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Inheritance of a Fertility Enhancer Factor from Pima Cotton when Transferred into Upland Cotton with *Gossypium Harknessii* Brandegee Cytoplasm[1]

R. H. Sheetz and J. B. Weaver, Jr.[2]

ABSTRACT

Present efforts towards the development of hybrid cotton in the United States are concentrated almost exclusively on the use of *Gossypium harknessii* Brandegee derived cytoplasmic male sterility. Fertility restoration is controlled by an incompletely dominant gene, leading to variable fertility in heterozygous F_1 upland × upland hybrid populations. However, *G. barbadense* L. apparently possesses a modifying gene(s) which enhances fertility expression when present in upland cytoplasmic male-sterile × upland restorer F_1 hybrids.

Self-pollinated seed, derived from a cross of male-fertile plants of *G. hirsutum* L. in *G. harknessii* cytoplasm × 'Pima S-4' (*G. barbadense*), followed by three backcrosses to non-restoring upland strains was planted in progeny rows in 1977. Individual fertile plants were selfed and testcrossed to six *G. harknessii*-derived cytoplasmic male-sterile strains to determine inheritance of the Pima enhancer factor. Testcrossed and selfed seed were planted in individual progeny rows in 1978 and segregation ratios analyzed.

Analysis of progenies showed that male fertility restoration in F_1 *G. hirsutum* hybrids in *G. harknessii* cytoplasm is enhanced by adding a genetic factor from the *G. barbadense* genome. Fertility in hybrid populations heterozygous for the restorer gene which possess the enhancer factor is comparable, if not superior, to that of the homozygous restorer lines which do not have the enhancer factor.

Data strongly indicate that the enhancer factor derived from *G. barbadense* is controlled by a single gene expressing dominance. However, there is some indication that the enhancer may, in some instances at least, express incomplete dominance. The symbol *E* is proposed to denote the *G. barbadense* gene for fertility enhancement.

***Additional index words:* *Gossypium barbadense* L., *G. hirsutum* L., Hybrid cotton, Pima enhancer factor, Cytoplasmic male sterility.**

EXPERIMENTAL evidence is widespread concerning the expression of heterosis for earliness, yield, and fiber properties in interspecific and intraspecific crosses in cotton (*Gossypium*) (4, 7, 10). Reported increases in yield have ranged from 0.8 to 47% over the better parent (4) and from 6 to 31% over the highest yielding cultivar used as a check (10). The significant economic potential of F_1 cotton hybrids has been demonstrated in India, where increases in yield have ranged from 25 to 50% over traditional cultivars (2). Since the initial release of the first *G. hirsutum* intraspecific hybrid ('Hybrid-4') in 1968 (1), total annual acreage planted to hybrid seed has increased dramatically, reaching present levels of approximately 750,000 ha (3).

Although production of F_1 hybrid cotton seed in India has depended upon hand emasculation and hand crossing, Indian breeders (8, 9) are currently directing their efforts toward utilizing the genetic male-sterile $^{ms}5,^{ms}6$. However, utilization of genetic male sterility requires identification and removal of male-fertile segregates in the female parent rows. This technique could be used in areas of the United States where cotton can be stubbed or ratooned, thereby spreading the initial roguing costs over a period of several years.

The most practical method for the production of F_1 hybrid cotton in the United States is through the development of a cytoplasmic male-sterility system along with an effective fertility restoration mechanism. Until recently, a consistent cytoplasmic male-sterile cotton was not available. Meyer (6) developed the first acceptable male-sterile line by transferring the *G. hirsutum* L. chromosomes into *G. harknessii* Brandegee cytoplasm. Through a backcross program to *G. hirsutum*, four lines were developed—two for male sterility (DES-HAMS 277 and DES-HAMS 16) and two for fertility restoration (DES-HAF 277 and DES-HAF 16).

Weaver and Weaver (11) indicated that a single gene, probably expressing partial dominance, (*Rf*) controls fertility restoration in this cytoplasmic male-sterile cotton. F_1 hybrid populations showed a wide range of male fertility expression. Pollen production in the F_1 (heterozygous for the restorer gene) was found to be much more variable and influenced by environmental conditions than in the homozygous restorer parent. Different flowers on the same plant varied in fertility from practically sterile to highly fertile. Throughout the flowering period, there is a certain percentage of flowers incapable of self-pollination. The male-sterile flowers will not produce cotton unless bee activity is adequate for cross-pollination from fertile flowers.

