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Mutations in Cotton Induced by Gamma-Irradiation of Pollen[1]

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THE M_1 plants from irradiated seed may be genetic chimeras, and it is desirable to study M_2 progenies from seed-bearing parts (bolls, panicles, or heads) to increase the probability of observing an induced change. Populations, therefore, may become very large in the M_2 generation. A further disadvantage of seed irradiation is that changes which are induced may be lost by diplontic selection which occurs in developing chimeral plants (2). The chimeric disadvantages encountered in seed irradiation are eliminated by treatment at the single cell stage, i.e., pollen, egg, or zygote. Irradiation of pollen is a most convenient and practical method of treating at the single cell stage for many plant species. However, disadvantages of pollen irradiation such as increased chromosomal aberration and difficulty of transport of induced changes are important considerations in deciding the best stage in the life cycle for irradiation.

Few studies have been conducted on the effects of seed or pollen irradiation in cotton. Horlacher and Killough (3, 4) reported plant color and leaf shape changes following X-irradiation of seed. Brown (1) found numerous chromosomal aberrations in cotton plants from seed exposed to gamma-irradiation at *Bikini*.

Genetic variability in upland cotton is substantial but not so great as in some other crops. Current commercial upland cotton varieties were derived from the somewhat limited pool of genetic variability in *Gossypium hirsutum* L. Hybridization of current commercial varieties with other *Gossypium* species, photoperiodic introductions, and primative varieties has increased the variability available to breeders. High fiber strength,[3] D_2 smoothness, nectariless, male sterility, and glandless seed are specific characters that have been isolated following hybridization and are currently in use to improve commercial cottons (5, 6, 7, 8, 11, 12). The success of such methods in the development of breeding stocks and increasing variability is recognized. However, variability from interspecific hybridization has been of limited value in applied breeding programs since desirable features from other species are frequently linked with undesirable agronomic characteristics. Success in breaking these associations has not been achieved to date in most instances. Due to the rather limited variability in upland cotton and difficulties in using that brought in by hybridization, there is need for other means of increasing variability and studying the potential use of such variability in applied programs. The study described herein was initiated to investigate the effectiveness of gamma-irradiation of cotton pollen in inducing potentially valuable mutants and increasing the variability in quantitative characters. The present paper is concerned with methods, the qualitative mutants induced, and their potential value as breeding and genetic stocks.

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[3] Kerr, Thomas. Transference of lint length and strength into upland cotton. Proc. Third Cotton Improvement Conference. 1951.

MATERIALS AND METHODS

T-92, a stable line that performed well in strain tests, was chosen for irradiation. It was selected from the backcross Coker 33–12 × Acala 5675[2] and is 1 of the 2 sib-lines of the Pope variety.

In 1956, two hundred extended flower buds were emasculated and bagged, and 50 buds were removed, placed in bags, and stored overnight in a household refrigerator. The following morning, the day of anthesis, the 50 detached buds were taken to the UT–AEC Agricultural Research Laboratory, where 10 were irradiated at each of the following cobalt-60 gamma-ray exposures: 400r–E1, 800r–E2, 1600r–E3, and 3200r–E4 (10). The remaining 10 were used for the nonirradiated control–E0. Forty emasculated flowers were pollinated for each exposure. Pollinated flowers were rebagged to prevent uncontrolled crossing. M is used to designate mutant generations; M_0 representing F_1 seed produced in 1956.

Bolls developing from crossed flowers were harvested and numbers of seed and motes recorded. Half of the E0 and E1 and all of the E2, E3, and E4 seed were planted in single seed hills (with a nurse marker stock to aid emergence) in 1957. Control and M_1 plants were carefully examined and notes taken on phenotypic characteristics of individual plants. All plants were self-pollinated by placing glassine bags on flower buds. Five open-pollinated bolls were taken at picking from each plant for laboratory determinations of lint percent, seed index, lint index, fiber length, fiber strength, and fiber fineness. Standard techniques were used in determining the above characteristics which are reported in conventional units of measurement.

In 1958, M_2 progenies were grown from the 336 M_1 plants which produced selfed seed. Environmental conditions were unfavorable for germination, and stands of many progenies were less than perfect (15 single plant hills spaced 2 feet apart). Individual plant notes were again taken. M_3 and M_4 progenies from selfed seed were grown in 1959 and 1960. Selection in these generations was based on phenotype and characters measured in the laboratory. M_3 and M_4 generations consisted of approximately 350 single-row 15-plant progenies. About 50 control progenies (E0) were grown each year, and selections were made in the manner described for the irradiated populations.

