Published July, 1977

Inheritance of Pollen Fertility Restoration in Cytoplasmic Male-Sterile Upland Cotton[1]

David B. Weaver and J. B. Weaver, Jr.[2]

ABSTRACT

Information regarding the inheritance of fertility restoration in cytoplasmic male-sterile cotton will be a valuable tool in commercial production of F_1 hybrid cottonseed. F_1, F_2, and testcross populations were planted in 1975 at two locations to determine the inheritance of the fertility-restoring factor in cytoplasmic male-sterile Upland cotton (*Gossypium hirsutum* L.). Fertility restoration in cytoplasmic male-sterile cotton is controlled by a single gene probably expressing partial dominance. Either some of the F_2 plants were misclassified or some minor modifying factor(s) was slightly deleterious to the growth and development of fertile plants in segregating populations. *G. barbadense* L. apparently has a factor that enhances pollen production in the F_1 hybrid. A breeding program is underway to transfer this factor(s) into Upland stocks. Fertile plants in segregating F_2 populations showed a significantly higher lint percentage than corresponding sterile plants. Higher micronaire was found for sterile plants than fertile plants.

Additional index words: ***Gossypium,*** **Cytoplasmic male-sterility, F_1 hybrid, Lint percentage, Micronaire.**

NUMEROUS studies have shown that hybrid Upland cotton (*Gossypium hirsutum* L.) is superior to nonhybrid cotton in seedling vigor, earliness, fiber properties, and yield (2, 3, 4). Cotton is a predominantly self-pollinated plant unless a high bee population is present. Therefore, hand crossing is currently the only available method for producing hybrid seed. The junior author (J. B. W.) visited India in 1973 and observed large production fields of hybrid seed produced by this method. Attempts have been made in cotton to utilize genetic male-sterility and gametocides (1, 7, 8), but these have not been successful on a commercial scale.

Until recently, a consistent, cytoplasmic male-sterile having desirable agronomic characters has not been available in cotton. In 1973, Meyer officially released the first acceptable cytoplasmic male-sterile line (5). Male-sterility was obtained by transferring chromosomes of Upland cotton into the cytoplasm of *G. harknessii* 'Brandagee', a wild, lintless species. None of the cultivars or strains within *G. hirsutum* or *G. barbadense* has the ability to restore fertility. A restorer line was developed by transferring genes from *G. harknessii* into the *G. hirsutum* genome. The exact inheritance of the fertility restoring factor is unknown, but Meyer (6) postulated two gene pairs, one dominant and one recessive, for restoring fertility.

Several fertility restorer strains obtained from Meyer have been evaluated in the cotton breeding program at the Univ. of Georgia. One line, designated as Code 37, appeared to be superior to all others in yield, fiber properties, and the ability to restore fertility. The purpose of this investigation was to determine the inheritance of fertility restorer genes in the Code 37 line.

MATERIALS AND METHODS

The restorer line released by Meyer (5) in 1973 known as DES HAF 277 (backcrossed seven times to *G. hirsutum,* the last three times with 'Delcot 277' as the recurrent parent) was planted in 1973 at the Plant Sciences Farm near Athens, Ga. A single plant, designated as Code 37, that appeared to be most fertile and showed agronomically acceptable characteristics, was selected and increased in Iguala, Mexico, during the winter of 1973-74.

Seed from individual plants in Mexico were planted in progeny rows in 1974 at Athens. Individual plants from several progeny rows, which appeared to be most fertile, were crossed with cytoplasmic male-sterile plants (derived from *G. harknessii* cytoplasm) to obtain F_1 hybrid seed. Some of these F_1 hybrids were testcrossed in the greenhouse during the winter of 1974-75 to a nonrestoring cultivar of *G. barbadense* ('Pima S-4') and also selfed to produce F_2 seed.

F_1, F_2, and testcross seed were planted in 1975 at the College Exp. Stn., Athens, and the Southeast Georgia Branch Exp. Stn., Midville, Ga. Segregation ratios were observed on F_2 and testcross (to Pima) generations. Individual plants on each segregating row were scored for fertility.

The difference between fertile and sterile plants was not always obvious in crosses involving a cytoplasmic male-sterile *G. hirsutum* and a restorer *G. hirsutum* (both with *G. harknessii* cytoplasm). Cytoplasmic male-sterile plants had previously been observed to have from 3 to 4 g less lint per 100 g of seedcotton than corresponding male-fertile plants. Therefore, lint percentage of individual plants was used to help determine the accuracy of scoring. Comparisons were made between lint percentage on a sterile plant and lint percentage from a fertile plant on the same row in the segregating F_2 population. This was done on 47 rows at Athens only, because essentially no bee activity at Midville resulted in male-sterile plants with little or no lint. Using 33 of these samples, determinations were made on micronaire value of fertile and sterile plants to determine if a relationship existed between micronaire and fertility.

