MUTATION BREEDING FOR

FIBER QUANTITY AND QUALITY

by

JINESH DAHYABHAI PATEL

(Under the Direction of ANDREW PATERSON/ PENG CHEE)

ABSTRACT

A total of 3,200 M5 mutant lines from *G. hirsutum* breeding line TAM94L25 were characterized for 20 phenotypic traits describing basic plant growth and development, including fiber quality analysis. A subset of 300 lines was selected for further studies. Three replications were grown at single locations in both Texas and Georgia to further evaluate fiber quality, and investigated relationships between trichome variation and fiber traits.

Several mutant lines showed substantial improvements over TAM94L25 in economically important traits including fiber elongation, fiber fineness, fiber strength, and fiber length. For each of the traits, multiple lines were identified that consistently had fiber qualities better than the parental line. Further, significant changes in fiber qualities associated with leaf and/or stem trichome mutations suggest that some common genes are involved in trichome and fiber development.

INDEX WORDS: TAM 94L25, Cotton, Mutant lines EMS, Fiber, Trichome

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BS, Anand Agricultural University, India, 2008.

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2011

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August 2011

DEDICATION

I would like to dedicate my thesis to my parents, sister, and Jiju. My papa Dr. D. M. Patel and my mummy Ranjenben D Patel have been very supportive concerning my aspirations. Their unwavering support, unconditional love, and sacrifices on my behalf have inspired me to accomplish this milestone today. My sister Dr. Nicky N Seth and Jiju Dr. Nirav G Seth have also been instrumental in my success – thank you for being there by my side.

ACKNOWLEDGEMENTS

It is a great pleasure to extend my gratitude to some of the individuals who helped me through my MS work.

First of all, I would like to thank Dr. Andrew Paterson who supervised and funded my MS research - his guidance and support has been genuinely instrumental.

Special thanks go to my co-major advisor Dr. Peng-Wah Chee for mentoring me as well as for serving as my academic advisor.

Also, I appreciate Dr. Cheolwoo Park and Dr. Dayton Wilde for serving as my committee members and helping me with invaluable suggestions - thank you.

I would also like to thank PGML members for their help in the field work and technical support. I am also thankful to Dr. Dick Auld, Dr. Robert Wright and the research crew at Texas Tech University for providing intellectual and material support for my research.

Finally, I would also like to thank Dr. Mukesh Rana, Dr. Rajib Bandopadhyay, Mr. Sameer Khanal and Mr. Rahul Chandnani for helping me in my academics as well as my research.

Your help have enabled me to earn my MS degree – I really appreciate and thank you all.

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Chapter 1

INTRODUCTION AND LITERATURE REVIEW

Cotton In A Nutshell

Cotton, also revered as white gold, is the world's leading naturally derived textile fiber. Historically, cultivated cotton has been extensively studied for its evolution and domestication which led to an allopolyploid species exploited for its spinnable seed fibers (Wendel *et al.* 2010). Belonging to the family *Malvaceae*, the genus *Gossypium L.* shows extensive global diversification with around 50 known species, including 45 diploids and 5 allopolyploids distributed over a wide geographic range in the arid and semiarid tropics (Vollesen 1987 ; Fryxell 1992). Diploids are differentiated into eight different genome types designated "A" through "G" and "K" based on karyotypic variability, meiotic chromosome pairing, and the fertility of interspecies hybrids; however, all the diploid species have the same chromosome number (n=13) (Endrizzi *et al.* 1985; Percival *et al.* 1999; Wendel *et al.* 1999). The allopolyploid species are the derivatives of A and D genome diploids and have a haploid chromosome number of 26 (Wendel and Cronn 2003). Interestingly, the centers of diversity for the A, B, E, and F genomes are in Africa; while the D genome cotton is native to the New World. Probably following the trans-oceanic dispersal of an A genome African diploid to the New World, it hybridized with a D genome New World species to give rise to an allotetraploid species either through the fusion of unreduced gametes or through chromosome doubling of a F1 interspecific hybrid. The nascent allopolyploids were reproductively isolated from the diploid progenitors,

with further speciation and radiation forming three clades and five species. Four species of this genus are cultivated, two A-genome diploids (*Gossypium arboreum L.* and *Gossypium herbaceum L.*) also known as Old World cotton, and two tetraploids (*Gossypium hirsutum L.* and *Gossypium barbadense L.*) also known as New World cotton. Each of these four species has been independently domesticated for fiber properties valuable in textiles (Brubaker *et al*. 1999; Butterworth *et al.* 2009).

Cotton is a highly profitable cash crop in two of the biggest cotton producing nations, viz. India and China, which account for almost 50% of the world's supply. It is also a leading cash crop in the U.S, where 18.4 million bales of cotton were produced from around 11 million acres planted in 2010 (National Cotton Council 2011). A large percentage (~40%) of raw cotton produced in the U.S. is exported, generating almost $ 3 billion and helping to curtail trade deficits. Within the U.S., the cotton industry benefits fertilizer and agrochemical companies, seed producers, farm power and machinery companies, as well as farm laborers. Even the byproduct and often-overlooked component from cotton, cottonseeds, have a major market as livestock and poultry feed, as well as a source of cottonseed oil. However, cotton fiber is the predominant commodity of interest, $2/3^{rd}$ of which provides raw materials to the textile industry while $1/3^{rd}$ is utilized for other purposes, such as home furnishings and various industrial products. Globally, the demand for cotton has been on the rise. Cotton production around the world experienced bad weather and natural calamities in 2010. These factors together with the soaring demand for cotton in the burgeoning economy of China, have led to an unprecedented recent rise of the commodity price.

Cotton Biology

Although wild cotton grows as a perennial plant, domestication and commercialization necessitated cultivated varieties to be grown as annual plants. Commercially cultivated varieties have a life cycle of 5 to 6 months depending upon the growing conditions. The agronomic growth stages can be divided into five parts: 1. Emergence (5 days after planting or DAP), 2. First Square (38 DAP), 3.First Flower (59 DAP), 4. Open Boll (116 DAP), and 5. Harvest (140 DAP). At anthesis, about 15 – 25% of the total epidermal layer cells start differentiating and developing into lint fibers which can be spun for commercial purposes (Basra and Malik 1984; Tiwari and Wilkins 1995; Kim and Triplett 2001). The remaining epidermal cells may not differentiate into fiber or may just form small "fuzz" fibers. The length of cotton fiber can reach up to 2.36 inches, making it one of the most exaggerated plant cell types (Kim and Triplett 2001; Lee *et al.* 2007). Cotton fiber development undergoes four distinct yet overlapping stages viz. fiber cell initiation, elongation, secondary wall biosynthesis, and maturation (Basra and Malik 1984). Much information regarding the timetable and expression patterns of candidate genes and/or proteins associated with cotton fiber initiation, elongation, and maturation is available (Turley and Ferguson 1996; Applequist *et al.* 2001; Wang et al. 2001; Ji et al. 2003; Arpat *et al.* 2004; Wilkins and Arpat 2005; Wu et al. 2006; Lee *et al.* 2007). Fiber initiation starts immediately after anthesis and continues up to 3 days post anthesis (DPA). Fiber elongation, one of the extensively studied periods of fiber development, overlaps with fiber initiation (Lee *et al.* 2007). During fiber elongation, the fiber cells expand with growth rates of 2 mm/day until they reach maximum length; the period is reported to extend between 5 to 20 DPA (Xu *et al.* 2008). Meantime, secondary wall biosynthesis initiates and continues up to 25 DPA wherein a large amount of cellulose deposition results in thickening of the secondary cell wall. Deposition of 3–6

μM of cellulose around the whole circumference of the fiber results in the highest percentage of targeted cellulose deposition known in plants, with around 90% of the total fiber weight being crystalline cellulose (Haigler 2007; Haigler *et al.* 2009). Fiber maturation succeeds secondary wall biosynthesis and lasts for 45-60 DPA. Cotton fiber matures as an eventual consequence of mineral accumulation and decreased water potential (John and Keller 1996).

Trichome/Fiber Relationship

Trichomes are fine outgrowths or appendages of the epidermal layer, mostly present in aerial parts and on roots as root hairs of the flowering plant. Trichomes can be unicellular or multicellular with or without glands (secretory organs) (Esau 2006). As indicated by its name, *Gossypium hirsutum L*. has coarse pubescence on the leaves and stems of mature plants. Trichomes in cotton are associated with advantages including increased tolerance of drought (Espigares and Peco 1995) and reduced attack of leaf hopper (Jenkins and Wilson 1996; Bourland *et al.* 2003), but also disadvantages including promotion of egg-laying by *Heliothis spp.* (Benedict *et al.* 1983; Treacy *et al.* 1986; Hassan *et al.* 1990), and increased attack of silverleaf white fly (Chu *et al.* 2000) , increased leaf trash in ginned cotton, and reduced fiber quality (Wanjura *et al.* 1976; Meredith *et al.* 1996). Different alleles responsible for variation of leaf and stem pubescence have been found, using chromosome cytological stocks (Endrizzi *et al.* 1984). Five different loci, t_1-t_5, were reported as having large impacts on leaf and stem trichome size or number (Lee 1985). Genetic mapping has clearly placed t_1 on chromosome 6 and t_2 on chromosome 25 (Wright *et al.* 1999; Lacape and Nguyen 2005). Eight trichome-related QTLs have been suggested in meta-analysis of polyploid cotton QTLs (Rong *et al.* 2007). Associations of the t_1 trichome locus with fiber quality have been shown in many studies (Simpson 1947;

Knight 1952; Kloth 1995; Rong *et al.* 2005; Desai *et al.* 2008). Two QTLs for lint percent, which increased trait phenotypic value, were at t_1 locus in the F_2 population of TM1×T586 (Guo et al. 2006). Nonenvironment-specific QTLs for lint percent, fiber length, fiber length uniformity, and fiber strength were identified within 5 cM of the t_1 locus region, suggesting that the t_1 locus might be a candidate gene for the QTLs (Wan et al. 2007). A linkage map based on 270 $F_{2:7}$ recombinant inbred lines derived from an upland cotton cross between T586 X Yumian 1 indicates two QTLs, *FL1* and *FU1* near the t_1 locus further supporting the Wan *et al.* (2007) hypothesis of a role of the t_1 locus in fiber development (Zhang et al. 2009). In genetic mapping and comparative analysis of seven fiber mutants, the association was extended to *sma-4(fbl)*, *Sus*, and perhaps t_2, also raising a question about whether the genes responsible for these traits might have similar functions due to close positional association (Rong *et al.* 2005). Desai *et al.* (2008) found the leaf pubescence mutation *glabrous* to be co-segregating with *sma-4(ha),* which suggested that glabrous and fiberless mutants may be influenced by gene/genes governing both trichome and fiber development (Desai *et al.* 2008). Therefore, it is imperative to further explore the genetics of simply-inherited traits such as trichomes in cotton or other botanical models such as Arabidopsis, which could be helpful to increase our understanding of lint fiber development.

Growth and development of trichomes in *Arabidopsis* has been deeply studied (Marks 1997; Schwab *et al.* 2000; Larkin *et al.* 2003; Schiefelbein 2003; Hulskamp 2004; Wang *et al.* 2007; Kryvych *et al.* 2008; Marks *et al.* 2009; Morohashi and Grotewold 2009; Uhrig and Hülskamp 2010). The leaf trichomes in cotton and *Arabidopsis* are morphologically the same, unicellular and branched, whereas lint fibers are unbranched and extremely elongated. Despite these morphological differences, trichomes and lint fibers might share similar developmental pathways (Hulskamp *et al.* 1994; Kim and Triplett 2001; Arpat *et al.* 2004; Rong et al. 2005; Wan *et al.*

2007; Desai *et al.* 2008). The probe Gate-4CE05 obtained from a cDNA library of 7-10 days postanthesis *G. arboreum* fiber and mapped to chromosome 6 of *G. hirsutum* showed significant homology with the Glabra1 (*GL1*) gene in *Arabidopsis* (Desai *et al.* 2008). Mutations in *GL1* can inhibit the production of trichomes in *Arabidopsis* (Larkin *et al.* 1994; Hauser *et al.* 2001; Karkkainen and Agren 2002). It has been shown that *GaMYB2*, a cotton MYB transcription factor that is highly expressed in developing lint fiber, can restore trichome production in *Arabidopsis gl1* mutants and also induces trichomes on Arabidopsis seed (Wang *et al.* 2004). This indicates potential similarity between the genetic regulation of *Arabidopsis* trichomes and cotton lint fiber. There is predominant expression of MYB genes in developing cotton fiber and trichomes (Loguercio *et al.* 1999; Cedroni *et al.* 2003; Suo *et al.* 2003; Desai *et al.* 2008; Lee *et al.* 2007; Machado *et al.* 2009; Zhang *et al.* 2010). *GhMYB109*, which is closely related to *GL1*, has been found to be specifically expressed in the cotton fiber initials and elongating fibers, indicating that it might have a role in fiber initiation and elongation (Suo *et al.* 2003). *GhMYB 25* was identified as differentially expressed between fiberless mutants and cotton having normal lint, and was more highly expressed in fiber initials than in adjacent epidermal cells (Lee *et al.* 2006; Wu *et al.* 2006). Reduction in the expression of *GhMYB25*, a low copy MYB transcription factor, caused condensed leaf trichome number and fiber growth that suggest a role of MYB genes in trichome and fiber development (Machado *et al*. 2009). A HD-Zip IV family transcription factor, *GbML1* when over expressed in *Arabidopsis* have increased the number of trichomes on stems and leaves. *GbML1* was identified as the first partner for *GbMYB25*, which is a key regulator of cotton fiber development (Zhang *et al.* 2010).

Fiber Characteristics

Over the years higher cotton yield has remained the single most important goal for breeders, the growers, and the industry. Breeders have historically focused on yield and yield influencing components such as biotic stresses. However, in the early twentieth century fiber quality started to be considered as important as yield and disease resistance due to enhancement in yarn processing efficiency and its influence on quality of the end product (May 2000). The U.S. Cotton Futures Act was passed in 1914 to determine fiber quality and in 1923, the U.S. Cotton Standard Act made it mandatory to classify cotton into different categories (Brown 1938; Ramey Jr 1980). The evolution of high throughput air jet spinning machines in the textile industry has necessitated this shift. These tools are as much as eight times faster and, hence, more productive than their old counterparts, but require better strength in fibers for efficient spinning. Fibers with greater strength and longer staple were much more desirable for spinning yarn, reducing waste (Bradow and Davidonis 2000). Raw cotton demand on a local basis has declined sharply in the USA with a number of textile mills relocating overseas to capitalize on lower taxes as well as lower labor costs. The U.S. Cotton Standard was replaced with Universal Cotton Standard to set quality standards for the international market (Brown 1938) as more raw cotton was exported. The stakeholders in the cotton business are aware of these facts and, hence, fiber quality has become a priority objective for cotton researchers in recent years.

