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Isogenic Lines in American Upland Cotton, *Gossypium hirsutum* L.: Preliminary Evaluation of Lint Measurements[1]

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Evaluation of isogenic lines of cotton

ABSTRACT

We backcrossed plants of eight dominant markers recurrently to a highly inbred line to develop a series of isogenic lines. After backcrossing six generations, we evaluated eight agronomic and physical properties of the lint to determine the degree of isogenicity, and to characterize the pleiotropic effect of the individual marker genes. After separating the segregating material by genotype, we evaluated them by comparing the variability between and within genotypes. Analyses revealed residual variability in some lines but not in others after six generations of backcrossing, which indicated progress toward isogenicity but that additional backcrossing was needed. Some markers displayed apparent pleiotropic effects and one displayed linkage.

INTEREST in the collection and study of cotton mutants began with the development of genetics as a science. Over the years, geneticists, breeders, and observant farmers have collected "freak" or "off-type" plants, either out of curiosity or with the thought that they might be of scientific or economic value. The extent to which mutants were studied varied widely, and mutants were left in diverse and variable genetic backgrounds. Since 1948, Regional Research Project S-1, "Genetics and Cytology of Cotton" has served to coordinate cataloguing of known mutants. An effort is being made to assemble all known mutants, to eliminate unnecessary duplications, to insure publication of the description and genetic behavior of each stock, and to place seed in the National Seed Storage Laboratory, Ft. Collins, Colorado.

One objective of a project initiated by the Texas Agriculture Experiment Station and contributing to the regional effort is to evaluate the influence of those mutant genes, which are simply inherited genetic markers, on the agronomic properties, which are usually quantitatively inherited. In recent years discovery of simply inherited traits with economic possibilities has emphasized the need for this information. However, marker stocks available in the early 1950's were inadequate for this purpose. Effects of the marker genes on the agronomic properties could not be separated from effects of their heterogeneous genetic backgrounds. Therefore, in 1957 we began a program of backcrossing stocks having simply inherited genetic markers to a common inbred line, TM-1. By this procedure, we expected to establish a series of isogenic lines in which the genotype and cytoplasm of the recurrent parent (TM-1) would be common to all lines. Each line would differ from the inbred only by a single marker gene pair. Such a series of isogenic lines would allow comparisons between normal and mutant alleles as well as between nonallelic markers. From the outset, we recognized that recurrent backcrossing could not establish completely isogenic lines. However, we thought that practical recovery of the recurrent genotype could be achieved and that this point could be recognized experimentally.

Eight dominant markers have undergone six generations of backcrossing in the isogenic lines program at College Station, Texas. This paper reports the results of our analysis of the measurement of agronomic and physical properties of lint in these eight lines.

MATERIALS AND METHODS

Eight dominant genetic markers were backcrossed as pollen parents to a standard inbred line (TM-1) for six generations. TM-1, selected from a cultivated variety, had been inbred 13 generations at the start of the program; inbreeding was continued by individual plant pedigree. In each backcross generation we selected heterozygotes as parents for the next backcross. Initially, the eight markers were present in two multiple marker lines. Pilose (H_2), yellow pollen (P), yellow petals (Y_1), and

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green lint (Lg) occurred in one line. Red plant body (R_1), okra leaf (L°), petal spot (R_2), and brown lint (Lc_1) occurred in the other. R_2 and Lc_1 are in linkage group I, L° and Lg are in linkage group II; R_1 is in linkage group III; H_2 is in linkage group IV; and P and Y_1 are independent loci. (See Kohel, Lewis and Richmond, 1965) for a review of linkages in Upland cotton.

Heterozygous plants in the sixth backcross generation were self-fertilized to produce seeds for a segregating generation in 1963. The eight SBC_6 lines were grown in two replications, and each line was represented by three entries. The three entries represented three BC_6 plants. Seeds were first planted in individual-seed containers in the greenhouse; and 2-week-old seedlings were then transplanted into field plots. Each entry was planted in a 9-meter (30-foot) row with 20 plants spaced 30.5 cm (1½ feet) apart. The total experiment included 8 marker lines with 3 entries in 2 replications; this equalled 48 plots containing 20 plants.

Segregating progenies in each plot were separated into mutant and normal classes. Genotypic differences were treated as split-plots and analysed accordingly. R_2, P, Y_1, and H_2 are completely dominant; therefore, mutant classes represented homozygous and heterozygous mutant genotypes, and normal classes represented the homozygous normal genotype. R_1, L°, Lc_1, and Lg segregating populations can be separated into three genotypic classes, so the mutant and normal classes represented the respective homozygous genotypes. R_1, L°, and Lc_1 were easily classified in the field but variability in Lg made classification difficult. Lint samples of Lg segregates were re-examined in the laboratory to assure that mutant and normal classes represented the respective homozygous genotypes.

