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Okra-leaf and normal cotton isolines with different backgrounds

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ABSTRACT

The results of a performance test of okra-leaf isolines of cotton (*Gossypium hirsutum* L.) are reported. The isolines were characterized by measurement of the effects of the qualitative characters on quantitative traits and by comparison with the performance of the recurrent parents. The variability of lines within each isoline was also determined. The okra-leaf isolines were significantly earlier than the normal-leaf isolines, as measured by the percentage of total seed cotton collected at first harvest (23.7 vs. 18.6%, respectively). However, okra-leaf isolines yielded significantly less total seed cotton (21.5 g/plant vs. 26.4, respectively, an 18.7% decrease).

INTRODUCTION

Okra-leaf (L_2^o) was described by Stephens, 1945; Green, 1953; Endrizzi and Kohel, 1966, as an old and common mutant that is present in certain race stocks and older cultivars of upland cotton, *Gossypium hirsutum* L. According to these authors, absence of dominance characterizes this leaf shape allele. The heterozygous okra-leaf has a phenotype similar to homozygous sea-island-leaf (L^eL^e). This leaf shape allele, along with sub-okra (L^u), super-okra leaf (L^s) and normal leaf (l_2), are members of an allelic series at the "L" locus on chromosome 15 of the "D" genome.

According to Jones (1982), cotton plants with okra-leaf have several agronomic characteristics which make them better adapted to narrow-row culture than plants with normal leaves. One of these characteristics is an open canopy produced by homozygous okra-leaf ($L_2^o L_2^o$), which results in decreased total leaf area. This decrease allows for better penetration of light and insecticides, earlier maturity, less boll rot, more resistance to banded-wing whitefly (*Trialeurodes abutilonea* Haldeman), to pink bollworm (*Pectinophora gossypiella* Saunders) and to the boll weevil (*Anthonomus grandis* Boheman). In addition, Jones (1982) has found no deleterious associations between okra and either fiber properties or fitness for harvesting. The resistance to boll rot and boll weevil has been attributed to the warmer, drier microclimate under the canopy of okra-leaf isolines than of normal-leaf cultivars.

However, as pointed out by Meredith Jr. and Wells (1986) the commercial use of okra-leaf cottons is limited due to a 5% yield disadvantage. Other reasons which determine the reluctance of commercial breeders in using the okra-leaf lines are: a) problems in identifying okra plants in segregating populations, and b) no yield advantage of okra-over normal-leaved lines. An exception was observed in areas of Louisiana, USA, where boll rot occurs frequently (Jones, 1982).

The okra-leaf trait was described by Ibrahim and Buxton, 1981 and Karami and Weaver, 1972, as a leaf shape that is deeply cleft with narrow lobes, in contrast with the normal broad leaf. The okra-leaf trait exhibits a large change in growth characteristics when compared with normal types. The plants homozygous for okra-leaf exhibit decreased total leaf area, have fewer nodes on the main plant axis, with resulting

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smaller production of floral buds (squares) and flowers (Andries *et al.*, 1969; Karami and Weaver, 1972; Kerby and Buxton, 1976) which suggests decreased yield potential.

The above-mentioned studies demonstrate the importance of investigating the effects of morphological mutants such as okra-leaf on different genetic backgrounds. Consequently a program was initiated in 1986 to develop isogenic lines (isolines) of cotton *G. hirsutum* L. with the qualitative genetic marker okra-leaf. The program was established to provide a series of lines with a common genetic background in which the effect of individual marker loci could be evaluated and characterized.