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

Fiber fineness, also known as micronaire, is a measure for determining maturity and/or fineness of cotton fibers. Air is blown through a certain weight of lint and fiber fineness is determined by calculating the resistance to this air flow. The unit used is Micronaire (Mic) (Smith 1947). Fibers having micronaire values of 3.4 or below are classified as immature, with the mature and desirable quality of fiber fineness considered to be between 3.5 and 4.9. Fibers having Mic 5.0 or above are considered to be coarse. Thick and coarse fibers are strong and can withstand the speed of spinning yarn but the yarn developed from this fiber is not stronger because fewer fibers are found in a particular cross section. Spinning immature fibers can affect the spinning rate, as they do not twist and cling well when spun, causing neps in the yarn and also affecting fabric dyeing and its appearance (Basra and Malik 1984; Hake *et al.* 1996). Mature but finer fibers are most desired for yarn, reducing the spinning speed as compared to coarse fiber but yielding an ultimate yarn product that is much softer and stronger with high quality (Grover and Hamby 1960). Fiber fineness is more important than fiber strength in determining yarn strength (Sattar and Hussain 1985).

Fiber strength is mostly contributed by cellulose present in secondary cell walls (Delmer and Amor 1995). It represents durability of fiber. It is measured by stelometer (Hertel 1953) reading or high volume instrumentation (HVI) (Benedict *et al.* 1999). A certain amount of force is required, expressed in grams, to break a bundle of fiber whose size is described in tex. Accordingly the unit used to determine fiber strength is grams/tex. Fibers having 20 grams/tex or below are considered very weak, 21 to 25 grams/tex are considered weak, 26 to 29 grams/tex are considered base, 30 to 32 grams/tex are considered strong and above 32 grams/tex are considered very strong (http://www.cottoninc.com). Stronger fiber can resist the high speed spinning of yarn and has a high correlation with yarn strength (Chee and Campbell 2009). Fiber

having substandard strength will break during yarn processing, which will cause damage and wasted product. As there is no end to the increase in speed of yarn spinning, the standard for fiber strength will keep on increasing in the future, which will require the cotton breeder to develop lines with high fiber strength.

Fiber length is the average of the longest 50 percent of fibers measured by HVI or fibergraph instrument (Hertel 1940). The units used are mm or inch. Fibers having length below 0.99 inch are considered short, 1 to 1.10 inch are considered medium, 1.11 to 1.26 are considered to be long and above 1.26 inch are considered to be extra long (http://www.cottoninc.com). Longer fiber will produce better and stronger yarn and improve the quality of fabrics as it will have greater resistance to friction caused by external forces (Broughton *et al.* 1992).

Fiber uniformity index is the ratio of the average length of all fibers to the average length of the longest 50 percent of fibers in the sample, and is measured in percentage. Fibers having below 79% are considered to have lower uniformity while the average is between 80-82% and 83% and above are considered to have higher uniformity. The fiber having higher uniformity produces less waste and higher quality of product.

Fiber elongation is the elasticity of cotton fiber and is measured simultaneously with fiber strength. Fibers having 5.8 or below are considered to have low elasticity, 5.9 to 6.7 is average, while 6.8 and above are consider to have high elasticity. Elongation contributes to yarn stretchiness but has nothing to do with yarn strength even though it helps to withstand high throughput textile processing (May 2000).

Fiber color is determined by two fiber characteristics, reflectance (Rd) or degree of grayness and degree of yellowness or +b value. The degree of grayness determines whiteness of cotton fiber expressed as percent reflectance (Rd). It ranges from 50 to 85%. Higher percentages are

more desirable. Units for expressing the yellowness of cotton fiber are Hunter's +b and it ranges from 5 to 18 where 5 is least yellow and most desirable.

Genotype X Environmental Effect

There have been abundant reports showing genotype x environment effects on fiber properties and yield (Pettigrew 2001; Paterson *et al.* 2003; Campbell and Jones 2005; Shen *et al.* 2006; Lacape *et al.* 2010). Genotype x environment effects have a large impact on fiber fineness but less effect on fiber length and fiber strength (Meredith 1984). The influence of night temperature on fiber properties and seed quality has been explained (Gipson and Joham 1968; Gipson and Joham 1969a, b). Night temperature of 15° C to 21°C was reported to be optimal for fiber length whereas micronaire was reported to be reduced when night temperature goes below 25°C (Gipson and Joham 1968; Gipson and Joham 1969b). Fiber length was reduced under moisture deficit, particularly when moisture stress was applied shortly after flowering (Bennett et al. 1967; Eaton and Ergle 1952, 1954; Marani and Amirav 1971; Marani 1973). Different QTLs for fiber length, strength, fineness, uniformity and fiber yellowness are affected by irrigated and rain fed conditions (Paterson *et al.* 2003). More carbohydrate deposition occurred in abundant sunlight than reduced light, thus developing fiber with higher strength (Pettigrew 2001). Reporting 8–24% variation in HVI fiber qualities caused by genotype x environment interactions, it has been suggested that information regarding genotype x environment interaction is important for cotton breeders to develop cultivars for adaptation to different locales (Campbell and Jones 2005).

Correlation Between Fiber Traits

Although fiber properties show largely additive gene effects, there is a possibility of inter-relationships between fiber properties. Correlations between traits can be a result of genetic linkage and/or pleiotropy (Chee and Campbell 2009). Correlation between lint yield and some fiber qualities is still controversial, but it is quite certain that lint yield and fiber strength are negatively correlated (Meredith 1994; Coyle and Smith 1997; Shen *et al.* 2007). A report of germplasm with high strength and high yield suggested that the correlation may be due to linkage rather than pleiotropy (Culp *et al.* 1979). It has also been reported that lint yield is negatively correlated with fiber length whereas it may have positive correlation with fiber elongation and micronaire (Meredith 1994). Lint yield was not consistently correlated with micronaire, length, or strength in a mutated population (Herring *et al.* 2004) and similar patterns have been reported in other populations (Green and Culp 1988).

Positive correlation was found for fiber strength to length and maturity and also for fineness to elongation (Basal and Smith 1997; Kloth 1998; May 2000). A fairly high correlation of r=0.57 was reported between fiber strength and fiber length in interspecific germplasm (Lacape *et al.* 2010). Length and strength were moderately correlated in all three generations of a mutant population (r = 0.46** to r = 0.58**) (Herring *et al.* 2004), showing that it may be possible to select mutant lines for improvement of both fiber strength and fiber length. Moreover, negative correlation is found between micronaire and fiber length (Herring *et al.* 2004) and also for elongation and fineness to strength.

Cotton Breeding And Genetics

Cotton genotypes are known to produce natural fibers rivaling the length, fineness, and strength of synthetic fibers, but are low-yielding and suffer other defects (Saha *et al.* 2006). Upland cotton germplasm has perhaps the lowest level of genetic diversity among major crop species, recently exacerbated in breeding programs by repeatedly crossing a few closely-related genotypes to generate new cultivars. A study conducted on 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.

While recent developments in cotton genomics have provided numerous essential resources, we lack one key component of an integrative approach to the identification of genes responsible for lint fiber morphogenesis and other important characteristics of the cotton plant, specifically an ample collection of well-characterized mutants suitable for dissecting the associated biochemical pathways. For instance a total of 432 QTLs mapped in 1 diploid and 10 tetraploid cotton populations, which included 224 fiber-related and 8 trichome-related QTLs, have been aligned using a high-density reference map and depicted in a CMap resource to simplify queries (RONG *et al.* 2007). Although a total of 36 QTLs for fiber length were identified in five diverse populations, there exist only four mapped mutants, viz. Li_1 (Griffee and Ligon 1929), Li_2 (Kohel *et al.* 1992; Narbuth and Kohel 1990), *Fbl* (Kearney and Harrison 1927), and T_1. Clearly, we lack qualitative mutants for most agriculturally-important cotton QTLs affecting fiber length and there would be much value in discovery of many more discrete fiber length mutants than are presently known.

Mutation Breeding

Nobel laureate Hermann J. Muller is the founder of mutation genetics. His pioneering work in fruit fly provided strong evidence that abundant genetic and chromosomal changes can be rapidly induced through x-ray irradiation (Muller 1928, 1946). Mutational research quickly spread from conventional fly genetics to agricultural species such as oat, barley, wheat, cotton and maize (Stadler 1928a, b, 1929, 1930; Horlacher and Killough 1931; Horlacher and Killough 1933). The history of mutation breeding can be viewed in two phases; first, during the late 1930s and second, after the Second World War when many countries including the U.S. started programs in coordination with international organizations such as FAO (Lonnig 2006). In recent years, induced mutation has been revived as an exciting tool for genetics research as well as for crop improvement endeavors. The successes in crop improvement have been significant with hundreds of varieties of induced-mutants of several agronomic crops released and being cultivated by farmers on millions of acres. Some achievements from mutation breeding played a significant role in the "Green Revolution", which precluded dire consequences of burgeoning world population growth by significantly increasing the yield of major staple crops like wheat and rice (http://www.iaea.org). With tremendous power to create variability, induced mutation is viewed as a promising tool to contribute to another "Green Revolution". Further, rapid advancement in allied scientific fields have enabled researchers to better understand the roles of genes and cognate proteins in many aspects of plant growth and development. Forward genetic studies are complemented by reverse genetic approaches with techniques such as Targeting Induced Local Lesions in Genomes (TILLING) (McCallum *et al.* 2000). The field of bioinformatics has coevolved with technological advancement to provide accurate and high-throughput analysis. Also, the post genomic era has seen a rapid decline in cost associated with

"omics" research. As such, increasing numbers of researchers are exploring mutational research to complement other crop improvement endeavors.

Several approaches including chemical, irradiation, and insertional have been developed to induce mutations. Each has advantages and disadvantages based largely on the objective of mutation. Mutation can be a large-scale deletion at the chromosomal level, for example, induced by non-ionizing (e.g. UV) or ionizing radiation (e.g. X and gamma rays, alpha and beta rays, fast and slow neutrons). In contrast, chemical mutagens most often only affect single nucleotide pairs to produce point mutations. Some of the most commonly used chemical mutagens in plant research include ethylmethane sulphonate (EMS), methylmethane sulphonate (MMS), hydrogen fluoride (HF), sodium azide, N-methyl-N-nitrosourea (MNU), and hydroxylamine. Chemical mutagens are more favored since single nucleotide changes can bring about mutations that are stable while mutations from physical mutagens frequently have negative if not lethal results (Parry *et al.* 2009). Ethyl methanesulfonate (EMS) is frequently favored as a chemical mutagen which can be used for both forward and reverse genetic studies (Kim *et al.* 2006). It is more efficient and effective than irradiation, due to a greater number of mutations as well as higher survival rates of the mutants (Favret 1960). EMS mutagenesis is based on mispairing and base changes due to induced chemical modification of nucleotides from biased alkylation of guanine (G) residues to form O6 -ethylguanine, which can pair with thymine (T) but not with cytosine (C). Subsequent DNA repair changes the nucleotide composition resulting in A/T (adenine/thymine) instead of the original C/G at the double stranded position (Greene *et al.* 2003). Methyl methanesulfonate, on the other hand, produces T/A to G/C transversion and A/T to G/C transitions (Krieg 1963; Kovalchuk *et al.* 2000; Greene *et al.* 2003).

Mutation Breeding In Cotton

The primary gene pool of the cultivated *Gossypium* spp. is narrow due to both evolutionary bottlenecks and human intervention. Evolutionary bottlenecks arose from the recent origin of polyploids, their reproductive isolation from the diploid progenitors, and consequent speciation. The evolutionary limitations are further exacerbated by intense selection concentrated on a handful of lines and specifically targeting a few major traits of interest. As such, allelic diversity in cultivated cotton is much lower than that of many major crop species. The diversity within the primary gene pool is limiting for conventional cotton breeding and application of molecular breeding tools and techniques. Also, understanding the physiological and genetic basis of various aspects of plant growth and development including responses to biotic and abiotic stimulus, fiber morphogenesis and others is limited by the paucity of allelic diversity. Although recent developments in cotton genomics have built a strong foundation for cotton researchers, one critical component of an integrated approach to identify and characterize agronomically important genes is still missing, specifically a broad collection of mutant lines which could be exploited to dissect the underlying biochemical pathways.

Auld *et al.* (2009) have presented a succinct review of the application of mutation in cotton species. Unlike several major crops, mutational breeding has not been established as one of the major tools in cotton improvement (Auld *et al.* 1998). Nevertheless, a broad spectrum of physical and chemical mutagens has been used sporadically in different species of *Gossypium* with varied responses (AULD *et al.* 2009). Typical of physical mutagenesis, radiation-induced mutants have shown a wide range of phenotypic variations (Horlacher and Killough 1931; Horlacher and Killough 1933). Physiological variants have also been generated using radiation-based approaches; some examples include: enhanced phosphorus uptake and improved drought

tolerance using radiophosphorus (Nazirov et al. 1979), and photoperiod insensitivity and cytoplasmic sterility using gamma rays (Raut *et al.* 1971; Ngematov *et al.* 1975). However, two major concerns viz. higher yields and better fiber quality characteristics, have yet to be realized from irradiation. Chemical mutagenesis, on the other hand, has shown a wide range of variation in different traits of interest including lint yield and fiber quality. The most commonly used chemical mutagens in cotton include sodium azide (Hussein *et al.* 1982; Larik *et al.* 1983), dimethyl sulfate, colchicine (Salanki and Parameswarappa 1968; Luckett 1989; Shi-Qi *et al.* 1991) and ethyl-methane sulfonate (Shattuck and Katterman 1982; Herring *et al.* 2004; Lowery 2007; Auld *et al.* 2009; Bechere *et al.* 2010). Bechere *et al.* (2010) registered four EMS-derived upland cotton mutants with elevated levels of imazamox tolerance (Bechere *et al.* 2010).

Mutation has also been employed in building genetic resources. Gao published a whole-genome radiation hybrid map of *G. hirsutum* L. (Gao *et al.* 2004) and *G. barbadense* (Gao *et al.* 2006), using gamma radiation to create mutants which were rescued with a wide cross to develop a mapping population, then genotyped with microsatellite markers to complement traditional linkage mapping. Gamma rays were used to fix post-zygotic mortality problems caused by gamete terminator genes on a *G. sturtianum* chromosome in [(*G. hirsutum* x *G. raimondii*)^2 x *G. sturtianum*] (HRS) trispecies hybrids having the glandless-seed and glanded-plant trait (Diouf et al. 2010) .

TILLING, an exciting technology that combines mutagenesis with high-throughput molecular analysis, is also being explored (Auld *et al.* 2009). In addition, researchers have expanded their interest in insertional mutagenesis, which is seen as an efficient strategy for direct isolation of genes of interest in several crop species.

