

CYTOPLASMIC MALE-STERILITY IN SUNFLOWERS

By

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Introduction

To produce commercial seeds of sunflower hybrids, cytoplasmic male sterility is not the only available method (5) (6), but it allows one to operate with the greatest security and the least amount of manual work.

For this reason, we have looked for this phenomenon following several approaches at the same time, which may be classified under two headings.

- 1) Study of the cultivated Helianthus annuus (intra-species program)
- 2) Study of the products of interspecific crosses, that include, with sunflower, two different species of the same genus:

--Helianthus tuberosus

--Helianthus petiolaris
(interspecies program)

Within the interspecific program too, several avenues are included

- a) Within a self-pollinated line segregating for male-sterility, study of the hypothesis of cytoplasmic mutation. Using this method since 1964, no supporting evidence could be demonstrated; male sterility was apparently only based on recessive gene action.
- b) A progeny study of open-pollinated male-sterile plants. This work begun in 1966, is based upon the hypothesis that two different cytoplasm occur within the same open-pollinated population; no conclusion has yet been reached.
- c) Hybridization of lines belonging to different populations, based on hypothesis on cytoplasmic difference between the two populations. Again, since 1966, we have not been able to reach a conclusion.

- d) Hybridization of two subspecies: Helianthus annuus subspecies bentii cularis (wild sunflower) and H. annuus subspecies macrocarpus (cultivated sunflower). It appears, within the limits of the genotypes used, that the two subspecies do not differ in the cytoplasm. We want to say, that in the inter-specific program, H. tuberosus and H. annuus do not differ in the cytoplasm, but that H. petiolaris and H. annuus do differ.

Methods and Materials

In 1964, we pollinated the wild H. petiolaris, of North American origin as all Helianthus, with the cultivated H. annuus.

The work of C. B. Heiser defines H. petiolaris as a close relative of H. annuus, both species being diploids ($2n=34$) and annuals. However, Heiser indicates that, at meiosis of the interspecific F_1 hybrids, the number of pairs varies from 7 to 15, the remainder of the chromosomes being associated in rings or in chains (2).

The crossing was carried out without emasculation using pollen of H. annuus (Armavirsky 93.45) on several heads of H. petiolaris (received from C. B. Heiser in January 1964 under number 6312), open pollinating being prevented by paper bags.

In 1965, we could distinguish easily two groups, within the progeny of these heads:

--Self-pollinated H. petiolaris, plants of low vigor with characteristics typical of this species: conical plant shape with the diameter of the base being equal to the height, numerous branches with equal sized heads, small and entire leaves.

-- F_1 hybrids of H. petiolaris and H. annuus, plants with increased vigor and characteristics intermediate to the two species: elongated habit with branching less numerous than that of H. petiolaris, with one apical and dominating head; the leaves differing only little from those of H. annuus (large, serrated). The hybrid F_1 plants, comprising about half of the total (10 out of 26) were then pollinated with different lines of H. annuus, always without emasculation.

In 1966, only nine plants resulted from five F_1 backcrossed plants, backcrossing being more difficult than the hybridization itself (2,4). In three progenies (comprising four plants) all the plants were male-fertile. One of three plants in the fourth progeny was male-sterile. One of two plants of the fifth progeny was male-sterile and was back-

crossed again with the sunflower line which has served before as the male parent. From this backcross, we obtain in 1967, 37 BC₂ plants, all male sterile, which were again backcrossed with their own paternal line and other lines of H. annuus.

The first generation grown in the greenhouse (BC₃) sown in August 1967, has yielded seven progenies totalling 39 plants which were all male-sterile, while the corresponding male lines were normal and fertile. A second generation grown in the greenhouse (BC₄), obtained from the first one and sown in the beginning of 1968, has yielded six progenies of eighty plants total, which were again all male steriles, while the male parents were normal and fertile.

In 1968, we observe the following results in the field:

--Of 45 BC₃ progenies, 41 totalling 714 plants are entirely male-sterile; four progenies are partially male-sterile (84 out of 98 plants); no progeny is entirely male-fertile.

--Of 19 BC₄ progenies, 14 are entirely male-sterile (553 plants in total), four are entirely male fertile (93 plants in total).

One BC₄ progeny was segregating for male sterility (56 male-steriles out of 57 plants).

Description of the Male-Sterility

Type one, pronounced, with anthers hidden.