Weaver and Weaver (11) noted that F_1 hybrids of an upland restorer line (homozygous for the restorer gene) × 'Pima S-4' (*G. barbadense* L., a non-restorer cultivar) were highly fertile under all conditions. This result led Weaver and Weaver (11) to postulate the existence in *G. barbadense* of a factor(s) that improved the expression of fertility in plants with *G. harknessii* cytoplasm. There have been similar indications of male fertility enhancement capacity in other cottons such as 'Sealand', a cultivar known to have genetic material from *G. barbadense*.

The purpose of this investigation was to determine the existence of a male fertility enhancer factor from *G. barbadense* (Pima enhancer factor) and to determine its mode of inheritance after three and four backcrosses to Upland cotton.

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[2] Former graduate assistant (Ring Around Products, Inc., P. O. Box 1629, Plainview, TX 79072.) and associate professor, Dep. of Agronomy, Univ. of Georgia, Athens, GA 30602.

MATERIALS AND METHODS

During the summer of 1973, F_1 hybrids of the cross of the fertility restorer line DES-HAF 277 × 'Pima S-4' had excellent fertility. F_1 hybrids of DES-HAF 277 × *G. hirsutum*, in general, produced little pollen. It was postulated that Pima S-4 had a factor(s) that enhanced pollen production. A backcross program was initiated at the University of Georgia. Plant Sciences Farm near Athens, Ga., to transfer the proposed Pima enhancer factor from *G. barbadense* into *G. hirsutum*.

Open-pollinated seed from the interspecific F_1 hybrid plants of DES-HAF 277 × Pima S-4 were grown at Iguala, Mexico, where the most fertile segregates were selected and backcrossed (BC_1) as females to upland non-restorer cultivars and strains. During the summer of 1974, the most fertile plants were selected from the BC_1 population and backcrossed to 'Dixie King' and other upland cultivars and strains. In 1975, the most fertile segregates in the BC_2 generation were selected and backcrossed to 'Dixie King nectariless,' 'McNair 612,' and an upland strain that carried the okra leaf, frego bract, and nectariless characters. In 1976, the BC_3 plants which showed the highest degree of fertility were self pollinated. F_2 seeds from the BC_3 plants were selected according to the amount of seed produced per plant and planted in individual progeny rows at the Plant Sciences Farm in 1977.

Nineteen BC_3 progeny rows were selected for use in making crosses onto cytoplasmic male-sterile upland strains. Individual plants within the 19 progeny rows were scored as male fertile or sterile. Fertile plants were identified with a white tag and steriles with a red tag. Fertile plants were assigned an individual number and were testcrossed an average of 25 times onto *G. harknessii*-derived cytoplasmic male-sterile strains of upland cotton. Crosses were made onto six different upland cytoplasmic male-sterile genotypes (DES 3967-ne, DES 16-ne, Dixie King-ne, Stoneville 7A-ne, DES 417-16-ne, and 'Coker 201' CMS) planted in an isolated block surrounded by soybeans [*Glycine max* (L.) Merrill]. The six different male-sterile strains were used to determine whether there was any effect of the female genotype on fertility levels in the F_1 hybrids. A total of approximately 6,000 hand crosses were made at random onto all male-sterile lines.

Hand cross-pollination was performed on open flowers which were then covered with a paper bag. Although the male-sterile flowers were not covered prior to cross-pollination, the plot was isolated sufficiently to insure that natural cross-pollination was essentially zero. An average of six self-pollinations were also made on each of the fertile plants used for crosses onto the male-steriles.

Unfavorable climatic conditions, coupled with a high bollworm (*Heliothis* zea Boddie) infestation during the summer and fall of 1977, resulted in a greatly reduced harvest of testcross and selfed seed. Nevertheless, 383 different testcrosses were obtained from the male-sterile strains. Of these testcrosses, 147 were selected for planting in 1978. The selection was based on the number of self-pollinated seed available from the male parent plants plus the availability of testcrosses onto at least two different cytoplasmic male-sterile strains.

In 1978, as soon as flower buds were produced, approximately 5,400 plants were initially scored for fertile vs. sterile. Those showing even the slightest degree of fertility were tagged as fertiles. Identification of completely male-sterile plants was done with relative ease.