TAM 94L25

The genotype selected for mutation was TAM 94L25, which was released by the Cotton Improvement Laboratory, Department of Soil and Crop Sciences, Texas Agricultural Experiment Station in 2002 (Smith 2003). Hybridization and pedigree selection at College Station, TX were the techniques for deriving TAM 94L25. TAM 94L25 resulted from an individual plant selected in the segregating F3 population of a cross between TAM 87G3-27 (Smith and Niles 1994) and TAM 87O3-37, a breeding line developed by G.A. Niles. TAM 94L25 was evaluated and compared with 'Tamcot Sphinx' (El-Zik and Thaxton 1996) and Deltapine 50 for several yield and fiber quality parameters. TAM 94L25 showed better micronaire reading and upper half mean length (UHM) compared to both the cultivars. Furthermore, TAM 94L25 had 3% higher lint percent and 38 kN m/kg greater bundle strength than Deltapine 50 (Smith 2003). Also, TAM 94L25 had acceptable fiber bundle strength of 32 gram/tex (Basal *et al.* 2009). Even though TAM 94L25 has above average fiber quality it was not released as a cultivar because of its low yield potential. Nonetheless, it has been used as a parental line for many germplasm lines which have been released, and for experimental purposes. Some examples are TAM 04 O-16L having long-staple with improved strength (Smith *et al.* 2011a), and nine germplasm lines (TAM A106-15 ELS,TAM A106-16 ELS, TAM B139–17 ELS, TAM B147-21 ELS, TAM B182-33 ELS, TAM C66-16 ELS, TAM C66-26 ELS, TAM C147-42 ELS, and TAM C155-22 ELS) with extra-long staple fiber (Smith *et al.* 2009; Smith *et al.* 2011b).

Mutagenesis in TAM 94L25 may help in developing lines with much higher fiber strength, longer fiber, and better fineness. It might also be possible to obtain lines which incorporate above average fiber quality with higher yield and lint percentage.

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Chapter 2

COMPARISON OF OUTSTANDING LINES WITH THE PARENTAL AND OTHER CONTROL LINES IN A MUTANT POPULATION

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[1] Jinesh D Patel, Robert Wright, Dick Auld, Rahul Chadnani, Valorie H. Goff, Jennifer Ingles, Andrew H Paterson. To be submitted to Theoretical and Applied Genetics

Abstract

A total of 3,200 M5 mutant lines developed by Dr. Dick Auld from *G. hirsutum* breeding line TAM 94L25 were characterized in 2008 for 20 phenotypic traits describing basic plant growth and development, including fiber quality. A small subset of 157 lines selected for improved fiber quality and 55 control lines were further studied. Altogether 22 lines for elongation, 22 lines for length, 17 lines for lint percent, 23 lines for fiber fineness, 21 lines for Rd value, 19 lines for strength, 21 lines for uniformity and 26 overall better performing lines were selected. Many lines were highly ranked in the selection for multiple fiber qualities. Three replications each at single locations in Texas and Georgia were grown to further evaluate fiber quality.

Several mutant lines showed substantial improvement over TAM 94L25 in replicated trials. For example, the strength of line 1793 was 37.1 gram/tex as compared to 31.97 gram/tex of TAM 94L25. Fiber fineness of line 2877 was 3.9 Mic as compared to 4.97 Mic of TAM 94L25. Fiber length of line 1903 was 1.3 inch (33.02 mm) as compared to 1.145 inch (29.01 mm) of TAM 94L25. Elongation of line 2925 was 8.7% as compared to 5.45% of TAM 94L25. Uniformity of line 2455 was 85.9% as compared to 83.3% of TAM 94L25. Rd value of line 1251 was 80.5% as compared to 77.1% of TAM 94L25. Lint percent of line 276 was 43.05 as compared to 40.85% of TAM 94L25. This indicates that genetic improvements for a wide range of fiber traits can be obtained from mutagenesis of elite cottons.

Introduction

Cotton is the world's leading textile fiber. Cotton is a highly profitable cash crop in two of the biggest cotton producing nations, viz. India and China, which account for almost 50% of the world’s supply. It is also a leading cash crop in the U.S, where 18.4 million bales of cotton were produced from around 11 million acres planted in 2010 (National Cotton Council 2011). A large

percentage (~40%) of raw cotton produced in the U.S. is exported, generating almost $ 3 billion and helping to curtail trade deficits.

Over the years, higher cotton yield has remained the single most important goal for breeders, the growers, and the industry. Breeders have historically focused on yield and yield influencing components such as biotic stresses. However, in the early twentieth century fiber quality started to be considered as important as yield and disease resistance due to enhancement in yarn processing efficiency and its influence on quality of the end product (May 2000). The U.S. Cotton Futures Act was passed in 1914 to determine fiber quality and in 1923, the U.S. Cotton Standard Act made it mandatory to classify cotton into different categories (Brown 1938; Ramey Jr 1980). The evolution of high throughput air jet spinning machines in the textile industry has necessitated this shift. These tools are as much as eight times faster and, hence, more productive than their old counterparts, but require better fiber strength for efficient spinning. Fibers with greater strength and longer staple were much more accepted for spinning yarn, reducing waste (Bradow and Davidonis 2000). Raw cotton demand on a local basis has declined sharply with a number of textile mills relocating overseas to capitalize on lower taxes as well as lower labor costs. The U.S. Cotton Standard was replaced with Universal Cotton Standard to set quality standards for the international market (Brown 1938) as more raw cotton was exported. The stakeholders in the cotton business are aware of these facts and, hence, fiber quality has become a priority objective for cotton researchers in recent years.

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

Cotton genotypes are known that produce natural fibers rivaling the length, fineness, and strength of synthetic fibers, but 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, recently exacerbated in breeding programs by repeatedly inter-crossing a few closely-related genotypes to generate new cultivars. A study conducted on 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.

While recent developments in cotton genomics have provided numerous essential resources, we lack one key component of an integrative approach to the identification of genes responsible for lint fiber morphogenesis and other important characteristics of the cotton plant, specifically an ample collection of well-characterized mutants suitable for dissecting the associated biochemical pathways. To fill this gap, we propose to characterize a random sample of 3,200 M5 mutant lines from *G. hirsutum* breeding line 94L25.

MATERIALS And Methods

Source of mutation lines

Dr. Auld developed a mutant population of TAM94L25 (Smith 2003) by treating seeds with the chemical mutagen EMS to their LD_{50}, using published techniques (Auld *et al.* 1992; Auld *et al.* 1998). About 3200 lines were generated and advanced by single boll descent to M5. A single boll descent risks duplicating a few mutants as a boll contains 20 to 30 seeds, but it is often a practical necessity in cotton.

In 2007, M4 population was grown in Lubbock, Texas (latitude 33N 34' 40.31" and 101W 51' 18.60'' longitude) and in 2008, the M5 population was grown in Watkinsville, Georgia (latitude 33N 51' 46.425" and 83W 24' 31.5756" longitude) with a completely randomized design (CRD). The soil type of the field near Watkinsville, GA is Appling Coarse Sandy Loam, and that of Lubbock, TX is Amarillo Fine Sandy Loam and Pullman Clay Loam. The sowing date in Texas was May 17, 2007, and Georgia was May 21, 2008. Seeds for each line were sown in 3 meter rows, spaced one meter apart. Thinning and weeding was done as necessary. A total of 74.45-74.45-74.45 kg/ha of NPK was applied as fertilizer and before sowing. Herbicide and pesticides were applied as required.

Eight morphological traits were examined, using a scoring scale from 0 to 3 where 0 is absence, 1 is present but less then parental type, 2 was similar to the parent, and 3 was more than the parent. The traits included plant stature, maturity, leaf nectaries, leaf gossypol gland numbers, stem gossypol gland numbers, leaf trichome numbers, stem trichome numbers and lint fiber.

Exactly 50 bolls (or as many as available, counting the exact number) were hand harvested from each progeny row to ensure a thorough representation of the fiber quality distribution. These samples were ginned in the laboratory using a 20-saw gin (DENNIS MFG. CO., INC., Texas), and about 50 gm of fiber was sent to the Fiber and Biopolymer Research Institute (FBRI) in Lubbock, Texas for High Volume Instrument (HVI) analysis of fiber properties. Data for harvested boll, and seed traits included average boll weight (grams), 1000 seed weight (grams), Lint % (lint weight/seed cotton weight x 100), 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).

Only about 5% of the 3200 lines were selected and evaluated further based on fiber properties. Two strategies were implemented to make the selection. First, ten lines with values in the upper extremes for each of the traits viz. lint percentage, fiber length, fiber strength, fiber elongation, length uniformity, and Rd value were selected, whereas ten lines with values in the lower extremes were selected for micronaire. As a second selection strategy, the field was stratified into plots consisting of 120 lines each and mean and standard deviation were calculated for each of these plots. Based on the observed statistical values, Z values were calculated for each of the lines for the concerned fiber traits. Lines were selected such that they show higher Z values than the rest for a particular fiber property. Stratification and Z-value based selection was adopted in order to mitigate the effects of micro environment. 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. In total, 22 lines for elongation, 22 lines for length, 17 lines for lint percent, 23 lines for Mic, 21 lines for Rd value, 19 lines for strength, 21 lines for uniformity and 26 overall better performing lines were selected. Many lines did repeat in the selection, outperforming for more than one trait, with the total of 171 selections only identifying 157 different lines.

In 2009, three replications of the selected lines were grown along with 55 randomly-selected control lines in both Watkinsville, Georgia and Lubbock, Texas in a completely randomized block design.

Planting was done on May 18th 2009 in Watkinsville, GA and May 20th 2009 at Lubbock, TX. A total of 35 seeds per row were planted. Weeding was done throughout the summer. Fertility and pest (weed and insect) management was maintained to maximize yield potential during the study.

Exactly 50 bolls were hand harvested for all three replications on October 19th 2009 at Lubbock and December 7th 2009 at Watkinsville, GA. The hand harvest of 50 boll samples served to determine lint % (lint/seed cotton X 100) or gin turnout (lint/burr cotton X 100) and the lint was used for HVI fiber analysis. Hand harvested cotton will have a higher genetic purity that machine harvested cotton (which will contain contamination from plot to plot). Because we harvested exactly 50 bolls we can also determine boll weight, seed per boll, or any other measure on a per boll scale. A John Deere cotton picker was used for harvesting, only harvesting seed cotton (lint+seed) so burrs do not confound estimates of total yield of the plots.

The hand harvested bolls were ginned using a 20-saw gin (DENNIS MFG. CO., INC., Texas), harvest scored boll and seed trait parameters were taken, and about 50 g of fiber was sent to the Fiber and Biopolymer Research Institute (FBRI) in Lubbock, Texas for High Volume Instrument (HVI) analysis of fiber properties.

Table 1 - Data collection summary

Generation	Lines	Replication	Location	Year
M4	3091	One replication	Texas	2007
M5	3168	One replication	Georgia	2008
M6	300	Three replications	Georgia	2009
M6	300	Three replications	Texas	2009
For lines tested in the M6 study, a total of 8 replication were available for data analysis, including 4 in Texas (1in 2007, 3 in 2009) and 4 in Georgia (1 in 2008, 3 in 2009)				

Statistical Analysis

Data were statistically analyzed using SAS software (SAS Institute Inc., SAS®9.2). Correlation was estimated using the command "PROC CORR". Most statistical analysis was done by command 'PROC ANOVA'. Means were separated using F tests and further by LSD tests at an alpha level of 0.05 or P value < 0.05. Graphs were developed using Sigma Plot.

Results

Ranges and means

Fiber fineness showed a range of 2.3 to 6.1 with the mean of 4.22 and SD of 0.5 for 2007, a range of -3.84 to +3.76 standard deviations from the mean value (Table 2). In 2008, the range was 3.3 to 5.8 with mean of 4.73 and SD of 0.37, or -3.86 to +2.89 standard deviations from the mean value (Table 2). In 2009, the range was 3.56 to 5.81 with mean of 4.77 and SD of 0.34, or -3.56 to +3.06 standard deviations from the mean value (Table 2).

Fiber length showed a range of 0.98 inch to 1.43 inch with a mean of 1.21 inch and SD of 0.06 for 2007, a range of -3.83 to +3.67 standard deviations from the mean value (Table 2). In 2008, range was between 0.96 inch to 1.37 inch with the mean of 1.16 inch and SD of 0.05, or -4 to +4.2 standard deviations from the mean value (Table 2). In 2009, the range was 0.93 inch to 1.36 inch mean of 1.17 inch with SD of 0.07, or -3.43 to +2.71 standard deviations from the mean value (Table 2).

Fiber uniformity showed a range of 74% to 91.9% with the mean of 83.75 and SD of 1.56 for 2007, a range of -6.25 to +5.22 standard deviations from the mean value (Table 2). In 2008, the range was 78.6% to 88.3% with the mean of 83.84 and SD of 1.24, or -4.23 to +3.6 standard

deviations from the mean value (Table 2). In 2009, the range was 79.4% to 88.2% with the mean of 83.93 and SD of 1.52, or -2.98 to +2.81 standard deviations from the mean value (Table 2).

Fiber strength showed a range of 21 to 39.6 gram/tex with the mean of 29.85 gram/tex and SD of 2.24 for 2007, a range of -3.95 to +4.35 standard deviations from the mean value (Table 2). In 2008, the range was 25 to 42.5 with the mean of 32.84 and SD of 2.63, or -2.98 to +3.67 standard deviations from the mean value (Table 2). In 2009, the range was 25.1 to 40.1 with the mean of 33.07 and SD of 2.42, or -3.29 to +2.9 standard deviations from the mean value (Table 2).

Fiber elongation showed a range of 3% to 10.2% with the mean of 5.57 and SD of 1.04 for 2007, a range of -2.47 to +4.45 standard deviations from the mean value (Table 2). In 2008, the range was 3.3% to 9.1% with the mean of 5.44 and SD of 0.89, or -2.4 to +4.11 standard deviations from the mean value (Table 2). In 2009, the range was 3.6% to 10.3% with the mean of 6.28 and SD of 1.12, or -2.39 to +3.59 standard deviations from the mean value (Table 2).

Fiber Rd value showed a range of 63.6% to 83.2% with the mean of 76.76 and SD of 2.3 for 2007, a range of -5.72 to +2.8 standard deviations from the mean value (Table 2). In 2008, the range was 66.6% to 82.7% with the mean of 75.27 and SD of 1.94, or -4.47 to +3.83 standard deviations from the mean value (Table 2). In 2009, the range was 71.3% to 83% with the mean of 78.32 and SD of 1.93, or -3.33 to +2.42 standard deviations from the mean value (Table 2).

In 2008, the range of lint percent was 27.59% to 67.46% with the mean of 39.22 and SD of 2.41, or -4.83 to +11.72 standard deviations from the mean value (Table 2). In 2009, the range was 31.47% to 54.67% with the mean of 39.32 and SD of 2.17, a range of -3.63 to +7.07 standard deviations from mean the value (Table 2).

In 2008, the range of 1000 seed weight was 82.6 g to 147 g with the mean of 111 g and SD of 9.75, or -2.91 to +3.69 standard deviations from the mean value (Table 2). In 2009, the range

was 94.73 to 138 with the mean of 115.3 and SD of 6.7, or -3.07 to +3.39 standard deviations away from the mean value (Table 2).

Seed cotton (yield) showed a range of 58.23 to 1504 with the mean of 735.04 and SD of 196.19 for 2009, a range of -3.45 to +3.92 standard deviations from the mean value (Table 2).

