SOURCES AND PRELIMINARY RESULTS ON INHERITANCE OF MALE STERILITY IN SUNFLOWERS

M. L. Kinman, Soil & Crop Sciences,
U. S. Department of Agriculture,
College Station, Texas.
E. D. Putt, Experimental Farm,
Research Branch, Canada Agriculture,
Morden, Manitoba.

Morden male-sterile - Putt and Heiser[1] reported that this male-sterile developed anthers and produced pollen but that the pollen grains were smaller than normal and failed to stain. Their data showed it to be controlled by a single recessive gene, designated ms_1 ms_1 for this discussion.

At College Station, Texas, sib crosses of this source and outcrosses to other lines were made. The progeny of these (and all others grown at College Station) were classified on visual appearance of the capitula without microscopic examination of anthers or pollen. Sibbed populations in the greenhouse gave 43 normal:13 male-sterile (3:1), but in the field there were 29 normal: 46 male-sterile, or a considerable excess of male-sterile plants.

In the F_2 from crosses involving five normal male parents, only 20 male-sterile plants appeared in a total of 840 plants in the field. Only 11 of 38 F_2 progenies produced any male-sterile segregates. Nine of these progenies segregated approximately 15 normal:1 male-sterile; one fit a 3:1 ratio and the remaining progeny consisted of only one male-sterile plant. Six F_2 progenies totaling 136 plants from the cross Morden male-sterile x CM 90RR contained only one male-sterile plant; and eight F_2 progenies, consisting of 160 plants from the cross Morden male-sterile x an inbred from Armavirsky 93.43, showed no male-sterile plants.

Either the ms_1 ms_1 genotype fails to produce male sterility in certain genetic backgrounds or cytoplasms, or classification was erroneous in this material. The latter appears unlikely, as does environmental suppression of this type of male sterility because an actual excess of male-sterile plants occurred in sibbed populations in the same nursery.

Bloomington male-sterile - The Bloomington male-sterile produces extremely reduced anthers and usually no pollen. If pollen does occur it is markedly clumped, the grains are uneven in size and fail to stain. This type was found at Bloomington, Indiana, by Dr. Heiser in ornamental red-type sunflowers. In the cross of Bloomington male-sterile x CM 201 in F_2 and F_3, 9:7 and 3:1 ratios were obtained at Morden in a manner indicating that one

---

1/ E. D. Putt and C. B. Heiser, Jr. Male sterility and partial male sterility in sunflowers. Crop Science 6:165-168. 1966.

or both of two recessive genes, ms_2 and ms_3, produce this type of male-sterility when in the homozygous condition as indicated by Putt and Heiser.

At College Station 3 normal: 1 male-sterile and a few 1:1 ratios occurred in backcrosses (i.e., sibs). In 53 F_2 progenies involving 15 normal male-fertile lines as male parents grown at College Station in 1966, there were 16 nonsegregating male-fertile progenies, 24 segregating 3 normal:1 male-sterile and 13 segregating 15:1. The nonsegreating populations ranged from two to 28 plants, and (except for three progenies totaling 49 plants in which two selections from 69-17 were the male parents) only crosses with relatively small F_2 populations failed to segregate. The data are presented by male parentage in Table 1.

These data suggest that a third recessive gene is required for expression of ms_2 or ms_3, that one of them is fairly common and that they do not function in certain genetic backgrounds or cytoplasms. Since no 9:7 ratios were obtained at College Station (nor in the original work at Bloomington), the dominant allele of either ms_1 or ms_2 may be rather rare (CM 201 and 77AB being unique in this regard).