RESULTS AND DISCUSSION

Numbers of bolls, seed, and motes produced with untreated and gamma-irradiated pollen are presented in Table 1. The largest numbers of bolls and seed were produced in the E1 treatment. Marked decreases in boll and seed production were noted at the E2, E3, and E4 treatment levels. Numbers of motes were greatest in the E0 and E1 treatments.

Numbers of seed planted and percentages of mature plants, phenotypic changes, and fertility changes in control and M_1 populations are presented in Table 2. Departures from control T-92 for leaf, boll, and general plant type characteristics were considered to be phenotypic changes. Plants producing less than 100 open-pollinated seed were considered fertility changes. Emergence and survival in the E1 and E2 treatments were considerably greater than in the E0 treatment (Table 2). Larger numbers of bolls and seed in the E1 (Table 1) and the better emergence and sur-

Table 1—Numbers of bolls, seed, and motes produced in cotton after pollination with untreated and gamma-irradiated pollen.

Irradiation treatment, r	Flowers pollinated	Bolls	Seed	Motes
E0 - 0	40	14	264	146
E1 - 400	40	20	568	132
E2 - 800	40	5	133	41
E3 - 1,600	40	2	39	16
E4 - 3,200	40	6	81	93

vival in the E1 and E2 (Table 2) possibly resulted from heterosis[4] (9).

Increasing the treatment rate from the E1 to the E3 level resulted in a progressive increase in phenotypic changes reaching 100% in the E3 treatment. Sterility increased markedly from the E2 to the E3 treatment. The E4 plants were normal for phenotype and seed production, and could have resulted from pollen which was not affected by irradiation. However, the possibility of contamination at emasculation or pollination cannot be overlooked.

Most of the gross phenotypic and fertility changes in the M_1 generation were probably the result of chromosomal aberrations induced by irradiation. Few gross phenotypic changes were noted in the M_2, and seed production was normal in most plants, indicating that the majority of induced aberrations were eliminated by one generation of selfing. Four changes which are considered to be mutants and three changes that are possibly due to chromosomal aberrations have resulted from these investigations. These have been studied through the M_4 generation, and are discussed in this paper.

Short internode mutant—Reduced-height plants were found in 3 M_3 sib progenies in the E1 treatment. One progeny was breeding true and 2 were segregating. The mutant was designated short internode. Progenies from short internode M_3 plants were true breeding in the M_4 generation. Plant heights were measured and nodes were counted in M_4 generation progenies breeding true for short and normal internode in the short internode family (progenies derived from a single M_1 plant). The cotyledonary nodes were used as the zero reference point in making these measurements.

Plant height, node number, and internode length in the short internode family are presented in Table 3. Differences between normal and short internode groups of plants were highly significant for characters measured. Plant height was reduced approximately one-third in short internode. The data indicate that the effect of internode length was greater than that of number of nodes in reducing plant height. The vegetative and fruiting branches in short internode plants were shortened in a manner similar to the main stem.

Mean values for 6 characters of selected plants in the short internode family are given in Table 4. Differences between the normal and short internode groups of plants were significant at the 1% level for lint percent, seed index, and lint index, and significant at the 5% level for fiber strength.

Improved cultural practices such as irrigation and high rates of fertilization frequently result in large plants. Mechanized production, especially picking, may be affected by such practices. The short internode mutant could be useful in reducing plant size to aid in mechanized production. The compact nature of the short internode plant results in a dense leafy condition. However, observations have indicated that in spite of this characteristic, there is adequate light penetration and aeration with no overlapping of the middles.

Current research with short internode is concerned with inheritance and linkage studies and the introduction of the character into southeastern commercial varieties. Seed stocks of the mutant are being increased for yield and other agronomic studies.

[4] Osborne, Thomas S. Past, present and potential uses of radiation in southern plant breeding. Proc. Ninth Oak Ridge Regional Symposium, Radiation in Plant Breeding. pp. 5–10. 1957.

Table 2—Numbers of seed planted and percentages of mature plants, phenotypic changes, and fertility changes in control and M_1 populations.

Irradiation treatment, r	Seed planted	Mature plants, %	Phenotypic changes, %	Fertility changes, %
E0 - 0	132	42	0	5
E1 - 400	284	77	38	16
E2 - 800	133	71	63	22
E3 - 1,600	39	51	100	95
E4 - 3,200	81	4	0	0

Table 3—Plant height, number of nodes, and internode length in short internode family, M_4 generation, 1960.