The range in fiber fineness, fiber strength, uniformity index, fiber elongation and fiber length was at least 6.62, 6.2, 5.79, 5.98 and 6.14 standard deviations respectively for any given year. In partial summary, across all measured traits we see a very wide range of values, much larger than could be explained by chance and presumably representing both + and - alleles present in the mutants.

Correlation between mutant generations

Correlation between mutant generations within traits varies from 0.046 to 0.86 (Table 3). All the correlations except those between Rd value of M4 vs. M5 and M4 vs. M6 were significant at $p<0.0001$ (Table 3). Correlation between M5 to M6 was greater than M4 to M6 for every fiber traits. This might suggest the mutational load has reduced and the lines are becoming more stable, or merely that there is a higher frequency of homozygosity.

Comparing different years and locations

Across the two locations in which lines were tested, the mean of Georgia data has significantly higher fiber fineness, fiber strength, fiber length, and uniformity index, whereas the mean of Texas data has significantly higher fiber elongation and Rd value (Table 4). Lint percent was similar for Georgia and Texas. This significant difference between locations suggests environmental effects on the quality of fiber. Texas is generally a much drier state than Georgia.

It has been suggested that many fiber traits are directly affected by water supply to plants at particular growth stages (Eaton and Ergle 1952; Bennett *et al.* 1967; Marani and Amirav 1971; Marani 1973; Paterson *et al.* 2003). Most means of fiber traits were also significantly different from each other across the three years of the study, except elongation, Rd value and Uniformity index (Table 4). For fiber strength, the ascending order of means was 2007, 2009 and 2008. For fiber length and fiber fineness, the ascending order of means was 2009, 2008 and 2007. This significant difference between years also suggests environmental effects on the quality of fiber.

Correlation between traits

Fiber fineness showed moderate to highly negative correlation (Tables 5.1, 5.2 and 5.3) to length (r=-0.19 in 2007, r=-0.49 in 2008 and r= -0.66 in 2009) and strength (r=-0.23 in 2007, r=-0.47 in 2008 and r=-027 in 2009), similar to another mutant population (Herring *et al.* 2004). Micronaire showed a negative correlation of r=-0.3 in 2009 with seed cotton, similar to a RIL population developed from an F2 population of an Upland cotton (*G. hirsutum L.*) cross 7235 X TM-1 (Shen *et al.* 2007) and contradicting the result obtain in a four-way cross population in *Gossypium hirsutum L.*(Qin *et al.* 2008). Micronaire is positively correlated with lint percent (r=0.4 in 2008 and r=0.45 in 2009). A positive correlation of r= 0.33 was seen between fiber fineness and lint yield in an M5 population of Paymaster HS 200 (Herring *et al.* 2004), and in other studies (Shappley *et al.* 1998; Wan *et al.* 2007; Qin *et al.* 2008).

Length shows positive correlation (Tables 5.1, 5.2 and 5.3) with strength (r=0.47 in 2007, r=0.58 in 2008 and r=0.51 in 2009), uniformity (r=0.28 in 2007, r=0.55 in 2008 and r=0.83 in 2009), seed cotton (r=0.45 for year 2009) and seed weight (r=0.3 in 2008 and r=0.21 in 2009). It shows negative correlation with elongation (r=-0.49 in 2007, r=-0.20 in 2008 and r=-0.60 in

2009) and lint percent (r=-0.38 in 2008 and r=-0.56 in 2009). It has been suggested in literature that length and strength are highly associated (Basal and Smith 1997; Kloth 1998; Herring *et al.* 2004; Wan *et al.* 2007) and this conclusion was supported by our study. Also, positive correlation between length and uniformity was confirmed as reported by others (Chee *et al.* 2005; Lacape *et al.* 2005). Our result shows a negative correlation of length to elongation which are similar to results in *G. hirsutum* germplasm (Shappley *et al.* 1998) but opposite to results from interspecific germplasm (Lacape *et al.* 2005). Additionally, negative correlation between length and lint percent was found in our results and *G. hirsutum* germplasm (Shappley *et al.* 1998), whereas another mutant population (Herring *et al.* 2004) showed no relationship. We found fiber length to be positively correlated to seed weight as shown for *G. hirsutum* germplasm (Shappley *et al.* 1998), whereas these traits were not correlated in an F2 population derived from interspecific hybrids between *G. hirsutum L.cv. Acala-44* and *G. barbadense L. cv. Pima S-7* (Mei *et al.* 2004).

Uniformity index shows positive correlation (Tables 5.1, 5.2 and 5.3) with strength (r=0.37 in 2007, r=0.54 in 2008 and r=0.51 in 2009) similar to BC1 and BC2 populations of *G. hirsutum* X *G. barbadense* (Lacape *et al.* 2005) and RILs originating from an upland cotton (Yumian 1 X T586) F2 population (Wan *et al.* 2007). Also we found positive correlation between Uniformity index and seed cotton (r=0.4 in 2009), which was not found in F2 population of an Upland cotton (*G. hirsutum L.*) cross 7235 X TM-1 (Shen *et al.* 2007). Uniformity index showed negative correlation with lint percent (r=-0.41 in 2008 and r=-0.51 in 2009) which is similar to the result of previous research (Shen *et al.* 2007; Qin *et al.* 2008).

A negative correlation is seen between fiber strength and +b value (r=-0.27 in 2008 and r=-0.19 in 2009) and lint percent (r=-0.48 in 2008 and r=-0.58 in 2009). Such negative correlation

between lint percent and strength has been shown by much research (Meredith 1994; Coyle and Smith 1997; Shen *et al.* 2007) but a positive correlation between lint percent and strength has been reported (Wan *et al.* 2007). We found a low to moderate positive correlation between fiber strength and seed weight (r=0.09 in 2008 and r=0.27 in 2009) which contradicts results from interspecific populations (Mei *et al.* 2004).

Seed cotton showed positive correlation to fiber yellowness (r=0.25) and negative correlation to elongation (r=-0.31) and Rd value (r=-0.11) in 2009 (Table 5.3). Elongation showed low negative correlation to Rd value (r=-0.09 in 2007, r=-0.13 in 2008 and r=-0.27 in 2009). Lint percent showed low positive correlation to +b value or fiber yellowness (r=0.28 in 2008 and r=0.17 in 2009), but low negative correlation to seed weight (r= -0.08 in 2008 and r=-0.29 in 2009).

Variation in the correlation between traits in different generations of the mutant population might be due to segregation of the genes which are responsible for the traits, or to differences in the environmental conditions between different years and location, which may change phenotypic correlations even though genetic correlations are still the same.

Comparison of selected lines to control lines

Strength

A total of 19 lines for fiber strength were selected based on having the high values and high Z scores in their microenvironments. The mean of lines selected for fiber strength was 35.64 g/tex, or about 10% higher than 32.58 g/tex of the control lines (Table 4). Lines 1793, 3023, 1919 and 3073 were particularly outstanding, with overall means across 8 replications of 37.1g/tex, 36.7g/tex, 36.7 g/tex and 36.6 gram/tex respectively, which is at least 14.48% higher than the

31.97 g/tex of the parental line (red lines in Figure 1). Each of these improvements was significant at $p < 0.0001$.

Fiber fineness

A total of 23 lines for micronaire were selected based on their low values and low Z scores in their microenvironments. The mean of lines selected for fiber fineness was 4.27 mic, which is 5.4% lower than the 4.78 mic of the control lines (Table 4). The overall mean of lines 2877, 3010, 3168, 2105 and 1903 for eight replications are 3.9 mic, 4.0 mic, 4.0 mic, 4.1 mic and 4.1 mic respectively which are at least 17.5% lower than 5.0 mic of the parental line (red lines in Figure 2). Each of these improvements was significant at $p<0.0001$.

Length

A total of 22 lines for length were selected based on their high values and high Z scores in their microenvironments. The mean of lines selected for fiber length was 1.24 inch, or about 6.9% higher than 1.16 inch of the control lines (Table 4). The overall mean of lines 1903, 3028, 2761 and 926 for eight replications are 1.3 inch, 1.27 inch, 1.26 inch and 1.26 inch respectively which is at least 10% higher than overall mean of the parental line (red lines in Figure 3). Each of these improvements was significant at $p< 0.0001$.

Fiber Elongation

A total of 22 lines for elongation were selected based on their high values and high Z scores in their microenvironments. The mean of lines selected for elongation was 7.31% or about 23% higher than the 5.31% of the control lines (Table 4). The overall mean of lines 2925, 2914, 2907 and 2958 for eight replications are 8.7%, 8.1%, 8.1% and 7.9% respectively, which is at least 45% higher than the overall mean of the parental line (red lines in Figure 4). Each of these improvements was significant at $p< 0.0001$.

Length uniformity index

A total of 21 lines for uniformity were selected based on their high values and high Z scores in their microenvironments. The mean of the selected lines was 84.93% which is significantly higher than the 83.77% mean of control lines (Table 4). The overall mean of lines 2455, 1767, 2466 and 1948 for eight replications are 85.9%, 85.9%, 85.8% and 85.8% respectively which is at least 3% higher than the average overall mean of the parental lines (red lines in Figure 5). Each of these improvements was significant at $p < 0.0001$.

Rd Value

A total of 21 lines for Rd value were selected based on their high values and high Z scores in their microenvironments. The mean of the selected lines was 78.95%, which is significantly higher than the 77.63% mean of control lines (Table 4). The overall mean of lines 1251, 2917 and 1237 for eight replications are 80.5%, 80.0% and 80.0% respectively which is at least 3.76% higher than the overall mean of the parental line (red lines in Figure 6). Each of these improvements was significant at $p < 0.0001$.

Lint percent

A total of 17 lines for lint percent were selected based on their high values and high Z scores in their microenvironments. The mean of the selected lines was 41.46%, which is significantly higher than the 39.85% mean of control lines (Table 4). The overall mean of lines 276, 77 and 383 for eight replications are 43.05%, 43.02% and 42.99% respectively, which is at least 5.24% higher than the overall mean of the parental line (red lines in Figure 7). Each of these improvements was significant at $p < 0.0001$.

Seed weight for 1000 seeds

Seed weight of the 300 selected lines ranges widely, from 94.73 g to 138 g. The mean of the parental lines was 117.9 g. Lines 1241, 696 and 697 had seed weight of 138 g, 136.91 g and 136.28 g respectively which is at least 15.59% higher than average seed weight of parental lines. Each of these improvements was significant at $p< 0.01$.

Seed cotton

Seed cotton yield had high variation within the 300 replicated lines. There were many selected lines which had much higher overall means than the parental lines. For example line 1251 selected for RD value (overall mean 969.58g), 2488 selected for lint percent (overall mean 921g), 1610 selected for fiber fineness (overall mean 918.5g), 790 selected for length uniformity index (overall mean 874.5g), 1097 selected for fiber strength (overall mean 869.81g), 1053 for elongation (overall mean 846.39g) and 1965 (833.56g) for fiber length, all had significantly higher yield than the 735.4g mean of the parental line. While much of the variation may be due to small plot size, it is also possible that some lines selected for different fiber qualities may help to increase fiber quantity.

Comparison of overall best lines and parental lines

A total of 26 overall better performing lines were selected by adding all the Z scores designated based on micro environment. The means of fiber elongation, fiber strength, and Rd value for the selected lines were significantly better than parental lines at $p < 0.005$ (Table 6). Mean fiber fineness for selected lines was significantly better than parental lines at $p< 0.0001$ (Table 6). There was no significant difference in lint percent, fiber length and length uniformity

index (Table 6), although a few individuals showed improvement of these fiber properties relative to the parental line (Table 7).

Discussion

An extensive range of values for fiber fineness, fiber strength, uniformity index, fiber elongation and fiber length of at least 6.62, 6.2, 5.79, 5.98 and 6.14 standard deviations respectively was seen for any given year. The range of values are consistently very large, much larger than could be explained by chance and presumably represent both + and - alleles present in the mutants. Our replicated studies lend support to a genetic basis for these differences. One might further explore whether these alleles are at new 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 identification of some markers for TAM 94L25 and its nearby inbred lines (Maleia *et al.* 2010).

Additionally, there were lines which showed significant improvements in fiber quality traits over the parental line for fiber length, fiber strength, fiber elongation, fiber fineness, lint percentage, and Rd value. Identified lines could be used as parental lines for further breeding programs. Further, the mutant lines showed an extensive range of seed weight (spanning 94.73 grams to 138 grams). The seeds can be further tested for oil content, and promising lines could be used for improving oil seed cotton. A significant negative correlation is seen between lint percent and seed weight, but there is no correlation seen between seed cotton and seed weight. A further in-depth study on seed weight, seed size, and number of fibers on seed surface might help to find mutant lines with appropriate seed size and high lint yield, which could ultimately increase the profit margin of the farmers.

There was substantial improvement in the mutant lines over TAM 94L25 in the replicated trials. As an illustration, the elongation of line 2925 showed an improvement of 59.6% over TAM 94L25, the parental line. Similarly, fiber fineness of line 2877 showed an improvement of 21.5% over TAM 94L25. Likewise, fiber strength of line 1793 showed an improvement of 16% and fiber length of line 1903 showed an improvement of 13.8%. Also, lint percent of line 276 showed an improvement of 5.39% over the parental line. Similarly, Rd value of line 1251 showed an improvement of 4.4% over TAM 94L25. Likewise, uniformity of line 2455 showed an improvement of 3.1% over the parental line. This indicates that genetic improvements for a wide range of fiber traits can be obtained from mutagenesis of elite cottons. Appreciable amount of improvement was observed in fiber elongation and fiber fineness as compared to the other traits. One of the reasons could be that fiber elongation and fiber fineness have comparatively shorter history of selection in breeding programs. As such, analysis and utilization of allelic variation for fiber elongation and fiber fineness has not been explored. Thus, any alteration in gene/s involved in fiber elongation and fiber fineness is likely to generate greater positive effects compared to similar alteration in gene/s for other fiber traits. The amount of variation introduced by chemical mutagenesis in these two traits is suggestive of the fact.

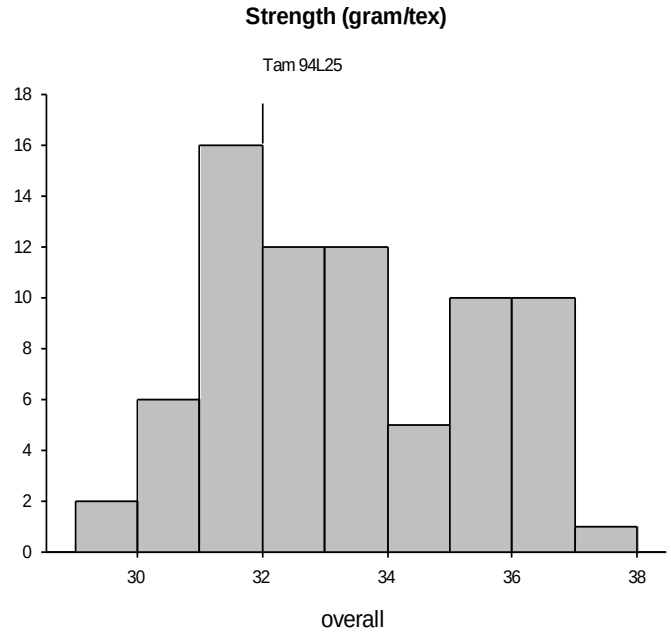
A line selected for one fiber trait sometimes conferred other attributes. For example, line 1903 selected for fiber fineness and for fiber length, had 17.18% higher fiber fineness, 13.43% higher fiber length, 2.18% higher uniformity, 13.74% higher fiber strength, than the average of the parental line, all significantly higher at $p<0.0001$. Another line 3010, selected for fiber fineness had 19.44% higher fiber fineness, 11.79% higher fiber length, 2.64% higher uniformity, 11.94% higher fiber strength, than the average of the parental line, all significantly higher at $p<0.0001$.

