CHEMICAL MUTAGENESIS AS A TOOL IN COTTON IMPROVEMENT

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Abstract

Chemical mutagenesis of *Arabidopsis* has had a tremendous impact on the application of molecular biology to higher plants. Our basic hypothesis is that chemical mutagenesis and subsequent selection can have an equivalent impact on developing new genes that enhance the fiber and other economically important traits of cotton (*Gossypium hirsutum* L.). To test this hypothesis, we have extensively evaluated a population of the cultivar HS 200 which was treated with 3% v/v Ethyl Methanesulfonate (EMS). The fiber of 1997 M_3 plants of this population had an extremely wide range in HVI fiber characteristics. The M_4 and M_5 generation of 115 selected lines were evaluated in 1996 and 1997 for HVI fiber characteristics. This process identified two mutant lines (202 and 271) which produce fiber approximately 0.7 inches longer than the parent cultivar. These mutants could be used to enhance fiber quality of cotton grown on the Texas High Plains.

Introduction

Cotton (*Gossypium hirsutum* L.) is one of the leading cash crops in the U.S. (Cotton Council, 1996). Nearly 20% of U.S. cotton production (3 million bales) occurs on the High Plains of Texas. The relatively short length and low strength of the fibers of the cultivars adapted in this area have limited the use of this cotton to low value markets. Chemical mutagenesis was used to develop a series of mutants with increased fiber length, increased fiber strength, and finer fibers. These characteristics should allow the development of cotton cultivars that could be utilized in higher value textile markets increasing the economic competitiveness of Texas cotton producers.

Chemical mutagenesis was utilized, because it allows the modification of fiber characteristics while leaving the lint yield and adaptation of proven cultivars intact. Chemical mutagenesis of *Arabidopsis* has developed many unique genes that have had a tremendous impact on the application of molecular biology in higher plants (Somerville and Browse, 1991). Chemically induced mutants do not require the extensive backcrossing, regulatory restrictions, and complex proprietary right considerations which currently limits commercialization of transgenically developed cotton cultivars. However, mutants can be protected by Plant Variety Protection or Plant Patents to allow exclusive commercialization that would benefit Texas cotton growers.

Modern upland cotton has a narrow germplasm base that often limits the success of breeding programs (Bowman et al., 1996). The existing fiber mutants currently described in cotton were obtained as spontaneous mutants found in breeding nurseries or selected from exotic germplasm lines (Kohel and McMichael, 1990; Kohel et al., 1992). There has never been a previous report of a single gene or mutation that significantly altered fiber quality in upland cotton. Chemical mutagenesis of cotton has historically had limited success in creating new genetic variation (Naivar, 1996). The allotetraploid chromosome configuration of commercial cotton and the potential for the oils found in the seeds to bind with chemical mutagens may have limited the success of earlier research.

Extremely high rates of chemical mutagens have been used to induce high value mutants in allotetraploid cultivars of canola (Brassica napas L.) (Auld et al., 1992; Tonnemaker et al., 1992). Preliminary studies at Texas Tech University indicated that successful mutagenesis in cotton would require exposing germinating embryos to similar high rates (3X to 5X of the predetermined LD_{50} dose) of mutagens (Krieg and Auld, 1993). Our approach was to use extremely high rates of Ethyl Methanesulfonate (EMS) on commercial cultivars of cotton adapted to the Texas High Plains.

On 6 June 1993, 10 kg lots of two cultivars (HS 26 and HS 200) were treated with 1, 3, and 5% v/v rates of EMS. HS 26 and HS 200 were selected for mutagenesis, since they are grown commercially on over one million hectares (2.5 million acres) on the Texas South Plains and produce high quality fiber. Poor emergence of the treated M_1 seed under field conditions near New Deal, TX indicated that cotton exposed to both the 3 and 5% v/v rates of EMS had emergence reduced by more than 95%. In the fall of 1993, one boll was harvested from each surviving M_1 plant, and the seed bulked to produce M_2 seed. The M_2 seed was increased near New Deal, TX during the 1994 growing season, and one boll from over 5,000 M_2 plants was harvested to obtain M_3 seed. The M_3 seed was planted on the Texas Tech University campus at Lubbock, TX in 1995. Four bolls were individually harvested from 1997 M_3 plants and the fiber analyzed using standard High-Volume Instrument (HVI) analyses at the TTU-International Textile Center (ITC). On 29 May 1996 and 24 May 1997, seed of the 115 selected M_3 lines and two check cultivars were planted at Lubbock, TX. Each line was planted in a single row 3.5 m in length, spaced 1 m apart. These studies were conducted as a randomized complete block with three replications. In the fall of 1996, plants were evaluated for

