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ORIGINAL PAPER

Alleles conferring improved fiber quality from EMS mutagenesis of elite cotton genotypes

Jinesh D. Patel · Robert J. Wright · Dick Auld · Rahul Chandnani · Valorie H. Goff · Jennifer Ingles · Gary J. Pierce · Manuel J. Torres · Andrew H. Paterson

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Abstract
Key message **Genetic improvements for many fiber traits are obtained by mutagenesis of elite cottons, mitigating genetic uniformity in this inbred polyploid by contributing novel alleles important to ongoing crop improvement.**
Abstract The elite gene pool of cotton (*Gossypium* spp.) has less diversity than those of most other major crops, making identification of novel alleles important to ongoing crop improvement. A total of 3,164 M_5 lines resulting from ethyl methanesulfonate (EMS) mutagenesis of two *G. hirsutum* breeding lines, TAM 94L-25 and Acala 1517-99, were characterized for basic components of fiber quality and selected yield components. Across all measured traits, the ranges of phenotypic values among the mutant lines were consistently larger than could be explained by chance (5.27–10.1 for TAM 94 L-25 and 5.29–7.94 standard deviations for Acala 1517-99-derived lines). Multi-year replicated studies confirmed a genetic basis for these differences, showing significant correlations between lines across years and environments. A subset of 157 lines selected for superior fiber qualities, including fiber elongation (22 lines), length (22), lint percent (17), fineness (23), Rd value (21), strength (19), uniformity (21) and multiple attributes in a selection index (26) were compared to 55 control lines in replicated trials in both Texas and Georgia. For all traits, mutant lines showing substantial and statistically significant improvements over control lines were found, in most cases from each of the two genetic backgrounds. This indicates that genetic improvements for a wide range of fiber traits may be obtained from mutagenesis of elite cottons. Indeed, lines selected for one fiber trait sometimes conferred additional attributes, suggesting pleiotropic effects of some mutations and offering multiple benefits for the incorporation of some alleles into mainstream breeding programs.

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J. D. Patel · R. Chandnani · V. H. Goff · J. Ingles · G. J. Pierce · M. J. Torres · A. H. Paterson (✉)
Plant Genome Mapping Laboratory, University of Georgia, Athens, GA 30602, USA
e-mail: paterson@plantbio.uga.edu

R. J. Wright · D. Auld
Plant and Soil Science Department, Texas Tech University, Lubbock, TX 79409-2122, USA

R. J. Wright
Texas AgriLife Research, 1102 East FM 1294, Lubbock, TX 79403, USA

M. J. Torres
Evolutionary Genomics Laboratory, San Francisco State University, San Francisco, CA 94132, USA

Introduction

Cotton (*Gossypium* spp.) is the world's leading textile fiber and a highly profitable cash crop. Cotton breeders have historically focused on yield and yield-influencing components such as biotic stresses, however, increasing attention is now being devoted to fiber quality due to enhancement in yarn processing efficiency and its influence on quality of the end product (May 2000). The US Cotton Futures Act was passed in 1914 mandating the determination of fiber quality of cotton produced in the US In 1923, the US Cotton Standard Act mandated the categorization, or

classification, of cotton sold in the United States (Brown 1938; Ramey 1980). The evolution of high throughput rotor spinning and subsequently air jet spinning machines in the textile industry, as much as eight times faster than their old counterparts, required better fiber strength for efficient spinning. Fibers with greater strength and longer staple are desirable for spinning yarns on newer technologies and these improved fiber properties also result in reduced waste in the spinning process (Bradow and Davidonis 2000). Globally, textile industries are using air jet spinning machines and improved fiber quality will maintain competitiveness of cotton with synthetic textiles in the overall fiber market.

The most commonly used industrial indices to test fiber quality include micronaire, elongation, length uniformity index, fiber length, strength, and color measured as reflectance (Rd) and yellowness (+b) (Mayfield 1995; Kohel 1999). Fiber length, strength and fineness are considered primary properties to determine fiber quality in textile processing (Kohel 1999).

Cotton genotypes that produce natural fibers rivaling the length, fineness, and strength of synthetic fibers are known, but they are low-yielding and suffer other defects (Saha et al. 2006). Upland cotton germplasm has one of the lowest levels of genetic diversity among major crop species. This has recently been exacerbated in breeding programs by repeatedly inter-crossing a few closely related genotypes to generate new cultivars. A study of more than 320 genotypes obtained from the US National Plant Germplasm System with 250 DNA markers reported that cotton has lower genetic variability than most major crops (Chee et al. 2004). Limited diversity hampers the ability of breeders to provide low-cost intrinsic genetic solutions to new requirements in agronomic or fiber quality, or challenges such as resistance to biotic and abiotic hazards.