TTU 202-1107-B and TTU 271-2155-C mutant lines developed from Paymaster HS 200 have been registered (Auld 2000). These lines had 8 to 9% longer fiber length and 5% higher fiber strength than Paymaster HS 200 but similar fiber fineness and uniformity (Auld 2000). The selected lines evaluated by us were much better than the parental lines. Research had shown that 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). Intercrossing among our mutant lines (1903 and 3010), or perhaps crosses of a mutant as one parent and an elite line as the other parent, may introduce novel alleles into the cotton gene pool that confer commercially important improvements.

Conclusion

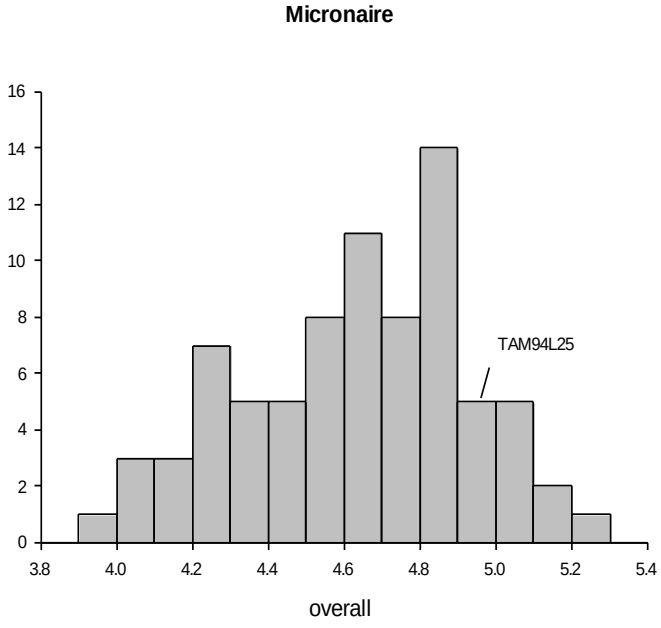
Much more variation than could be explained by non genetic factors was observed in fiber elongation, fiber strength, fiber fineness, fiber elongation and fiber length. Lesser but still useful variation was also seen in fiber uniformity and Rd value. Molecular study of such mutant populations might help to reveal new QTLs for fiber quality or perhaps contribute to finding genes involved in the complex pathway of fiber development.

Figures



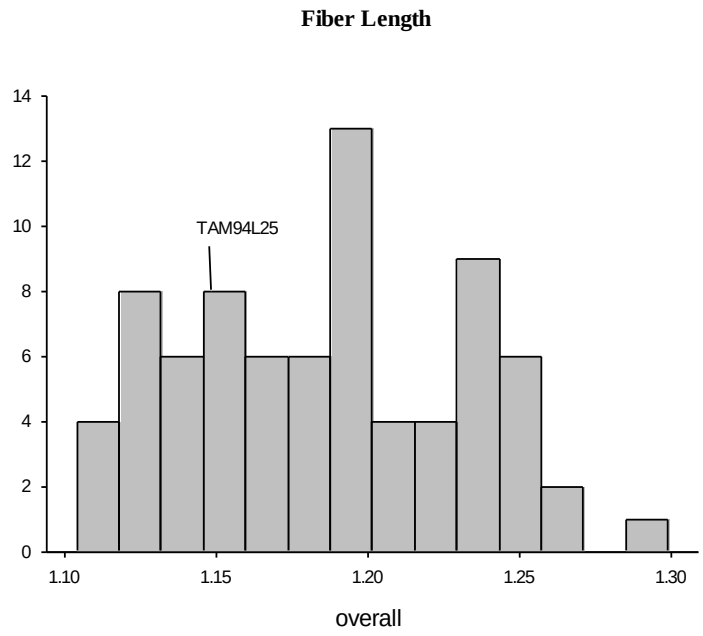
Number	Overall	GA_09	TX_09	2009
1793	37.1	37.2	37.1	37.2
1504	37.0	35.7	38.4	37.1
3023	36.7	35.2	37.3	36.2
1919	36.7	36.3	38.0	37.2
3073	36.6	35.1	36.4	35.8
3251	33.0	33.9	32.9	33.4
3249	32.0	32.9	31.3	32.1
3250	32.0	33.1	31.2	32.2
3246	31.8	32.6	31.1	31.9
3247	31.7	32.7	30.4	31.6
3248	31.3	31.4	30.7	31.1
956	30.6	30.9	30.9	30.9
526	30.6	31.5	29.4	30.5
915	30.4	30.4	31.0	30.7
3170	29.9	30.5	29.7	30.1
84	29.5	30.3	30.6	30.4

Figure 1 -Distribution of lines for fiber strength



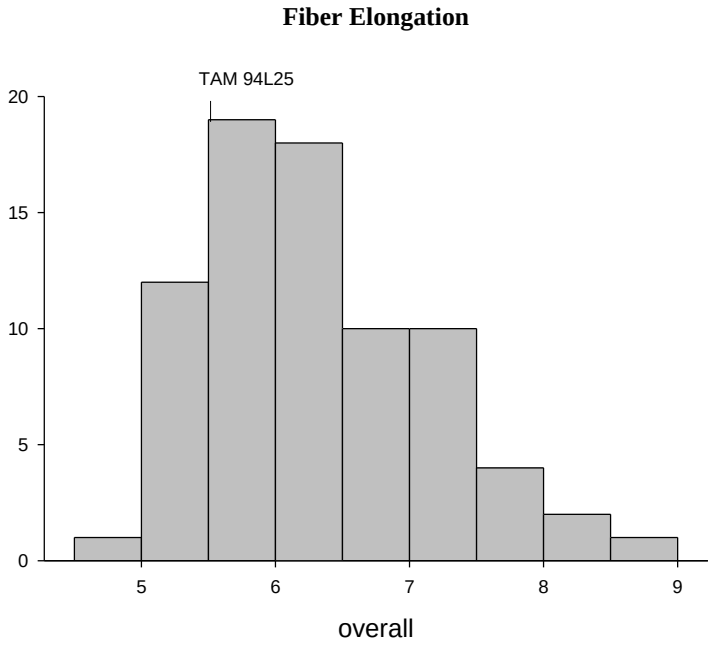
Number	Overall	GA_09	TX_09	2009
2877	3.9	4.3	4.2	4.3
3010	4.0	4.1	4.4	4.3
3168	4.0	4.0	4.3	4.2
2105	4.1	3.9	4.6	4.3
1903	4.1	4.2	4.2	4.2
3250	4.9	4.8	4.9	4.9
3251	4.9	4.8	5.1	4.9
3247	5.0	5.0	5.0	5.0
3248	5.0	4.9	5.0	5.0
3246	5.0	4.8	5.2	5.0
3249	5.0	5.1	5.1	5.1
187	5.1	4.7	5.7	5.2
3170	5.2	5.0	5.4	5.2
2985	5.2	5.1	5.1	5.1
84	5.3	5.1	5.4	5.2

Figure 2 - Distribution of lines for fiber fineness



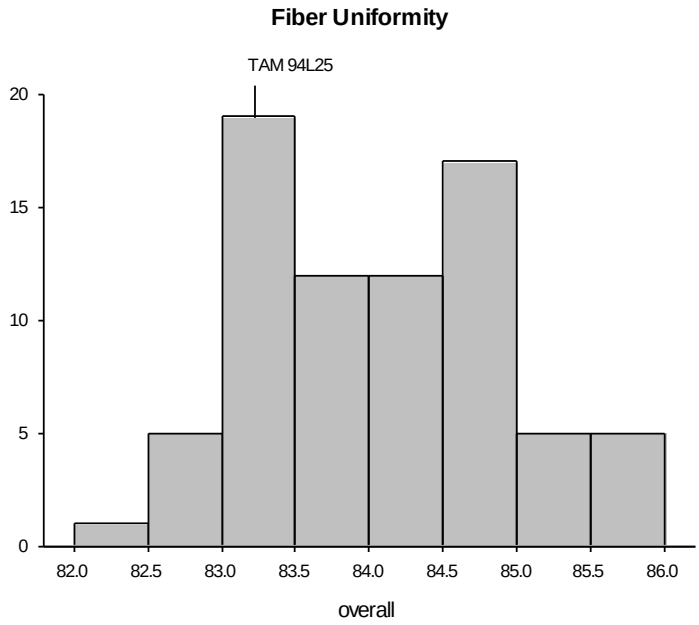
Number	Overall	Ga_09	TX_09	2009
279	1.10	1.15	1.08	1.12
84	1.11	1.15	1.07	1.11
526	1.11	1.14	1.08	1.11
748	1.11	1.15	1.06	1.11
3246	1.13	1.17	1.07	1.12
3247	1.13	1.17	1.08	1.12
3249	1.14	1.19	1.10	1.15
3250	1.14	1.21	1.08	1.15
3248	1.16	1.21	1.11	1.16
3251	1.17	1.24	1.11	1.18
926	1.26	1.30	1.17	1.24
2761	1.26	1.31	1.20	1.25
3028	1.27	1.29	1.21	1.25
1903	1.30	1.32	1.24	1.28

Figure 3 - Distribution of lines for fiber length



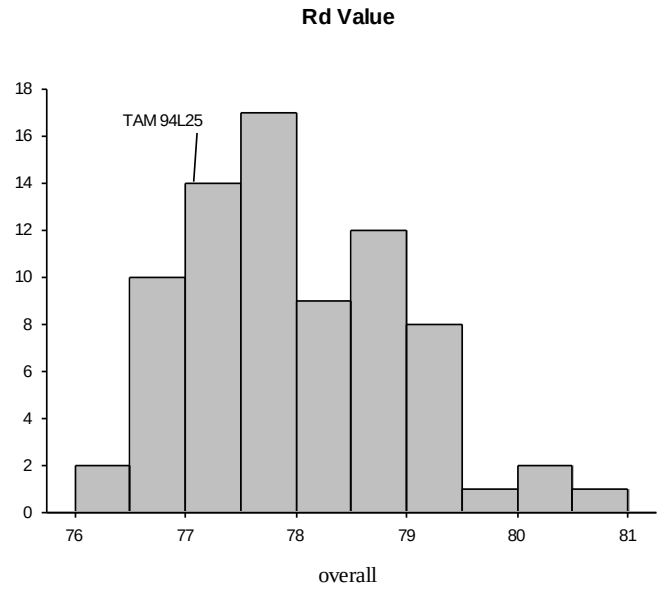
Number	Overall	GA_09	TX_09	2009
294	4.8	4.2	5.8	5.0
880	5.1	4.7	6.1	5.4
3247	5.2	4.7	6.0	5.4
3248	5.4	4.9	6.2	5.6
3249	5.4	4.9	6.3	5.6
3251	5.5	4.6	6.4	5.5
3246	5.5	4.8	6.5	5.7
3250	5.7	4.8	6.9	5.8
410	7.7	6.7	8.4	7.6
923	7.8	6.7	8.3	7.5
2958	7.9	6.8	8.4	7.6
2907	8.1	7.0	8.5	7.7
2914	8.1	7.6	8.2	7.9
2925	8.7	7.4	9.8	8.6

Figure 4 - Distribution of lines for fiber elongation



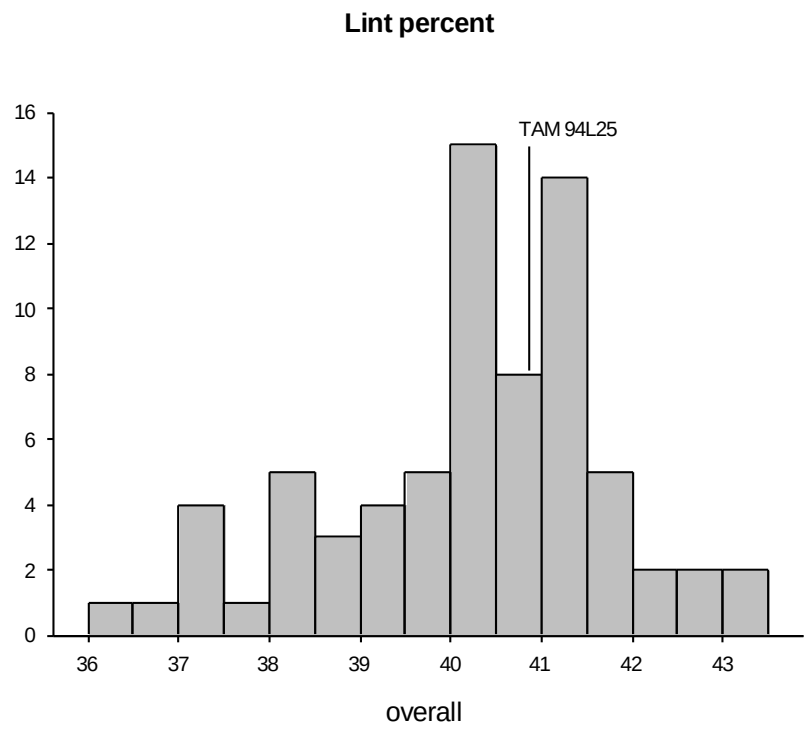
Number	Overall	GA_09	TX_09	2009
1419	82.5	83.3	82.0	82.7
956	82.5	82.7	82.1	82.4
3246	83.0	83.8	81.6	82.7
3250	83.2	84.4	82.1	83.2
3249	83.3	84.2	82.4	83.3
3248	83.4	84.5	82.2	83.4
3247	83.4	84.8	81.8	83.3
3251	83.5	84.2	82.8	83.5
1948	85.8	86.4	84.2	85.3
2466	85.8	86.7	84.4	85.5
1767	85.9	86.3	85.1	85.7
2455	85.9	86.0	85.1	85.6

