Published September, 1968

Analysis of a Genetic Double Recessive Completely Male-Sterile Cotton[1]

J. B. Weaver, Jr.[2]

ABSTRACT

Genetic analysis of a male-sterile character in cotton (*Gossypium hirsutum* L.) showed that the male-sterile expression was conditioned by two pairs of homozygous recessive alleles. Tests of six varieties of cotton showed that they had a homozygous dominant constitution at the two loci. The varieties 'Lankart 57' and 'Gregg' apparently have homozygous dominant genes at only one of the two loci. It is proposed that the appearance of a single male-sterile plant from an F_2 population of a cross involving Lankart 57 occurred as a gene mutation. Tests indicated that the genes at these two loci are independent of the previously reported ms_2 gene. The new genes have been assigned the symbol ms_5 and ms_6. This is the second published report of a complete male-sterile that is inherited as a recessive character and the first to be inherited as a double recessive.

Additional index words: **cytoplasmic-genetic sterility, glandless cotton, nectariless cotton, ratooning, hybrid cotton, *Gossypium hirsutum*.**

TWO independently inherited partially male-sterile genes were reported by Justus et al (2, 3). These recessive genes have been assigned the symbols ms_1 and ms_3. Richmond and Kohel (8) reported the discovery of the first completely male-sterile line in the F_3 generation of a cross of 'Texas 86' × 'D&PL 14.' The symbol ms_2 was assigned to this recessive gene. These workers later reported (4) that no cytoplasmic interaction existed after extensive investigation. Allison and Fisher (1) have published preliminary data which indicated that complete male-sterility in an Acala line was due to a dominant gene, Ms_4. Meyer and Meyer (6, 7) have reported a form of cytoplasmic-genetic sterility developed from certain interspecific crosses in *Gossypium*. The expression of sterility within any one genotypic-cytoplasmic combination was influenced by environmental factors however.

The purpose of this paper is to report the discovery of a completely male-sterile condition that is inherited as a double recessive.

MATERIALS AND METHODS

In 1954 a strain of cotton known as 'Stoneville 20' was crossed with a nectariless species (*G. tomentosum*) using Stoneville 20 as the female parent. In 1957 a nectariless plant in the F_3 generation of the above hybrid was crossed with 'Empire WR.' The nectariless × Empire WR hybrid was advanced to the F_3 generation and a nectariless plant was crossed with the stormproof variety 'Gregg' in 1959. This hybrid was advanved to the F_2 generation during the winter of 1959-60 at Iguala, Mexico. Nectariless segregates were crossed with 'Lankart 57' during the summer of 1960, and this cross was advanced to the F_2 generation during the winter of 1960-61. One completely male-sterile plant was found in the 1961 nursery at Bogart, Georgia, in approximately 800 F_2 plants from the cross with Lankart 57. The nursery row in which this male-sterile plant was found was 1-339, and the designation MS 1-339 was used in test crosses. The male-sterile plant was fairly productive, stormproof, nectariless, and had glandless leaves. Some of the fertile segregates in

[1] Journal Series Paper No. 260, University of Georgia College of Agriculture Experiment Station, College Station, Athens, Ga. 30601. Received March 21, 1968.

[2] Formerly Research Agronomist, DeKalb Agricultural Association, Inc., Bogart, Ga., Presently Asst. Prof. of Agronomy, University of Georgia, Athens, Ga. 30601.

this group were glandless also, although none of the original parents involved in these crosses was glandless.

The original male-sterile plant in this study was transplanted to a greenhouse where it flowered profusely and produced completely male-sterile flowers throughout the winter and following summer.

F_1 plants of MS 1-339 cross pollinated with the varieties Gregg, Empire WR, and Lankart 57 were observed in the greenhouse during the winter of 1961-62. All of the F_1 hybrids were fertile and self-pollinated seed was obtained.

Genetic Analysis

Two F_2 families were scored for male-sterility at Iguala, Mexico, during the winter of 1962-63. A population of 138 F_2 plants from the cross MS 1-339 × 'Austin' produced 130 fully male-fertile plants and eight completely male-sterile plants. Another group of 115 plants from the cross MS 1-339 × 'Coker 137' produced 108 fertile and seven male-sterile plants. The X^2 value for a 15:1 ratio was .05 and .005 respectively, with a probability value of .90-.80 and .95-.90, respectively.