MATERIAL AND METHODS

Isolines carrying okra-leaf (L_2^o) from one source and the normal allele (l_2) were synthesized by a multiple backcross method that had AFC, REBA and ACALA 4-42 cultivars as the recurrent parents. AFC, REBA and ACALA 4-42 cultivars were introduced in their 12th generation of inbreeding. The okra-leaf source was obtained from a cross between a frego (fg) line that was found in a plant from a production field of upland cotton in Patos, Paraíba, in 1978 and the okra-leaf Stoneville-7A cultivar. This cultivar was obtained from the collection maintained at the University of Arizona, USA, which traces to the original mutant okra-leaf found in the Stoneville-7A cultivar. A frego bract plant in the 9th generation of inbreeding was crossed as female to Stoneville-7A, also in the 9th generation of inbreeding, in 1983. A frego bract and okra-leaf plant was selected in the F4 generation, in 1985. The cultivars AFC, REBA and ACALA 4-42 were crossed as females, all of them in the 12th generation of inbreeding, to the frego bract and okra-leaf plant, in 1986. For each of seven generations a heterozygous plant was backcrossed to AFC, REBA and ACALA 4-42 cultivars. One heterozygous plant for leaf shape was then selected and selfed, from which S_1 seeds were recovered. These S_1 seeds were then grown at the Federal University of Ceará, Brazil, where segregating S_1 plants of okra-leaf (L_2^o) and normal-leaf (l_2) were selfed. S_2 seeds were recovered and grown as S_2 plants. From these S_2 plants four plants of each type in each cross were randomly selected and selfed to provide S_3 (sub-lines) seeds for a performance test at the Experimental Station of Pentecoste, Ceará, Brazil, under irrigation, in 1992. Excellent insect control was maintained at this location, and thus we avoided confounding the test variables with the effect of insect susceptibility.

Each entry (isolines or genotypes of okra-leaf and their normal counterparts) and the AFC, REBA and ACALA 4-42 recurrents were grown in three-row (sub-line) plots. Each plot consisted of three 5-m rows, with a spacing of 0.80 m between rows and 0.20 m between plants, with 75 plants in each plot. Five plants per plot were used in the analyses. The test was replicated four times. The four sub-lines within each entry were grown to estimate the genetic variability within each isoline in the AFC, REBA and ACALA 4-42 recurrent parent backgrounds. Ten agronomic characters were measured: (1) days to first flower initiation; (2) number of vegetative branches; (3) number of nodes of first fruiting branch; (4) height at the end of the harvest; (5) percentage of first harvest; (6) yield of seed cotton/plant; (7) boll size (mean weight of seed cotton); (8) lint percentage (ratio of lint to seed cotton expressed as a percentage); (9) number of bolls/plant; (10) seed index (weight (g) of 100 seeds).

RESULTS AND DISCUSSION

An ANOVA model was used to test the genetic variability among sub-lines within isolines (Table I). In addition to the characterization of the isolines, we were interested in the variability that remained within each genotype following seven generations of backcrossing. The ANOVA (Table II) showed no significant variation among sub-lines within entries for five of the 10 characters measured: (1) number of vegetative branches; (2) number of nodes; (3) percentage of first harvest; (4) seed cotton yield, and (5) number of bolls, indicating that genetic stability had been attained for these five agronomic and morphological characters. Significant variation among sub-lines within entries were found for the other morphological and agronomic characters measured: (1) date of first flower; (2) height measured at last harvest; (3) boll size; (4) fiber percentage, and (5) seed index, (Table II). Kohel and Richmond (1971) found significant variation within entries even after eight generations of backcrossing to the parental lines, and thus we confirmed variation within entries in this test. The results presented here were not unusual as the cultivars AFC, REBA and ACALA 4-42 were probably not stable.

The significant variability of sub-lines within genotypes (entries) emphasizes the inherent difficulties involved in attempts to develop a true set of isolines. However, if the observed variability does represent linkages, as it should, it does provide some guidelines for the use of genetic markers for the mapping of agronomic characters. The indicated associations reflect

Table I - ANOVA model to test the genetic variability among sub-lines within isolines of cotton introgressed with okra-leaf source in three genetic backgrounds.