Figure 5 - Distribution of lines for uniformity



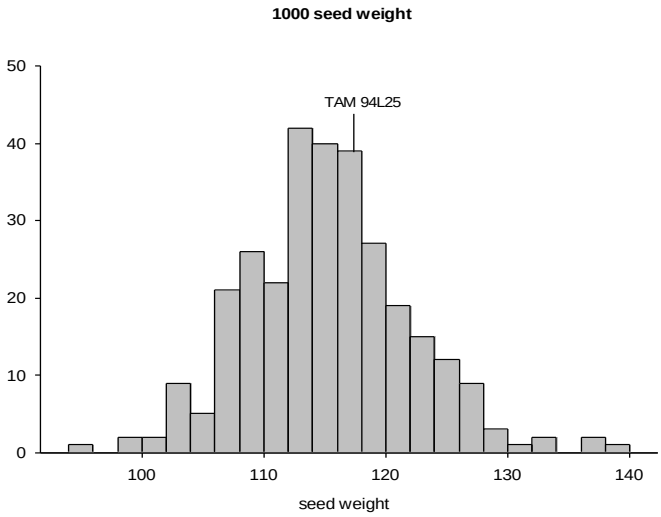
Number	Overall	GA_09	TX_09	2009
3249	76.5	75.7	79.4	77.6
3247	76.5	74.8	78.6	76.7
3250	76.9	75.1	79.4	77.3
3251	77.2	76.6	78.9	77.8
3246	77.4	76.3	79.6	78.0
3248	78.1	77.3	79.7	78.5
1237	80.0	78.6	81.5	80.1
2917	80.0	79.0	80.8	79.9
1251	80.5	79.2	82.2	80.7

Figure 6 - Distribution of lines for fiber Rd value



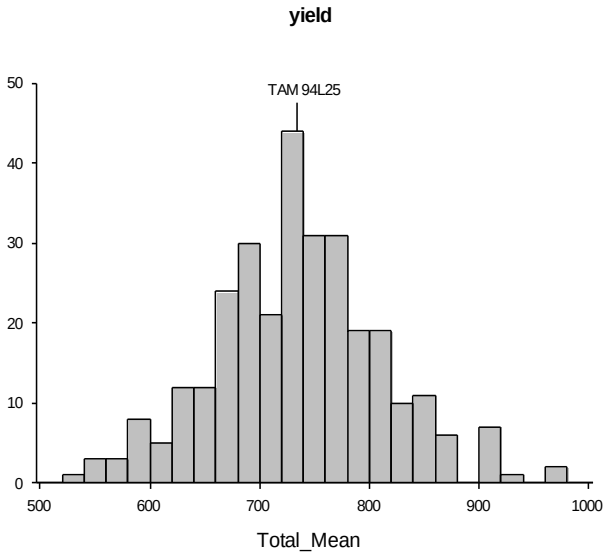
Number	Overall	GA_09	TX_09	2009
2535	36.31	35.81	37.31	36.56
1504	36.82	36.97	37.56	37.27
2191	37.02	37.03	37.82	37.43
3251	40.14	38.66	41.26	39.96
3246	40.74	40	41.12	40.56
3247	40.76	39.73	41.56	40.65
3250	40.98	39.9	41.84	40.87
3248	41.01	41.42	40.6	41.01
3249	41.49	41.09	42.01	41.55
383	42.99	42.53	42.97	42.75
77	43.02	42.57	42.87	42.72
276	43.05	42.52	42.98	42.75

Figure 7 - Distribution of lines for Lint percent



Number	1000 Seed Wt
2143	94.73
1288	98.73
1161	99.44
492	100.60
3069	101.30
3248	113.13
3247	114.60
3249	115.67
3246	118.32
3250	121.53
3251	124.20
880	131.13
2345	132.33
769	133.00
697	136.28
696	136.91
1241	138.00

Figure 8 - Distribution of lines for 1000 seed weight



sample	TOTAL MEAN	TOTAL stdev	Tx Mean	Tx Stdev	GA Mean	GA stdev
3247	651.24	138.37	649.53	125.28	652.96	162.35
3248	711.87	83.92	672.37	97.23	751.37	39.86
3250	742.76	155.70	709.28	151.21	776.23	155.00
3249	754.41	161.74	732.19	177.59	776.63	146.94
3251	761.61	259.55	665.75	42.20	857.47	329.64
3246	790.48	193.37	696.12	131.65	884.83	205.98
790	874.52	294.63	733.91	94.98	1015.13	415.52
383	900.29	237.49	727.85	132.33	1072.73	129.59
294	900.99	261.66	741.24	125.68	1060.73	211.24
1227	904.22	289.30	663.50	39.36	1144.93	132.17
2030	910.80	251.73	714.00	202.31	1107.60	71.47
331	917.01	364.75	717.75	182.61	1116.27	337.96
1610	918.50	334.91	688.24	213.41	1148.77	158.82
2488	921.12	320.18	736.74	119.25	1105.50	302.81
182	963.27	349.02	696.40	220.68	1230.13	269.45
1251	969.58	330.10	794.13	165.01	1145.03	336.74

Figure 9 - Distribution of lines for Seed cotton

Tables:

Table 2- Range, mean and standard deviation for traits by year

	2007		2008		2009	
Variable	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**
LINT_%	N.A	N.A	39.22(2.41)	27.6-67.5	39.3(2.17)	31.5-54.7
YIELD	N.A	N.A	N.A	N.A	735.0(196.19)	58.23-1504
MIC	4.22(0.5)	2.3-6.1	4.73(0.37)	3.3-5.8	4.77(0.34)	3.6-5.8
LENGTH	1.21(0.06)	0.98-1.43	1.16(0.05)	0.96-1.37	1.17(0.07)	0.93-1.36
UNIF	83.75(1.56)	74-91.9	83.84(1.24)	78.6-88.3	83.93(1.52)	79.4-88.2
STRENGTH	29.85(2.24)	21-39.6	32.84(2.63)	25-42.5	33.07(2.42)	25.1-40.1
ELON	5.57(1.04)	3-10.2	5.44(0.89)	3.3-9.1	6.28(1.12)	3.6-10.3
Rd	76.76(2.3)	63.6-83.2	75.27(1.94)	66.6-82.7	78.32(1.93)	71.9-83
1000 SEED WT	N.A	N.A	111(9.75)	82.6-147	115.3(6.7)	94.73-138

Number of lines for 2007 = 3091, 2008 = 3168 and 2009 = 1800

Table 3 - Trait correlations between generations using Pearson Correlation Coefficient

Generation	Mic	Length	Uniformity	Strength	Elongation	Rd value	Lint_%
M4 vs. M5	0.23*	0.59*	0.32*	0.43*	0.67*	0.046	N.A
M4 vs. M6	0.40*	0.69*	0.48*	0.53*	0.72*	0.072	N.A
M5 vs. M6	0.77*	0.86*	0.62*	0.84*	0.75*	0.38*	0.64*

Traits correlated at p < 0.0001 are having '*'

Table 4 - F test for mean comparison followed by LSD for pair wise comparison

	Selected	Control	Significance	GA	TX	Significance	2007	2008	2009	Significance
Fiber strength	35.64	32.58	***	33.72	33.02	*	30.85	34.77	33.51	***
Fiber length	1.24	1.16	***	1.21	1.16	***	1.24	1.19	1.18	***
Fiber fineness	4.27	4.78	***	4.6	4.7	*	4.1	4.48	4.73	***
Fiber elongation	7.31	5.9	***	5.73	6.91	***	5.97†	6.09†	6.4	***
Uniformity	84.93	83.77	***	84.83	83.33	***	84.37†	84.81†	83.93	***
Rd Value	78.95	77.63	***	76.85	79.3	***	77.1†	76.73†	78.27	***
Lint Percent	41.46	39.85	***	40.44	40.07		N.A	41.28	40.05	***

Traits means having * and * in significance cell are having significant mean difference at p < 0.0001 and at p < 0.005 respectively. For year means marked with '†' are not significantly different to another.**

Correlation between traits using Pearson's Correlation Coefficient

Traits and their abbreviation**:** LEN= fiber length, ELON= fiber elongation, Rd= reflectance value, STR= strength, UNIF= length uniformity index, lint %= lint percent, Mic= fiber fineness (micronaire)

Table 5.1-Year 2007

	Mic	LEN	UNIF	STR	ELONG	Rd
Mic	1					
LEN	-0.19*	1				
UNIF	0.28*	0.27*	1			
STR	-0.23*	0.47*	0.37*	1		
ELON	0.04	-0.49*	0.07	-0.28*	1	
Rd	-0.12*	0.16*	-0.02	0.07	-0.09*	1
B	-0.01	0.04	0.01	0.0	-0.14*	0.06

Traits correlated at p < 0.0001 are having '*'. Number of lines = 3091

Table 5.2-Year 2008

	Mic	LEN	UNIF	STR	ELON	Rd	B	lint%	seed_wt
Mic	1								
LEN	-0.49*	1							
UNIF	-0.31*	0.55*	1						
STR	-0.47*	0.58*	0.54*	1					
ELON	-0.20*	-0.20*	0.13*	-0.05	1				
Rd	-0.32*	0.24*	0.17*	0.15*	0.18*	1			
B	0.16*	-0.14*	-0.16*	-0.27*	-0.13*	-0.1*	1		
lint%	0.4*	-0.38*	-0.41*	-0.48*	-0.23*	-0.16*	0.28*	1	
seed_wt	0.13*	0.3*	0.16*	0.09*	-0.29*	0.035	0.1*	-0.08*	1

Traits correlated at p < 0.0001 are having '*'. Number of lines = 3168

Table 5.3 -Year 2009

	MIC	LEN	UNIF	STR	ELON	Rd	b	lint%	seed_wt	S.C
MIC	1									
LEN	-0.66*	1								
UNIF	-0.46*	0.83*	1							
STR	-0.27*	0.51*	0.51*	1						
ELON	0.34*	-0.6*	-0.45*	-0.02	1					
Rd	0.13*	-0.38*	-0.34*	0.05	0.55*	1				
B	0.02	0.09*	0.06	-0.19*	-0.27*	-0.31*	1			
lint%	0.45*	-0.56*	-0.51*	-0.58*	0.16*	0.08	0.17*	1		
seed_wt	-0.1*	0.21*	0.1*	0.27*	-0.05	0.013	0	-0.29*	1	
S.C	-0.30*	0.45*	0.40*	0.04	-0.31*	-0.11*	0.25*	-0.06	0	1

Traits correlated at p < 0.0001 are having '*'. Number of lines = 1800

Table 6 - F test for mean comparison followed by LSD for pair wise comparison for overall best lines

	Selected	Control	Significance	GA	TX	Significance	2007	2008	2009	Significance
Fiber strength	32.87	31.96	*	33.08	32.32	*	30.33	32.64†	33.02†	***
Fiber length	1.17	1.15		1.19	1.13	***	1.21	1.15†	1.16†	***
Fiber fineness	4.71	4.98	***	4.72	4.79		4.16	4.73†	4.84†	***
Fiber elongation	6.04	5.46	*	5.26	6.68	***	5.36†	5.38†	6.11	***
Uniformity	83.94	83.31		84.59	83.01	***	83.78	83.94	83.82	
Rd Value	78.95	77.63	*	76.49	79.60	***	78.35†	75.53	78.23†	***
Lint Percent	40.19	40.85		40.26	40.39		N.A	42.42	39.96	***

Traits means having * and * in significance cell are having significant mean difference at p < 0.0001 and at p < 0.005 respectively. For year means marked with '†' are not significantly different to another.**

Table 7- Few overall best lines compared with the parental lines

Number	Lint %	Yield	MIC	LEN	UNIF.	STR	ELON.	Rd	+b
2243	40.86	696.86	4.58	1.22	84.81	32.99	6.46	78.64	7.96
2384	39.44	690.08	4.64	1.19	85.14	34.36	6.69	77.88	7.87
1787	36.03	694.35	4.28	1.21	85.35	36.33	5.71	78.21	7.79
2030	41.08	910.80	4.64	1.19	84.34	34.20	5.79	78.97	7.90
2091	38.78	639.64	4.54	1.20	84.74	35.33	5.33	78.33	8.16
1571	40.14	766.72	4.70	1.23	84.98	35.94	6.05	76.46	7.60
1842	39.49	728.25	4.61	1.20	84.73	35.29	6.04	77.07	8.01
2058	40.22	723.01	4.40	1.20	83.50	33.10	5.71	78.90	8.07
3246	40.74	790.48	5.03	1.13	83.01	31.84	5.51	77.41	8.27
3247	40.75	651.24	4.99	1.13	83.40	31.67	5.20	76.54	8.46
3248	41.00	711.87	5.01	1.16	83.36	31.29	5.40	78.13	8.09
3249	41.48	754.41	5.03	1.14	83.33	32.03	5.43	76.46	8.30
3250	40.98	742.76	4.89	1.14	83.24	31.97	5.73	76.87	8.23
3251	40.14	761.61	4.95	1.17	83.51	32.97	5.49	77.16	8.30

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Chapter 3

IMPACT OF TRICHOME MUTATIONS ON COTTON FIBER QUALITY

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Abstract

A total of 3,200 M5 mutant lines developed by Dr. Dick Auld from *G. hirsutum* breeding line 94L25 were characterized in 2008, for 20 phenotypic traits describing basic plant growth and development, including fiber quality analysis. A subset of 106 lines with leaf and stem trichome variations and 55 control lines were further studied for investigating association between trichome variation and lint fiber development.

Means for micronaire, lint percent and fiber strength were significantly higher for the parental line than mutant lines with lower stem trichome number. Means for fiber elongation value were significantly higher for lines having lower stem trichome number than the parental line. Mean for Rd value differed significantly between lines with lower and higher stem trichome numbers than the parent. For leaf trichome variation, means for micronaire and Rd value were significantly higher in the parental line than in lines having higher leaf trichome number. In summary, mutants in leaf and /or stem trichome development are often found to have altered lint fiber characteristics, suggesting common genetic factors acting in the development of these similar organs.

Introduction

Cotton, also revered as white gold, is the world's leading naturally derived textile fiber. Historically, cultivated cotton has been extensively studied for its evolution and domestication which led to an allopolyploid species exploited for its spinnable seed fibers (Wendel *et al.* 2010). Cotton is a highly profitable cash crop in two of the biggest cotton producing nations, viz. India and China, which account for almost 50% of the world’s supply. It is also a leading cash crop in the U.S, where 18.4 million bales of cotton were produced from around 11 million acres planted

in 2010 (National Cotton Council 2011). A large percentage (~40%) of raw cotton produced in the U.S. is exported, generating almost $ 3 billion and helping to curtail trade deficits.

At anthesis, about 15 – 25% of epidermal layer cells start differentiating and developing into lint fibers which can be spun for commercial purposes (Basra and Malik 1984; Tiwari and Wilkins 1995; Kim and Triplett 2001). The remaining epidermal cells may not differentiate into fiber or may just form small "fuzz" fibers. The length of cotton fiber can reach up to 2.36 inches, making it one of the most exaggerated plant cell types (Kim and Triplett 2001; Lee et al. 2007). Cotton fiber development undergoes four distinct yet overlapping stages viz. fiber cell initiation, elongation, secondary wall biosynthesis, and maturation (Basra and Malik 1984). Much information regarding the timetable and expression patterns of candidate genes and/or proteins associated with cotton fiber initiation, elongation, and maturation is available (Turley and Ferguson 1996; Applequist et al. 2001; Wang et al. 2001; Ji et al. 2003; Arpat et al. 2004; Wilkins and Arpat 2005; Lee et al. 2006; Wu et al. 2006 ; Lee et al. 2007).

