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Analyses of a Dominant Male-Sterile Character in Upland Cotton. II. Genetic Studies[1]

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ABSTRACT

A plant in an okra leaf, frego bract, nectariless strain of upland cotton (*Gossypium hirsutum* L.) had one branch bearing male-sterile flowers. Crosses between male-sterile and male-fertile plants resulted in populations segregating 1:1 for fertile and sterile plants. Populations from selfed F_1 and F_4 fertile plants contained only fertile plants. These data suggested that a dominant gene controlled the sterility expression. Viability tests disclosed that a few viable pollen grains were produced in male-sterile flowers. Six male-sterile plants produced self-pollinated seed; the resulting progenies gave a ratio of one sterile to one fertile plant, rather than 3:1 as expected. It was theorized that the pollen grains carrying the dominant gene for male sterility were less competitive than pollen grains carrying the recessive allele or non-viable. Testcross populations of sterile plants in the F_2 generation segregated 1 fertile:1 sterile, indicating that no plant was homozygous dominant for male sterility. Cytological observations had revealed that the new dominant gene differed from Ms_4 and Ms_7 is microsporogenesis breakdown. The gene symbol Ms_{10} is proposed for this new dominant gene conditioning male sterility.

Additional index words: Gossypium hirsutum L., Pollen viability.

GENETIC studies of upland cotton (*Gossypium hirsutum* L.) revealed a number of genes conditioning male sterility (1, 3, 4, 9, 10, 12, 14). Justus and Leinweber (3) were the first to assign a symbol to a recessive gene (ms_1) for male sterility. Another recessive gene, ms_2, was reported by Richmond and Kohel (10). The recessive gene ms_3 conditions partial male sterility in cotton (4). Allison and Fisher (1) assigned the gene symbol Ms_4 to the first dominant male-sterile character found in cotton. Weaver (12) assigned the gene symbols ms_5 and ms_6 to two recessive genes causing male sterility. A second dominant male-sterile was given the gene symbol Ms_7 (14). Rhyne and Rhyne (9) reported two pairs of recessive genes conditioning indehiscent anthers in cotton; the gene symbols ms_8 and ms_9 were later assigned by Kohel (5).

Meyer (6) reported a cytoplasmic-genetic male-sterile using *G. hirsutum* L. genome in a *G. harknessii* Brandegee cytoplasm. Negmatov et al. (8) induced cytoplasmic male sterility in cotton with gamma irradiation. The purpose of this paper is to report the occurrence of another dominant male-sterile character in upland cotton.

MATERIALS AND METHODS

In 1972, J. B. Weaver, Jr. discovered an upland cotton plant with one branch bearing male-sterile flowers. Other branches on the same plant bore only male-fertile flowers. The plant was found in an okra leaf, frego bract, nectariless strain. Crosses were made to the sterile flowers using sib plants as the pollen source. In the segregating populations from these initial crosses, sterile plants were crossed to pollen sources of no known sterile background. The F_1 generation from sterile × fertile plants, open-pollinated seed from male-sterile plants, and selfed seed from fertile plants of F_1 and F_4 generations were grown in the field in 1976. Plants were scored for sterility during the flowering season. Bags to insure self pollination were attached to at least five squares (flower buds) of each plant to aid in their classification.

An effort was made to self-pollinate a number of male-sterile plants by rubbing the anthers between the fingers and applying the pollen, if present, to the stigma. Crosses were again made to sterile plants utilizing 'Coker 201' as the pollen source. Selfed seeds were obtained from fertile F_1 plants. Open-pollinated seed from two male-sterile plants and all self-pollinated seed were planted in the greenhouse during the winter of 1976-77. Plants were scored for fertility or sterility and crosses were made to sterile segregates using Coker 201 and 'Coker 310' as pollen sources.

Pollen viability tests were performed on sterile and fertile segregates in the greenhouse. Coker 201 and Coker 310 were used as controls in these tests. The testing solution combined one part of a 10% solution of 2,3,5-Triphenyl-2H-tetrazolium chloride to five parts of a 60% sucrose solution (11). The remainder of the open-pollinated seed, selfed seed from fertile F_1 plants, and testcross seed were planted in the field in 1977. Pollen viability tests were performed to confirm classifications. The field work in 1976 was performed at the University of Georgia Plant Sciences Farm near Athens. The 1977 field work was performed at the Louisiana Agricultural Experiment Station, Baton Rouge.

