HOW TO PRODUCE HYBRID SUNFLOWER SEEDS BY INDUCING MALE STERILITY WITH GIBBERELLIC ACID

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Summary

The author reviews briefly the different means proposed to obtain hybrid seed of sunflower. He recalls more particularly the data concerning the use of Gibberellic acid. Then he gives an account of his own experiments and shows how the results allow to define the rules to follow to produce commercial hybrid seed. The interest of chemical sterilization is emphasized in order to do easily numerous top-crosses and to obtain non expensive three ways hybrids.

Introduction

The sure advantage to expect from heterosis in sunflower incited many research workers to prospect the means allowing an economic commercial hybrid seed production.

They generally admit that the ideal solution is using a cytoplasmic male sterility. But it does not seem, presently, that such a sterility would be sufficiently mastered.

P. LECLERCQ (3) described a cytoplasmic male sterility resulting from crossing H. petiolaris NUTT. and H. annus L. Recently the problem of fertility restoration seemed to be solved. But until now, in approach of a better solution, the breeders recommend to use the different types of chromosomic male sterility found in sunflower, many of which have been described particularly by V. V. VULPE (14).

E. P. PUTT and Ch. B. HEISER (6) showed that partially male sterile strains can be bred. These one, in presence of highly pollen producers, can give progenies with about 85% of hybrid plants. V. VRANCEANU (13), 1967, thought that nuclear androsterility would not be able to solve the problem of hybrid seed as long as a better phenotypic stability could not be obtained from a cytoplasmic factor. This author had observed a good stability only for a character of partial fertility like those described by E. P. PUTT and Ch. B. HEISER.

However, P. LECLERCQ (4) pointed out a male sterility gene sufficiently stable and closely linked with a character for hypocotyle colour. On this basis the production of hybrid seed started in France using as female

parent the progeny of a sib-cross having 50% male sterile plants; these ones being sorted according to their hypocotyl colours.

At last, a quite different way was exploited; the chemical sterilization of the female parent. Let us cite, among many others, W. SCHUSTER's works. As soon as 1956, this author reported (8) that phytohormones related to I.A.A. could produce male sterile flowers, but insufficiently and with damage to the germinative ability of the seed. In 1961, essaying several substances, he recognized (9) that Gibberellic acid was the less delusive of them all. In a more recent paper W. SCHUSTER (10) records the results he obtained since 1963 until 1967. He mentions that sunflowers treated at the beginning of the bud formation (0,50 to 0,25 mgr. a plant) appear as male sterile and can furnish hybrids for "top-crosses" tests. The reduction of female fertility can be corrected also by adding to gibberelline some others substances without decrease of male sterility; or else plants treated with gibberellin alone and giving 100% male sterile would have only 39% female fertile flowers instead of 90%. Moreover, the author thinks that strains and varieties respond very differently according to the surrounding conditions.

M. SPIROWA (11) who had already confirmed first W. SCHUSTER's results, gave (12) 1968, an account of more recent experiments leading to some interesting conclusions:

1. Gibberellin really appears as the most active and the most supple, among all chemical agents tested; but the yield of seed is half reduced.
2. "What seems the most important in artificial inducing male sterility, is not the choice of strains and varieties, but chiefly of the propitious time".

When, instead of treating only ten plants, as in his first experiments he undertook to treat some forty or fifty he obtained relatively less male sterile plants. But we must say that persistent rains had not permitted to treat at the opportune moment.

On the other hand, A. V. ANASCENKO (1) 1967, published much more encouraging data following a broad experimentation on 76 strains or varieties. The best results had been observed when plants were treated at the "rosette" stage with a solution of 50 p.p.m. of gibberellin giving 0,5 to 1,5 mg by plant. In these conditions one could obtain 100% male sterile plants with a seed yield reaching 70 to 75% of the normal. A. V. ANASCENKO then concluded "this method offers high possibilities as well to the plant breeder as to produce hybrids on a large scale". In 1968, the author gave some complimentary informations (2) on his experiments. He also indicated that 113 hybrids obtained in 1966 had been cultivated next year with yields often twice higher than the checks.

The object of the present paper is to give an account of our essays realized in France since 1963 and to compare the results to those reviewed formerly and finally to show how it is possible to use gibberellin successfully.