In the present study, we evaluated 3,164 M_5 mutant lines in two different populations for fiber and other attributes, further testing a subset in replicated trials to better assess their potential value in breeding and/or functional genomics. We describe a rich resource of potential new alleles that may contribute to cotton improvement, and may provide foundational material for dissecting biochemical pathways associated with economically important components of fiber quality.

Materials and methods

Source of mutation lines

TAM 94L-25 (Smith 2003; PI631440) and Acala 1517-99 (Cantrell et al. 2000; PI612326) seeds were treated with the chemical mutagen EMS as described by Auld et al. (1992, 1998). Populations were advanced by single boll descent to M_5.

In 2007, the M_4 populations of Acala 1517-99 (1,527 lines) and TAM 94L-25 (1,564 lines) were grown in Lubbock, TX (latitude 33 N34′40.31″ and 101 W51′18.60″ longitude) in a completely randomized design (CRD). Similarly in 2008, the M_5 populations of Acala 1517-99 (1,580 lines) and TAM 94L-25 (1,584 lines) were grown in Watkinsville, GA (latitude 33 N51′46.425″ and 83 W24′31.5756″ longitude) in a completely randomized design (CRD). Soil types were Amarillo Fine Sandy Loam (fine-loamy, mixed, superactive, thermic aridic paleustalfs) and Pullman Clay Loam (fine, mixed, superactive, thermic torrertic paleustolls) (TX); and Appling Coarse Sandy Loam (fine, kaolinitic, thermic typic kanhapludults) (GA). Sowing dates were May 17, 2007 (TX), and May 21, 2008 (GA). Seeds for each line were sown in 3 m rows (progeny rows), spaced 1 m apart. Fertility, irrigation and pest (weed and insect) management was maintained to maximize yield potential during the study.

In each year, 50 bolls were hand harvested from each progeny row to ensure a thorough representation of the fiber quality distribution. These samples were ginned using a 20-saw gin (DENNIS MFG. CO., INC., and ~50 g of fiber from each row was sent to the Fiber and Biopolymer Research Institute (FBRI) in Lubbock, TX for High Volume Instrument (HVI) analysis. Data for harvested boll and seed traits included average boll weight (g), 1,000 seed weight (g), seed cotton (seed wt + lint wt), lint % (lint weight/seed cotton weight × 100), and naked seed (reduced linters). HVI fiber quality traits included upper half mean fiber length (LEN), micronaire (MIC), bundle strength (STR), length uniformity index (UNIF), elongation (ELONG), reflectance (Rd value), and yellowness (+b).

About 5 % of the 3,164 lines were selected for replicated evaluation of fiber properties. Two strategies were implemented to make selections. First, ten lines with the highest values for each of the traits, viz. lint %, LEN, STR, ELONG, UNIF, and Rd value were selected, whereas ten lines with the lowest values were selected for MIC (low values for this measure of fiber fineness being preferred). As a second selection strategy, to mitigate the effects of micro-environment, the field was stratified into approximately equal sized subplots of 120 lines each and mean and standard deviation were calculated for each of these plots. *Z* scores were calculated for each of the lines for the fiber traits, selecting lines with the highest *Z* scores for a particular fiber property. To accommodate the possibility that some lines had good overall fiber properties but did not stand out for any particular fiber qualities, an additional group of lines were selected by the sum of *Z* values of all fiber properties. These lines were expected to have two or more improved fiber qualities. Many lines did exhibit

Table 1 Comparing selected vs. randomly selected vs. parental lines

TAM 94-L25	Selected	Control	parental
STR	33.1 a	31.7 b	31.9 b
LEN	1.22 a	1.15 b	1.14 b
MIC	4.3 a	4.9 b	5.0 c
ELONG	7.7 a	5.8 b	5.5 b
UNIF	84.5 a	83.4 b	83.4 b
Rd	79.1 a	77.4 b	76.4 b
LINT %	42.2 a	40.7 b	41 b

Same letters indicate no significant difference among cells

overall improved fiber properties. This process resulted in 171 total selections that included only 157 different lines (54 TAM 94L-25, 103 Acala 1517-99) including selections for ELONG (11, 11), LEN (6, 16), lint % (10, 7), MIC (5, 18), Rd value (11, 10), STR (2, 17), UNIF (7, 14) and multiple attributes (6, 20).

As primary controls for evaluation of the lines selected for fiber properties, we selected a subset of random mutant lines from each population (27 TAM 94L-25, 22 Acala 1517-99). Such a subset of mutant lines offers a control for both the mutagenesis process and subsequent population development history (for example, instability that is widely reported in elite cotton genotypes), together with the further advantage over using the source genotype that such a group of lines may more thoroughly sample the genetic variation inherent to cotton cultivars. Six wild-type TAM 94L-25 plots per rep were compared to the 27 TAM 94L-25 random mutant lines, and differed only slightly in one trait (MIC: Table 1), thus wild-type and random mutant lines were pooled for comparison to selected mutant lines to increase statistical power.