Extensive F_2 data were obtained at Bogart during the summer of 1963. Table 1 shows the segregation in the first backcross F_2 generation with variety 'Deltapine Smoothleaf' as the recurrent parent. From a total of 1510 plants scored, 1408 were found to be fertile and 102 male-sterile, a ratio which does not deviate significantly from the 15:1 ratio expected for a character controlled by two pairs of recessive genes. Family number 2 showed more male-steriles than expected and family number 11 produced all fertiles.

Table 2 shows data from backcross families with the variety Empire WR as the recurrent parent. Again the observed phenotypic ratio corresponds very closely to that expected for a character determined by two pairs of recessive genes.

A 15:1 segregation ratio in F_2 populations was obtained from MS 1-339 crossed with each of the varieties 'Stoneville 213,' Austin, 'Paymaster 101,' and 'Auburn 56.'

MS 1-339 was crossed with the two varieties (Gregg and Lankart 57) that were involved in the pedigree of the material immediately prior to the discovery of the MS 1-339 male-sterile. An F_2 of MS 1-339 × Lankart 57 produced 27 fertile plants and 16 male-sterile plants. The X^2 value for a 3:1 ratio was 3.4 with a corresponding P value of .10-.05. The F_2 generation of MS 1-339 × Gregg gave 122 fertile plants and 33 male-sterile plants. The X^2 value for a 3:1 ratio was 1.14 with a corresponding P value of .30-.20.

One hundred and eight individual male-fertile F_2 plants of MS 1-339 × Coker 137 were advanced to F_3 families and scored for male-sterility. If male-sterility is controlled by two pairs of recessive genes the expected number of non-segregating families is 7/15 of 108 or 50 families. There were 41 F_3 families, with an average population of 79 plants, that produced no male-sterile plants. Two additional families that had only one male sterile plant each should be included in the non-segregating group. The male-sterile plant in these two cases bloomed very late in the season and the male-sterility most likely was environmentally induced. A 3:1 ratio should have likewise been found in 4/15 of 108 or 29 families.

Within the 64 families that segregated for male-sterility there was a range from a low of 1.3% male-steriles to a high of 35.6% male-steriles. According to Mather (5) the ambiguous ratio for 3:1 and 15:1 is $\sqrt{3(15)}$:1 or 6.7:1. Thus, the dividing proportion is $1/(6.7+1) = .13$ or 13%. There were 29 families found with 13% or more male-sterile plants. Six of these families gave a X^2 value greater than expected at the .05 level of probability for a 3:1 segregation. The X^2 value for heterogeneity was 38.67 with a corresponding probability of .10-.05. The total number of male-sterile plants found in these 29 families was 570 while the expected number was 624.

Less than 13% male-steriles were found in 35 families. In two families the X^2 value was greater than expected at the .05 level of probability for a 15:1 segregation. The X^2 value for heterogeneity was 44.56 with a corresponding probability of .05-.02. The total number of male-sterile plants for these 35 families was 162 while the expected number was 196.

Table 1. Segregation resulting from [(MS 1-339 × Deltapine Smoothleaf) F_2 *ms* × Deltapine Smoothleaf] F_2.

Family number	Number of plants: Male fertile	Male sterile	Total	χ^2 (15:1)	P
1	97	7	104	.04	.90-.80
2	77	10	87	4.07	.05-.02
3	72	5	77	.01	.95-.90
4	78	7	85	.57	.50-.30
5	84	10	94	3.10	.10-.05
6	93	4	97	.75	.50-.30
7	105	6	111	.14	.80-.70
8	117	9	126	.17	.70-.50
9	96	6	102	.02	.90-.80
10	92	8	100	.52	.50-.30
11	77	0	77	5.13	.05-.02
12	121	8	129	.00	.99-.98
13	102	6	108	.09	.80-.70
14	93	10	103	2.10	.20-.10
15	104	6	110	.12	.80-.70
Pooled	1,408	102	1,510	.65	.50-.30
Heterogeneity				16.26	.30-.20

Table 2. Segregation resulting from [(MS 1-339 × Empire WR) F_2 *ms* × Empire WR] F_2.