Seed cotton from individual plants was harvested and ginned and measurements were made of lint index (grams of lint on 100 seeds); lint percent (the percentage of lint in a given quantity of seed cotton); and lint quantity (grams of lint per plant). For purposes of analysis, data were pooled by genotypic class and expressed as a per plant average. Samples of lint from plants of the same genotypic class within a plot were pooled. Length, strength, fineness, and elongation were measured on the pooled sample. Length was measured at the 2.5% and 50% span-lengths (inches) by the digital fibrograph. Strength (T_1) was measured with ⅛-inch gauge length on the Stelometer and expressed as grams per grex. Fineness, measured by the Micronaire (Mic), represents an approximate ratio of weight per unit length; the larger the value the coarser the individual fiber. Elongation (E_1) is the measure of elongation at the time of break-loading compared to the initial length; this is expressed as a percentage.

RESULTS AND DISCUSSION

Results of the analyses of variance of lint property data are shown in Table 1. Elongation was the only property that showed no statistically significant differences among lines or between genotypes. For all other properties, differences among marker lines, between genotypic classes, and the interaction between these sources of variation were significant. Differences among marker lines were expected because of pleiotropic effects of the mutant genes. Significant differences between genotypes and the (line × genotype) interaction indicated differences between mutant and normal genotypic classes as a result of separation of the effects of marker loci and background genotypes. Differences among marker lines and genotypic classes are illustrated in Figure 1. Not all the markers would be expected to influence lint properties equally. R_2, Y, and P were placed first because they affect flower pigment and were expected to have the least direct influence on lint properties. R_1 causes the production of red pigment throughout the plant and was suspected to have more influence than the flower characters. Lg, H_2, and Lc_1 were presented in that order because of their affect on length. Lg and Lc_1 were expected to influence the lint properties because of the direct effect of pigment deposition in the fibers. H_2 influences the production of hairs on the plant surface, therefore, an influence on lint properties might be expected since, botanically, the cotton fiber is a seed hair.

Table 1. Analyses of variance of lint measurements of Upland cotton.

		Mean squares							
		Lint				Length			
Source	DF	index	percent	quantity	E_1	2.5%	50%	T_1	Mic
Lines	7	2.01**	57.41**	99.45**	0.90	.0208**	.0337**	.1305**	1.9499**
Replications	1	1.39*	0.03	36.63	2.73	.0032	.0016	.2593**	0.0417
Error (a)	37†	0.31	4.04	28.07	0.71	.0021	.0027	.0120	0.1226
Genotypes	1	3.20*	42.53**	218.70**	0.70	.0858**	.1254**	.5719**	1.4211**
G × L	7	2.26**	56.87**	56.71**	0.55	.0210**	.0300**	.1100**	1.8000**
Error (b)	38†	0.65	5.68	4.50	0.80	.0008	.0012	.0099	0.0555

*, ** F ratio exceeds the .05 and .01 levels of significance, respectively.
† Two degrees were lost due to missing observations.

Table 2. Analyses of variance of lint measurements for normal F_2 segregates of Upland cotton.

		Mean squares							
		Lint				Length			
Source	DF	index	percent	quantity	E_1	2.5%	50%	T_1	Mic
Lines	7	.5458**	10.19*	63.31	0.46	.0016	.0012	.0272*	.1766*
Replications	1	.9408**	0.12	0.52	5.81**	.0050	.0027	.1365**	.0070
R × L	7	.1165	3.35	17.17	0.44	.0008	.0008	.0070	.1430
Within	30†	.2692	3.68	29.31	0.74	.0014	.0014	.0100	.0741

*, ** F ration exceeds the .05 and .01 levels of significance, respectively.
† Two degrees were lost due to missing observations.

Two criteria were selected for evaluation of the degree of isogenicity of these lines. The first was an analysis of differences among entries within normal segregates. The second was an analysis of the within line source of variability.

The analyses of variance of normal segregates are shown in Table 2. Lint quality, 2.5% and 50% span-length, and elongation (E_1) showed no significant differences among entries within the normal segregates. Lint index, lint percent, strength (T_1), and fineness (Mic) were significantly different among lines.

With reference to fineness, Duncan's multiple range test indicated that only the L° line was significantly different from the other lines. Inspection of the data revealed that one of the three SBC_6 entries, originating from a single BC_6 plant, had an obviously lower Mic value than the others. The other two L° entries were within the range of the remainder of the population. The same SBC_6 line was the major contributor to the differences in lint per cent among entries. Thus the BC_6 population must have been segregating, and this divergent entry needed to be eliminated from the isogenic line.

