULTS AND DISCUSSION

The occurrence of male fertile plants was detected in 5.7% of the cytoplasmic male sterile lines under investigation (Table 1), their frequency being higher in the first backcross generations (BC_1—BC_5).

The male sterility sources CF I-CF V presented a similar percentage of progenies with fertile plants (4.08-7.32%), indicating that their S cytoplasm is identical.

In the case of the inbred lines with S cytoplasm, in which the occurrence of pollen fertility was observed just from the first backcross, the segregation ratios indicate the existance in the genotype of B lines of a small number of recessive genes which determine this phenomenon (Table 2). Thus, the occurrence of male fertile plants within the cytoplasmic male sterile lines V-5317 A, O-7640 A and R-8840 A has proved to be controlled by two complementary recessive genes, the generations F_1 being completely sterile and the segregation ratios in BC_1 significantly close to the theoretical ratio 1 fertile: 3 sterile. The segregation ratio 1:7 of the line O-7602 A suggests the effect of three recessive complementary genes. As

TABLE 1

The number of cytoplasmic male sterile lines in which pollen fertility occurred in BC_1—BC_9

Cytoplasmic male sterility sources		Number of cms lines analysed	Backcross generations									
			BC_1	BC_2	BC_3	BC_4	BC_5	BC_6	BC_7	BC_9	Total	%
CF I	Cern.-Ce-2-1-1-C7-1	116	1	1	1	1	1	—	—	1	6	5.17
CF II	A-9345-M_2-1-3-3-C1-1	110	1	2	1	2	—	1	1	—	8	7.27
CF III	V-8883-M_6-2-3-2-1-C4-2	41	—	1	—	1	1	—	—	—	3	7.32
CF IV	H (112-121)-5-1-1-1-1-C3-1	196	3	2	1	2	2	1	—	—	11	5.61
CF V	C 1957-M_6-1-1-2-2-C3-1	98	1	—	1	1	1	—	—	—	4	4.08
	Total	561	6	6	4	7	5	2	1	1	32	5.70*

* percent weighted mean

TABLE 2

Segregation ratios within 6 cytoplasmic male sterile lines in which pollen fertility occurred in BC_1

Lines	Fertile: partial fertile: sterile plants			
	F_1	BC_1		
		observed ratio	expected ratio	P %
V-5317 A	0:0:22	28:0:69	1:3	0.30-0.50
O-7640 A	0:0:24	22:0:49	1:3	0.10-0.30
R-8840 A	0:0:21	12:0:51	1:3	0.10-0.30
O-7620 A	0:0:42	6:0:44	1:7	0.90-0.95
OS 2-12662 A	0:0:22	5:24:20		
P-6415 A	0:0:23	9:26:14		

regards the inbred lines OS-2-12662 A and P-6415 A, the heredity of pollen fertility is much more complicated, due to the presence of a great number of partial fertile plants in BC_1, in addition to the male fertile and sterile plants.

Examining the inbred lines with sterile cytoplasm in which pollen fertility occurred in more advanced backcrosses (BC_2—BC_9), it is obvious that the small or very small number of fertile plants observed in most cases demonstrates the existence of at least 3-4 genes or of polygenes. A part of the segregating progenies comprised also a big proportion of partial fertile plants, which confirms the polygenic nature of the investigated phenomenon.

For the genetic study of the occurrence of pollen fertility in advanced backcross generations, we used ten fertile plants identified in BC_1 and BC_5 in each of the cytoplasmic male sterile lines S-1358 A and P-1380 A. The S_1 selfing generation presented only segregating progenies, with the predominance of fertile and partial fertile plants. Sixteen to twenty four progenies for each of the two lines were studied in the subsequent generations S_2-S_4. The self pollination was clearly accompanied by a continuous increase of fully fertile progenies (Fig. 1). Starting from S_3, fertile plants prevailed among segregating progenies. Taking into consideration that as the number of segregating gene pairs increases, a greater number of selfing generations is necessary for achieving homozygosity, one may sup-

pose that the occurrence of pollen fertility in the male sterile line P-1380 A has a more complex genetic background than the other line S-1358 A.

The mating study presented in Table 3 comprised:

— two groups of five fully fertile sublines selected in the third selfing generation from the male sterile lines S-1358 A and P-1380 A;
— the male sterile lines V-2612 A and EL-8455 A which have shown a complete and stable sterility troughout 12 and 14 backcross generations, respectively;
— the fertile maintainer lines V-2612 B and EL-8455 B.

The sublines originating from S-1358 A and P-1380 A proved to be very different concerning their pollen fertility restoration ability. So, the sublines S-1358 A-3-1-2, S-1358 A-9-4-1, S-1358 A-11-16 and P-1380 A-9-1-1 brought forth to entirely or almost entirely fertile progenies that could be used as restorers in breeding works. The hybrids of the subline S-1358 A-6-10-3 with V-2612 (A and B) and EL-8455 (A and B) were made up mostly of fertile plants, while those of the sublines S-1358 A-5-6-4 and P-1380 A-1-4-1, of sterile plants. As for the other sublines, the ratio fertile: partial fertil: sterile plants varied considerably, being in many cases close to 1:1:1. It is obvious that, by self pollination and selection among such genotypes, one can obtain both pollen fertility restorers and sterility maintainers.