Experiments and Results in Montpellier

Having formerly found at the same time as Ov. SCHIFFRISS (7) the feminizing influence of gibberellin on castorbean (R. communis) (5) we were naturally led to essay it on sunflower. In 1963, we had treated plants of the variety "VNIIMK 6540" at various time since 3 days after getting up until 85 days, with solutions of gibberellin (100 or 500 p.p.m.) or 2, 4 D (6 or 12 p.p.m.).

In no case 2, 4 D had been able to furnish male sterile plant. On the contrary gibberellin had allowed to get good proportions of plants entirely male sterile or giving rare pollen. So the 500 p.p.m. dose seemed to be too much high. Moreover, the latest application made when plants had 15 leaves, was the most satisfactory.

In 1964 and during the following years, sprayings were limited to the upper leaves cluster instead of all the plant as in the preceding year. The gibberellin concentration were lowered to 5, 10, or 20 p.p.m. and applied once or twice. There were also treatments with I.A.A., or T.I.B.A. or M.H., with or without gibberellin. Only the treatments including Gibberellin gave more or less male sterile plants.

Plants of the variety VNIIMK 6540 had on the average 18 leaves at the time of treatments but with a V.C. of 22%. Each observed plant received a note according to the pollen absent (100), rare (50) or normally present (0). So, a note of average efficiency could be calculated for each treatment. The best result (68% of efficiency) was obtained when plants were sprayed once with gibberellin at 20 p.p.m. having 18 leaves. From numerous recorded data we might conclude:

1. The latest treatments (18 leaves at least) were the most satisfactory and we had to experiment still more late sprayings;
2. In these cases the seed yield, in spite of a concentration of 20 p.p.m. of gibberellin was higher than the yield obtained with only 5 p.p.m. sprayed much more early (82 gr of seed by plant in the first case, against 66 gr in the second);
3. A complete male sterility could be obtained with an acceptable decrease of seed yield as indicated by A. V. ANASCENKO.

Table 1 Seed Yields by Head According to the Efficiency of the Treatments.

Efficiencies	0	25%	50%	75%	100%
Average yield (gr)	84	67	71	66	61
Yield % Control	100	80	85	79	73

However, it was evident that perfect results could be obtained only by the use of very homogenous inbred lines.

In that purpose, several strains were sprayed once during the summer

1965 and often we obtained 100% male sterile plants which gave us hybrid seeds enough to realize trials with rather broad plots (50 m^2 each and 4 repications). So, we were sure that hybrid seed with good germinative ability could be harvested. Unfortunately all the progenies did not behave in the same manner because treatments were made at a badly definite stage.

Principally, A. V. ANASCENCO's results incited us to make again trials upon new basis during summer 1968.

Essay n° 1: Its aim was to verify if very low concentrations of gibberellin could change the proportions of the different phenotypes in the progenies of genetically male sterile plants crossed with a brother supposed heterozygous. There were 3 Russian strains (nos. 30, 82, 125) sent from Leningrad in 1967, a mixture of male sterile strains from the variety "Peredovik" and another strain likely selected in Montpellier from the variety VNIIMK 1646 (strain n° D 34-2-3).

These progenies were sown the 26th of March and all sprayed on 14th of May (this date, we know today, was too early). The table (2) indicates the proportions of entirely male sterile plants in each case according to the concentrations of gibberellin.

Table 2 - Percent of Male Sterile Plants According to the Progenies and Concentrations of Gibberellin Solutions.

Progenies	Giberellin Concentrations			
	0	5 p.p.m.	10 p.p.m.	20 p.p.m.
	%	%	%	%
Russian 125	23/53 = 43,4	23/37 = 62,2	26/36 = 72,2	26/36 = 72,2
Russian 30	14/37 = 37,9	20/43 = 46,5	26/43 = 60,5	29/41 = 70,7
Peredovik ms	58/140= 41,5	39/85 = 54,9	53/85 = 62,4	56/87 = 64,4
Russian 82	43/95 = 45,2	25/72 = 34,7	36/70 = 51,4	35/71 = 38,5
D 34-2-3	26/121= 21,5	30/82 = 36,6	24/81 = 29,6	29/80 = 36,3

We can see that light doses of gibberellin sometimes accentuated clearly the feminity of the progenies. Yet we must remark that the opening of heads ranged on 10 days at least in a same strain and on the other hand the choiced time for spraying was too early. In the table 3 one can see that the progenies most easily converted seem to be the most early flowering except "Russian n° 30" and that the % of male sterile plants is higher among plants which flowered before the 15th of Juin:

Table 3 - Percent Male Sterile Plants According to the Dates of Flowering.