Family number	Number of plants: Male fertile	Male sterile	Total	χ^2 (15:1)	P
1	84	6	90	.03	.90-.80
2	87	8	95	.78	.50-.30
3	85	5	90	.08	.80-.70
4	91	2	93	2.67	.20-.10
5	71	2	73	1.53	.30-.20
6	82	5	87	.04	.90-.80
7	107	6	113	.17	.70-.50
8	103	8	111	.17	.70-.50
9	81	4	85	.34	.70-.50
10	75	8	83	1.63	.30-.20
11	72	8	80	1.92	.20-.10
12	93	5	98	.22	.70-.50
Pooled	1,031	67	1,098	.04	.90-.80
Heterogeneity				9.54	.70-.50

Test for Allelism with ms_2

Seventeen F_1 plants were studied from crosses of MS 1-339 × pollen parents that were heterozygous for the ms_2 male-sterile gene. In each instance these F_1 hybrids were completely male-fertile, which indicated the MS 1-339 genes and the ms_2 were nonallelic.

Table 3 shows the segregation in the F_2 and F_3 generation from one of the above mentioned crosses. The MS 1-339 is assumed to have the genetic constitution of $ms_5ms_5ms_6ms_6Ms_2Ms_2$ while the male parent should have been $Ms_5Ms_5Ms_6Ms_6Ms_2ms_2$. It should be noted that all F_1 plants carried the MS 1-339 genes in the heterozygous condition and one-half should have been heterozygous for the ms_2 gene. Thus, it is probable

that the F_1 plant from which family number 3 was derived did not carry the ms_2 gene. Data from test crosses involving individual male-sterile plants within families 1, 2, and 4 with pollen plants known to be heterozygous either for ms_2 or MS 1-339 will be required before it can be determined whether an individual male-sterile plant is devoid of pollen because it is homozygous for the ms_2 gene or the MS 1-339 genes. Whether or not a given plant can be homozygous recessive for both sets of male-sterile genes is unknown. In families 1, 2, and 4 there is a deficiency in the expected number of male-sterile plants, whereas, if the F_1 plants were heterozygous for both sets of male-sterile characters, more than one-fourth of the plants should have been male-sterile. There is a possibility that the ms_2 locus is closely linked to either the ms_5 or ms_6 locus.

Extensive data collected in 1962 and 1963 demonstrate a deficiency in the number of male-sterile plants obtained in F_2 populations involving the ms_2 gene when the plants are grown under field conditions. For example, 11,857 F_2 plants scored in 1962 gave an average of 17.81% male-steriles instead of the expected 25%. Therefore, under field conditions at least, it would be rather difficult to determine the effects of both sets of genes combined if one studied F_2 data.

Agronomic Properties

The male-sterile plants in the F_2 and F_3 generation appeared to be as productive as their fertile sibs and no apparent deleterious factor was observed as being associated with male-sterility derived from MS 1-339.

During the summer of 1963, a test was conducted to determine the date of first bloom observed on male-sterile plants versus fertile sibs. Table 4 shows the weekly average percent of male-sterile plants found in MS 1-339 material as compared to ms_2 segregating families. In arriving at these averages, only those families in the MS 1-339 groups that segregated for male-sterility were included. In the case of the MS 1-339 families there was no appreciable change in the percent of male-steriles for the 1st week as compared with the 5th week. In contrast the ms_2 gene being backcrossed into Coker 100A and D&PL Smoothleaf produced 5.67 and 7.88% male-steriles during the 1st week and 24.2 and 27.87% male-steriles respectively during the 5th week. The seed was sown thickly within the row, which accounts for the fact that some plants had their first bloom 5 weeks earlier than others. Data from Table 4 indicated that the male-sterile plants of the ms_2 type do not bloom as early as the fertile sibs and that male-sterile plants resulting from the MS 1-339 genes bloom essentially as early as their fertile sibs.

Table 3. Segregation of F_2 and F_3 families from the cross of MS 1-339 × F_1 of (ms_2ms_2 × Empire WR).

Family	Number of plants: Generation	Male fertile	Male sterile	Total	Proposed segregation ratio	χ^2	P
1	F_2	32	7	39	3:1	1.0	.50-.30
2	F_2	49	9	58	3:1	2.78	.30-.05
3	F_2	61	4	65	15:1	.09	.99-.95
4	F_2	44	8	52	3:1	2.56	.30-.05
5	F_2	29	2	31	15:1	.00	.99-.95
6	F_2	32	0	32	none		
7	F_2	20	0	20	none		
8	F_3	29	6	35	3:1	1.15	.30-.05

DISCUSSION

The ms_2 gene was discovered by Richmond and Kohel (8) from a cross in which they were making a systematic search for cytoplasmic-genetic male-sterility. In contrast, the MS 1-339 male-sterile was discovered as a chance observation in an F_2 population where the primary interest was transfer of the nectariless genes into Upland stocks.