The hybrids obtained by crossing the ten fertile sublines originating from S-1358 A and P-1380 A with the male sterile lines V-2612 A and El-8455 A were very similar as pollen fertility concerned to the hybrids obtained by crossing the same sublines, used as female parents, with the fertile maintainers V-2612 B and EL-8455 B. Considering the two categories of hybrids as direct and reciprocal hybrids, one can assume that the cytoplasm of the sublines originating from S-1358 A and P-1380 A and that of the male sterile lines V-2612 a and EL-8455 A is identical. Because the lines V-2612 A and EL-8455 always produce wholly male sterile progenies, it is obvious that the occurrence of male fertile plants in the cytoplasmic male sterile lines S-1358 A and P-1380 A is due exclusively to the nuclear factors. Similar results were also obtained and in the case of crosses between the sublines S-1358 A-3-1-2, P-1380 A-1-4-1 and the other sublines.

In support of these data, it is worth mentioning the fact that the

TABLE 3

Number of male fertile: parcial fertile: sterile plants in F_1 generation of crosses performed with 10 fully fertile sublines, selected from the inbred lines S-1358 A and P-1380 A

Fully fertile sublines*) selected from cytoplasmic male sterile lines	V-2612A fully sterile throughout 12 back-cross generations	V-2612 B	EL-8455A fully sterile throughout 14 backcross generations	EL-8455B	S-1358A 3-1-2 (emasculated)	S-1358A 3-1-2	P-1380A 1-4-1 (emasculated)	P-1380A-1-4-1
	♀	♂	♀	♂	♀	♂	♀	♂
S-1358 A-3-1-2	41:0:0	36:0:0	40:2:0	21:0:0	37:0:0	21:0:0	36:0:0	
S-1358 A-5-6-4	0:0:43	0:0:32	0:2:19	0:3:37	26:6:3	31:3:5	7:14:19	9:11:12
S-1358 A-6-10-3	18:2:13	18:3:18	16:7:11	19:4:14	36:2:0	37:1:3	24:4:10	28:3:6
S-1358 A-9-4-1	22:0:0	39:0:0	19:0:0	36:0:0	39:0:0	42:0:0	37:2:0	20:0:0
S-1358 A-11-1-6	38:1:0	32:0:0	31:5:0	38:2:0	42:0:0	41:0:0	36:2:0	38:0:0
P-1380 A-1-4-1	0:0:40	0:0:39	0:2:36	0:1:39	20:2:0	21:0:0	32:4:0	38:0:0
P-1380 A-3-2-9	17:6:19	14:11:9	8:16:16	11:12:7	19:1:1	36:4:0	28:5:4	31:6:4
P-1380 A-7-11-2	13:16:10	18:11:11	12:18:8	12:12:14	37:0:0	38:2:1	25:10:2	30:2:8
P-1380 A-9-1-1	42:0:0	35:3:0	36:5:0	39:1:0	29:0:0	41:0:0	20:2:0	37:3:0
P-1380 A-14-7-4	10:6:21	14:3:16	7:12:20	11:18:11	21:0:0	36:3:0	35:3:4	31:4:6

* When used as female parent, the respective plants were emasculated manually.

occurrence of pollen fertility has never been noticed in very late generations of backcrossing (BC_{10}-BC_{15}). This finding attests the fact that the sterile cytoplasm "petiolaris" has a considerable stability throughout a great number of generations and confirms the theory of selfreproduction of the plasmic factor and its transmissibility only through maternal line. So, if pollen would contribute with small amounts of cytoplasm to the zygote formation, the sterile line A could become male fertile after a greater number of backcross generations, owing to the dose of hereditary particles received repeatedly from the normal, fertile cytoplasm of the B counterpart.