Plant type	Plants	Height, in.	Nodes	Internode length, in.
Normal internode	74	41	20	1.98
Short internode	130	25	17	1.49
Mean difference		16**	3**	0.49**

** Significant at 1% level.

Table 4—Mean values for 6 characters of selected plants in short internode, marcel leaf, and stipple leaf families, M_4 generation, 1960.

Family and group	Plants	Lint, %	Seed index	Lint index	Length UHM	Strength T_1	Fineness Mic.
Short internode:							
Normal internode	14	42.4	12.4	9.09	1.09	1.63	4.28
Short internode	50	43.5	10.8	8.30	1.08	1.54	4.32
Mean difference		1.1**	1.6**	0.79**	0.01	0.09*	0.04
Marcel leaf:							
Normal leaf	50	41.8	12.3	8.75	1.12	1.85	4.19
Marcel leaf	63	39.1	11.4	7.30	1.10	2.52	4.19
Mean difference		2.7**	0.9**	1.45**	0.02**	0.67**	0
Stipple leaf:							
Normal leaf	16	42.7	14.3	10.62	1.15	1.79	4.59
Stipple leaf	23	40.9	10.6	7.28	1.07	1.70	3.93
Mean difference		1.8**	3.7**	3.34**	0.08**	0.09*	0.66**

* Significant at 5% level. ** Significant at 1% level.

Marcel leaf mutant—Wavy leaf plants were found in the E2 treatment in 5 M_3 sib progenies. One progeny was breeding true and 4 were segregating. The mutant was designated marcel leaf because of the characteristic waviness of the margin of the leaf. Progenies from marcel leaf M_3 plants were true breeding in the M_4 generation. High T_1 fiber strength was associated with marcel leaf mutant plants in the M_3 generation, and a relatively large number of plants were selected for laboratory analyses in the M_4. These data, summarized in Table 4 with other laboratory data, show that T_1 fiber strength of the marcel leaf group of plants is outstandingly high. Differences between the normal and marcel groups of plants were significant at the 1% level for lint percent, seed index, lint index, fiber length, and fiber strength.

Field observations indicate that yield of the marcel leaf mutant would be very low. It is also late and reduced in height and has long, sharp pointed bolls. The association of high strength and poor agronomic type in this mutant is of considerable theoretical interest, since fiber strength is an important quality characteristic of cotton. Breeders of upland cotton have been successful in combining good strength with high yield in a number of varieties. However, efforts to combine outstanding strength with good agronomic type have not been successful. The association of high fiber strength with low yield has been noted by many breeders, particularly those working with triple species hybrids.[3] Fiber strength is influenced by environment, and conditions which place the plant in physiological stress, such as lack of adequate moisture, may result in an apparent increase in strength.[3]

The foregoing general observations regarding the association of strength and yield indicate that a portion of fiber strength is genetic and that some progress through breeding without sacrificing yield is possible. However, any con-

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dition, genetic, cytological or environmental, which results in physiological stress may cause increased fiber strength and a decrease in yield. The introgression of germ plasm from other species could cause physiological stress and account for the relation between high strength and poor agronomic type in triple hybrid materials.

It is not likely that the association of marcel leaf, high strength, and low yield is a genetic linkage of specific mutants brought about by irradiation. Irradiation probably caused some change, perhaps a small deletion, which acts as a true breeding recessive resulting in the marcel leaf phenotype. The deletion may also cause some metabolic disorder which results in high strength and low yield.

Preliminary observations indicate that F_1's involving marcel leaf are good agronomic types with fiber strengths intermediate to the parent lines. Within the marcel leaf family normal plants with intermediate strength always segregate the marcel leaf phenotype. Fiber strengths of materials heterozygous for marcel leaf need to be studied further. The utilization of marcel leaf as a parent in commercial hybrids for fiber strength is a possibility.

Current research with marcel leaf is concerned with inheritance and linkage studies and the introduction of the character into southeastern commercial varieties. Seed stocks of the mutant are being increased for yield and other agronomic studies.

Stipple leaf mutant—Plants with leaves exhibiting small irregular, dark green, depressed, water-soaked-like spots were found in 3 M_3 sib progenies in the E2 treatment. These spots were most pronounced on the leaf undersurface. One progeny was breeding true for the condition and 2 were segregating. The mutant was designated stipple leaf. Progenies from stipple leaf M_3 plants were true breeding in the M_4 generation.

Means for 6 characters of selected plants in the stipple leaf family are given in Table 4. Differences between the normal and stipple leaf groups of plants were significant at the 1% level for lint percent, seed index, lint index, fiber length, and fiber fineness and significant at the 5% level for fiber strength.