In 2009, three replications of the selected lines (using seed produced in GA only, to assure consistency) were grown along with control lines in a randomized complete block design with three replications in both locations (RCB), with 35 seeds per row planted on May 18 (GA) and May 20 (TX). The soil type, plot size and spacing, fertility, irrigation and pest management were as described above. Exactly 50 bolls were hand harvested from each plot on October 19 (TX) and December 7 (GA), and used to measure seed wt., lint wt., seed cotton wt., lint %, burr cotton wt. (weight of seed, lint, and burrs), and gin turnout (lint/burr cotton × 100). Burr cotton weight and gin turnout were only measured in Texas. All hand-harvested 50 boll samples were ginned on a tabletop, eight-saw gin and analyzed for HVI fiber quality. In GA, seed cotton yields were also measured by mechanical harvesting of each plot using a modified spindle cotton picker while in Texas a modified cotton stripper was used to harvest and measure burr cotton yields. Lint yield per plot was extrapolated by multiplying either the seed cotton yield with the calculated lint % (GA) or the burr cotton yield with the calculated gin turnout (TX) for each plot.

Statistical analysis

Data were analyzed using SAS software (SAS Institute Inc., SAS®9.2). Correlations were estimated using PROC CORR. Statistical analysis was accomplished using PROC GLM. Means were separated using the Fisher Protected LSD tests at an alpha level of 0.05. For comparison between years, the single replications from 2007 (TX) and 2008 (GA) were compared with means of six replications in 2009 (3 TX, 3 GA). Comparison between locations used all four replications per location from TX (1 from 2007 and 3 from 2009) and GA (1 from 2008 and 3 from 2009). Comparison between selected lines and control lines used data from all four environments (TX 2007 and 2009, GA 2008 and 2009). Year, location, genotype and selection (i.e., fiber trait selected mutant vs. control lines) were considered fixed variables.

Results

Ranges and means

Across all measured traits in both mutagenized populations a wide range of values were obtained, larger than could be explained by chance. For example, in 2007 Acala 1517-99 mutant lines showed high variation in MIC (−3.89 to +3.38 standard deviations (SD) from the mean), LEN (−3.97 to +3.49 SD), STR (−3.64 to 3.71 SD), UNIF (−6.49 to 2.68 SD) and ELON (−2.72 to 4.06 SD) (Table s1). Similar patterns of high variation were seen again in 2008 and 2009 (Table s1). Likewise, in 2007 TAM 94 L-25 mutant lines showed high variation in MIC (−3.54 to +3.77 SD from mean), LEN (−2.86 to +2.57 SD), STR (−4.59 to 3.36 SD), UNIF (−3.23 to 5.76 SD) and ELON (−2.10 to 4.45 SD) (Table s1), with similar patterns of high variation in 2008 and 2009 (Table s1).

High frequencies of qualitative variations in discrete traits reflected the efficacy of mutagenesis in the populations, and suggest the wide range of phenotypes to reflect the presence of some mutations increasing and others decreasing the values of the quantitative fiber traits. For example, qualitative variations in leaf and stem trichome densities were found in 26 Acala 1517-99 mutants and 80 TAM 94L-25 mutants; and naked seeds were found in 35 Acala 1517-99 mutants and 2 TAM 94L-25 mutants (details of these mutants are being published under separate cover).

Table 2 Correlations between mutant generations using Pearson Correlation Coefficient

Generation	MIC	LEN	UNIF	STR	ELONG	Rd	LINT %
ACALA 1517-99 mutant lines							
M_4 vs. M_5	0.36*	0.59*	0.28*	0.43*	0.50*	–	–
M_4 vs. M_6	0.48*	0.69*	0.42*	0.57*	0.61*	–	–
M_5 vs. M_6	0.76*	0.83*	0.54*	0.73*	0.55*	0.37*	0.54*
TAM 94L-25 mutant lines							
M_4 vs. M_5	0.37*	0.63*	0.23*	0.41*	0.74*	0.05	–
M_4 vs. M_6	0.47*	0.69*	0.40*	0.46*	0.77*	0.07	–
M_5 vs. M_6	0.74*	0.78*	0.41*	0.69*	0.84*	0.35*	0.78*

Traits correlated at $p < 0.0001$ are having '*'

M# no. of mutant generation

Correlation between mutant generations

For the 157 lines selected for fiber qualities and the 55 controls, correlation coefficients across generations reflected increasing homozygosity for mutations. Correlation coefficients of fiber traits across mutant generations ranged from 0.28 to 0.83 for Acala 1517-99, and 0.05 to 0.84 for TAM 94L-25 (Table 2), all significant at $p < 0.0001$ except Rd value in TAM 94L-25 for the M_4 generation. In both mutant populations, correlation with the replicated M_6 generation data was greater for M_5 than M_4 for all traits except fiber elongation in Acala 1517-99, consistent with the expected higher frequency of homozygosity in M_5 than M_4.