Progenies	Number of Plants Having Flowers Before 15/6	% Male Sterile Plants Flowering	
		Before 15/6	After 15/6
Russian 125	68/109 = 62%	51/61 - 79%	21/41 = 51%
Russian 30	39/127 = 31%	30/39 = 77%	45/88 = 51%
Peredovik ms	169/257= 66%	100/169=59%	48/88 = 55%
Russian 82	71/213 = 33%	33/71 = 47%	63/142= 44%
D 34-2-3 ms	56/243 = 35%	38/85 = 45%	45/158= 29%

In the best condition (Russian 125) one month separated the day of spraying and the half flowering time.

Essay n° 2: A very late sowing (the 8th of May) of a normally fertile strain from Peredovik gave us an extremely heterogenous culture. This allowed us to treat very differently grown plants. The dates for spraying were, according to the cases, 20th or 28th of June or 8th of July. The developmental stages were defined as following:

ε = the bud is not perceptible with naked eye
eε = the bud can be guessed
e = "star stage" with young bracts clearly visible
ea = intermediate stage between e and a
a = "garlic grain stage", the young head more or less out having a diameter of about 1 cm
oa = intermediate between a and o
o = "onion stage", 4-5 cm diameter.

The efficiencies of the treatments were judged as formerly indicated. To obtain larger effectives, the stages eε and e were joined in one class in the table n° 4 which gives the effectives and the average notes for efficiency.

Table 4 - Effects of Gibberellin Solutions on the Male Sterility of Sunflower According to the stage and to the Time of Spraying.

Gibb. Concent.	Developmental Stages					
	ε	eε + e.	ea	a	ao	o
10 p.p.m.	43 8,1%	19 2,6%				
20 p.p.m.	49 17,3%	55 54,5%	16 (93,7%)	5 (80%)		
40 p.p.m.	54 27,8%	48 58,3%	20 85,0%	21 45,2%	15 (3,3%)	25 0
60 p.p.m.	8 (12,5%)	38 76,3%	19 89,5%	17 46,0%	26 15,4%	19 0
80 p.p.m.		1	3	12 (54,2%)	19 8,0%	24 0

This table shows the greatest importance of the time choiced to realize the treatment than the concentration of gibberellin, as M. SPIROWA had already pointed out. This fact appears still better in the table n° 6 where the results are given according to the numbers of days which separate the spraying and the opening of the head.

The lower line of the table gives the effeciencies observed for all the concentrations together (except 10 p.p.m.). These data allowed to draw a very regular curve (Fig. 1) which shows that, in the conditions of our experiment the most suitable time for a given plant was on average 23 days before flowering. The spread of the curve is explained partially by the remanence of the gibberellin, in case of too early treatments (though increasing the concentrations does not seem to correct markedly a too early spraying). About the treatments given less than 20 days before the flowering, we may think that the head which were sterilized had a little diameter so that they required less time to flower. That is the reason why we could not record a good fitting between the developmental stages and the times until the flowerings. On average these times for each stage were the following:

ε = 24 days
eε = 22,3 "
e = 21,9 "
ea = 20,5 "
a = 19 "