The stamens, devoid of pollen, only reach half the normal size. That is the reason that they do not protrude out of the corolla. Moreover, while anthers are normally joined over their entire length, anthers of male-sterile plants are free at the top, and only more or less joined at the base.

Type two, weak, with anthers exposed.

In certain progenies, the anthers are of normal length, and do not protrude out of the corolla; however, they are devoid of pollen, and male-sterility is again complete. In both cases, the styles and seeds are normal.

Study of Restorer Genes

Supposing that male-sterility is based on interaction of the cytoplasm of H. petiolaris with the genotype of H. annuus, one might look for restorer genes in two ways.

1. Using male-fertile plants BC₂ and BC₃, with cytoplasm of H. petiolaris, whose ancestors have invariably been male-fertile since F₁ origin. Such plants are now at disposal and one may assume that these contain the proper restorer genes of H. petiolaris.
2. Explore the possibility that restorer genes occur in the species H. annuus. This second method possibly offers some chance of success and is based on our first results in which 4 out of 45 BC₃ and five out of 19 BC₄ progenies, all arising from male-sterile parents, are not entirely male-sterile. It is interesting to note, however, the suddenness with which the progenies become stabilized in BC₄, in view of the fact that of 19 BC₄ progenies from male-sterile BC₃ parents, only one is segregating, 56 out of 57 plants being male-steriles, and four progenies are entirely male-fertile. It is feared that such a sudden stabilization of the fertility restoring ability is really due to a cytoplasmic mutation, changing cytoplasm of H. petiolaris into cytoplasm of normal H. annuus, under the influence of repeated backcrosses to H. annuus.

Summary and Conclusion

Two male-sterile BC₁ plants [Helianthus petiolaris x H. annuus] x H. annuus were repeatedly backcrossed with H. annuus and have yielded BC₃ and BC₄ progenies which, for the most part, were entirely male-sterile (55 progenies, totalling 1267 plants out of 64 progenies) cytoplasmic male-sterility is suggested.

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DISCUSSION

Kinman: The same mechanism did not work for me. I think I had the restorer gene in the cytoplasm all the time. I am wondering if you have any comment to make on this.

Leclercq: I was reading some reports on the use of Helianthus petiolaris as a female to transfer some resistance to diseases. One can ask why cytoplasmic male sterility behaves this way. It is quite possible at the time of the cross there was a cytoplasmic mutation.

The second possible explanation is that in H. petiolaris there are different cytoplasm and by transfer one may or may not find it. This problem raises many questions.

Kinman: I gather from your summary that you did not have good restorers.

Leclercq: I have not put in the paper all the story. It is more complicated than I was reading because I used sunflowers that were heterozygous for genetic male sterility and in some cases this made for a more complex situation.

Kinman: I understood you to say that you did not get good restorers yet.

Leclercq: As restorers, I could not find them in sunflowers.

Kinman: Perhaps they are in the material I sent you three weeks ago which has H. petiolaris cytoplasm with two back-crosses to H. annuus. These were all fertile. This would be a good place to look for the restoration gene.

Leclercq: Yes, it is quite possible.

Putt: Just a comment on the female fertility. Was it normal or reduced?

Leclercq: Yes. In my observations, female fertility is quite normal, quite complete.

Oldemeyer: Is there any other obvious cytoplasmic effects in this material that you backcrossed with? Secondly, will bees visit a male sterile flower? What has been your observation?

Leclercq: Yes, From my observations, several bees have visited the male sterile plant. In some male steriles the styles are retracted after fertilization more quickly than styles of male fertile plants because, I suppose, when bees arrive at male sterile heads they immediately pollinate. They cannot gather pollen.

Johnson: In a genetic male sterile is the red color also associated in the seeds and is this color water soluble?

Leclercq: Yes. To realize red seeds, it is necessary that the plant is red and the head is red - but when plant and head are red, seed coat is not always red. There are, I suppose, two different genes in action. In order to have red seed coat, we have to have two dominant genes: one to give red color to the plant and one to give red color to the seed coat.

Johnson: Which type of system is in the genetic male sterile - the link marker?

Leclercq: This color that is linked to male fertility is the basic color that gives red color to plants.

Johnson: The other question: I understand the background of the red plant is 'Petite Noire'. What is the origin of the red plants?

Leclercq: They came from an old population that has been cultivated in France for many years in the West Region near Chartres that was very bad for oil production.