Correlation between traits

In both mutant populations, MIC showed a significant negative correlation (Tables s2.1, s2.2, s2.3) with both LEN and STR, similar to another mutant population of cotton (Herring et al. 2004). Also in both populations, MIC showed a moderate negative correlation with seed cotton (yield) and lint yield, similar to that reported by Shen et al. (2007) using a RIL population developed from an Upland cotton (*G. hirsutum L.*) cross, 7235 X TM-1, but contradicting findings from a four-way cross-population (Qin et al. 2008). In both of our mutant populations, MIC was positively correlated with lint %, consistent with an M_5 population of Paymaster HS 200 (Herring et al. 2004), and other studies (Shappley et al. 1998; Wan et al. 2007; Qin et al. 2008).

In both mutant populations, LEN shows positive correlation (Tables s2.1, s2.2, s2.3) with STR, UNIF, seed cotton, lint yield (Table s2.3) and 1,000 seed weight (Table s2.2, s2.3); and negative correlation with ELONG (Table s2.1, s2.2, s2.3) and lint % (Table s2.2 and s2.3). This finding is consistent with prior evidence that fiber LEN is closely related to STR (Basal and Smith 1997; Kloth 1998; Herring et al. 2004; Wan et al. 2007); and to UNIF (Chee et al. 2005; Lacape et al. 2005). Our positive correlation of LEN to seed weight (Table s2.2 and s2.3) is consistent with findings for *G. hirsutum* germplasm (Shappley et al. 1998), whereas these traits were not correlated in an F_2 population derived from interspecific hybrids between *G. hirsutum* L.cv. Acala-44 and *G. barbadense* L.cv. Pima S-7 (Mei et al. 2004). Our negative correlation of LEN to ELONG is similar to results in *G. hirsutum* germplasm (Shappley et al. 1998) but opposite to results from interspecific germplasm (Lacape et al. 2005). Our negative correlation between LEN and lint % is consistent with findings from *G. hirsutum* germplasm (Shappley et al. 1998), whereas another mutant population (Herring et al. 2004) showed no relationship.

In both mutant populations, UNIF shows positive correlation (Table s2.1, s2.2 and s2.3) with STR, similar to RILs originating from an Upland cotton (Yumian 1 X T586) and F_2 population (Wan et al. 2007). In both mutant populations, UNIF showed positive correlation between seed cotton and lint yield (Table s2.3), which was not found in F_2 population of an Upland cotton (*G. hirsutum L.*) cross 7235 X TM-1 (Shen et al. 2007). UNIF showed negative correlation with lint % in both populations (Table s2.2, s2.3), similar to previous research (Shen et al. 2007; Qin et al. 2008).

In both mutant populations, negative correlation is found between STR and lint % (Table s2.2, s2.3), consistent with much prior research (Meredith 1994; Coyle and Smith 1997; Shen et al. 2007) albeit with exceptions also reported (Wan et al. 2007). We found moderate positive correlation between STR and seed weight in both population (Table s2.2 and s2.3) that contradicts results from interspecific populations (Mei et al. 2004). STR showed low positive correlation with seed cotton and lint yield in the TAM 94L-25 mutant population (Table s2.3).

In both mutant populations, seed cotton showed a very strong positive correlation with lint yield whereas it showed positive correlation to fiber yellowness (+b value) and negative correlation to MIC, ELONG and Rd value in 2009 (Table s2.3). Lint % showed low negative correlation to seed weight in both population (Table s2.2 and s2.3).

In both mutant populations, lint yield showed a positive correlation with fiber yellowness and negative correlation with ELONG (Table s2.3).

Variation in the correlation between traits in different generations of the mutant population might be due to segregation of the genes responsible for the traits, or to differences in the environmental conditions between different years and locations.

Comparing years and locations

Significant differences among years and locations for many fiber traits as well as climatic variation exemplify the broad range of environments reflected in the conclusions of the study (Tables 3, 4). For example, rainfall varied widely between the locations and growing seasons—at 11.3 inch (2007) and 7.64 inch (2009) in TX (http://www.wunderground.com), versus 15.7 inch (2008) and 36.4 inch (2009) in GA (http://www.georgiaweather.net). For both mutant populations, trials in GA had significantly higher average STR, LEN, and UNIF, whereas TX trials had significantly higher average ELONG and Rd value (Table 4). Lint % was similar for GA and TX in the Acala 1517-99-derived mutant population, but significantly higher in GA for the TAM 94L-25-derived mutant population. MIC was similar for GA and TX in the TAM 94L-25-derived mutant population, but significantly higher in GA for the Acala 1517-99-derived mutant population.