Source of variation		d.f.	Expected mean squares	F
a. Replications	R - 1	3	$\sigma^2 + ES\sigma_R^2$	
b. Entries (isolines)	E - 1	5	$\sigma^2 + R\sigma_S^2 + S\sigma_{ExR}^2 + RS\sigma_E^2$	b/(c + d - e)
c. Reps x entries	(E - 1) (R - 1)	15	$\sigma + \sigma$	c/e
d. Sub-lines (within entries)	E (S - 1)	18	$\sigma + \sigma$	d/e
e. Reps x sub-lines (within entries)	E (S - 1) (R - 1)	54	σ	
Total	ERS - 1	95		

Where: E = 6; S = 4 and R = 4.

the chromosome location of some loci controlling agronomic traits. They do so to the extent that variability existed in the original material used in the backcrossing. The marker locus came from two marker lines (okra-leaf and frego bract). It is not possible to characterize such marker lines agronomically, but the separate origin of these lines makes it probable that they are different from AFC, REBA and ACALA 4-42 recurrent lines.

The comparisons among genotypes (entries) and with AFC, REBA and ACALA 4-42 recurrent parents provided the opportunity to measure the pleiotropic or tight linkage effects of the marker locus. The variability within each genotype was further analyzed to determine which genotypes contributed significantly to the variability among sub-lines (Table III).

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 variability. Comparisons were made between the okra-leaf and normal biotypes extracted from the same segregating population of each variety. No selection other than that for okra-leaf type was made in establishing the biotypes of each variety. The base population in each case was considered adequate for a representative sample. Thus, differences associated with the two leaf types could be attributed to the genetic factor for leaf types or to other genetic factors closely linked with it. The contrasting leaf types were considered to be near-isogenic populations.

The morphological characters as affected by leaf type in the three varietal backgrounds are summarized in Table IV. The okra-leaf biotype of each variety had fewer days to first flower than its normal leaf strain, except in the REBA background. The okra-leaf biotype of ACALA 4-42 background had more vegetative branches than its normal counterpart. The okra-leaf biotype also had less nodes than its normal counterpart and less height at the end of the harvest, although in the REBA background the situation was reversed.

Table II - Mean squares for the 10 agronomic and morphological characters measured from ANOVA for okra and normal cotton entries. Pentecoste, Ceará, Brazil, 1992.

Source of variation	d.f.	Days to 1st flower	No. of vegetative branches	No. of nodes	Height (cm)	1st harvest (%)	Yield g per plant	Boll weight (g)	No. of bolls per plant	Fiber (%)	Weight 100 seeds (g)
a. Reps	3	42.59	3.01	0.48	1,653.60	29.08	979.84	1.86	60.81	1.09	1.66
b. Entr.	5	9.54	0.85	2.79**	767.40*	204.98	317.34	10.74**	52.53**	132.05	12.34**
c. R x E	15	3.73	0.24	0.29	83.73	100.18	35.11	0.15	7.72	0.65	0.51
d. Sub	18	5.50*	0.45	0.41	172.07*	156.58	64.04	0.52**	4.27	10.92**	2.37**
e. R x S	54	2.82	0.29	0.25	76.77	86.78	39.75	0.16	4.77	0.88	0.48
Total	95	-	-	-	-	-	-	-	-	-	-
C.V. (%)	-	3.15	79.19	9.01	12.81	44.11	26.35	8.30	26.28	2.34	5.64

*, ** Significant at 0.05 and 0.01 probability levels, respectively, by F test.
Reps = Replications; C.V. = coefficient of variation; Entr. = entries; Sub = sub-lines.

Table III - Mean squares for the five morphological characters which showed significant variation among sub-lines within entries measured from ANOVA for okra and normal leaf cotton isolines. Pentecoste, Ceará, Brazil, 1992.