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phenotypic characteristics, and three plants from each row were individually harvested to obtain fiber for HVI analysis. Additionally, in both years of the study 1 m of each row was harvested to provide an estimate of lint yield and bulk harvested fiber for HVI analyses. During 1997 additional trials with the two fiber mutants and selected controls were conducted at New Deal and Shallowater, TX.

Discussion

Evaluation of fiber from nearly 2,000 M_3 lines from HS 200 treated with 3% v/v EMS showed significant variation for micronaire, length, and strength (Table 1). The extremely large range in fiber characteristics in these populations exceeded the variation expected for individual plant selection from a cultivar such as HS 200. Based on these data, 115 lines with divergent fiber properties of micronaire, length, or strength were selected for inclusion in the 1996 and 1997 field evaluations.

The 1996 HVI fiber analyses identified two mutant lines that had average fiber length which exceeded 1.2 inches (Table 2). Length of 1996 bulk grown fiber was not expected to be equivalent to the length observed in 1995 which reflects lint harvested from the four most mature bolls produced on the plant. These lines had lint yields equivalent to HS 200 under furrow irrigated conditions at Lubbock, TX.

In 1997, four random bolls were harvested in mid-September for preliminary fiber quality tests (Table 2). Bulk harvested fiber samples will not be available until later this spring. Once again, the fiber samples harvested from the mutant lines consistently averaged more than 1.25 inches in length and were significantly longer than similarly harvested fibers of HS 200 and HS 26.

Analyses of 1997 data show that at both Lubbock and New Deal, TX the mutant lines 271 and 202 had significantly longer fiber than HS 200 and PM 280 (Table 3). PM 280 is a cultivar with enhanced fiber quality selected from HS 200. Because HS 200 was not included as a check cultivar at the Littlefield, TX location, the results were less clear. However, again at this location both mutant lines produced fiber over 1.20 inches in length. Fiber strength and micronaire were not statistically significant at any of the three locations with the exception of micronaire at Lubbock, TX where 271 was equivalent to HS 200.

During the 1998 growing season, these two mutant lines will be tested at a minimum of six locations. These tests will help confirm genetic stability and provide a more accurate estimate of the impact of environment on fiber length in these two mutants. Putative mutants for increased fiber strength and other fiber properties will also be evaluated in 1998. Additional studies have been initiated to determine the inheritance of these traits. Crosses between these fiber mutants and a broad range of cultivars will be made to determine if this mutation could be used to enhance fiber quality of Acala and Pima varieties. Crosses have also been made between the two mutant lines to see if fiber length is conditioned by different and perhaps additive alleles which would allow selection of even longer fibers.

Summary

Chemical mutagenesis was utilized to develop two mutant lines of upland cotton with enhanced fiber length. These mutant lines may represent a significant enhancement in both the perceived and economic fiber quality of West Texas cotton. It may be possible for textile mills to utilize cotton produced from these genotypes as a substitute for imported higher value, extra long stable, domestic cotton. However, the current grading and pricing schedule does not even recognize that this quality of fiber can be grown in this region. Consequently, textile manufacturing and marketing studies of longer fiber cotton must be conducted prior to the initiation of commercial production of these mutants if growers are to receive full value of this improved class of cotton.

References

Auld, D.L., M.K. Heikkinen, D.A. Erickson, J.L. Sernyk and J.E. Romero. 1992. Rapeseed mutants with reduced levels of polyunsaturated fatty acids and increased levels of oleic acid. Crop Sci. 32:657-662.

Bowman, D.T., O.L. May and S.D. Calhoun. 1996. Genetic Base of upland cotton cultivars released between 1970 and 1990. Crop Sci. 36:577-581.

Cotton Council. 1996. Cotton counts its customers: The quality of cotton consumed in final end uses produced in the United States National Cotton Council of America.

Kohel, R.J. and S.C. McMichael. 1990. Immature fiber mutant of upland cotton. Crop Sci. 30:419-421.