Across the years of the study, for the Acala 1517-99-derived mutant lines means were significantly different for STR (2007 < 2009 < 2008), LEN (2009 < 2008 < 2007), Rd value (2008 < 2009), and lint % (2009 < 2008) (Table 4). For the TAM 94L-25-derived mutant lines means were significantly different for Rd value (2008 < 2007 < 2009), and lint % (2009 < 2008) (Table 4).

Comparison of selected lines to control lines

Analyses of variance (Table 3) showed that selected lines showed significant ($p < 0.0001$ in all but one case <0.0005) improvement over control lines for all fiber traits. Details of the efficacy of selection for individual traits and outstanding lines that differ significantly from controls are as follows.

Fiber strength (STR)

A total of 17 and 2 lines were selected from Acala 1517-99 and TAM 94L-25 mutant populations, respectively. For Acala 1517-99, the mean of lines selected for STR was 35.9 g/tex, or 6.21 % higher than the 33.8 g/tex of control lines (Table 4). Lines 1793 and 3023 were particularly outstanding, with overall means across four environments of 37.1 and 36.7 g/tex, respectively, or 8.3 % higher than the control lines. For TAM 94L-25, the mean of lines selected for STR was 33.1 g/tex, or 4.41 % higher than the 31.7 g/tex of the control lines (Table 4). Line 1097 was particularly outstanding, with an overall mean across four environments of 33.6 g/tex, 5.9 % higher than the control lines.

Micronaire (MIC)

A total of 18 and 5 lines were selected from Acala 1517-99 and TAM 94L-25 mutant populations, respectively. For Acala 1517-99, the mean of lines selected for micronaire was 4.2 MIC units, or 11 % better than the 4.7 of control lines (Table 4). Lines 3010 and 3168 were particularly outstanding, with overall means across four environments of 4.0 and 4.0 MIC units, respectively, 15 % better than control lines. For TAM 94L-25, the mean of lines selected for micronaire was 4.3 MIC, or 12 % better than the 4.9 of control lines (Table 4). Lines 2877 and 1162 were particularly outstanding, with overall means across four environments of 3.9 and 4.1 MIC units, respectively, 16 % better than control lines.

Fiber length (LEN)

A total of 16 and 6 lines were selected from Acala 1517-99 and TAM 94L-25 mutant populations, respectively. For Acala 1517-99, the mean of lines selected for LEN was 1.24 inches, or 4.20 % higher than the 1.19 inches of control lines (Table 4). Lines 1903 and 3028 were particularly outstanding, with overall means across four environments of 1.30 inches and 1.27 inches, respectively, 7.20 % improved over the control lines. For TAM 94L-25, the mean of lines selected for LEN was 1.22 inches, or 6.09 % higher than the 1.15 inches of control lines (Table 4). Lines 926 and 2888 were particularly outstanding, with overall means across four environments of 1.26 and 1.25 inches, respectively, 8.60 % improved over control lines.

Fiber elongation (ELONG)

A total of 11 lines were selected from both Acala 1517-99 and TAM 94L-25 mutant populations, respectively. For Acala 1517-99, the mean of lines selected for ELONG was 7 %, or 15 % higher than the 6.1 % of control lines (Table 4). Lines 2984 and 1712 were particularly outstanding, with overall means across four environments of 7.5 and 7.3 %, respectively, 20 % higher than control lines. For TAM 94L-25, the mean of lines selected for ELONG was 7.7 %, or 33 % higher than the 5.8 % of control lines (Table 4). Lines 2925 and 2907 were particularly outstanding, with overall means across four environments of 8.7 and 8.1 %, respectively, 40 % higher than control lines.

Table 3 Analysis of variance for fiber and yield traits in two mutant populations