Source of variation	d.f.	Days to first flower	Height (cm)	Boll weight (g)	Fiber (%)	Weight 100 seeds (g)
Sub-lines (within entries)	18	5.50	172.07	0.52	10.92	2.37
Sub-line 1 - AFC-N	3	2.76	3.84	0.12	0.62	1.12
Sub-line 2 - AFC-O	3	5.82	254.87*	0.17	3.19*	0.75
Sub-line 3 - REBA - N	3	4.14	354.27**	1.40**	0.97	2.41**
Sub-line 4 - REBA - O	3	2.88	238.09*	0.28	33.61**	0.07
Sub-line 5 - ACALA - N	3	11.70*	90.46	1.02**	9.98**	4.79**
Sub-line 6 - ACALA - O	3	5.72	108.89	0.12	17.16**	5.09**
Reps x sub-lines (within entries)	54	2.82	76.77	0.16	0.88	0.48

*,** Significant at 0.05 and 0.01 probability levels, respectively, by F test.
O = Okra-leaf isoline; N = normal-leaf isoline.

Two methods were used to study earliness: (1) the percentage of the total crop at first harvest determined for all plots, and (2) date of first flower. The okra-leaf isolines were earlier than the normal counterpart in the three genetic backgrounds. Twenty-four percent of the crop was open in the okra-leaf plots vs. 19% at 106 days after planting. The earliness of the okra-leaf biotypes can be attributed not only to the openness of the plant canopy, but also to the fact that the okra-leaf biotypes fruited at a faster rate than did the normal-leaf plants of all three genetic backgrounds.

Comparisons of the okra-leaf and normal-leaf biotypes for yield, boll size, number of bolls per plant, lint percentage and seed index are given as an average of the three varieties in Table V. The two leaf-shape isolines differed in earliness, yield and number of bolls per plant. The two leaf shapes did not differ in boll weight, fiber percent and seed index, but okra-leaf plants produced lower weight per boll, lower fiber percentage and lower seed index.

The okra-leaf character reduced the leaf area per plant to such an extent that it might be suspected of causing a reduction in yield. The openness of the plant canopy in the okra-leaf plots allowed better air exchange and more sunlight to penetrate to the lower leaves and bracts to the point that the okra-leaf plants were able to compensate for the loss of leaf surface area.

The okra-leaf isolines were significantly earlier than the normal-leaf isolines, as measured by percentage of total seed cotton produced at first harvest (Table V). However, when the criterium used to measure earliness was the date of first flower, there were no significant differences between okra-leaf and normal-leaf isolines (Table IV). Okra-leaf yielded significantly less total seed cotton (Table V).

Table IV - Morphological characters as influenced by leaf type in three varietal backgrounds of cotton in Pentecoste, Ceará, Brazil, 1992.

Biotypes	Morphological characters			
	Days to first flower	No. of vegetative branches	No. of nodes	Height (cm)
AFC - Okra	52.60	0.65	5.03	66.05
AFC - Normal	52.88	0.81	5.15	70.66
REBA - Okra	54.38	0.31	6.01	78.43
REBA - Normal	54.23	0.63	6.01	71.69
ACALA - Okra	52.74	1.01	5.48	57.88
ACALA - Normal	53.43	0.68	5.61	65.74
Avg. okra	53.24	0.66	5.51	67.45
Avg. normal	53.51	0.71	5.59	69.36
Avg. check variety	49.80	0.20	5.45	56.05
% O/N	99.50	92.96	98.57	97.25
% O/C	106.46	330.00	101.08	116.90
% N/C	106.94	355.00	102.57	119.19

O = Okra-leaf isoline; N = normal-leaf isoline; C = normal-leaf control.

Table V - Agronomic characters as influenced by leaf type in three varietal backgrounds of cotton in Pentecoste, Ceará , Brazil, 1992.