Kohel, R.J., E.V. Narbuth and C.R. Benedict. 1992. Fiber development of ligon lintless-2 mutant of cotton. Crop Sci. 32:733-735.

Krieg, D.R. and D.L. Auld. 1993. Development of cotton mutants with improved fatty acid composition. Proc. 7th Annual Southwest Consortium on Plant Genetics and Water Resources Symposium. Riverside, CA. p. 10.

Naivar, K.S. 1996. Fiber quality parameters and within-boll yield components of *Gossypium arboreum* . Putative mutant lines. M.S. Thesis. Texas A&M University. 71 p.

Somerville, C.R. and J. Browse. 1991. Plant lipids: Mutants, metabolism, and membranes. Science 252:80-87.

Tonnemaker, K.A., D.L. Auld, D.C. Thill, C.A. Mallory-Smith and D.A. Smith. 1992. Development of sulfonylurea-resistant rapeseed using chemical mutagenesis. Crop Sci. 32:1387-1391.

Table 1. Mean, range and standard deviation of micronaire, length and strength of fiber of 1977 M3 plants of HS 200 grown at Lubbock, TX in 1995. HVI Analyses was conducted at the International Textile Center at Texas Tech University.

	Mean	Range	Standard Deviation
Micronaire	4.6	2.8-5.8	0.40
Length (in.)	1.14	0.97-1.38	0.05
Strength (g/tex)	29.8	23-41	2.3

Table 2. Fiber characteristics of two mutant lines (202 and 271) selected for increased fiber length at Lubbock, TX during the 1995, 1996, and 1997 growing seasons.

		HIV Analyses					
Entry	Year	Micronaire	Length	Index	Strength	Yield	HS200 (A)
			---in---	--%--	-- g/tex—	lbs/Ac	----%----
202	1995	4.9	1.31	86.0	32.0	---	---
	1996	4.5	1.21	80.6	30.6	1521	93
	1997	4.5	1.27	84.3	38.3	---	---
		4.6	1.26	83.6	33.6		
271	1995	4.7	1.38	80.0	35.0	---	---
	1996	4.4	1.24	80.3	31.3	1564	95
	1997	4.2	1.26	82.7	36.3	---	---
		4.4	1.29	81.0	34.2		
HS 200	1995	4.6	1.14	82.8	29.8	---	
	1996	5.2	1.13	82.6	30.6	1642	---
	1997	4.4	1.19	83.7	35.7	---	---
		4.7	1.15	83.0	32.0		
HS 26	1995	---	---	---	---	---	
	1996	4.8	1.12	84.7	28.6	1210	74
	1997	4.6	1.15	83.3	37.0	---	---
		4.7	1.14	84.0	32.8		

*1995 and 1997 data was taken on fiber from 4-5 primary bolls and represents optimum fiber development and maturity. 1996 data is the average of cotton harvested from three replicated rows of each line and represents fiber quality expected under commercial production conditions on the Texas South Plains.

Table 3. Fiber length, strength and micronaire of three check varieties and two fiber mutants as determined by HVI analyses at the International Textile Center on fiber grown on the Texas South Plains in 1997.

	Texas South Plains		
Variety	Lubbock	New Deal	Littlefield
Fiber Length:	----------inches----------		
HS 26	1.15 b†	1.13 b†	1.13 c†
HS 200	1.19 b	1.16 b	--
PM 280	1.17 b	1.17 b	1.18 bc
271	1.26 a	1.25 a	1.20 ab
202	1.27 a	1.23 a	1.23 a
CV	2.4%	2.0%	2.0%
Fiber Strength:	----------g/tex----------		
HS 26	37.0 a†	34.0 a†	30.0 a†
HS 200	35.7 a	33.0 a	--
PM 280	37.3 a	33.0 a	37.0 a
271	36.3 a	34.7 a	31.0 a
202	38.3 a	35.3 a	32.0 a
CV	2.7%	4.7%	7.5%
Micronaire:			
HS 26	4.6 b†	4.4 a†	4.0 a†
HS 200	4.4 bc	3.7 a	--
PM 280	5.1 a	4.1 a	3.8 a
271	4.2 c	4.0 a	3.5 a
202	4.5 b	4.0 a	3.8 a
CV	2.9%	5.8%	7.4%

†Means within a data column of an individual index not followed by the same letter differ at the 0.05 level of probability Fisher's Protected Least Significance Difference Test.