Each of the varieties Lankart 57 and Gregg apparently carried one of the pairs of recessive genes that resulted in the development of the MS 1-339 male-sterile plant. This conclusion is drawn from the fact that both of these varieties produced approximately a 3:1 ratio of fertile and male-sterile in the F_2 generation. It is assumed that the other pair of genes arose as a gene mutation. There is the remote possibility that the species *G. tomentosum* may have contributed one of the pairs of recessive genes. However, this does not seem probable since male-sterility did not appear in the F_2 generation following the cross made with Gregg in 1959 and since only one plant was male-sterile in a population of 800 F_2 plants in 1961.

These new genes, apparently the first to be inherited as a double recessive, are designated ms_5ms_6. Gene symbols ms_1 through ms_3 have previously been assigned to recessive genes and Ms_4 to a dominant gene.

No special significance is attached to the fact that the original male-sterile plant was glandless leaf. Glandless leaf plants have been found by the author in other F_2 populations involving the Gregg variety.

The discovery of this genetically controlled completely male-sterile strain of cotton represents another step toward the day when male-sterility will be utilized on a commercial basis in providing the advantages of heterosis to cotton producers. The MS 1-339 genes show the most promise of any male-sterile factor that has been extensively investigated. The MS 1-339 male-sterile has two advantages over the ms_2 male-sterile. First, the MS 1-339 factor does not appear to be deleterious, which makes it superior from the standpoint of a female parent on which F_1 seeds are produced. Secondly, the fact that it is inherited as a double recessive in most of the varieties offers considerable promise for its utilization in the production of F_2 seed for commercial sale, since only one out of 16 plants is male-sterile. Therefore, the presence of pollen vectors in the farmer's field of F_2 hybrid plants

Table 4. Comparison of date of first bloom of families segregating for ms_2 male-sterility and MS 1-339.

Week	[(ms_2 × Coker 100 A) F_2 ms × Coker 100 A] F_2: Total plants	% male sterile	[(ms_2 × Smoothleaf*) F_2 ms × Smoothleaf] F_2: Total plants	% male sterile	MS 1-339 × Austin F_3: Total plants	% male sterile	MS 1-339 × Coker 137 F_3: Total plants	% male sterile
1st	405	5.67	317	7.88	1,862	10.52	162	9.87
2nd	684	11.11	535	9.71	1,364	11.29	532	11.09
3rd	933	15.11	860	14.18	1,082	15.34	1,313	11.72
4th	798	18.42	880	18.18	813	14.88	1,838	11.91
5th	219	24.20	165	27.87	658	13.37	1,666	11.94

* D & PL Smoothleaf.

is not nearly as crucial as would be the case if 1 out of 4 plants was male-sterile.

Stroman (9) has proposed ratooning of F_1 plants in Peru so that more than one crop of F_2 seed could be harvested. Ratooning of male-sterile plants in areas where such a practice is possible would provide an excellent method of producing F_1 seed.

LITERATURE CITED

1. Allison, D. C., and W. D. Fisher. 1964. A dominant gene for male-sterility in Upland cotton. Crop Sci. 4:548-549.
2. Justus, Norman, and C. L. Leinweber. 1960. A heritable partially male-sterile character in cotton. J. Hered. 51:191-192.
3. ————, James R. Meyer, and Jean B. Roux. 1963. A partially male-sterile character in Upland cotton. Crop Sci. 3:428-429.
4. Kohel, R. J., and T. R. Richmond. 1963. Tests for cytoplasmic genetic interaction involving a genetic male-sterile stock of the genotype ms_2ms_2. Crop Sci. 3:351-362.
5. Mather, K. 1951. The measurement of linkage in heredity. John Wiley & Sons, Inc., New York.
6. Meyer, James R., and Vesta G. Meyer. 1961. Cytoplasmic male-sterility in cotton. Genetics 46(8):883.
7. Meyer, Vesta G., and James R. Meyer. 1965. Cytoplasmically controlled male sterility in cotton. Crop Sci. 5:444-448.
8. Richmond, T. R., and R. J. Kohel. 1961. Analysis of a completely male-sterile character in American Upland cotton. Crop Sci. 1:397-401.
9. Stroman, G. N. 1961. An approach to hybrid cotton as shown by Intra and Inter-specific crosses. Crop Sci. 1:363-366.