This same effect was present in the measure of lint index, but the seven lines, other than l, fell into four overlapping significant classes by the multiple range test. Table 1 and Figure 1 serve to illustrate that there was a definite genotypic effect and a genotype × line interaction for lint index; but that the normal segregates also remained variable. Inspection of the entries did not reveal any obvious individual entry deviations. Therefore, the background genotype remained variable in some lines after six generations of backcrossing to the inbred line. Duncan's multiple range test of strength values for normal segregates indicated that l, r_2, and lc_1 were significantly different from the remaining lines. As in lint index, there were no obvious deviations among entries; therefore, the variability must have resulted from residual variation of the background genotypes.

The original markers and their resulting multiple marker lines used in the isogenic program were present

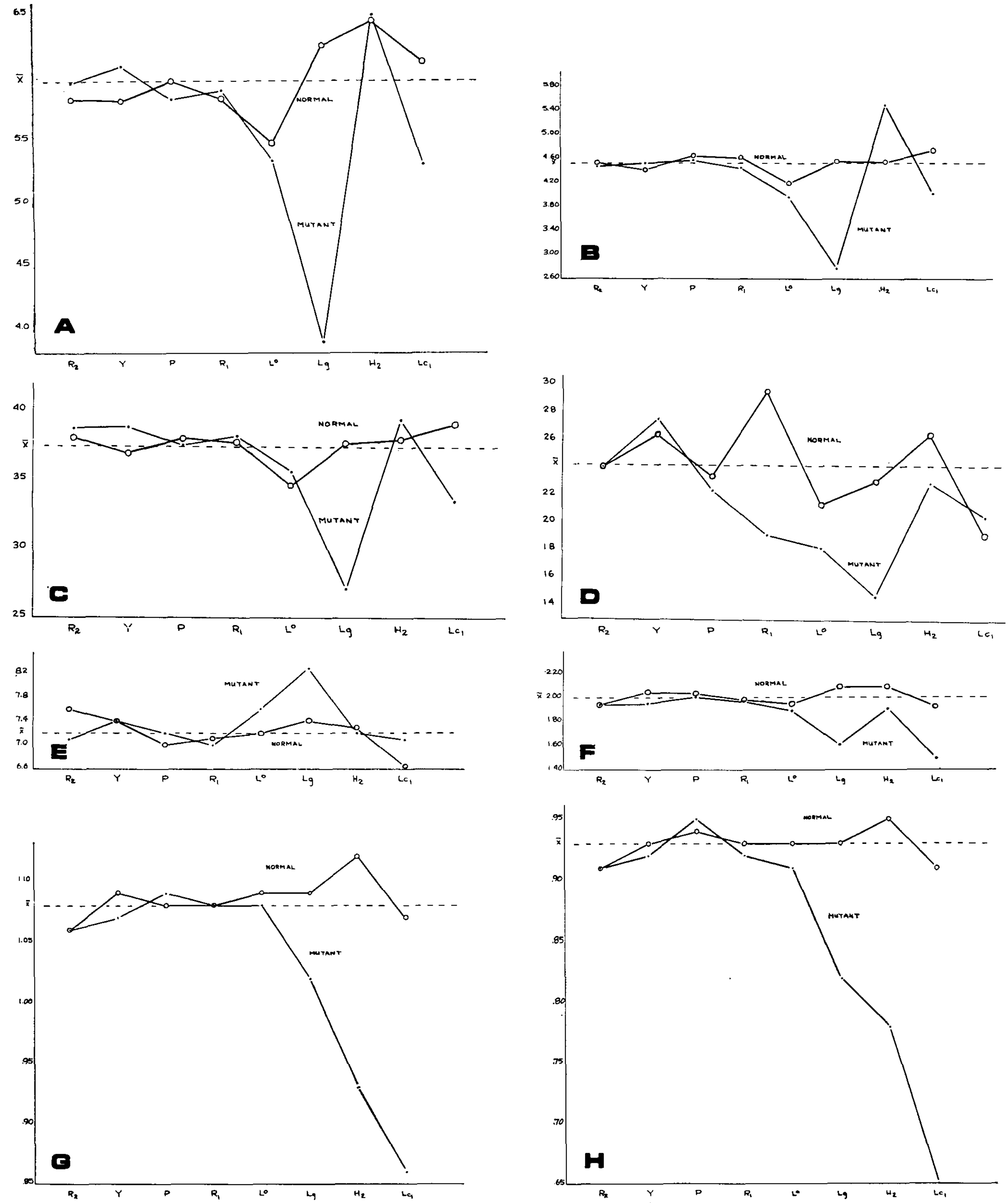

Figure 1. The mean lint measurements are plotted for each genetic marker line. Open circles connected by lines represent values for the normal classes and the closed circles represent the mutant classes. The dotted line is the mean of all the normal classes combined. (A) Lint index, (B) Micronaire values, (C) lint percent, (D) lint quantity (grams), (E) elongation (percent), (F) strength (grams per grex), (G) 2.5% span-length (inches), (H) 50% span-length (inches).

in unselected genetic backgrounds that did not represent the same high degree of agronomic refinement as that present in the inbred line. Therefore, nonsignificance among lines within normal segregates of four of the eight measurements reflected progress toward isogenicity.

