Published September, 1982

Inheritance Studies of Bract Size in Cotton[1]

D. T. Bowman and J. E. Jones[2]

ABSTRACT

Bracts in Upland cotton, *Gossypium hirsutum* L., have been implicated in byssinosis, a lung disorder associated with textile mill workers. Before initiating a breeding program with the objective of reducing bract trash in machine-harvested seedcotton, inheritance studies were performed on the trait bract size or bract surface area.

In the first study, heritability was calculated from regression of 83 F_3 plot means on F_2 plant values from a cross involving a small bract parent and a nectariless normal bract breeding line. Correlation coefficients were calculated to determine heritability in standard units. Highly significant regression and correlation coefficients of 0.69 and 0.75, respectively, were computed for heritability estimates.

In the second study a seven parent diallel was used and analyzed by the procedures outlined by Jinks and Hayman. The experiment included the seven parents, their 21 F_1's, and their 21 F_2's and was performed at two locations for 2 years.

Partial failure of the assumptions of no epistatis, no multiple allelism, and independent gene distribution was observed in the three general tests. Epistasis and multiple allelism were tested and considered negligible. Discrepancies were noted between populations for estimates of frequency of alleles and dominance gene effects. Partial dominance was expressed at most loci where dominant alleles governed bract surface area. Narrow-sense heritability estimates indicated that bract size heritability was primarily additive although dominance gene effects contributed significantly. Parents were identified with bract size and levels of dominance suitable for cultivar breeding programs.

Additional index words: Byssinosis, Cultivar, Diallel analysis, *Gossypium hirsutum* L., Heritability.

BRACTIOLES or involucral bracts of cotton plants, *Gossypium* spp., along with stems, leaves, capsules, and weedy material are commonly a part of the nonlint content of machine-harvested seedcotton. In 1966, Corley (5) found that bracts comprised 12% of the total trash in machine-harvested cotton and that an average of 40% of the fine trash content was composed of bract material. In support of Corley's work, Morey et al. (17) found an average of 43% of the visible wastes in raw cotton extracted by Shirley Analyzer techniques were derived from bracts. The nonlint or trash, if not removed at the gin, must be extracted at the textile mill. The latter process pulverizes a portion of the trash which is then emitted into the air as microscopic dust particles. This dust is associated with a lung disorder, byssinosis, in susceptible mill workers (4). Ayer (3) suggested that the bract was the active agent in cotton dust involved in byssinosis. Hitchcock et al. (11) found that bracts contained a steam-volatile component which released histamine after in vitro application to chopped human lung tissue. It was thought that the histamine-releasing agent was the active material causing byssinosis. Thus, bracts are implicated in byssinosis; although at present there is not published evidence that bracts are micronized into respirable cotton dust (17).

Theoretically, a reduction in bract size would reduce bract trash content in machine-harvested cotton, thus reducing microscopic dust and thereby decreasing the incidence of byssinosis. Screening studies by Milam et al. (16) and Jones and Milam (14) indicate considerable genetic variability for bract surface area in Upland cotton.

The objectives of the two studies reported herein were to investigate the genetic nature of bract size prior to the initiation of a breeding program.

MATERIALS AND METHODS

Study One

In 1977 the bract surface area or bract size was determined on 327 individual plants of the F_2 generation from a cross involving HR-26-Sm, a cotton with small, flared bracts, and Stoneville 7Ane$_1$ BC$_7$, a cotton with bract size more typical of modern Uplands. HR-26-Sm is an F_5 generation line developed by M. J. Lukefahr from a cross between small bracted *G. hirsutum* race stock T718 and La. 213-19556, an inbred line of 'Stoneville 213'. Lukefahr further crossed HR-26-Sm (plant 2) with Stoneville 7Ane$_1$ BC$_7$, a breeding line obtained from W. R. Meredith. The F_2 and F_3 generations of this cross, which is the basis of this study, stem from F_1 seed furnished to us by M. J. Lukefahr in 1976.

The F_2 population was grown spaced one plant per 0.5m. Five bracts per plant were collected from the first, second, or third mature bolls on the sympodial branches in the middle of the plant. Bract size was measured on a Hayashi Denko Area Meter, Model AAm-5[3] in cm^2.

In 1978, 83 random F_3 progenies obtained from self-pollinated F_2 plants were planted in unreplicated single-row plots, 5.49m long with 12 hills per plot and approximately two plants per hill. The only restriction on selection of F_2 parental plants was the availability of at least 20 self-fertilized seed. 'Deltapine 61' was used as the commercial check, being replicated four times within the site. The Stoneville 7Ane BC$_7$ parent was replicated twice. Seed of the HR-26-Sm parent was not available for the study. Twenty bracts, one per plant, were collected from each plot using the same procedure as in 1977.