The fertile segregates in all rows were classified as weakly or strongly fertile. Determination of fertility was difficult due to the variation in the amount of pollen in different flowers on the same weakly fertile plants throughout the season. Fertility level observations were made for each plant at different dates. Those plants which at any time showed inferior fertility were classified as weakly fertile and those showing high fertility throughout the summer as strongly fertile.

The easiest differentiation of weakly and strongly fertile plants could be made during the period between 2 hours before flower opening and mid-dehiscence. Fertility determination was based on visual appearance of anthers (number and size) and the amount of pollen observable by rubbing 5 to 10 anthers between the thumb and index fingers. This seemingly subjective method of determination permitted a high degree of accuracy if carried out during the morning hours. Beyond mid-dehiscence, accuracy declined, mainly because the observer could not be certain whether low pollen content was due to low fertility per se or simply to pollen shedding and removal by bees.

Blooming rates began to deminish relatively early in the season because of dry conditions. Accurate classifications could not be made on all fertile plants within every row. Nevertheless, counts were obtained on a sufficient number of fertile plants within each family to consider these data representative of each population.

RESULTS AND DISCUSSION

Strongly fertile plants selected in the third backcross population are presumed to have carried the Pima fertility enhancer factor(s). This assumption is based on the facts that: (i) the *G. harknessii* restorer gene (*Rf*) would have to have been present in the heterozygous condition for the individual plant to show any degree of fertility; (ii) the presence of the restorer gene in the heterozygous condition normally leads to variable and weak fertility in association with the *G. hirsutum* genome. Furthermore, this gene(s) would have to be in the heterozygous condition since each of the three backcrosses had been made onto non-Pima material. Assuming the Pima enhancer factor to be a single dominant gene (*E*) not linked to *Rf*, the genotype of these highly fertile BC_3 plants would theoretically be *EeRfrf*.

When selfed, the strongly fertile BC_3 plants should segregate into nine different genotypic classes of which five (*EERfRf, EERfrf, EeRfRf, EeRfrf,* and *eeRfRf*) should show strong fertility, one (*eeRfrf*) weak fertility, and three (*EErfrf, Eerfrf,* and *eerfrf*) complete sterility. Phenotypic segregation in the 1977 progeny rows should then fit a 12:4 ratio of fertile to sterile plants (10 strongly fertile:2 weakly fertile:4 sterile). Segregation ratios determined on individual progeny rows are presented in Table 1. Non-significant deviations from the expected 12:4 ratio were obtained for 17 of 19 progeny rows. However, the chi square value for goodness of fit to a 12:4 ratio in the entire population (302 plants) was highly significant.

Table 1. Segregation ratios of fertile (F) and sterile (S) phenotypes in selfed BC_3 generations in 1977 progeny rows.

Progeny no.	Observed		Expected (12:4)		
	F	S	F	S	χ^2†
7-415	6	1	5.25	1.75	0.04†
7-420	9	4	9.75	3.25	0.23
7-423	9	5	10.50	3.50	0.85
7-429	4	2	4.50	1.50	0.00†
7-438	10	4	10.50	3.50	0.09
7-439	4	0	3.00	1.00	0.33†
7-445	4	0	3.00	1.00	0.33†
7-456	6	1	5.25	1.75	0.04†
7.467	13	10	17.25	5.75	4.18*
7-468	14	14	21.00	7.00	9.33**
7-470	6	4	7.50	2.50	0.53†
7-472	12	5	12.75	4.25	0.57
7-475	14	5	14.25	4.75	0.01
7-480	18	6	18.00	6.00	0.00
7-481	19	10	21.75	7.25	1.39
7-482	9	6	11.25	3.75	1.80
7-483	15	5	15.00	5.00	0.00
7-484	16	9	18.75	6.25	1.61
7-491	14	9	17.25	5.75	2.44
Total	202	100	226.50	75.50	10.60**

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.
† Yates correction factor used (12).

Table 2. Segregation ratios of fertile (F), sterile (S), strongly fertile (St.F), and weakly fertile (WF) phenotypes in grouped testcrossed and selfed progenies, listed by proposed genotype.

