

CHEMICALLY INDUCED MUTATION TO IMPROVE FIBER TRAITS IN UPLAND COTTON

Efrem Bechere
USDA-ARS
Stoneville, MS

Abstract

Modern Upland Cotton has a narrow germplasm base that often limits the success of breeding programs. Chemically induced mutations can be used to develop mutant populations and individual mutants with improved fiber traits that can be exploited by cotton breeders. The objective of this research was to develop improved fiber quality population and individual plants from the germplasm MD 15 through the use of chemical mutagenesis. About 5,000 seeds of the germplasm MD 15 were mutagenized with 3.2 % volume by volume ethyl methanesulfonate (EMS). One boll plant⁻¹ was bulk harvested and a sample of seeds from this bulk was planted in the M₂ (2009), M₃ (2010), and M₄ (2011) generations. This allowed for three generations of selfing which increased homozygosity and additive genetic variance. In 2011, the bulked M₄ generation was labeled MD 15 M₄ and registered as an improved population of MD 15 for fiber traits (Reg. No. GP-957, PI 665638). Starting in 2011, individual mutant plants were selected on the basis of fiber length, strength, and uniformity. Outstanding plants that performed better than both the best checks and random checks were identified. Chemically induced mutations were effective in producing much needed diversity in upland cotton.

Introduction

Intensive breeding for narrowly defined fiber-quality parameters required for processing cotton has further narrowed the gene pool of commercial cotton germplasm (Bowman et al., 1996). Recent studies have shown that chemical mutagenesis can be an effective tool to create a wide range of phenotypic variation in both diploid and tetraploid *Gossypium* populations (Auld et al., 2000; Bechere et al., 2009). Chemical mutagenesis was used to develop a series of mutants with increased fiber length and strength (Herring et al., 2004), reduced short fiber content (Peabody et al., 2002) and improved herbicide tolerance (Bechere et al., 2009). These characteristics should allow the development of cotton cultivars that could be utilized in higher-value textile markets, increasing the economic competitiveness of US cotton production in international markets. The objectives of this research were to develop an improved population and individual plants of mutants for fiber quality through the use of chemical mutagenesis.

Materials and Methods

An enhanced fiber quality okra-leaf germplasm MD 15 (PI 642769) developed in 2005 (Meredith, 2006) was mutated with 3.3 % v/v of ethyl methanesulfonate (EMS) in 2008. About 5000 seeds were imbibed for 16 to 20 h in aerated distilled water. The seeds were then rinsed and placed in aerated distilled water, and the 3.2% v/v EMS was pipetted into the solution, where it was allowed to mix with the seed for 2 h to produce the M₀ generation. The seeds were then removed and rinsed several times to remove any residual mutagens. The treated seeds were hand planted in the field at Lubbock, TX in 2008 to produce the M₁ plants. In December 2008, the M₁ seeds were harvested by removing one boll per plant from the surviving 2000 mutant plants (40%). The bolls were ginned, the seeds were mixed, and a sample of seeds from this bulk was planted at Stoneville, MS in 2009 to produce the M₂ generation. In 2010, the M₃ generation was generated with the same procedure. This allowed for three generations of selfing, which increased homozygosity and additive genetic variance. About 3000 seeds from a random sample of seeds from the bolls harvested were planted in each generation. In 2011, one boll from each plant was bulked, labeled MD 15 M₄ and registered as an improved population (Bechere et al., 2012). During 2011, 2012 and 2013, individual mutants selected on the basis of fiber length, uniformity, and fiber strength from the MD 15 M₄ bulk plus the best quality check for the above traits and a randomly selected check were planted in three replications to test for fiber quality. Plots were planted in 12.2-m long rows in a randomized complete block design with 1 m spacing between rows.

Fifty random bolls were hand-picked from each plot for fiber quality, lint percentage, and seed index, and the remaining cotton was machine harvested for estimating lint yield. High-volume-instrument (HVI) analysis was performed from 12 g of grab samples from the 50-boll sample of each entry for fiber length, fiber strength, and micronaire at Starlab (Knoxville, TN) and the Fiber and Biopolymer Research Institute at Lubbock, TX. Statistical analysis was performed on the fiber-quality data with Proc Mixed procedure (SAS Inst., 2008) with replication and

genotype as fixed effects. Sample variances were analyzed separately for the mutants and check. This model included the fixed effect Rep, and the residual model error included only sample variance for the genotype. F-tests were then constructed to determine if sample variance differed between the genotypes. The sample variance of the mutant was divided by the sample variance of the check to get the F-value. If this F-value was higher than the 95% confidence interval, then the difference between the sample variances were considered to be significant.

Results and Discussion

Development of improved mutant population

Mean, range, and sample variance results for the check MD15 and the mutants for 2010 and 2011 are given in Table 1. Significant differences in sample variances were observed for fiber length (2010), fiber strength (2011), lint percentage (2010 and 2011), micronaire (2010 and 2011), and seed index (2010) between the MD 15 mutants and MD 15. Higher variances were accompanied by higher values for most of the traits (Table 1). Overall, chemical mutagenesis created new extremes at both ends of the spectrum in most traits. The most consistent effect of the mutagen on variances appeared to be on the lint percentage and micronaire in both 2010 and 2011. Means of fiber strength for MD 15 mutants increased by 41 kN m kg⁻¹ when compared with the means for MD 15 (data not shown). An improved mutant population designated as MD 15 M4 was developed and registered (Bechere et al., 2012).