Bloomington male-sterile x Morden male-sterile - The cross of a male-sterile plant of the Bloomington type x a normal plant heterozygous for the single gene conditioning the Morden male-sterile was made at Morden where all generations were grown and classified. There were five F_2 progenies. Three of these segregated for both types of male sterility and gave a total of 48 normal:19 Morden male-sterile:20 Bloomington male-sterile plants in 1964. At first we assumed that both recessive genes conditioning the Bloomington sterile were present and that the observed ratios fit a 27:16:21 ratio, which would be expected if ms_1 ms_1 were epistatic to both ms_2 ms_2 and ms_3 ms_3. That the original Bloomington-type male-sterile plant carried both ms_2 and ms_3 was indicated by 9:7 ratios in the two F_2 populations which did not segregate for the Morden male-sterile; F_3 lines derived from these two populations produced mostly 9:7 and 3:1 ratios as expected. However, upon closer study and Chi-square analyses of the three F_2 populations which produced both types of male sterility and of F_3 lines derived from them which segregated for both types of sterility, it was determined that only two genes for male sterility were present, and ratios of 9 normal:3 Morden male-sterile:4 Bloomington male-sterile were obtained (observed ratio 191:73:80 with Chi-square of 1.57 and P of .25-.50). This indicates that at least one of the Bloomington male-sterile genes, and perhaps both, is epistatic to the Morden male-sterile gene. Since no 3-loci ratios were obtained and since two F_3 lines from one of the F_2's which produced no Morden male-steriles did segregate for Morden male-sterile-type plants, the possibility that one of the Bloomington male-sterile genes (ms_2 or ms_3) is allelic and dominant to the Morden male-sterile gene (ms_1) cannot be ruled out on the basis of present evidence.

HA 55 male-sterile - This source of male sterility appeared in the ornamental-type nursery at College Station in 1964. The line had a pedigree of T 57002-B-6-4-1-1 and was later given the permanent accession HA 55. It traces to a natural cross of a dwarf-internode line selected from the Morden number 45-47 x an unknown male parent, probably an orange petal line tracing

Table 1 Segregation of Bloomington-type male sterility in F_2 at College Station, Texas, in 1966

Male-fertile male parent	Number of Crosses	Number of F_2 progenies	Observed pooled ratio Male-fertile	Observed pooled ratio Male sterile	Assumed ratio	Chi-square	P
HA 59	1	1	20	1	15:1	0.124	.75-.70
CM 90RR sel.	1	1	8	2	3:1	0.133	.75-.70
953-88-3-14 sel.	1	1	11	1	15:1	0.088	.90-.75
953-88-3-15 sel.	5	5	37	11	3:1	0.111	.75-.70
953-102-1-1-41-3 sel.	2	5	60	8	13:3	2.178 1/	.20-.10
953-102-1-1-41-13 sel.	5	5	47	6	15:1	2.326 1/	.20-.10
69-17 sel.	4	6	91	5	15:1	0.178 2/	.70-.50
CM 91 sel.	1	1	10	0	15:1?	0.667	.50-.30
Ienissei-10 sel.	1	7	147	3	63:1	0.187 3/	.70-.50
CM 307 sel.	1	5	89	9	15:1	1.439 4/	.25-.20
CM 308 sel.	1	1	2	0	?	-----	-------
CM 310 sel.	2	2	21	6	3:1	0.111	.75-.70
CM 314 sel.	1	1	8	3	3:1	0.030	.90-.75
T 63006-2-1-1	1	8	129	41	3:1	0.071	.90-.75
T 63006-4-6-1	1	4	38	12	3:1	0.027	.90-.75
Total	28	53	718	108			

1/ Probably not homogeneous, appears to be mixture, populations segregating 3:1 & 15:1
2/ Probably not homogeneous, appears to be mixture, populations segregating 3:1 & 15:1 and nonsegregating.
3/ Probably not homogeneous, appears to be mixture, populations segregating 15:1 and nonsegregating.
4/ Probably not homogeneous, appears to be mixture, populations segregating 3:1 and nonsegregating.

to Hopi. HA 55 has dwarf internodes, orange petals, resistance to some races of rust and ornamental branching growth habit, which is probably recessive branching. Phenotypically, the male-sterile feature is similar to the Bloomington male-sterile.