Fiber traits	Source of variance	DF		MS		F	
		ACALA 1517-99	TAM 94L-25	ACALA 1517-99	TAM 94L-25	ACALA 1517-99	TAM 94L-25
STR	SVC[a]	1	1	248.31	35.87	54.7**	14.55*
	Year	2	2	320.27	9.14	70.56**	3.71
	Loc	1	1	16.58	1.02	3.65	0.42
	Loc × SVC	1	1	30.80	0.03	6.78	0.01
	Year × SVC	2	2	38.85	8.09	8.56	3.28
	Error	142	126	4.54	2.47		
LEN	SVC	1	1	0.20	0.14	154.2**	60.06**
	Year	2	2	0.08	0.09	63.04**	38.63**
	Loc	1	1	0.14	0.08	105.21**	34.73**
	Loc × SVC	1	1	0.00	0.00	0.06	0
	Year × SVC	2	2	0.02	0.01	11.45**	3.34
	Error	138	142	0.00	0.00		
MIC	SVC	1	1	10.64	4.83	98.82**	34.94**
	Year	2	2	5.27	2.63	48.95**	19.03**
	Loc	1	1	1.87	0.20	17.38**	1.43
	Loc × SVC	1	1	0.00	0.04	0.02	0.29
	Year × SVC	2	2	0.69	0.51	6.45	3.68
	Error	145	138	0.11	0.14		
ELONG	SVC	1	1	43.11	176.03	118.56**	359.19**
	Year	2	2	16.45	4.01	45.24**	8.18**
	Loc	1	1	0.00	65.64	0	133.94**
	Loc × SVC	1	1	2.01	0.49	5.53	1
	Year × SVC	2	2	6.45	12.20	17.73**	24.9**
	Error	122	162	0.36	0.49		
UNIF	SVC	1	1	53.98	60.49	48.44**	53.46**
	Year	2	2	13.98	18.29	12.55**	16.17**
	Loc	1	1	48.60	36.52	43.61**	32.27**
	Loc × SVC	1	1	0.14	0.17	0.13	0.15
	Year × SVC	2	2	10.23	11.18	9.18*	9.88**
	Error	129	146	1.11	1.13		
Rd	SVC	1	1	87.52	196.71	71.35**	69.07**
	Year	1	2	25.05	47.22	20.42**	16.58**
	Loc	1	1	101.16	119.36	82.48**	41.91**
	Loc × SVC	1	1	0.03	0.27	0.02	0.1
	Year × SVC	1	2	85.13	44.08	69.41**	15.48**
	Error	90	157	1.23	2.85		
LINT %	SVC	1	1	266.16	98.64	45.17**	85.54**
	Year	1	1	210.23	61.89	35.68**	53.67**
	Loc	1	1	3.81	14.74	0.65	12.78*
	Loc × SVC	1	1	0.00	0.35	0	0.3
	Year × SVC	1	1	234.96	24.57	39.88**	21.3**
	Error	81	123	5.89	1.15		

[a] SVC (selected lines vs. control (randomly selected mutant) lines. In column "F", *, and ** represent significance with p values of 0.0005 and 0.0001, respectively

Table 4 Comparison of selected and control lines for fiber qualities across selection methods, environments, and years

Fiber trait	Top 10 lines	*Z* value lines	Control	Sign.	GA	TX	Sign.	2007	2008	2009	Sign.
Acala 1517-99 mutant lines											
STR	35.9†	36†	33.8	*	35.2	34.42	*	31.4	37.3	34.9	*
LEN	1.25	1.24	1.19	*	1.24	1.18	*	1.25	1.23	1.2	*
MIC	4.2	4.4	4.7	*	4.63	4.4	*	4.1†	4.2†	4.6	*
ELONG	6.9†	7.1†	6.1	*	5.9	6.9	*	6.0	6.3†	6.5†	*
UNIF	85.2†	85.2†	84.3	*	85.4	83.7	*	84.8†	85.6	84.5†	*
Rd	79.0†	78.6†	78.0	*	77.2	79.7	*	–	77.6	78.4	*
LINT %	40.3†	40.9†	38.7	*	39.1	39.2		–	40.6	38.9	*
TAM 94L-25 mutant lines											
STR	–	33.1	31.7	*	32.2	31.4	*	30.1	32.0†	32.0†	*
LEN	1.23†	1.21†	1.15	*	1.19	1.14	*	1.23	1.16†	1.15†	*
MIC	–	4.3	4.9	*	4.8	4.8		4.1	4.8†	4.9†	*
ELONG	7.9	7.1	5.8	*	5.6	6.9	*	6.0†	5.9†	6.4	*
UNIF	84.5†	84.5†	83.4	*	84.3	82.9	*	84.0†	84.1†	83.5	*
Rd	79.4†	78.9†	77.4	*	76.5	79.1	*	77.1	76.0	78.2	*
LINT %	42.4	41.9	40.7	*	41.3	40.8	*	–	41.8	40.9	*

Traits means having '*' in Sign. cell are having significant mean difference at $p < 0.05$, respectively. For years mean marked with '†' are not significantly different to each other

Sign. significance, *GA* Watkinsville, GA, *TX* Lubbock, TX

Length uniformity index (UNIF)

A total of 14 and 7 lines were selected from Acala 1517-99 and TAM 94L-25 mutant populations, respectively. For Acala 1517-99, the mean of lines selected for UNIF was 85.2 %, or 1.07 % higher than the 84.3 % of control lines (Table 4). Lines 1767 and 2455 were particularly outstanding, with overall means across four environments of 85.9 and 85.9 %, respectively, 1.9 % higher than control lines. For TAM 94L-25, the mean of lines selected for UNIF was 84.5 %, or 1.32 % higher than the 83.4 % of control lines (Table 4). Lines 790 and 2868 were particularly outstanding, with overall means across four environments of 85.0 and 85.5 %, respectively, 2 % higher than control lines.