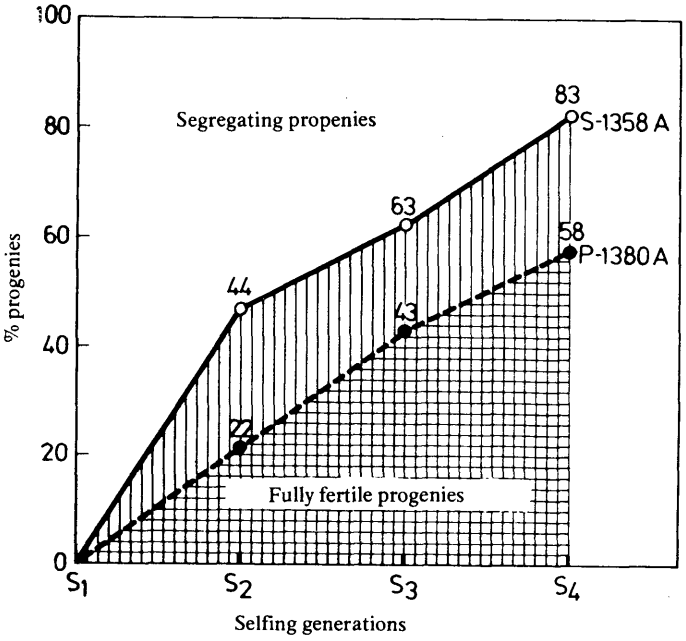

Figure 1. Proportion of segregating and fully fertile progenies obtained by repeated self pollination of fertile plants occurring within the male sterile lines S-1358 A and P-1380 A.

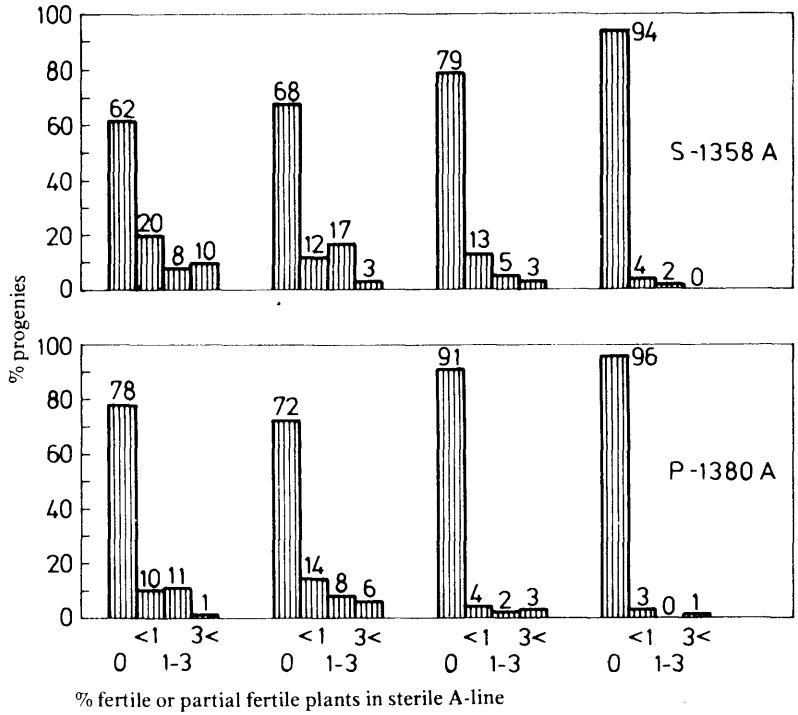

Figure 2. Effect of repeated selection of the individual crosses A x B with entirely sterile progenies, within the male sterile lines S-1358 A and P-1380 A.

Our results infirm the explanations anticipated by LECLERQ (1971) who considers that the occurrence of male fertile plants in cytoplasmic malesterile lines of sunflower is the result of the mutation or transformation of the "sterilising S" cytoplasm into a "fertilizing F" one. In fact, the changing effect of the "unstabilized S cytoplasm" mentioned by Leclercq is the result of the differing from plant to plant concentration of the polygenes that determine the occurrence of pollen fertility in sunflower lines with S cytoplasm.

The repeated promotion of the individual crosses A x B with fully sterile progenies has proved very efficient for selection of inbred lines with complete and stable cytoplasmic male sterility. So, as shown in Figure 2, after four generations of individual selection within the inbred lines S-1358 A and P-1380 A, the rate of fully sterile progenies increased from 62 to 94% and from 78 to 96% respectively.

CONCLUSIONS

The occurrence of male fertile plants in sunflower cytoplasmic male sterile lines is conditioned by the existence of the recessive restorer genes or of polygenes in the genotype of B lines. The smaller the number of these genes, the more this phenomenon appears in earlier generations of backcrossing and vice versa. Thus, the occurrence of pollen fertility in BC_1 is determined by two or three complementary recessive genes, while in later backcross generations the fertility restoration is the result of the different concentration of polygenes into the genotype of B lines.

The presence of a limited number of complementary recessive genes in the genotype of B lines permits the early identification, in BC_1 or BC_2, of male fertile plants within the A lines and the elimination of these lines at the beginning of the breeding works. The occurrence of pollen fertility in later backcross generations or during the increase of fundation seed of the A line generates greater difficulties both in sunflower breeding works and in hybrid seed production.

That is why both the conversion of the inbred lines with normal cytoplasm to cytoplasmic male sterility and the subsequent maintenance of these lines should be performed only on the basis of the individual crosses A x B, accompanied by the selection of fully sterile progenies. Such a methodology for maintaining and increasing the seedstock of A and B lines involves the periodical performance of a great number of pair crosses A x B, the study of their progenies and the selection for further multiplication only of the individuale B that assure the maintenance of a complete male sterility of the A line.

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