The regression of the F_3 plot means on the F_2 plant values were calculated to determine heritability. To minimize any genotype-environment interaction that may exist, the correlation coefficient was also calculated to determine heritability in standard units.

Study Two

The second study consisted of a seven parent diallel using the following lines or cultivars:

1. MoBw 51849,

[1]Contribution from the Dep. of Agronomy, Louisiana Agric. Exp. St., Baton Rouge, LA 70803. Received 21 Dec. 1981.

[2]Former research associate (presently assistant professor, Crop Science Dep., North Carolina State Univ., Raleigh, NC 27650), and professor Dep. of Agronomy, Louisiana Agric. Exp. Stn., Baton Rouge, LA 70803.

[3]Mention of a trademark or a proprietary product does not constitute a guarantee or a warranty of the product by the Louisiana Agric. Exp. Stn. and does not imply its approval to the exclusion of other products that may also be suitable.

2. 'LSS' (Pak) M71-010,
3. NCJ-9 (B-5)23790-1796-1167-657,
4. 4S-180 (Greece)-1766-1149-636,
5. La. DS1S 12513-245-1667-1015,
6. Coker NF 73-809-060,
7. La. 16ne-24-1-845-103-63-57.

Parents and crosses among parents will be identified by the number and the appropriate number combination, respectively. The parents used in this study were specifically chosen for their bract size based on previous screening studies (14, 16). Though they represent a wide range of bract sizes (4.1 to 8.7 cm^2), they do not constitute a random sample of all Uplant cotton cultivars and breeding lines. Thus, inferences derived herein are applicable only to the parents, crosses, and generations studied.

Diallel crosses among parents, bulking reciprocal full sibs, were made at the Perkins Road Agronomy Farm in Baton Rouge, La. in the summers of 1977 and 1978 and in the greenhouse during the winter of 1978 and at Iguala, Mexico during the winter of 1977. The experiments were conducted at the Perkins Road Agronomy Farm in Baton Rouge and at the Dean Lee Agricultural Center in Alexandria, La. in 1978 and 1979 and included the seven parents, their $21F_1$'s, and their $21F_2$'s.

The experiments were conducted in a randomized, complete block design with four replications at each location with the exception of Baton Rouge in 1978 where data were obtained only from three replications. Plots in 1978 were single rows 5.5m long and 1.0m apart. In 1979 at Alexandria, the plots were again single rows 5.5m long and 1.0m apart, while at Baton Rouge, the plots were single rows 5.0m long and 1.0m apart. Twelve hills, spaced approximately 0.5m apart with two plants per hill, were planted in each plot. Adjustments and weighted averages were made for plots containing less than 24 plants. La. 13212R^s, a strain with red plant color, was planted in skips to compensate for differential spacing between plants caused by missing hills; skips were minimal in both years. The plots were bordered on each end by a 1.8m plot of La. 13212R^s except at Baton Rouge in 1978, which had 1.5m plots of La. 13212R^s. The red leaf plants used as borders and as fillers within plots were removed after maturity and prior to harvest. A minimum of one border row was planted on each side of the experiments at both locations.

Cultural practices regarding fertilization, cultivation, and pest control normally used for cotton production were performed at both locations.

Twenty-four bracts per plot, one per plant, were collected in the same manner as in Study One. Preliminary studies with Deltapine 61 revealed that the coefficient of variation for bract area measurements did not decrease substantially with more than 20 bracts per plot in the nonsegregating population.

Data analyses followed the procedure as outlined by Jinks and Hayman (7, 8, 9, 10, 12, 13) and were analyzed on a plot-mean basis. All effects were assumed fixed. Environmental variance estimates (E_0, E_1, and E_2) were obtained from multiple observations within each plot and averaged across entries. Statistical significance from zero was determined by the Student's t-test.

Notations and their definitions as used in this study are those of Hayman (9, 10). Estimates of the degree of dominance were defined by Crumpacker and Allard (6). Formulae for narrow-sense heritability estimates were defined for the F_1 by Crumpacker and Allard (6) and for the F_2 by Verhalen and Murray (18).