Male genotype	Testcrossed to CMS†								Selfed							
	Progenies‡	Plants observed		χ^2	Progenies‡	Plants observed		χ^2	Progenies‡	Plants observed		χ^2	Progenies‡	Plants observed		χ^2
		F	S			St.F	WF			F	S			St.F	WF	
	no.				no.				no.				no.			
EERfRf	18(13)	2,055	15	(Exp. 1:0) 0.10	18(11)	1,613	25	(Exp. 1:0) 0.38	16(16)	277	0	(Exp. 1:0) 0.00	16(12)	175	10	(Exp. 1:0) 1.86
EeRfRf	3(0)	415	14	Exp. 1:0) 0.45	3(3)	165	135	(Exp. 1:1) 3.00	3(3)	51	0	(Exp. 1:0) 0.00	3(1)	31	6	(Exp. 1:0) 0.97
eeRfRf	1(0)	12	2	(Exp. 1:0) 0.28	1(0)	1	11	(Exp. 0:1) 0.08	0(0)							
EERfrf	16(13)	876	807	(Exp. 1:1) 2.82	16(7)	579	26	(Exp. 1:0) 1.11	14(14)	98	34	(Exp. 3:1) 0.04	13(6)	71	10	(Exp. 1:0) 1.23
EeRfrf	7(6)	283	275	(Exp. 1:1) 0.11	7(7)	79	81	(Exp. 1:1) 0.02	5(5)	50	15	(Exp. 3:1) 0.12	5(5)	29	10	(Exp. 10:2) 2.26
eeRfrf	2(2)	41	33	(Exp. 1:1) 0.86	2(1)	2	13	(Exp. 0:1) 0.27	2(2)	30	8	(Exp. 3:1) 0.31	2(2)	8	9	(Exp. 1:2) 1.44

† Cytoplasmic male-sterile; assumed genotype is *eerfrf*.

‡ Number in parenthesis is the no. of progenies which conformed to the expected ratio.

If a few plants of the *eeRfrf* genotype were misclassified as strongly fertile in the BC_3 generation and selfed, segregation of weakly fertile genotypes would be expected to increase in the 1977 progeny rows derived from these plants. Furthermore while classifying the plants during 1977, the senior author did not realize that *eeRfrf* plants produce some flowers with essentially no pollen and could have misclassified some weakly fertile plants as steriles. The classification of weakly fertile plants as male-sterile is a logical explanation for the significant deviations from the expected 12:4 ratio in progeny rows 7-467 and 7-468. Misclassification could also have occurred on weakly fertile segregates in the selfed progeny of the genotype *EeRfrf*.

In the BC_3 F_2 generation, six possible male-fertile genotypes could have been used as pollinator parents: *EERfRf, EeRfRf, eeRfRf, EERfrf, EeRfrf,* and *eeRfrf*. It is assumed that the cytoplasmic male-sterile strains were of the genotype *eerfrf*. Although six different male-sterile strains were used as female parents, an analysis of the segregation ratios and degree of fertility observed in the testcross progenies indicated no consistent differences attributable to the male-sterile lines. Therefore, the testcross progenies from the cytoplasmic male-sterile strains were grouped for statistical analysis of the proposed genotypes for the pollinator parent.

Fertile-to-sterile segregation ratios, pooled for the six male genotypes, are presented in Table 2. Forty-three progenies of the 47 testcross families included in the pooled data showed non-significant deviations ($P>0.05$) when tested for goodness of fit to these ratios.

The unexpected appearance of some sterile segregates in populations expected to be completely fertile is not without precedent. This phenomenon has been previously observed in hybrid populations heterozygous for the restorer gene. The junior author proposes that there may be an occasional loss during meiosis of the *G. harknessii* chromosome segment carrying the restorer gene. This result would lead to small deviations from the expected in all-fertile populations. Although the male-sterile crossing plot was isolated, some outcrossing may have occurred that was not immediately apparent in F_1 populations. This outcrossing would also tend to produce small deviations from expected ratios.

Table 2 also shows the number of plants that were scored for degree of fertility. As previously noted, accurate classifications for degree of fertility could not be made on all fertile plants within every row. However, non-significant chi-square deviations were obtained for strongly vs. weakly fertile plants in all 47 families. Total χ^2 values were also non-significant for all groups of proposed genotypes.