Rd value

A total of 11 and 10 lines were selected from Acala 1517-99 and TAM 94L-25 mutant populations, respectively. For Acala 1517-99, the mean of lines selected for Rd value was 78.8 %, or 1.03 % better than the 78.0 % of control lines (Table 4) although no single line was significantly different from controls. For TAM 94L-25, the mean of lines selected for Rd value was 79.1 %, or 2.22 % higher than the 77.4 % of control lines (Table 4). Lines 1251 and 2917 were particularly outstanding, with overall means across four environments of 80.5 and 80.0 %, respectively, 3.4 % better than control lines.

Lint percent (lint %)

A total of 7 and 10 lines were selected from Acala 1517-99 and TAM 94L-25 mutant populations, respectively. For Acala 1517-99, the mean of lines selected for lint % was 40.5 %, or 4.65 % higher than 38.7 % of control lines (Table 4). Lines 1524 and 3168 were particularly outstanding, with overall means across four environments of 41.4 and 41.4 %, respectively, 6.98 % higher than control lines. For TAM 94L-25, the mean of lines selected for lint % was 42.2 %, or 3.69 % higher than the 40.7 % of control lines (Table 4). Lines 77 and 276 were particularly outstanding, with overall means across four environments of 43.0 and 43.1 %, respectively, 5.65 % higher than control lines.

Seed cotton and lint yield

Seed cotton and lint yields had high variation within the replicated lines. In both populations, there were many selected lines that had much higher overall yield than the control lines. For example, in the Acala 1517-99 mutant population, line 1610 selected for MIC (overall mean 918.5 g), line 1965 (833.6 g) for LEN and 2488 selected for lint % (overall mean 921.0 g), all had significantly higher yields than the 711 g mean of control lines. Line 2488 also had significantly higher lint yield (overall mean 380 g) than the 271 g mean of control lines. Similarly, in the TAM 94L-25 mutant population, line 1251 selected for RD value (overall mean 969.6 g), 790 selected for UNIF (overall mean 874.5 g), 1097 selected for STR (overall mean 869.8 g) and 1053 for ELONG (overall mean 846.4 g), all had significantly higher yield than the 755.1 g mean of the control lines. Line 1251 also had significantly higher lint yield (overall mean 411 g) than the 304 g mean of control lines. While the small plot sizes in this study may have been inadequate to evaluate changes in yield, it is also possible that some lines selected for other fiber qualities may also enhance yield.

Table 5 Comparison of Acala 1517-99 lines selected for overall performance with control lines

Line	LINT %	Yield	L.yield	MIC	LEN	UNIF	STR	ELONG	Rd
1787	36.0	551.2	252	4.3*	1.21	85.4	36.3*	5.7	78.2
Control	38.7*	711.0	271	4.7*	1.19	84.3	33.8*	6.1	78.0
3168	41.0*	565.6	264	4.0*	1.15	83.2	30.2	6.1	78.0

Yield seed cotton, *L.yield* lint yield. The value in each fiber quality column is mean of four environments

Overall best lines in selection index

A total of 20 and 6 lines were selected from Acala 1517-99 and TAM 94L-25 mutant populations, respectively, for overall performance, quantified by summing Z scores for each of the eight fiber parameters (see "Materials and methods"). Two lines selected from Acala 1517-99 showed significant improvements over control lines for multiple traits. Line 1787 showed improvement in MIC and STR, and line 3168 showed improvement in lint % and MIC (Table 5). None of the lines selected from TAM 94L-25 for overall performance showed multiple trait improvements.

Comparison of selection strategies

In both populations, lines were selected using two different strategies, specifically absolute values of fiber attributes, and Z scores relative to 'nearest neighbor' groups in the field (see "Materials and methods"). Most fiber traits showed no significant difference between the means of lines chosen using the respective selection strategies (Table 4).

Discussion

Across all measured traits, the range of phenotypic values among the mutant lines is consistently larger than could be explained by chance (5.29–7.94 standard deviations for Acala 1517-99 and 5.27-10.1 for TAM 94 L-25). Our multi-environmental replicated studies confirm a genetic basis for these differences. One might further explore whether these alleles are at new quantitative trait loci (QTL) or existing ones by crossing such extreme lines to a new genetic background and conducting QTL mapping. This work might be made easier by the identification of some markers for TAM 94L-25, Acala 1517-99 and their closely related inbred lines (Maleia et al. 2010).