RESULTS AND DISCUSSION

Study One

Bract surface area of the F_2 plants selected for use in this study ranged in 1977 from 4.3 to 9.5 cm^2 with a mean of 7.1 cm^2. Bract surface area of F_3 plot means had a nearly identical range, 4.2 to 9.6 cm^2, but with a slightly smaller mean, 6.9 cm^2. The coefficients of variability were roughly the same, 18 and 17% for the F_2 and F_3 populations respectively. The commercial check, Deltapine 61, in 1978 had a range of 6.7 to 8.3 cm^2 and a coefficient of variability of 8%.

Table 1. Combined analysis of bract surface area for the parents.

Source	df	Mean squares
Years	1	1.4502
Locations	1	9.5759**
Year × Location	1	0.0898
Parents	6	28.5734**
Years × Parents	6	0.2778
Locations × Parents	6	0.4207
Years × Locations × Parents	6	0.2513
Residual	66	0.2301

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

Heritabilities based on regression of F_3 plot means on F_2 plant values and calculated in standard units were 0.69 and 0.75, respectively. The close agreement of these two values indicates a relatively small affect of any genotype-environment interaction on the phenotypic variability present in this population. These moderately high estimates of heritability indicate that selection for small bracts on an individual plant basis in early segregating generations would be effective.

Study Two

An analysis of variance for bract surface area on a plot mean basis revealed highly significant differences among the parents (Table 1); therefore we conducted a detailed analysis of the gene action involved with this trait.

The diallel analysis was based on the assumptions as follows:

1. Diploid segregation,
2. No reciprocal effect,
3. No epistasis,
4. No multiple allelism,
5. Homozygous parents,
6. Independent gene distribution,
7. No genotype-environment interaction.

G. hirsutum, an amphidiploid, segregates in a diploid manner (15) thus satisfying the first assumption. The second assumption was not tested. The fifth assumption, homozygous parents, was not fulfilled in the strictest sense; the coefficient of inbreeding was less than 1.0 for all parents. The parents were considered sufficiently inbred to satisfy this assumption although some loci for the trait under consideration may have been heterozygous.

Test of Assumptions. The third, fourth, and sixth assumptions of no epistasis, no multiple allelism, and independent gene distribution were first subjected to three general tests as outlined by Allard (1), Hayman (8), and Jinks and Hayman (13). Partial failure of the assumptions were detected in the first test (Table 2) by a significant array source of variation in the F_2 population at Baton Rouge in 1979, in the second test (Table 3) by three (Wr, Wr') regression coefficients being significantly different from 0.5, and in the third test (Table 3) by two (Vr, Wr) regres-

Table 2. Analyses of variance of (Wr-Vr) values.

Source	df	F_1 Mean squares				F_2 Mean squares			
		Alexandria		Baton Rouge		Alexandria		Baton Rouge	
		1978	1979	1978	1979	1978	1979	1978	1979
Rep	3	0.0247	0.0158	0.0167	0.0043	0.0548	0.3091**	0.0175	0.0581
Array	6	0.0096	0.0148	0.0101	0.0033	0.0221	0.0888	0.0179	0.1065*
Error	18	0.0083	0.0334	0.0081	0.0042	0.0242	0.1214	0.0116	0.0337

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

Table 3. (Wr, Wr') and (Vr, Wr) regression coefficients and their 95% confidence intervals.

Population	Location	Year	(Wr, Wr')	(Vr, Wr)
F_1	Alexandria	1978	0.483 ± 0.067	0.871 ± 0.243
	Alexandria	1979	0.304 ± 0.147	0.831 ± 0.390
	Baton Rouge	1978	0.392 ± 0.013	0.582 ± 0.463
	Baton Rouge	1979	0.483 ± 0.098	0.917 ± 0.219
F_2	Alexandria	1978	0.447 ± 0.113	0.704 ± 0.228
	Alexandria	1979	0.422 ± 0.080	0.752 ± 0.281
	Baton Rouge	1978	0.374 ± 0.083	1.006 ± 0.263
	Baton Rouge	1979	0.371 ± 0.183	0.646 ± 0.291

Table 4. Genotype by environment analysis of the dominance components of variation.

Source	df	Mean squares
Years	1	0.0034
Locations	1	0.1814*
Years × Locations	1	0.0030
Dominance	1	0.7022**
Years × Dominance	1	0.0264
Locations × Dominance	1	0.0107
Years × Locations × Dominance	1	0.0156
Arrays	6	0.0506*
Years × Arrays	6	0.0137
Locations × Arrays	6	0.0307
Dominance × Arrays	6	0.0093
Years × Locations × Arrays	6	0.0514*
Years × Dominance × Arrays	6	0.0038
Locations × Dominance × Arrays	6	0.0048
Years × Locations × Dominance × Arrays	6	0.0012
Residual	143	0.0229

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

sion coefficients being significantly different from 1.0. Although certain data sets failed to meet expectations in the second and third tests, they did approach their criteria.