RESULTS AND DISCUSSION

F_1 hybrids between cytoplasmic male-sterile plants and the Code 37 restorer line showed a wide range of fertility expression. Some plants displayed fertility comparable to 'Coker 201' (a widely grown cultivar) but this did not occur consistently. Enough pollen was shed in most cases to insure adequate boll set.

Segregation ratios determined on individual rows of F_2 plants in Athens are presented in Table 1. According to Meyer's hypothesis (6), the segregation ratio of a cross between a cytoplasmic male-sterile line and a restorer line in the F_2 generation should be 13 fertile to three sterile (two gene pairs, one dominant and one recessive). Applying the chi-square (χ^2) test for goodness of fit on the basis of a 13:3 ratio, a highly significant deviation from this expected ratio was found in F_2 populations at both Athens ($\chi^2 = 30.8$, $P = 0.01$) and Midville ($\chi^2 = 37.4$, $P = 0.01$). These data obviously do not fit Meyer's two gene hypothesis. Therefore the data were tested for goodness of fit assuming

[1] Contribution from the Dep. of Agronomy, College Exp. Stn., Univ. of Georgia, Athen, GA 30602. Submitted by the senior author in partial fulfillment of the M.S. degree. Received 2 Aug. 1976.

[2] Graduate research assistant and associate professor of Agronomy, Univ. of Georgia.

Table 1. Segregation ratios of fertile (F) and sterile (S) phenotypes in F_2 populations of cytoplasmic male-sterile × restorer lines at Athens and Midville, Ga., 1975. Tested to a 3:1 ratio.

F_2 population no.	Observed		Expected		
	F	S	F	S	X^2¶
1†‡	16	4	15	5	0.27
2	13	4	12.8	4.2	0.01
3	36	11	35.2	11.8	0.07
4	20	7	20.2	6.8	0.01
5	22	15	27.8	9.2	4.23*
6	29	15	33	11	1.93
7	8	3	8.2	2.8	0.02
8	10	5	11.2	3.8	0.56
9	11	4	11.2	3.8	0.02
10§	23	5	21	7	0.76
11§	15	9	18	6	2.00
12	16	9	18.8	5.2	1.64
13	11	5	12	4	0.33
14	10	3	9.8	3.2	0.02
15	13	7	15	5	1.07
16	10	5	11.2	3.8	0.56
17	7	5	9	3	1.77
18	21	3	18	6	2.00
19	13	3	14.2	4.8	0.48
20	10	6	12	4	1.33
21	5	0	3.8	1.2	1.66
22	15	1	12	4	3.00
23	5	3	6	2	0.67
24	10	5	11.2	3.8	0.55
Total	349	140	366.8	122.2	3.44
Total for Midville#	407	164	428.2	142.8	4.20*

* Significant at 0.05 probability level. † All crosses advanced to F_2 by selfing. ‡ CMS × restorer line. § Restorer × nonrestorer. ¶ X^2 value needed for significance at 0.05 level is 3.84.
# Represents 40 different F_2 populations, most being identical to F_2 populations at Athens.

a single dominant gene was present for fertility restoration.

Pollen production in F_1 hybrid plants (heterozygous for the restoring factor) is not as consistent and is much more environmentally influenced than pollen production in plants homozygous for the restoring factor. It was obvious that F_1 hybrids as a group produced less pollen than the homozygous restorer parent. These observations indicated that the restorer gene may be incompletely dominant. Because of the difficulty in determining the difference between a homozygous and heterozygous plant on an individual plant basis in segregating F_2 populations, all fertiles were grouped in the same phenotypic class.

When the observed ratios were tested for fit assuming monogenic control for fertility, 23 of 24 F_2 rows at Athens showed a nonsignificant deviation from the expected. Applying the χ^2 test for fit to a 3:1 ratio to the entire F_2 population at Athens (489 plants), a nonsignificant χ^2 of 3.44 (P=0.05-0.10) was obtained. At Midville, 37 of 40 individual rows showed nonsignificant deviations from the expected 3:1 ratio. The total population at Midville (407 fertile and 164 sterile) showed a significant deviation from the expected 3:1 ratio with a total χ^2 of 4.20 (P=0.025 to 0.05). A highly significant (P=0.005) χ^2 of 7.65 was obtained when data from the two locations (1,060 plants) were combined.

Data from individual rows of testcross populations of F_1 (cytoplasmic male-sterile × restorer line) × Pima S-4 are presented in Table 2. Pima S-4 was chosen as the testcross parent because it had been previously observed that Pima S-4 had a factor or factors that improved expression of fertility in plants with *G. harknessii* cytoplasm. Phenotypic expression of differences between fertile and sterile plants in segregating testcross progenies was much more pronounced when Pima S-4 was used as the male parent as compared with nonrestoring Upland parents. Fertile segregates in the populations had much larger flowers than the steriles, and also had a much more pronounced petal spot. These obvious differences permitted a high degree of accuracy in scoring for fertility. The expected segregation ratio, assuming one dominant gene, from these testcrosses is one fertile to one sterile, since the hybrid should be heterozygous for the restoring factor and Pima S-4 carries no genes per se for fertility restoration. All rows showed a statistically nonsignificant deviation from the expected 1:1 ratio and the entire population (235 plants) gave a nonsignificant (P=0.05 to 0.10) χ^2 of 3.57 (Table 2). The data from the F_1, F_2, and testcross generations indicated that fertility restoration was conditioned by an allele at a single locus probably expressing partial dominance.