Trichomes are fine outgrowths or appendages of the epidermal layer, mostly present in aerial parts and on roots as root hairs of the flowering plant. Trichomes can be unicellular or multicellular with or without glands (secretory organs) (Esau 2006). As indicated by its name, *Gossypium hirsutum L*. has coarse pubescence on the leaves and stems of mature plants. Trichomes in cotton are associated with advantages including increased tolerance of drought (Espigares and Peco 1995) and reduced attack of leaf hopper (Jenkins and Wilson 1996; Bourland *et al.* 2003), but also disadvantages including promotion of egg-laying by *Heliothis spp.* (Benedict *et al.* 1983; Treacy *et al.* 1986; Hassan *et al.* 1990), and increased attack of silverleaf white fly (Chu *et al.* 2000) , increased leaf trash in ginned cotton, and reduced fiber quality (Wanjura *et al.* 1976; Meredith *et al.* 1996). Different alleles responsible for variation of

leaf and stem pubescence have been found, using chromosome cytological stocks (Endrizzi *et al.* 1984). Five different loci, t_1-t_5, were reported as having large impacts on leaf and stem trichome size or number (Lee 1985). Genetic mapping has clearly placed t_1 on chromosome 6 and t_2 on chromosome 25 (Wright *et al.* 1999; Lacape and Nguyen 2005). Eight trichome-related QTLs have been suggested in meta-analysis of polyploid cotton QTLs (Rong *et al.* 2007). Associations of the t_1 trichome locus with fiber quality have been shown in many studies (Simpson 1947; Knight 1952; Kloth 1995; Rong *et al.* 2005; Desai *et al.* 2008). Two QTLs for lint percent, which increased trait phenotypic value, were at the t_1 locus in the F_2 population of TM1×T586 (Guo et al. 2006). Nonenvironment-specific QTLs for lint percent, fiber length, fiber length uniformity, and fiber strength were identified within 5 cM of the t_1 locus, suggesting that the t_1 might be a candidate gene for the QTLs (Wan et al. 2007). A linkage map based on 270 $F_{2:7}$ recombinant inbred lines derived from an upland cotton cross between T586 X Yumian 1 indicates two QTLs, *FL1* and *FU1* near the t_1 locus, further supporting the Wan *et al.* (2007) hypothesis of a role of t_1 in fiber development (Zhang et al. 2009). In genetic mapping and comparative analysis of seven fiber mutants, the association was extended to *sma-4(fbl)*, *Sus*, and perhaps t_2, also raising a question about whether the genes responsible for these traits might have similar functions due to close positional association (Rong *et al.* 2005). Desai *et al.* (2008) found the leaf pubescence mutation *glabrous* to co-segregate with *sma-4(ha),* which suggested that glabrous and fiberless mutants may be influenced by gene/genes governing both trichome and fiber development (Desai *et al.* 2008). Therefore, it is imperative to further explore the genetics of simply-inherited traits such as trichomes in cotton or other botanical models such as Arabidopsis, which could be helpful to increase our understanding of lint fiber development.

Growth and development of trichomes in *Arabidopsis* has been deeply studied (Marks 1997; Schwab *et al.* 2000; Larkin *et al.* 2003; Schiefelbein 2003; Hulskamp 2004; Wang *et al.* 2007; Kryvych *et al.* 2008; Marks *et al.* 2009; Morohashi and Grotewold 2009; Uhrig and Hülskamp 2010). The leaf trichomes in cotton and *Arabidopsis* are morphologically the same, unicellular and branched, whereas lint fibers are unbranched and extremely elongated. Despite these morphological differences, trichomes and lint fibers might share similar developmental pathways (Hulskamp *et al.* 1994; Kim and Triplett 2001; Arpat *et al.* 2004; Rong et al. 2005; Wan *et al.* 2007; Desai *et al.* 2008). The probe Gate-4CE05 obtained from a cDNA library of 7-10 days postanthesis *G. arboreum* fiber and mapped to chromosome 6 of *G. hirsutum* showed significant homology with the Glabra1 (*GL1*) gene in *Arabidopsis* (Desai et al. 2008). Mutations in *GL1* can inhibit the production of trichomes in *Arabidopsis* (Larkin *et al.* 1994; Hauser *et al.* 2001; Karkkainen and Agren 2002). It has been shown that *GaMYB2*, a cotton MYB transcription factor that is highly expressed in developing lint fiber, can restore trichome production in *Arabidopsis gl1* mutants and also induces trichomes on *Arabidopsis* seed (Wang *et al.* 2004). This indicates potential similarity between the genetic regulation of *Arabidopsis* trichomes and cotton lint fiber. There is predominant expression of MYB genes in developing cotton fiber and trichomes (Loguercio et al. 1999; Cedroni *et al.* 2003; Suo et al. 2003; Lee *et al.* 2007; Desai *et al.* 2008; Machado *et al.* 2009; Zhang *et al.* 2010). *GhMYB109*, which is closely related to *GL1*, has been found to be specifically expressed in the cotton fiber initials and elongating fibers, indicating that it might have a role in fiber initiation and elongation (Suo *et al.* 2003). *GhMYB 25* was identified as differentially expressed between fiberless mutants and cotton having normal lint, and was more highly expressed in fiber initials than in adjacent epidermal cells (Lee *et al.* 2006; Wu *et al.* 2006). Reduction in the expression of *GhMYB25*, a low copy MYB transcription

factor, caused condensed leaf trichome number and fiber growth that suggest a role of MYB genes in trichome and fiber development (Machado *et al*. 2009). A HD-Zip IV family transcription factor, *GbML1* when over expressed in *Arabidopsis* have increased the number of trichomes on stems and leaves. *GbML1* was identified as the first partner for *GbMYB25*, which is a key regulator of cotton fiber development (Zhang *et al.* 2010).

Chemical mutagenesis such as with ethyl methane sulfonate (EMS) mostly causes single base pair changes which can cause a partial to complete loss of gene or modify the function of gene. Mutagenesis may be a good way to test whether disruption function of a gene affects trichomes separately from lint fiber development, or affects both at once. Chemical mutagens are more favored since single nucleotide changes can bring about mutations that are stable while mutations from physical mutagens frequently have negative if not lethal results (Parry *et al.* 2009). Ethyl methanesulfonate (EMS) is frequently favored as a chemical mutagen which can be used for both forward and reverse genetic studies (Kim *et al.* 2006). It is more efficient and effective than irradition due to a greater number of mutations as well as higher survival rates of the mutants than the irradiation method (Favret 1960). EMS mutagenesis is based on mispairing and base changes due to induced chemical modification of nucleotides from biased alkylation of guanine (G) residues to form O6 -ethylguanine, which can pair with thymine (T) but not with cytosine (C). Subsequent DNA repair changes the nucleotide composition, resulting in A/T (adenine/thymine) instead of the original C/G at the double stranded position (Greene *et al.* 2003).

Here we tried to address a question asked for decades, whether genes responsible for trichome variation also affect lint fiber development. By evaluating 3200 lines develop through chemical mutagenesis, we identified a substantial collection of stem trichome mutants, and a smaller

number of leaf trichome mutants. By evaluating fiber characteristics of these lines in replicated field trials, we tested the hypothesis that perturbation of trichome development also alters lint fiber development.

Materials and Methods

Source of Mutation lines

Dr. Auld developed a mutant population of TAM94L25 (Smith 2003) by treating with the chemical mutagen EMS to their LD_{50}, using published techniques (Auld *et al.* 1992; Auld *et al.* 1998). About 3200 lines were generated and advanced by single boll descent to M5. A single boll descent risks duplicating a few mutants as a boll contains 20 to 30 seeds, but it is often a practical necessity in cotton.

In 2007, M4 population was grown in Lubbock, Texas (latitude 33N 34' 40.31" and 101W 51' 18.60'' longitude) and in 2008, the M5 population was grown in Watkinsville, Georgia (latitude 33N 51' 46.425" and 83W 24' 31.5756" longitude) with a completely randomized design (CRD). The soil type of the field near Watkinsville, GA is Appling Coarse Sandy Loam, and that of Lubbock, TX is Amarillo Fine Sandy Loam and Pullman Clay Loam. The sowing date in Texas was May 17, 2007, and Georgia was May 21, 2008. Seeds for each line were sown in 3 meter rows, spaced one meter apart. Thinning and weeding was done as necessary. A total of 74.45-74.45-74.45 kg/ha of NPK was applied as fertilizer and before sowing. Herbicide and pesticides were applied as required.

Eight morphological traits were examined, using a scoring scale from 0 to 3 where 0 is absence, 1 is present but less then parental type, 2 was similar to the parent, and 3 was more than the parent. The traits included plant stature, maturity, leaf nectaries, leaf gossypol gland

numbers, stem gossypol gland numbers, leaf trichome numbers, stem trichome numbers and lint fiber.

Exactly 50 bolls (or as many as available, counting the exact number) were hand harvested from each progeny row to ensure a thorough representation of the fiber quality distribution. These samples were ginned in the laboratory using a 20-saw gin (DENNIS MFG. CO., INC., Texas), and about 50 gm of fiber was sent to the Fiber and Biopolymer Research Institute (FBRI) in Lubbock, Texas for High Volume Instrument (HVI) analysis of fiber properties. Data for harvested boll, and seed traits included average boll weight (grams), 1000 seed weight (grams), Lint % (lint weight/seed cotton weight x 100), 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).

A total of 106 lines with leaf and stem trichome variations (i.e. that did not match the parental line), were grown along with 49 randomly selected mutant lines and six replications of the parental line (TAM 94L25) in 2009.

Planting was done on May 18th 2009 in GA and May 20th 2009 in TX. A total of 35 seeds per row were planted. Weeding was done throughout the summer as needed. Fertility and pest (weed and insect) management were consistent with commercial cotton production. Trichome variations observed in each replication of the trial confirmed the phenotypes observed in single-row M5 plots.

Exactly 50 bolls were hand harvested for all three replication on October 19th 2009 at Lubbock and December 7th 2009 at Watkinsville. The hand harvest of 50 boll samples served to

determine lint % (lint/seed cotton X 100) or gin turnout (lint/burr cotton X 100) and the lint was used for HVI fiber analysis. Hand harvested cotton will have a higher genetic purity that machine harvested cotton (which will contain contamination from plot to plot). Because we harvested exactly 50 bolls we can also determine boll weight, seed per boll, or any other measure on a per boll scale. A John Deere cotton picker was used for harvesting, only harvesting seed cotton (lint+ seed) so burrs do not confound estimates of total yield of the plots.

The hand harvested bolls were ginned using a 20-saw gin (DENNIS MFG. CO., INC., Texas), harvest scored boll and seed trait parameters were taken, and about 50 g of fiber was sent to the Fiber and Biopolymer Research Institute (FBRI) in Lubbock, Texas for High Volume Instrument (HVI) analysis of fiber properties.

Statistical Analysis

Data were statistically analyzed using SAS software (SAS Institute Inc., SAS®9.2). Correlation was estimated using the command "PROC CORR". Most statistical analysis was done by command 'PROC ANOVA'. Means were separated using F tests and further by LSD tests at an alpha level of 0.05 or P value < 0.05.

Results

Stem trichome variation was classified into four levels, with 0 for lines which had no trichomes, 1 for lines which have trichomes but less than the parental line, 2 for lines which are similar to the parental lines and 3 for lines having more trichomes than the parental line. There were no lines designated 0, 87 lines designated 1 and 13 lines designated 3.

For leaf trichomes only lines similar to or with more trichomes than parental lines were observed. A total of 12 lines having more leaf trichomes were found.

Relationship between trichome variation and fiber qualities

Fiber strength showed a weak correlation of r= 0.17 in 2009 to stem trichome level, significant at $p<0.0001$. Seed weight shows a weak negative correlation to stem trichome level (r=-0.1 in 2008 and r=-0.18 in 2009), significant at $p<0.0001$. Leaf and stem trichome level share a weak correlation of r=0.15 in 2008 and r=0.29 in 2009, significant at $p<0.0001$ (Table 8), which is similar to the result obtain in another study of *G. hirsutum* (HORNBECK and BOURLAND 2007).

Comparison between different levels of leaf/stem trichomes

Mean lint percent was 1.4% and fiber strength was 1.6% higher of lines with parental stem trichomes than reduced stem trichomes, which are significant differences (Table 9). Mean fiber elongation was 4.55% and fiber fineness was 3% higher of lines with reduced stem trichome than parental stem trichomes, which are significant differences (Table 9). Mean Rd value of lines with reduced stem trichomes was 0.66% higher than those with parental stem trichomes and 1.01% higher than lines with increased stem trichomes, each of which is significant difference (Table 9). Mean fiber elongation of lines with increased stem trichome was 4.72 % higher than lines with parental trichomes, which is significant difference (Table 9). Mean length and uniformity index did not show significant differences between any levels of stem trichome (Table 9).

Mean Rd value was 1.06% higher in lines with parental leaf trichomes than lines having increased leaf trichomes, a significant difference (Table 9). Mean fiber fineness of lines having increased leaf trichomes was 4.3% higher than for lines having parental type leaf trichomes, a significant difference (Table 9). Means of other fiber qualities showed no significant difference between the two levels (Table 9).

Discussion

These results suggest that genes functioning in stem trichome development also function in fiber development. For 5 of the 7 fiber traits measured, lines with mutations affecting stem trichome development also had altered fiber quality. While the small number of leaf trichome mutants found offered only minimal statistical power to resolve differences, nonetheless we found 2 of the 7 fiber traits measured to be altered in lines with mutations affecting leaf trichome development.

Mutant lines having reduced stem trichomes showed significant differences from the parental lines for fiber fineness, fiber strength, fiber elongation, lint percent, and Rd value. Even though fiber strength of lines designated as level 1 stem trichomes (less than parental) was on average significantly lower than the parental lines, there were four level 1 lines viz. line 1097 (33.6 gram/tex), line 1793 (37.1 gram/tex), line 2370 (35.3 gram/tex), and line 3149 (36.3 gram/tex) which were selected for increased fiber strength. It has been indicated that fiber strength might be controlled by 2–3 major genes (Meredith 2005; Chee and Campbell 2009). If a low number of genes are responsible for much variation in fiber strength, then mutants responsible for lowering stem trichomes in these four lines might have different consequences than in other lines, resulting in increased fiber strength. Indeed, this suggests that our studies might have underestimated the degree to which trichomes and lint fibers are influenced by the same genes. Moreover, this indicates that it is possible to sort occasional useful mutants from a pool of mutants that are not desirable on average. Alternatively, two independent mutations could be responsible for the stem trichome and lint strength phenotypes, a hypothesis that could be tested by crossing the lines with different genetic backgrounds and genetically mapping fiber strength and trichome variation. This work might be made easier due to identification of some

markers for TAM 94L25 and its nearby inbred lines (Maleia *et al.* 2010). Also, such lines can be used for improving fiber strength with an advantage of having less stem trichomes. Reduced trichome number or "smooth" stems reduces the attraction of major pest of cotton, which may reduce pest attack (Wright et al. 1999). Similarly, 14 more lines were selected for different fiber qualities like lint percent, fiber length, fiber fineness, fiber elongation, length uniformity index, and Rd value. Thus, a breeding program that requires improving fiber qualities and a smooth variety might use one of these lines as a parent. Finally, studying these lines closely might further clarify the association between these fiber qualities and trichome variation.