Some lines in each population showed significant improvements in fiber quality traits over the control lines for LEN, STR, ELONG, MIC, lint %, and Rd value. Crosses between these superior lines and elite germplasm have been made to further investigate the merit of the presumed mutant alleles in practical improvement of cotton fiber quality.

Variation in seed size and composition warrants further investigation. Acala 1517-99 mutant lines showed a range of 94.70–132.3 g, and TAM 94L-25 mutant lines showed a range of 98.70–138.0 g, for 1,000-seed weight. A significant negative correlation is seen between lint % and 1,000 seed weight, perhaps suggesting mutation of genes related to allocation of photosynthate between seeds and lint. A further in-depth study on seed weight, seed size, and number of fibers on the seed surface might help to find mutant lines with appropriate seed size and high lint yield. Such evaluation would also need to consider any impacts of reduced seed size on germination and stand establishment. In addition, we did not evaluate seed for oil content or oil quality, but this would be another area worthy of further study toward improving the value of cotton as an oilseed.

Relatively greater improvement was observed in both mutant populations for ELONG and MIC than the other traits. One reason for this difference could be that ELONG and MIC have comparatively shorter histories of selection in breeding programs (Zhang et al. 2011), with fewer genetic loci near fixation for favorable alleles in elite cotton germplasm. Thus, there may be a higher probability that alteration in gene(s) involved in ELONG and MIC could generate positive effects than for other fiber traits.

The present study differs from conventional breeding studies, in that phenotypic correlations are more likely to represent pleiotropic effects of individual mutants. Lines selected for one fiber trait sometimes conferred other attributes. For example, Acala 1517-99-derived mutant line 1903 selected for MIC and LEN had 12 % higher MIC and 9.60 % higher LEN, and also had 7.09 % higher STR than the average of the control lines, all significant at $p < 0.05$. Line 3010 selected for MIC had 15 % higher MIC, and also had 7.95 % higher LEN, 1.38 % higher UNIF, and 5.39 % higher STR, than the average of the control lines, all significant at $p < 0.05$. Line 1793 selected for STR had 9.30 % higher STR, and also had 5.9 % higher MIC and 5.21 % higher LEN than control lines, all significant at $p < 0.05$. Similarly, TAM 94L25-derived mutant line 926 selected for LEN had 8.97 % higher LEN, 1.74 % higher UNIF and 6.05 % higher STR than the average of the control lines, all significant at $p < 0.05$. Line 1162 selected for MIC had 17 % higher MIC, 6.58 % higher LEN, 5.53 % higher STR and 10 % higher ELONG than the control lines, all

significant at 0.05 %. Line 2888 selected for LEN had 8.60 % higher LEN and 9.9 % higher MIC than the control lines, all significant at $p < 0.05$.

Further work on the selected lines may introduce novel alleles into the elite cotton gene pool, building on prior successes from mutagenesis of other cotton germplasm. TTU 202-1107-B and TTU 271-2155-C mutant lines developed from Paymaster HS 200 have been registered (Auld 2000), with 8–9 % longer LEN and 5 % higher STR than Paymaster HS 200 but similar MIC and UNIF (Auld 2000). New germplasm lines TTU 0774-3-3 derived from TTU 202-1107B (Auld 2000) X Acala 1517-95 (Cantrell et al. 1995) and TTU 0808-1-6-1 derived from TTU 1722 (Auld et al. 1998) X NM24052 (Tatineni et al. 1996) were much better than the mutant parental lines, TTU 202-1107B and TTU 1722 (Bechere et al. 2007). In a like manner, some of our selected lines or intercrosses among them may yield commercially important improvements.

Qualitative mutants have long been recognized as a valuable resource for dissecting pathways and identifying genes that can influence a trait, and the lines described herein may be useful in clarifying the biochemical and molecular basis of variation in cotton fiber qualities. Genes segregating for qualitative mutants may also frequently segregate for more subtle QTL alleles (Robertson 1985). For example, the qualitative mutant that was central to the 'Green Revolution' for rice also segregates for subtle variants that had been under selection since domestication of one rice subspecies (Asano et al. 2011; Paterson and Li 2011). Thanks to successes with reverse genetic approaches such as TILLING (McCallum et al. 2000) and massively parallel re-sequencing approaches, it is becoming routine to identify discrete mutations (Blumenstiel et al. 2009) and we anticipate further analysis of these lines to seek specific mutations responsible for the observed phenotypes.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All experiments were conducted in compliance with the current laws of the USA.

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