Table 2. Segregation ratios of fertile (F) and sterile (S) phenotypes in testcross populations of F_1 (cytoplasmic male-sterile × restorer) × Pima S-4 at Athens, Georgia, 1975. Tested to a 1:1 ratio.

Testcross pop. no.	Observed		Expected		
	F	S	F	S	X^2†
1	8	9	8.5	8.5	0.06
2	11	5	8	8	2.25
3	7	15	11	11	2.91
4	8	8	8	8	0
5	7	6	6.5	6.5	0.08
6	5	9	7	7	1.14
7	10	10	10	10	0
8	10	13	11.5	11.5	0.39
9	5	6	5.5	5.5	0.09
10	7	10	8.5	8.5	0.53
11	8	8	8	8	0
12	10	14	12	12	0.67
13	2	8	5	5	3.60
14	5	11	8	8	2.25
Totals	103	132	117.5	117.5	3.57

† X^2 value needed for significance at P = 0.05 level is 3.84.

In comparing the totals of the two F_2 populations and the testcross population, a definite trend was observed. In all three cases, the number of male-sterile plants observed was slightly higher than the number expected. Likewise, the number of observed fertiles was slightly less than expected. Since χ^2 is based on sample size instead of percentage, significant χ^2 values were obtained for the Midville F_2 population and the combined Athens and Midville F_2 populations. Inasmuch as the differences between total observed and expected are so consistent, it is postulated that the restoring factor is controlled by a single dominant or partially dominant gene with some minor factor(s) that is slightly deleterious to the growth and development of fertile plants. This factor(s) is probably closely linked to the fertility restoring factor on the segment of the *G. harknessii* chromosome that carries the fertility-restoring gene.

The authors have observed at least two deleterious characters in populations carrying genes for fertility restoration. One of these is cracked root, which causes the roots to be severely cracked and underdeveloped. Preliminary data collected by the junior author suggest the cracked root character was controlled by a

single dominant gene in Upland cotton. Another deleterious factor is characterized by dwarfed and distorted terminal leaves. Although neither of these characters was observed in Code 37, other deleterious factors could still be associated with fertility restoration in this line. Continued backcrossing and selection should eliminate any unfavorable effects associated with fertility restoration unless these characters are so closely linked that an unusually large population is required to isolate the desired recombinants.

A significant difference existed between lint percentage of fertile and sterile plants. Of 47 pairs of plants measured, only 4 showed a higher lint percentage for steriles than fertiles. Mean lint percentage for fertiles was 37.2 and the mean for steriles was 33.3. Comparing the means for the two samples, a highly significant ($P=0.01$) t value of 5.93 was obtained. A difference was also found for micronaire. Only 4 of 33 pairs of plants showed a higher micronaire value for fertiles than for steriles. Mean micronaire value for steriles was 4.27 and 3.94 for fertiles, a difference of 0.33 micronaire units. A significant ($P=0.05$) t value of 2.29 was obtained. Low micronaire value in fertiles may have been inherited from the restorer line, which was predominantly 'Delcot 277', an inherently low micronaire cotton. These data indicate our scoring for male fertility was reasonably accurate.

REFERENCES

1. Eaton, Frank M. 1957. Selective gametocide opens way to hybrid cotton. Science 126:1174-1175.
2. Hawkins, B. S., H. A. Peacock, and W. W. Ballard. 1965. Heterosis and combining ability in Upland cotton—effect on yield. Crop Sci. 5:543-546.
3. Jones, J. E., and H. D. Loden. 1951. Heterosis and combining ability in Upland cotton. Agron. J. 43:514-516.
4. Kime, P. H., and R. H. Tilley. 1947. Hybrid vigor in Upland cotton. J. Am. Soc. Agron. 39:308-317.
5. Meyer, V. G. 1973. Fertility restorer genes for cytoplasmic male-sterility from *Gossypium harknessii*. Beltwide Cotton Prod. Res. Conf. Proc. p. 65. (Abstr.).
6. ———. 1975. Male-sterility from *Gossypium harknessii*. J. Hered. 66:23-27.
7. Richmond, T. R., and R. J. Kohel. 1961. Analysis of a completely male-sterile character in American Upland cotton. Crop Sci. 1:397-401.
8. Weaver, J. B., Jr. 1960. The present status of male-sterile cotton. Beltwide Cotton Prod. Res. Conf. p. 79-80.