The original F_6 progeny row had 20 normal and five male-sterile plants suggestive of a good 3:1 ratio. In 1965, nine selfed progenies had 166 male-fertile and 39 male-sterile plants. In the greenhouse in 1965-66, six sibbed progenies gave a ratio of 19 normal:29 male-sterile plants. In the 1966 nursery, 14 sibbed progenies gave 212 normal and 80 male-sterile plants. Also, in 1966 the F_2 of 60 crosses involving 23 normal lines as males on male-sterile HA 55 plants were grown at College Station. Among these, 20 crosses segregated 13:3, 16 segregated 15:1, eight segregated 3:1, two segregated 63:1, seven were not homogeneous in that different progenies gave different ratios and seven produced no male-sterile plants. The original assumption that the male sterility of HA 55 is conditioned by a single gene appears erroneous. Present evidence indicates that a dominant gene and two (or possibly three) recessive genes are required for expression of the male sterility in this source.

F_1 plants resulting from crossing HA 55 male-sterile plants with plants heterozygous for Bloomington-type male sterility (and reciprocals) and with plants heterozygous for Peredovik-21 male sterility were male-fertile in the 1966 nursery at College Station.

Morden male-sterile x HA 55 male-sterile - In 1965, the F_2 generation of two crosses involving Morden male-sterile plants as females and HA 55 plants known to be heterozygous for male sterility were grown in the nursery at College Station. Cross No. 1 consisted of 12 F_2 progenies of single F_1 plants and totaled 242 plants. Only one progeny (containing 27 plants) failed to produce any HA 55-type steriles (it did produce five Morden-type male-sterile plants); omitting this progeny resulted in 220 normal:20 Morden male-sterile:37 HA 55 male-sterile. Cross No. 2 consisted of 12 F_2 progenies with a total of 290 plants. Two progenies failed to produce any male-sterile plants of either type. All other progenies produced at least one HA 55-type male-sterile; although, three others failed to produce any Morden-type male-steriles. Omitting the two progenies lacking HA 55-type steriles resulted in 208 normal:9 Morden male-sterile:32 HA 55 male-sterile. The total corrected observed ratio was 428 normal fertile:29 Morden male-sterile:69 HA 55-type male-sterile plants. Far too few progenies failed to produce HA 55 male-steriles indicating that the Morden gene may also be involved in the HA 55 male-sterile. Observed ratios fit a 51:4:9 ratio quite well (Chi-square = 0.98 with a P value of .75-.50), but this does not make sense genetically unless gene "a" in the homozygous recessive condition gives the Morden male-sterile in the presence of "B-" or "bb" and "cc" while "aa" in the presence of "B-" and "C-" gives the HA 55 male-sterile.

Peredovik-21 male-sterile - In the 1965 nursery at College Station, two S_2 progenies of Peredovik-21 (selections 2 and 4) produced male-sterile plants of the Bloomington type; i.e., no pollen was shed. The progeny of P-21-2 consisted of a single male-sterile plant which was pollinated with bulked pollen from normal plants in the progeny of P-21-4 which flowered at the same time. P-21-4 produced five plants of which one was male-sterile

and it was sib-pollinated with bulked pollen of its normal sibs which flowered at the same time. In the greenhouse, P-21-(2-1 x -4-B) produced only normal fertile plants. The seven self-pollinated plants produced 54 normal:13 male-sterile plants in the 1966 nursery which is an almost perfect fit to a 13:3 ratio. Progeny of P-21-4-1# produced two male-sterile and four normal plants in the greenhouse. The male-steriles (selection 1 and 2) were sib-pollinated with pollen from normal but presumably heterozygous individual sibs. P-21-4-1#-1x5 gave a progeny of 22 normal:19 male-sterile plants for a good fit to a 1:1 ratio in the 1966 nursery, while P-21-4-1#-2x3 gave 38 normal:27 male-steriles. This also fit a 1:1 ratio, but fit a 5:3 ratio better. However, the self-pollinated male parents of these sibbed populations (P-21-4-1#-3 and P-21-4-1#-5) segregated 20:2 and 12:1; values which do not fit 3:1 or 13:3 ratios; these with P-21-4-4-1 (25:1) gave a good fit to a 15:1 ratio. Five other first and second generation selfed progenies of P-21-4 gave ratios which when pooled fit a 13:3 ratio more closely than the 3:1 ratio. The evidence suggests that two homozygous recessive genes and a dominant gene are required for expression of the Peredovik-21 type of male sterility.