The chi-square test for goodness-of-fit was performed on segregating populations. Yates' (15) correction factor was used when the expected subclass number was less than 25.

RESULTS AND DISCUSSION

Frequency distributions of male-fertile and sterile plants in F_1 progenies grown in the 1976 field nursery and 1976-77 greenhouse are shown in Table 1. A 1:1 ratio was observed, which suggested one dominant gene controlling the sterility expression. There was no significant deficiency of sterile plants as has been reported in other male-steriles (13).

Due to the nature of microsporogenesis breakdown in this particular male-sterile, which was later learned

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Table 1. Frequency distributions of F_1 progenies from crosses of the male-sterile cotton plant with several fertile pollen sources.

Family	Observed		Expected		χ^2	P
	Fertile	Sterile	Fertile	Sterile		
			1976 nursery			
5-1442	58	43	50.5	50.5	2.23	0.14
5-2020	16	20	18	18	0.25	0.64
5-2021	111	95	103	103	1.24	0.27
5-2022	25	21	23	23	0.19	0.68
5-2023	51	48	49.5	49.5	0.09	0.80
5-2024	152	138	145	145	0.68	0.43
5-2025	130	119	124.5	124.5	0.49	0.49
Pooled	543	484	513.5	513.5	3.39	0.07
			1976–77 greenhouse			
6-369-17	12	17	14.5	14.5	0.55	0.47
6-369-25	24	20	22	22	0.20	0.67
Pooled	36	37	36.5	36.5	0.01	0.92

in cytological studies (2), it was difficult to classify 52 plants in the 1976 field nursery during the flowering period. Sixteen flowers on male-sterile plants were recorded to have shed a small amount of pollen in 1976. The remaining plants in question, even though they did not show evidence of pollen shed, contained some pollen in the anthers. It was not determined at that time whether the pollen was viable. It was necessary to observe boll set under the self-pollination bags to confirm the phenotype. The majority of these plants in question were eventually classified as sterile. In the greenhouse and in the 1977 field nursery, this problem was not encountered. Plants in the greenhouse were used for the cytological studies (2) in the spring of 1977. These cytological studies showed the production of shrunken, shriveled pollen grains in male-sterile anthers, but the male-sterile plants were not difficult to classify. Microenvironmental differences may influence the amount of pollen production, even nonviable pollen, and anther dehiscence to some degree. Thus, the occasional production of detectable amounts of pollen made it difficult to classify some male-sterile plants.

Viability tests performed in the spring of 1977 showed that a very high percentage of pollen produced by the male-sterile plants was nonviable. In a sample of 20 flowers from male-sterile plants, eight viable pollen grains were found among more than 1,100 examined. These pollen grains were obtained by squashing the anthers on the day of flowering. Nonviable pollen grains were shriveled and misshapen. Fertile segregates averaged 75% viable pollen as compared to the checks, which averaged 87% viable pollen.

In F_1 male-sterile plants, 10 bolls from 297 selfed flowers set seed. F_2 progenies from these plants should have segregated three sterile plants: one fertile plant if a dominant gene conditions sterility. The selfed progenies included only 65 plants that flowered. The selfed progeny from plant 5-2024 and the pooled total showed highly significant deviations from the expected 3:1 ratio (Table 2). The heterogeneity chi-square was nonsignificant, indicating that the data from the six families could be pooled. The observed data were not significantly different from an expected 1:1 ratio ($\chi^2 = 0.38$). This result indicates a significant deviation from the expected genotypic frequency of functional pollen.

Table 2. Observed and expected frequencies of F_2 progenies from male-sterile cotton plants.

Plant	Observed		Expected (3:1)		χ^2	P
	Sterile	Fertile	Sterile	Fertile		
5-2024	14	16	22.5	7.5	11.37	0.005
5-2025	3	0	2.25	0.75	0.11	0.74
6-369-14	6	5	8.25	2.75	1.48	0.23
6-369-19	3	3	4.5	1.5	0.89	0.37
6-369-24	6	4	7.5	2.5	0.53	0.48
6-369-43	3	2	3.75	1.25	0.07	0.81
Pooled	35	30	48.75	16.25	14.40	0.005
Heterogeneity					4.45	0.49

Table 3. 1977 nursery data of observed and expected frequencies of progenies from open-pollinated sterile cotton plants and crosses of sterile plants in the F_2 generation.