The second method of evaluating isogenicity was an analysis of within-line variability. The within-line variability among lines, between genotypes, and the (lines $\times$ genotype) interaction was not significant for any of the lint measurements. The lack of significance indicated that any variability due to segregation (as detected with the analysis of normal segregates) was uniform in the population.

Results indicate progress toward isogenicity. The theoretical work of Hanson (3) suggests that the linkage block around each marker locus in this study should be about 26 to 37 units. It is no wonder that significant background variability was found. Hanson's (3) work points out the need for caution in interpreting single gene pleiotropic effects, even when dealing with isogenic lines.

Although not an intrinsic part of this study, some observations as to the effect of the mutant genotypes on lint properties are in order. R_2, Y_1, and P did not affect the plant in any way that would suggest a direct action of fiber development. Fiber measurements plotted in Figure 1 illustrate that normal and mutant classes are grouped together consistently. This indicates the effective elimination of most of the variability in background genotype, and the lack of any effects from pleiotropy or linkage. We presumed that R_1 might have influenced lint properties because of the general occurrence of anthocyanin pigment throughout the plant. However, no differences were detected due to this mutant character, with the possible exception of a slight loss in yield.

L° was considered likely to influence lint development because it reduces total leaf area and consequently might affect plant growth. L° influenced Mic, lint index, lint percent, and lint quantity. As we pointed out in the discussion about the normal classes, the significant deviations were attributed primarily to a single entry for Mic values and to a lesser extent to the same entry for the other measurements. In the L° class, the three entries were uniform for all measurements. The effects of L° could be due to some general physiological effect, but the occurrence of a fine-fibered entrant in the normal class suggests that instead it was caused by linkage.

Lg and Lc_1 were expected to influence the lint properties because of pigment deposition in the fibers. Conrad (1) and Conrad and Neely (2) reported a 10- to 30-fold increase in wax content associated with Lg. Hull (4) reported a reduction in lint percent, and Neely (7) reported a reduction in lint index associated with Lg. Richmond (8) reported a reduction in weight of fiber per unit length (a measure similar to the Micronaire value used in this study) associated with Lc_1 and Lg. All of these workers used populations segregating in early generations and they did not have available material that approached an isogenic line in purity. However, the effects of Lg are so pronounced that not much precision is required to detect these differences. The deposition of pigment was associated with short weak fibers, and Lg fibers were fine. These effects were reflected in lint percent and lint index. The Lg lint also displayed a high degree of elasticity, whereas the Lc_1 lint displayed a slight reduction in elasticity compared with normal segregates.

H_2 influences the production of numerous short epidermal hairs on the plant. Lint is a type of epidermal hair. This effect is apparently reflected in lint because H_2 caused deviations toward coarse short lint. In earlier studies, Simpson (9) and Lee (6) reported the association of short coarse lint with H_2, so this association is probably a pleiotropic effect or very close linkage.

Even after isogenic marker lines have been established, they must be characterized in terms of their effects on the genotype (or phenotype) of the recurrent parent. Comparative study of a series of isogenic marker lines may reveal that certain alleles have no visible or measurable effects, while others may have minor to major effects on vigor, yield, lint properties, and the like, of the recurrent parental line. Usually, these effects may be assigned to pleitropy, but part or all of them could be ascribed to linkage. Once the individual isogenic marker lines have been characterized in terms of the pleiotropic effects of the various marker genes, they will be ready for use in linkage studies and various other genetic investigations involving quantitative, agronomic or economic characters.

Data presented in this paper have helped to determine the precautions necessary in handling material of this kind. Other characters remain to be evaluated. Additional backcross generations are suggested, and should be followed by self-fertilizing or intermating and then by self-fertilizing. The self-fertilization process should include the opportunity to increase the total population size. Once the populations have been sufficiently increased, they should be evaluated intensively for agronomic and lint properties. Results of tests reported herein have identified specific entries that need to be eliminated. Intensive evaluation may eliminate other entries or indicate the need to apply positive selection pressure.

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