Segregation of Peredovik-21 male sterility has been studied only in its original genetic and cytoplasmic background. However, only male-fertile F_1 progeny were produced when plants heterozygous for this source of male-sterility were used as male parents in crosses with Morden, Bloomington and HA 55 male-steriles.

Other sources of male sterility - In the 1966 nursery at College Station, two additional male-steriles were found. The S_3 progeny Peredovik-23-1-1 contained two plants one of which was male-sterile while the other was a normal male-fertile plant. The male-sterile plant was sib-pollinated, and the normal was selfed. Phenotypically, this one is similar to the Bloomington type.

In the same nursery, Smena-3-02-4 had three plants. Two were male-sterile of the Morden type (anthers but no apparent pollen). These were sib-pollinated to the normal plant which was self-pollinated.

It should be noted that these two new male-steriles and also the Peredovik-21 male-sterile are in highly self-incompatible lines which are difficult to maintain by self-pollination in the field at College Station, Texas.

At Morden a male-sterile of the Bloomington type has been located in an inbred line out of the Russian variety VNIIMK 16.46. It has been crossed with CM 201, the line which has been used as a normal in genetic studies of male sterility at Morden. F_2 populations from this cross are growing in the 1966 nursery.

Possible evidence of cytoplasmic influence on male sterility - Thirty-three F_1 crosses involving the Morden male-sterile as female parents and a wide range of normal male parents were grown at Morden in 1964. Most produced only normal male-fertile plants. However, the three plants with three sources of Helianthus petiolaris as male parents produced zero pollen, 1% normal pollen and 3% normal pollen. Of 11 F_1 plants with the Chicago Wild as the male parent, one produced no pollen, and one had only 50% normal pollen. One of 11 plants with 69-17 as the male parent had only 25% normal pollen. The F_2 of crosses in which plants heterozygous for the Morden male-sterile gene were used as male

parents with 17 inbred lines, 4 Russian varieties and 6 sources of wild H. annuus were grown at Morden in 1965 as individual F_2 progenies. Seven of these crosses produced no male-sterile plants in F_2. When male parents were CM 5RR and CM 222, four F_2 progenies with nine to 15 plants each produced no male-steriles. This may suggest that homozygous ms_1 ms_1 does not produce male sterility in some cytoplasms. Larger populations of these two crosses are being grown in 1966.

A few male-sterile or partially male-sterile (sectorial chimeras) plants appeared in the F_1 generation of crosses involving both Morden and Bloomington male-sterile x normal lines in the 1965-66 greenhouse at College Station. This could be attributed to either cytoplasmic or environmental influences.

Discussion

Crosses among the four sources of male sterility which have been hybridized to date (i.e., Morden, Bloomington, HA 55 and Peredovik-21) produce normal male-fertile F_1 progeny. This indicates that the expression of the male-sterile phenotype is conditioned by at least one different gene in each source. From the two crosses that have been examined in advanced generations (Bloomington male-sterile x Morden heterozygote and Morden male-sterile x HA 55 heterozygote), it does appear that these three sources of genetic male sterility have at least one locus (and probably additional loci) in common. When additional crosses have been analyzed, it may be possible to establish a genetic system involving a group of loci and specific alleles which govern the expression of the various sources and types of genetic male sterility. Analyses of such hybrids and others involving a wide range of material within Helianthus annuus and of interspecific hybrids involving closely related species may reveal one or more cytoplasmic-genic systems involving male sterility. This may allow the utilization of cytoplasmic male sterility in production of sunflower hybrids.

The search for a useable form of cytoplasmic male sterility will be time consuming and possibly unfruitful. In the meantime, it may be possible to utilize some form of genetic male sterility for production of commercial F_1 hybrids especially if used in conjunction with the self-incompatibility system now being utilized to some extent, with the partial male sterility (pollen abortion) reported by Putt and Heiser or with both. For example, the Peredovik-21 male-sterile family is segregating for self-incompatibility. Some sibbed lines segregating 1:1 for male sterility with high self-incompatibility of the male-fertile plants already have been tentatively identified. Since a dominant gene appears necessary for expression of male sterility in the HA 55 and in Peredovik-21 sources, it should also be possible to produce populations segregating 3 male-sterile:1 normal for use as the female parent of hybrids.

* * * * * *