Since all data sets did not consistently conform to all three general tests, specific tests were then performed to determine the validity of the three assumptions. The first specific test was a chi-square analysis to detect epistasis (7). The pooled chi-square was nonsignificant (236.6 at 315 df), suggesting that there was not a significant amount of epistasis among loci controlling bract surface area. The second specific test was an analysis of (Wr_1-$2Wr_2$) values to detect multiple allelism (7). This test revealed a nonsignificant amount of multiple allelism as evidenced by nonsignificant array sources of variation across all data sets.

Independent gene distribution was not specifically tested and by deduction cannot be considered valid because of partial failure of the three general tests for several data sets.

The seventh assumption of no genotype-environment interactions was tested using procedures outlined by Allard (2). Nonsignificant interaction terms involving parents suggested that the additive effects were constant among environments, thus fulfilling the additive portion of this assumption (Table 1). This supports the conclusion drawn in the first study regarding genotype-environment interactions.

The dominance components of variation were also analyzed for genotype-environment interactions (Table 4). Significance of the dominance, arrays, and years by locations by arrays sources of variation suggested either partial or overdominance for the mean degree of dominance, differences in dominance among the parents and inconsistency of dominance relationships among parents across environments, respectively. The dominance portion of this assumption was therefore not fulfilled and was evident in estimates of dominance shown in Table 5. The insignificance of all interactions involving dominance provided additional evidence that epistasis was not a significant factor in the inheritance of bract surface area.

A more complex genetic system than the simple system described by Hayman (10) is theorized for traits that do not conform to the assumptions. He stated that even though a trait has not met all the assumptions, it is still possible to estimate population parameters and genetic components. However, such estimates would not be as reliable as if all assumptions had been met. Considering that the data appeared to show only minor violations of the assumptions, the authors proceeded with the genetic analysis.

Estimates of Genetic and Environment Parameters. All estimates of environmental variance (E_0, E_1, I_2) were significantly different from zero (Table 5). The average values for respective F_0, F_1, and F_2 populations were 0.37, 0.40, and 0.47. Since the environmental variance estimates for the F_2 population is normally found to be intermediate between F_0 and F_1 populations, an inadequate number of bracts may have been collected from the segregating population.

All estimates of additive gene effects (D) were positive and highly significantly different from zero indicating a substantial amount of the inheritance of bract surface area was additive in nature (Table 5).

Estimates of the relative frequency of dominant vs. recessive alleles in the parents (F) were positive and highly significantly different from zero in the F_1 population, suggesting a predominance of dominant alleles in the parents (Table 5). However, in the F_2 population, one estimate was highly significantly different from zero in a negative

Table 5. Mean environmental and genetic parameter estimates for F_1 and F_2 populations for bract surface area.

	F_1				F_2			
	Alexandria		Baton Rouge		Alexandria		Baton Rouge	
Parameter	1978	1979	1978	1979	1978	1979	1978	1979
E_0	0.37*	0.37*	0.35*	0.37*	--	--	--	--
E_1	0.37**	0.54**	0.34**	0.33**	--	--	--	--
E_2	--	--	--	--	0.46**	0.56**	0.42**	0.44**
D	1.53**	2.38**	1.40**	1.88**	--	--	--	--
F	1.26**	3.15**	1.20**	2.19**	-0.28	0.36	-0.79**	-0.25
H_1	1.02**	3.06**	0.64**	1.83**	-3.91**	0.54	-3.31**	-2.85**
H_2	-0.58**	0.06	-0.62**	-0.33**	2.30**	7.35**	2.73**	2.44**

*,** Significantly different from zero at the 0.05 and 0.01 levels of probability, respectively.

Table 6. Mean genetic estimator ratios for F_1 and F_2 populations and their 95% confidence intervals for bract surface area.

		F_1		F_2	
Estimator	Year	Alexandria	Baton Rouge	Alexandria	Baton Rouge
Dominance 1	1978	0.61±.14	0.37±.18	-0.67±.10	-0.64±.20
	1979	1.15±.15	0.94±.09	-0.06±.23	-0.40±.16
Dominance 2	1978	0.75±.09	0.49±.17	--	--
	1979	1.06±.07	0.96±.05	--	--
Dominance 3	1978	0.72±.13	0.56±.11	0.20±.01	0.29±.07
	1979	1.65±.37	0.96±.12	0.47±.09	0.32±.06
Heritability	1978	0.55±.01	0.63±.08	0.71±.05	0.66±.14
	1979	0.62±.07	0.66±.06	0.60±.05	0.70±.07

manner, while all others were not significantly different from zero.