Selfed progenies of the BC_3F_2 plants should segregate (fertile:sterile) for each of the six proposed genotypes as follows: *EERfRf*, 1:0; *EeRfRf*, 1:0; *eeRfRf*, 1:0; *EERfrf*, 3:1; *EeRfrf*, 12:4; *eeRfrf*, 3:1. Segregation data are presented in Table 2. Due to poor germination, no selfed progenies were obtained from eight of the 47 pollinator parents.

Strongly to weakly fertile segregation ratios in these selfed progenies are also presented in Table 2. F_2 populations arising from any of the first four genotypes (*EERfRf, EeRfRf, eeRfRf,* and *EERfrf*) should segregate 1 strongly fertile:0 weakly fertile. Those from *EeRfrf* would be expected to segregate 10 strongly fertile:2 weakly fertile, and those from *eeRfrf*, 1 strongly fertile:2 weakly fertile. Of the 39 progenies analyzed, 38 did not deviate significantly from the expected ratios. Pooled χ^2 values for all groups of proposed genotypes were nonsignificant.

According to the hypothesis, if all fertile genotypes in the 1977 progeny rows are pooled, genotypic segregation should be as follows: 1 *EERfRf*, 2 *EeRfRf*, 1 *eeRfRf*, 2 *EERfrf*, 4 *Eerfrf*, and 2 *eeRfrf*. The 47 progenies analyzed and grouped according to genotype showed the following frequencies: 18 *EERfRf*, 3 *EeRfRf*, 1 *eeRfRf*, 16 *EERfrf*, 7 *EeRfrf*, and 2 *eeRfrf*. As previously pointed out, selection of progenies for planting in 1978 was made by applying two criteria. One of these was the amount of selfed seed obtained from each pollinator parent. Application of this criterion implies that the 47 progenies did not represent a random sample from the original population. Therefore, they should not be expected to segregate according to the hypothesis.

The most significant increases in frequency occurred in genotypes *EERfRf* and *EERfrf*, both homozygous for the enhancer factor. We speculate that these increases may indicate a higher degree of fertility in plants homozygous for the enhancer factor, regardless of the heterozygous or homozygous condition of the restorer gene. Hence more selfed seed were obtained from these two genotypes. If this were the case, *E* would also be incompletely dominant, leading in an F_2 population to the segregation of three phenotypic classes of fertility: very strongly fertile (*EERfRf* and *EERfrf*); strongly fertile (*EeRfRf*, *eeRfRf* and *EeRfrf*); weakly fertile (*eeRfrf*). Further investigation may help to elucidate such a mode of action. Differentiation between these phenotypes will be extremely difficult unless a new technique is devised to determine fertility levels. If the homozygote for the enhancer factor is indeed superior in male fertility, it would be advantageous for the F_1 commercial hybrid populations to have the gene in the homozygous condition. This result could be achieved by transferring the enhancer factor into the cytoplasmic male-sterile lines used in commercial hybrid seed production.

Another area that may warrant further investigation is the possible association of the red plant character (R_1) with fertility restoration in upland cotton. Meyer (5) reported higher fertility in red leaf than in green F_1 hybrids in *G. arboreum* L. and *G. anomalum* Wawr. ex Wawr. & Peyr. derived cytoplasmic male-sterile plants. We have observed that fertility tended to be higher in F_1 hybrids in which the male parent had the R_1 gene for red plant color. A disproportionate frequency of red plants was also observed among the pollinator parents selected for strong fertility in the BC_3 F_2 generation. Red upland strains were among those utilized in the first backcross only. The persistence of large proportions of red plants after three cycles of backcross selection may indicate some degree of association between these characters. However, no evidence was observed for linkage between R_1 and *Rf* (fertility restorer). Although R_1 has no restoring properties *per se*, it may, when present in the hybrid genome, have some minor effect on fertility enhancement.

The gene symbol *E* is proposed to denote male-fertility enhancement in *Gossypium* hybrids utilizing *G. harknessii* cytoplasm. Incorporation of this gene into present upland restorer lines should permit the commercial production of completely fertile F_1 cotton hybrids. The fertility enhancer gene will eliminate the need for high levels of natural cross-pollination in F_1 hybrids that have only the restorer gene and should permit the production of upland × Upland F_1 hybrids from cytoplasmic male-sterile cotton.

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