Current work with stipple leaf is concerned with inheritance and linkage studies. This mutaut does not appear to be of any practical value. However, since it is easily identified, it should be a good marker, and might be of use in genetic studies.

Female sterile mutant—An M_3 progeny in the E2 treatment was observed segregating plants which were essentially sterile. Bolls were generally shed about 10 days after flowering. However, an occasional open-pollinated seed developed. The plants appeared normal in all other respects. Two of these plants have been observed through 2 seasons in the greenhouse and 2 in the field by transplanting.

The sterility of these two plants was checked by selfing and crossing. Seed were not obtained in hand made crosses using these two plants as females. However, the plants were apparently fully male fertile. A few seed, as many as 3 or 4 per plant per season, were produced following open-pollination in the field. Segregation in F_2 and backcross populations indicates that the female sterile mutant is conditioned by a simple genetic factor.

Leathery leaf aberrations—Three aberrant types designated leathery leaf-1, -2, and -3 were observed. Phenotypically the three types are similar in many ways. Leaves are thick, leathery feeling, and dark green, while the bolls are large; and all three types are late maturing.

Leathery leaf-1 was isolated from an M_3 progeny in the E2 treatment, and leathery leaf-2 and -3 from separate M_3 progenies in the E1. Normal and leathery leaf plants were observed in all progenies from selfed leathery leaf-1 and -2 plants. Population sizes were not large enough to establish definite ratios. Preliminary data for crosses of these 2 leathery leaf types with T-92 indicate differential male and female transmission of the aberrancy. Leathery leaf-3 appears to be true breeding on the basis of a limited popnlation.

SUMMARY

Gamma-irradiation of pollen at 400, 800, 1600, and 3200r was investigated as a method of inducing potentially valuable mutants in cotton. Stimulative or heterotic effects from irradiation were indicated in that boll set and seed production were greater in the M_0 400r treatment than in the control. Higher emergence and seedling survival from 400r and 800r treatments in the M_1 generation indicated a similar effect.

Plant height was rednced approximately one-third in a short internode mutant derived from the 400r treatment. It may be of value as a breeding stock to reduce plant size. Marcel leaf, stipple leaf, and female sterile mutants were isolated from the 800r treatment. Marcel leaf is associated with very high T_1 fiber strength and could be of value as a breeding stock. Stipple leaf and female sterile are easily classified and could be useful as genetic markers.

Three leathery leaf conditions, 1 from the 400r treatment and 2 from the 800r treatment, are similar phenotypically. Preliminary data on their behavior indicate that chromosomal aberrations may be involved.

These investigations indicate good probabilities of obtaining qualitative mutants following gamma-irradiation of cotton pollen at 400r and 800r. Changes in quantitative characters of economic importance may have been indnced in these studies, and this possibility is being investigated.

LITERATURE CITED

1. Brown, M. S. Cotton From Bikini. J. Her. 41:115–121. 1950.
2. Gaul, Horst. Present aspects of induced mutations in plant breeding. Euphytica 7:275–289. 1958.
3. Horlacher, W. R., and Killough, D. T. Progressive mutations induced in *Gossypium hirsutum* by radiations. The American Naturalist 67:532–538. 1933.
4. ———————, and ———————. Radiation induced variation in cotton. J. Her. 22:253–262. 1931.
5. Justus, Norman, and Leinweber, C. L. A heritable partially male-sterile character in cotton. J. Her. 51:191–192. 1960.
6. Meyer, James R. Origin and inheritance of D_2 smoothness in upland cotton. J. Her. 48:249–250. 1957.
7. ———————, and Meyer, Vesta G. Origin and inheritance of nectariless cotton. Crop Sci. 1:167–169. 1961.
8. McMichael, Scott C. Combined effects of glandless genes gl_2 and gl_3 on pigment glands in the cotton plant. Agron. J. 52:385–386. 1960.
9. Osborne, Thomas S. Mutation production by ionizing radiation. Proc. Soil and Crop Sci. Soc. of Florida 17:91–107. 1957.
10. ———————, and Lunden, A. O. The cooperative plant and seed irradiation program of the University of Tennessee. Int. J. of Appl. Irrad. and Isotopes 10:198–209. 1961.
11. Rhyne, C. L., Smith, Frank H., and Miller, P. A. The glandless leaf phenotype in cotton and its association with low gossypol content in the seed. Agron. J. 51:148–152. 1959.
12. Richmond, T. R., and Kohel, R. J. The analysis of a completely male-sterile character in American Upland cotton. Crop Sci. 1:397–401. 1961.

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