Female parent	Observed		Expected (1:1)		χ^2	P
	Fertile	Sterile	Fertile	Sterile		
5-2024	3	7	5.0	5.0	0.95	0.37
5-2025	7	5	6.0	6.0	0.08	0.79
6-369-13	7	3	5.0	5.0	0.95	0.36
6-369-17	3	3	3.0	3.0	0.00	>0.99
6-369-25	3	3	3.0	3.0	0.00	>0.99
5-2024-10	3	6	4.5	4.5	0.44	0.50
5-2024-14	3	5	4.0	4.0	0.25	0.64
5-2024-16	4	4	4.0	4.0	0.00	>0.99
5-2024-17	4	4	4.0	4.0	0.00	>0.99
5-2024-22	5	3	4.0	4.0	0.12	0.74
5-2024-23	2	2	2.0	2.0	0.00	>0.99
5-2024-25	2	3	2.5	2.5	0.00	>0.99
5-2024-27	4	4	4.0	4.0	0.00	>0.99
5-2024-30	4	6	5.0	5.0	0.10	0.75
5-2025-2	8	3	5.5	5.5	1.45	0.25
6-369-14-3	7	8	7.5	7.5	0.00	>0.99
6-369-14-4	4	2	3.0	3.0	0.33	0.59
6-369-14-5	5	5	5.0	5.0	0.00	>0.99
6-369-14-6	1	9	5.0	5.0	4.90	0.03
6-369-14-7	3	4	3.5	3.5	0.00	>0.99
6-369-14-8	3	7	5.0	5.0	0.60	0.46
6-369-24-5	3	3	3.0	3.0	0.00	>0.99
6-369-43-4	3	8	5.5	5.5	1.46	0.24
Pooled	91	107	99	99	1.30	0.25
Heterogeneity					18.55	0.67

The 1977 field data are shown in Table 3. The first five entries are progenies from open-pollinated male-sterile plants and the remaining 18 are testcrosses of sterile plants in the F_2 generation. Pollen which fertilized the sterile plants should be homozygous recessive at the locus for this particular male sterility. Testcross seed were obtained from only 18 of the 35 sterile plants in the F_2 generation. A high infestation of the cotton bollworm (*Heliothis zea* Boddie) permitted only a limited number of plants to be classified. Segregation for sterility in the testcross populations indicated that the plants were not homozygous dominant for sterility. The junior author observed the progeny of a sterile plant for an earlier generation, which did not seem to segregate for sterility. One plant, 6-369-14-6, deviated significantly from the expected ratio, but the heterogeneity chi-square value indicated that all families could be pooled. Also, one family of 20 would deviate by chance alone from the expected ratio at the 0.05 probability level.

We theorize that the pollen grains carrying the dominant male-sterile gene may be either nonviable or that their pollen tube is less vigorous (competitive) than pollen grains carrying the recessive allele. This con-

dition could explain why the number of male-sterile plants was deficient in the F_2 generation.

In 1976 and 1977, all 1,501 F_2 and F_5 plants from selfed fertile F_1 and F_4 plants were also fertile. This result supports the theory that a dominant gene conditions male sterility.

Gross morphology of male-sterile plants was similar to that of fertile plants except that anthers were reduced in size, a difference that was more obvious in the greenhouse than in the field. Cytological studies (2) revealed this dominant male sterile to be different from Ms_4 and Ms_7 in microsporogenesis breakdown. Microsporogenesis breakdown in the new dominant male-sterile cotton mutant occurred in the post-meiotic stage except for a few degenerating sporogenous cells, which distinguishes it from Ms_4. Microsporogenesis breakdown occurs in the premeiotic stage in Ms_4 (1, 7). Pollen development in the new dominant male-sterile proceeded through exine and intine formation, which distinguishes it from the Ms_7 dominant male-sterile. Microsporogenesis breakdown in the Ms_7 occurs both in the meiotic stage and post-meiotic stage of pollen development (7). In some instances, degeneration of the dyad and the tetrad occurs or microspores degenerate during development of the pollen wall in the Ms_7. The gene symbol Ms_{10} is proposed for this new dominant gene.

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