Table 1. Mean, range, and sample variance for MD 15 check germplasm and MD 15 mutants grown at Stoneville, MS during 2010 and 2011.

Traits	<u>Number</u>		<u>Mean</u>		<u>Range</u>		<u>Sample variance</u>	
	2010 (M3) [†]	2011 (M4)	2010 (M3)	2011 (M4)	2010 (M3)	2011 (M4)	2010 (M3)	2011 (M4)
<u>Lint percentage</u>								
MD 15 check	30	150	37.9	36.4 a [‡]	35.8-39.9	30.0-41.1	1.50 b	2.78 b
MD 15 M3/M4	150	150	38.2	35.0 b	25.0-38.2	30.0-41.0	5.16 a	3.87 a
<u>Fiber length (mm)</u>								
MD 15 check	30	150	31.8	31.5	30.5-33.3	29.0-33.8	0.0009 b	0.0019
MD 15 M3/M4	150	150	32.0	31.8	28.7-34.0	26.7-34.0	0.0016 a	0.0020
<u>Fiber strength (kN m kg⁻¹)[§]</u>								
MD 15 check	30	150	324	389 b	242-378	298-407	13.62	15.16 b
MD 15 M3/M4	150	150	324	430 a	245-388	307-539	8.86	17.01 a
<u>Micronaire</u>								
MD 15 check	30	150	4.2	4.5 a	4.0-5.0	3.0-5.8	0.06 b	0.22 a
MD 15 M3/M4	150	150	4.5	4.1 b	3.0-5.9	3.2-5.2	0.26 a	0.13 b

[†]M3 and M4 refer to third and fourth mutant generations

[‡]Numbers followed by different letters are statistically different from each other at the 0.05 probability level.

[§]Measured with stelometer in 2010 and high-volume instrument in 2011.

Development of improved individual mutant plants

Tables 2 and 3 present 3-yr data for mutant individual plants selected based on fiber length, strength and uniformity, compared to the best check and randomly selected check. Best checks are the best individual plants selected from the check germplasm for the particular trait. Random checks are random individual plants selected from the check germplasm. Quality data on the most recently released germplasm from Stoneville, MD25-26ne and MD25-87 (Meredith, 2013) are also presented for comparison purposes. The strongest performance of the mutants was in fiber strength. Three mutants, MD 15-61, MD 15-138, and MD 15-31 consistently gave stronger fibers than all the checks during all the three test years (Table 2). Average of three years data indicated that the mutant MD 15-31 combined high fiber length (34 mm), strong fiber (404 kN m kg⁻¹) (Table 2) and high fiber uniformity (88 %) (Table 3).

Table 2. Fiber length and strength for some selected individual M5 mutants grown at Stoneville, MS

Mutant-Selection	Length (mm)				Mean
	2010 (M2) Unreplicated	2011 (M3)	2012 (M4)	2013 (M5)	
MD 15-89	34.0	34.3	35.6	32.5	34.1
MD 15-31	33.3	33.8	33.5	33.3	33.5
MD 15-13	33.3	33.3	33.3	32.5	33.2
MD 15-16 (Best Ck)	33.0	32.5	32.8	32.5	32.7
MD 15-9 (Random Ck)	31.8	31.8	30.0	32.0	31.4
MD 25-26ne			33.0	32.5	32.8
MD 25-87			32.3	32.0	32.2
SG 747			29.5	30.7	30.1
LSD (0.05)		0.03	0.03	0.06	

Mutant-Selection	Strength (kN m kg ⁻¹)				Mean
	2011 (M3)	2012 (M4)	2013 (M5)		
MD 15-61	434	403	384		407
MD 15-138	426	424	397		416
MD 15-31	402	419	392		404
MD 15-8 (Best Ck)	395	403	380		393
MD 15-15 (Random Ck)	408	378	349		378
MD 25-26ne		340	341		341
MD 25-87		380	365		373
SG 747		300	295		298
LSD (0.05)	3.3	2.0	2.2		

Best Ck = Best individual plant selected from the check germplasm for the particular trait.

Random Ck = Individual plant randomly selected from the check germplasm.

Table 3. Fiber uniformity and lint yield for some selected individual M5 mutants grown at Stoneville, MS

Mutant-Selection	Fiber Uniformity (%)			Lint Yield (lbs/acre) 2013 (M5)
	2012 (M4)	2013 (M5)	Mean	
MD 15-31	87.5	88.9	88.2	1145
MD 15-61	87.5	88.3	87.9	1284
MD 15-138	88.0	87.7	87.9	1227
MD 15-13	87.7	87.5	87.6	965
MD 15-89	87.0	86.6	86.8	840
MD 15-9 (Random Ck)	85.2	87.6	86.4	1229
MD 15-8 (best Ck)	87.1	87.1	87.1	1384
MD 25-26ne	87.3	88.2	87.8	1960
MD 25-87	87.4	88.5	88.0	1820
SG 747	85.7	87.4	86.6	1698
LSD (0.05)	0.9	1.3		233

Conclusion

Cotton breeders can exploit chemical mutagenesis to create the much needed diversity in fiber quality. However chemical mutagenesis does not appear to work on more complicated traits controlled by many genes such as lint yield. In 2014, molecular analyses of these mutants will be carried out to add to the understanding of the basis for the improved fiber quality. Some of these germplasm will be ready for release in 2015.

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