One estimate of dominance gene effects (H_1) in the F_1 population (Alexandria - 1979) was greater than the estimate of additive gene effects (D), while the absolute value of the other three estimates were smaller than the estimates of additive gene effects; thus additive gene effects seemed to be more important than dominance gene effects (Table 5). All H_1 estimates in the F_1 population were highly significantly different from zero, indicating that a significant amount of dominance gene effects was involved in bract surface area. H_1 estimates in the F_2 population were either not different from zero or highly significantly different from zero in the negative direction.

The second estimate of dominance gene effects corrected for gene distribution (H_2) appeared either negative or equal to zero in the F_1 population (Table 5). F_2 estimates of H_2 were uniformly positive and highly significantly different from zero, agreeing with H_1 estimates in the F_1.

Investigations of Genetic Systems. The estimates of the mean degree of dominance (Dominance 1, 2, and 3) were generally in the partial dominance range although all estimates for the F_1 population at Alexandria in 1979 were in the overdominance range (Table 6). The F_1 estimates at Baton Rouge in 1979 were not significantly different from one. Of the 24 estimates of the mean degree of dominance four could not be calculated, three were in the overdominance range, while 14 were in the partial dominance range. Except for four estimates that could not be calculated, all but one estimate were significantly different from zero. The overall mean degree of dominance was estimated at 0.49; thus partial dominance was expressed at most loci where dominant alleles were found. The results agree with the interpretation of the analysis of the genotype-environment interaction of the dominance components of variation where significant dominance variation suggested either partial dominance or overdominance.

Narrow-sense heritability estimates were all significantly different from zero and were similar in value to each other. The values ranged from 0.55 to 0.71 and averaged 0.64. These estimates revealed the major portion of heritability for bract surface area was additive in nature. Thus, additive gene effects were more important than dominance gene effects, although dominance gene effects contributed significantly to the inheritance of bract surface area. The heritability estimate in this second study (0.64) is in agreement with that from the first study (0.69).

Direction of dominance was calculated from (Vr + Wr) correlations with parental means (10). A high correlation suggests that most dominant alleles act in the same direction and most recessive alleles act in the opposite direction, while a small or insignificant correlation suggests that approximately equal proportions of dominant and recessive alleles act in both directions. Correlation coefficients ranged from — .62 to .69 and no coefficient was significantly different from zero. There appears to be equal proportions of dominant and recessive alleles acting in both directions. The wide range of correlation coefficients reinforce the conclusion drawn from the genotype-environment analysis that dominance relationships among parents were not constant among environments.

The quantity (Vr + Wr) was used to estimate the order of dominance of parents. From the F_1 data the parental order of dominance from dominant to recessive was estimated as: 4, 6, 7, 5, 3, 2, 1. In the F_2 the order of dominance from dominant to recessive was estimated as: 4, 3, 1, 6, 2, 7, 5. In addition to dominance relationships among parents not being constant among environments they were also not constant between generations. The order of performance of the parents averaged over all environments from largest bracts to smallest was: 7, 6, 5, 4, 2, 3, 1.

With respect to the objective of reducing bract size, a breeder should be interested in those parents with small bract surface area combined with a minimum amount of dominance if his objective is a cultivar. LSS (Pak) (No. 2) should be a desirable parent in a cultivar development program. Even though MoBW (No. 1) and NCJ-9 (No. 3) would also appear to make desirable parents in a cultivar development program, they were inconsistent with

respect to their position in the order of dominance between populations.

The nature of inheritance of bract surface area as revealed in the current studies suggests the use of recurrent selection as a means for concentrating favorable alleles in a breeding population. The moderately high estimates of narrow-sense heritability in both studies suggest that selection for small bract on an individual plant basis would be effective. The insignificance of genotype environment interaction suggests that substantial testing over years and locations is not necessary to identify desirable lines in a cultivar development program.

ACKNOWLEDGMENT

The authors thank Dr. M. J. Lukefahr, Entomologist, USDA-ARS, Weslaco, Tex. for furnishing F_1 seed from which the segregating populations used in the first study were derived. The authors appreciate the advice and suggestions on the data analysis given by K. L. Koonce, Jerry Baker, Laval Verhalen, and Jerry Quisenberry. The authors acknowledge the assistance of Jude Brand, Gregg Marshall, Roy Vidrine, William Day, Danny Aguillard, Doug Traylor, Billy Kingery, and Salvador Martinez.

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