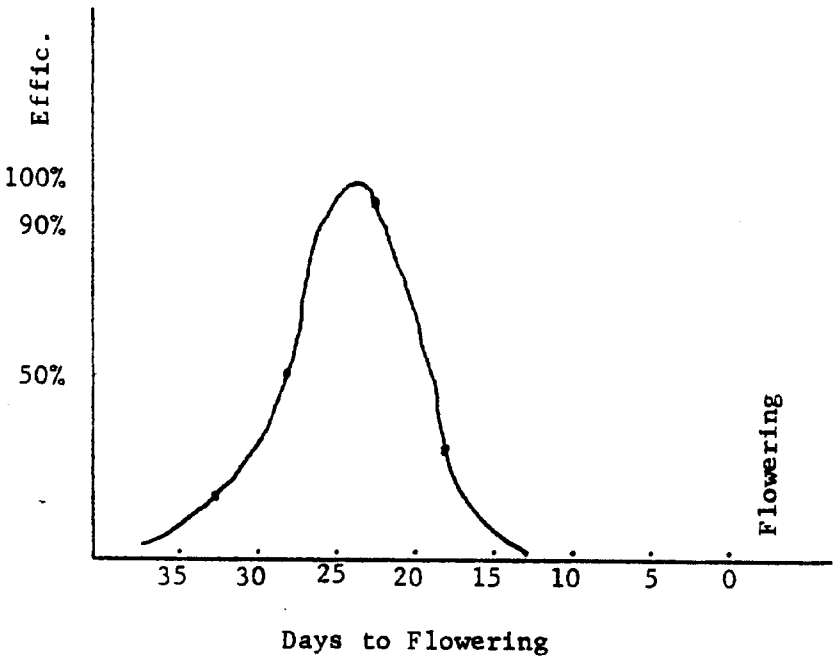

Table 5 - Efficiencies of Gibberellin Treatments According to the Duration Between Spraying and Flowering

Gibb. Concentr.	Duration (days) From Spraying to Flowering					
	Less Than 15 d.	15 d. to 19	20 to 24 d.	25 to 29 d.	30 to 34 d.	More Than 34 d.
10 p.p.m.				7 (0,7%)	26 3,8%	28 0%
20 p.p.m.		5 (60%)	34 100%	42 50%	23 10,9%	22 2,2%
40 p.p.m.	22 0%	43 22,1%	35 95,7%	40 58,8%	35 25,7%	12 (8,3%)
60 p.p.m.	21 0%	42 31%	39 91,0%	18 58,3%	3 (16,7%)	
80 p.p.m.	25 2%	33 31,8%	2 (75%)			
All Conc. Gathered	68 0,7%	123 29,3%	110 95,0%	107 51,9%	87 14,9%	62 2,4%

After this experiment we may finally assert that a male sterility as high as 95% could be certainly obtained by spraying plants twice : first at a badly defined stage "ε" then, at the stage "e" to "ea". For each of these treatments the concentration of gibberellin solution must not exceed 20 p.p.m..

It could be probably lowered till 10 p.p.m. for some progenies with natural tendency to male sterility.

Conclusions

The experiments conducted at the Station for Plants Improvement of I.N.R.A., in Montpellier (France) allow to confirm the results obtained by several foreign workers, particularly A.V. ANASCENKO who had shown the advantage of treatments given when the young head is clearly visible. Like this author, we verified that all varieties could be sterilized by this way and give easily hybrid seed. Else, since three years we were using this method to obtain seeds for ours top-crosses. During last summer some hybrid seeds were harvested, in two private farms. The proceeding we advise has to respect the following rules:

1. Give great care for sowing and thinning the female parent so as to get a culture as uniform as possible.

2. Choice a male parent flowering a little earlier than the female parent (3 to 5 days before). So it can serve as indicator for the time of spraying, and will furnish a lot of pollen at the beginning of the flowering of the female parents (in fact, they are the outer flowers of the head which have to be fertilized).

3. Treat firstly the female parent when the male parent reaches the "star stage", then another time 5 days later.

4. Use a 20 p.p.m. Gibberellin solution and on the occasion of the spraying eliminate the heads which would seem too big. Spray gibberellin very quickly on the upper part of the plants.

Proceeding so, it is reasonable to expect a seed yield nearly 70% of the normal without treatment. That is to say for an inbred line, and taking into the account of the surface let to the male parent, the yield of hybrid seed would be of 7 to 10 qx an hectare (or cwt. an acre). Moreover, the great advantage of the chemically inducing male sterility resides in the ability to produce by this means three ways hybrid seed. On one hand female parent would give about 14 cwt. of good seeds an acre. On the other hand, there would not have to fear the least risk of defective sterilization.

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