Biotypes	Agronomic characters					
	% yield at 1st harvest	Yield g/plant	Boll weight (g)	No. of bolls per plant	Fiber (%)	Seed index
AFC - O	20.61	20.87	4.11	7.65	37.34	11.45
AFC - N	19.78	21.64	3.91	11.33	36.59	11.38
REBA - O	23.09	23.57	4.45	8.01	39.38	12.99
REBA - N	17.35	25.48	4.95	9.11	40.46	12.68
ACALA - O	27.27	19.97	5.50	5.86	43.29	11.76
ACALA - N	18.64	32.08	6.00	7.90	43.35	13.48
Avg. O	23.66	21.47	4.69	7.17	40.00	12.07
Avg. N	18.59	26.40	4.95	9.45	40.13	12.51
Avg. Chek	58.14	23.86	5.57	6.90	42.96	11.25
% O/N	121.43	81.33	94.75	75.87	99.68	96.48
% O/C	40.69	89.98	84.20	103.91	93.20	106.79
% N/C	31.97	109.62	88.87	126.98	93.41	110.07

O = Okra-leaf isoline; N = normal-leaf isoline; C = normal-leaf control.

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RESUMO

Um programa foi iniciado em 1986 com o objetivo de desenvolver linhagens isogênicas de algodão, *Gossypium hirsutum* L., envolvendo o marcador genético qualitativo, okra-leaf. A finalidade do programa era proporcionar uma série de linhagens com um fundo genético comum no qual o efeito individual do marcador pudesse ser avaliado e caracterizado. As linhagens assim desenvolvidas podiam fornecer uma base para uso mais eficiente do marcador em estudos genéticos e agronômicos. Os resultados de um ensaio de desempenho das linhagens isogênicas são descritos, sendo as mesmas caracterizadas por medidas dos efeitos de um caráter qualitativo sobre caracteres quantitativos e pela comparação com o desempenho dos progenitores recorrentes. A variabilidade dentro de cada linhagem foi também determinada. As linhagens okra-leaf foram significantemente mais precoces do que as linhagens de folha normal de acordo com o critério da percentagem de algodão em rama da primeira colheita em relação ao total da colheita (23,66 versus 18,59%). Com relação à produção total, observou-se que as linhagens okra-leaf produziram menos algodão em rama (21,47 g/planta *versus* 26,40, um decréscimo de 18,67%).

REFERENCES

Andries, J.A. , **Jones, J.E.**, **Sloane, L.W.** and **Marshall, J.G.** (1969). Effects of okra leaf shape on boll rot, yield, and other important characters of upland cotton, *Gossypium hirsutum* L. *Crop Sci. 9*: 705-710.

Endrizzi, J.E. and **Kohel, R.J.** (1966). Use of telosomes in mapping three chromosomes in cotton. *Genetics 54*: 535-550.

Green, J.M. (1953). Sub-okra; a new leaf shape in upland cotton. *J. Hered. 44*: 229-232.

Ibrahim, M.E. and **Buxton, D.R.** (1981). Early vegetative growth of cotton as influenced by leaf type. *Crop Sci. 21*: 639-643.

Jones, J.E. (1982). The present state of the art and science of cotton breeding for leaf-morphological types. *Proceedings of Beltwide Cotton Production Research Conferences* (Brown, J., ed.). Memphis, TN. 5-7 January. National Cotton/ Council, Memphis, TN, pp. 93-97.

Karami, E. and **Weaver, J.B.** (1972). Growth analysis of American upland cotton, *Gossypium hirsutum* L., with different leaf shapes and colors. *Crop Sci. 12*: 317-320.

Kerby, T.A. and **Buxton, D.R.** (1976). Fruiting as affected by leaf type and population density. *Proceedings of Beltwide Cotton Production Research Conferences* (Brown, J., ed.). Las Vegas, NV. 5-7 January. National Cotton/Council, Memphis, TN, pp. 67-70.

Kohel, R.J. and **Richmond, T.R.** (1971). Isolines in cotton: Effects of nine dominant genes. *Crop Sci. 11*: 287-289.

Meredith Jr., W.R. and **Wells, R.** (1986). Normal vs. okra leaf yield interactions in cotton. I. Performance of near-isogenic lines from bulk populations. *Crop Sci. 26*: 219-222.

Stephens, S.G. (1945). A genetic survey of leaf shape in new world cottons - a problem in critical identification of alleles. *J. Genet. 56*: 313-330.

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