Lines with reduced stem trichomes and lines with increased leaf trichome number relative to the parental line have each shown significant improvement in fiber fineness. Similarly, lines with either reduced or increased stem trichomes have better fiber elongation than the parental lines. The subset of lines showing enhanced fiber fineness and fiber elongation co-associated with changes in trichome characteristics compared to the parental lines can be crossed to appropriate phenotypic background to identify location of gene/s responsible for leaf/stem trichome variation. One could expect two possible outcomes: i. identified gene/s could map at or close to the reported trichome variation genes or ii. novel genes involved in changes in trichome characteristics may be identified. Furthermore, QTL analysis for fiber traits would further characterize the nature of association between the identified trichome variation genes and the fiber elongation and fiber fineness.

In this study, six lines were observed which had higher stem and leaf trichomes compared to the parental lines. Studies have revealed that locus t_1 through t_5 are responsible for trichome variation in cotton (Wright *et al.* 1999; Zhang *et al.* 2000). A QTL, QSP_1 on chromosome 23 was associated with stem trichomes and was also expected to have some association with variation of

trichome on leaf veins (Wright *et al.* 1999) , whereas a QTL on D03 (now known to be chromosome 17) had a strong effect on stem trichome variation (Lacape and Nguyen 2005; Wang *et al.* 2006). It remains unknown whether we have discovered additional mutations at these known loci, or mutations at previously-unknown trichome/fiber related loci. An intensive study of crosses between lines having higher stem and leaf trichome number and other genetic backgrounds, using method described in previous studies (Wright *et al.* 1999; Lacape and Nguyen 2005; Nawab *et al.* 2011), is needed to clarify whether we have rediscovered known genes or found new ones.

If we have found new mutations, a natural next step after mapping them would be to determine their proximity to orthologs of the many genes known to be involved in growth and development of trichome in *Arabidopsis* (Marks 1997; Hulskamp 2004; Kryvych *et al.* 2008; Marks *et al.* 2009; Morohashi and Grotewold 2009; Uhrig and Hülskamp 2010), using the cotton genome sequence that is expected to be available in the near future. Additional candidates for new trichome/fiber mutations might include MYB genes, which show predominant expression in developing cotton fiber and trichomes (Loguercio *et al*. 1999; Cedroni *et al.* 2003; Suo et al. 2003; Lee *et al.* 2007; Desai *et al.* 2008; Machado et al. 2009).

EMS is known to cause only single base pair change instead with very few deletion of DNA fragment. Thus, sequencing candidate genes or ESTs that may be responsible for trichome and/or fiber development in our EMS treated mutation population might help us to develop array of SNPs which can be correlated to trichome and/or fiber variation. These will ultimately lead us to the knowledge of overlapping genes for trichome development and fiber development, if any.

Table – 8 Correlation between trichome variation and fiber traits

2008	Mic	LEN	UNIF	STR	ELON	Rd	+B	lint%	seed_wt	S.C	leaf_t	stem_t
leaf_t	0.01	-0.01	0	0.01	0.01	-0.02	0.01	0.02	0.017	N.A	1	
stem_t	0.02	0.04	0.03	0.03	-0.05	-0.04	-0.04	-0.01	-0.1*	N.A	0.15*	1
Traits correlated at p < 0.0001 are having '*'. Number of lines = 3168												
2009												
leaf_t	0.07	-0.02	-0.02	0	0.01	-0.09*	0.02	0.04	-0.07	-0.02	1	
stem_t	0.04	0.05	0.05	0.17*	-0.01	-0.12*	0.03	0.02	-0.18*	0	0.29*	1
Traits correlated at p < 0.0001 are having '*'. Number of lines = 300 (6 replication)												

Table 9 - Relationship between stem or leaf trichome variation, and fiber quality

	stem trichome			**Leaf trichome**	
	1	2	3	2	3
Lint %	39.3†*	39.85†*	39.79†	39.53†	39.74†
Mic	4.64†*	4.78†*	4.7†	4.82*	4.69*
Length	1.17†	1.17†	1.17†	1.17†	1.17†
Unif	83.71†	83.77†	83.73†	83.78†	83.73†
Strength	32.08†*	32.61†*	32.46†	32.64†	32.28†
Elong	6.2*†	5.93*	6.21*†	6.14†	6.1†
Rd	78.07*	77.56*†	77.29*†	77.88*	77.06*

Means having '*' are significantly different; means having '†' are not significantly different.

Conclusion

In summary, leaf and stem trichome variation was often closely associated with variation in cotton lint fiber attributes, suggesting that genes functioning in trichome development also function in fiber development. In some cases, some mutant lines showed significant increases and others decreases, in the same fiber trait, suggesting that both + and – mutant alleles for trichome and fiber qualities are present in the population. Thus, the average behavior of groups of mutants may mask variation of potential utility. For example, reduced stem trichomes were associated generally with lower fiber strength, but a few lines having lower stem trichomes had significantly higher fiber strength than the parent. It seems possible to develop smooth varieties with better fiber qualities by just using a single parent for crossing from our mutant line. More generally, this collection of mutants may offer new combinations of traits that are of value, in addition to specific mutations that may reveal previously-unknown genes.

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Chapter 4

DISCOVERING ANOMALISM IN COTTON MUTANT POPULATION

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ABSTRACT

A total of 3,200 M5 mutant lines developed by Dr. Dick Auld from *G. hirsutum* breeding line 94L25 were characterized in 2008. Eight morphological traits were examined, using a scoring scale from 0 to 3 where 0 is absence, 1 is present but less then parental type, 2 was similar to the parent, and 3 was more than the parent.

There were 44 mutant lines having less stature and 214 lines having more stature than the parental lines. Four mutant lines were found to mature earlier and eight lines to mature later than the parent. Three lines were found with triple nectaries whereas the parental line has single nectaries. A total of 12 lines were found having leaf and 100 having stem trichome variation differing from the parent, and 37 lines having naked to semi naked seed phenotypes. On average, the lint percent of naked seed was significantly lower than the parent but its Rd value and elongation were significantly higher. A few naked seed lines with similar lint percent as the parent, might warrant further evaluation.

Introduction

Cotton is the world's leading textile fiber. Cotton is a highly profitable cash crop in two of the biggest cotton producing nations, viz. India and China, which account for almost 50% of the world's supply. It is also a leading cash crop in the U.S, where 18.4 million bales of cotton were produced from around 11 million acres planted in 2010 (National Cotton Council 2011). A large percentage (~40%) of raw cotton produced in the U.S. is exported, generating almost $ 3 billion and helping to curtail trade deficits. Upland cotton, *Gossypium hirsutum*, is result of an interspecific hybridization between an A- and D-genome diploid species about 1-2 million years ago (Wendel and Cronn 2003). The agronomic growth stages can be divided into five parts: 1.

Emergence (5 days after planting or DAP), 2. First Square (38 DAP), 3.First Flower (59 DAP), 4. Open Boll (116 DAP), and 5. Harvest (140 DAP).

Unlike several major crops, mutational breeding has not been established as one of the major tools in cotton improvement (Auld *et al.* 1998). Nevertheless, a broad spectrum of physical and chemical mutagens has been used sporadically in different species of *Gossypium* with varied responses (Auld *et al.* 2009). Typical of physical mutagenesis, radiation induced mutants have shown a wide range of phenotypic variations (Horlacher and Killough 1933; Horlacher and Killough 1931). Physiological variants have also been generated using radiation-based approaches; some examples include: enhanced phosphorus uptake and improved drought tolerance using radiophosphorus (Nazirov et al. 1979), and photoperiod insensitivity and cytoplasmic sterility using gamma rays (Raut *et al.* 1971; Ngematov *et al.* 1975). However, two major concerns viz. higher yields and better fiber quality characteristics, have yet to be realized from irradiation. Chemical mutagenesis, on the other hand, has shown a wide range of variation in different traits of interest including lint yield and fiber quality. The most commonly used chemical mutagens in cotton include sodium azide (Hussein *et al.* 1982; Larik *et al.* 1983), dimethyl sulfate, colchicine (Salanki and Parameswarappa 1968; Luckett 1989; Shi-Qi *et al.* 1991) and ethyl-methane sulfonate (Shattuck and Katterman 1982; Herring *et al.* 2004; Lowery 2007; Auld *et al.* 2009; Bechere *et al.* 2010). Bechere *et al.* (2010) registered four EMS-derived upland cotton mutants with elevated levels of imazamox tolerance (Bechere *et al.* 2010). Thus mutagenesis is an effective tool to create a broad range of phenotypic variation in cotton species and will help us to develop a prodigious germplasm in cotton.

Materials And Methods

Dr. Auld developed a mutant population of TAM94L25 (Smith 2003) by treating with the chemical mutagen EMS to their LD_{50}, using published techniques (Auld *et al.* 1992; Auld *et al.* 1998). About 3200 lines were generated and advanced by single boll descent to M5. A single boll descent risks duplicating a few mutants as a boll contains 20 to 30 seeds, but it is often a practical necessity in cotton.

In 2007, M4 population was grown in Lubbock, Texas (latitude 33N 34' 40.31" and 101W 51' 18.60'' longitude) and in 2008, the M5 population was grown in Watkinsville, Georgia (latitude 33N 51' 46.425" and 83W 24' 31.5756" longitude) with a completely randomized design (CRD). The soil type of the field near Watkinsville, GA is Appling Coarse Sandy Loam, and that of Lubbock, TX is Amarillo Fine Sandy Loam and Pullman Clay Loam. The sowing date in Texas was May 17, 2007, and Georgia was May 21, 2008. Seeds for each line were sown in 3 meter rows, spaced one meter apart. Thinning and weeding was done as necessary. A total of 74.45-74.45-74.45 kg/ha of NPK was applied as fertilizer and before sowing. Herbicide and pesticides were applied as required.

Eight morphological traits were examined, using a scoring scale from 0 to 3 where 0 is absence, 1 is present but less then parental type, 2 was similar to the parent, and 3 was more than the parent. The traits included plant stature, maturity, leaf nectaries, leaf gossypol gland numbers, stem gossypol gland numbers, leaf trichome numbers, stem trichome numbers and lint fiber.

Exactly 50 bolls (or as many as available, counting the exact number) were hand harvested from each progeny row to ensure a thorough representation of the fiber quality distribution. These samples were ginned in the laboratory using a 20-saw gin (DENNIS MFG. CO., INC.,

Texas), and about 50 gm of fiber was sent to the Fiber and Biopolymer Research Institute (FBRI) in Lubbock, Texas for High Volume Instrument (HVI) analysis of fiber properties. Data for harvested boll, and seed traits included average boll weight (grams), 1000 seed weight (grams), Lint % (lint weight/seed cotton weight x 100), 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).

Statistical Analysis

Data were statistically analyzed using SAS software. Most Statistical analysis was done by command 'PROC ANOVA' command of SAS 9.2 (SAS Institute Inc., SAS®9.2). Means were separated using F tests and further by LSD tests at an alpha level of 0.05 or P value < 0.05.

Results and Discussion

The height (stature) of parental plants was 37 inches, with 214 mutant lines having height approximately 42 inches or more designated level 3 (more stature), and 44 mutant lines having height approximately 32 inches or less designated level 1 (less stature).

Maturity was noted based on flowering stage, boll development stage and open bolls. Four mutant lines with flowering stage, boll formation and open bolls before the parental line were designated level 1 (early maturity), whereas eight lines still in flowering or boll formation when the bolls of parental lines were already open, were designated level 3 (late maturity).

Stem trichome density varied widely, with no lines that were completely lacking stem trichomes, but 87 that had less trichomes than the parental line, and 13 having more trichomes

than the parental line. A total of 12 mutant lines had higher leaf trichome density than the parental line.

A nectary is a glandular structure, which secretes sugar and other organic compounds (ESAU 2006). Most cotton species exhibit nectaries on the midrib at approximately one-third of the way from the petiole junction to the leaf apex and also usually have nectaries on the lower part of bracts (Rudgers et al. 2004). Being secretory ducts, nectaries may attract pollinators which help in crossing, but at the same time they also may attracts insect pests (Henneberry et al. 1977; Wilson et al. 1980; Adjei-Maafo et al. 1983). The nectariless trait was identified in *G. tomentosum* (Meyer and Meyer 1961) and has been genetically mapped (Waghmare et al. 2005; Ashraf and Ahsan 2008). In three mutant lines, multiple (but not all) plants had three leaf nectaries instead of the single nectaries seen in the parent (Figure 10 & 11). Four seeds were grown to examine the heredity of phenotype, but this phenotype was not seen in progeny.

Naked seed cotton does not contain seed fuzz, potentially reducing the cost of separating lint from the seed and also helping to reduce ginning trash. A total of 37 naked seed lines were found in the mutant population. Lint percent of parental lines was 15% higher than naked seed lines, which is statistically significant. Mean elongation and Rd value of naked seed lines were respectively 31.29% and 3% higher than the parental line. Fiber length, strength and fineness were not significantly different in parental and naked seed lines. Until now four genes, *N1, fl1, n2 and N3,* responsible for naked seed production are reported (Turley and Kloth 2002; Turley and Kloth 2008). A detailed study has been done on the effect of different combinations of these genes on lint percent, suggesting a wide range of differences, but with all combinations having at least 16.5% lower lint percent than the parental line having normal fuzz (DP569) (Turley and Kloth 2008) which matches our result. Although the average lint percent of the parental line was

significantly higher, a few of our naked seed lines showed higher lint percent than the parental line, similarly to another mutant study (Lowery 2007). Such lines might be worthy of further study to develop germplasm with naked seed and better lint percent. Also, scrutiny of these lines in detail might help in identifying new genes which are responsible for higher lint percent or cloning the existing or new genes for naked seed.

Orange colored cotton fiber (Figure 12) was observed in a very small amount of fiber in one mutant line. Also, there were many mutant lines having different shades from yellow to brown colored fiber. *Lc1*, a dominant mutant gene has been reported for brown fiber (Zhang et al. 2005; Zhang et al. 2009). Two seeds were grown from the line having orange colored cotton fiber, but the phenotype was not reproduced.

Similarly, minute mutant seed were seen in a few lines (Figure 13), much smaller than even an immature seed, although showing all the features of mature seeds including normal fuzz on the seed. Such seeds never germinated.

Even traits like orange fiber and minute mutant seed that were not reported in the progeny, indicate that there might be variation in genes which needs to interact with environmental factors to develop such traits.

The generation that was screened for mutants was M5, which means mutant load had already been reduced and there is less chance of observing variation or abnormality than there might have been in earlier generations. Future screening of mutant plants for discrete morphological abnormalities should be done in much earlier generations.

Table 10 - Naked Seed lines V.S. Parental line

	lint%	MIC	LEN	UNIF.	STR	ELON.	Rd
NS lines	35.97***	4.89	1.15	84.40	31.66	6.21***	76.11*
P lines	41.38	5.03	1.15	83.75	31.57	4.73	73.90

'***' denotes significant difference at p<0.0001 and '*' significant difference at p<0.005

Figure 10 - Leaf with triple nectaries

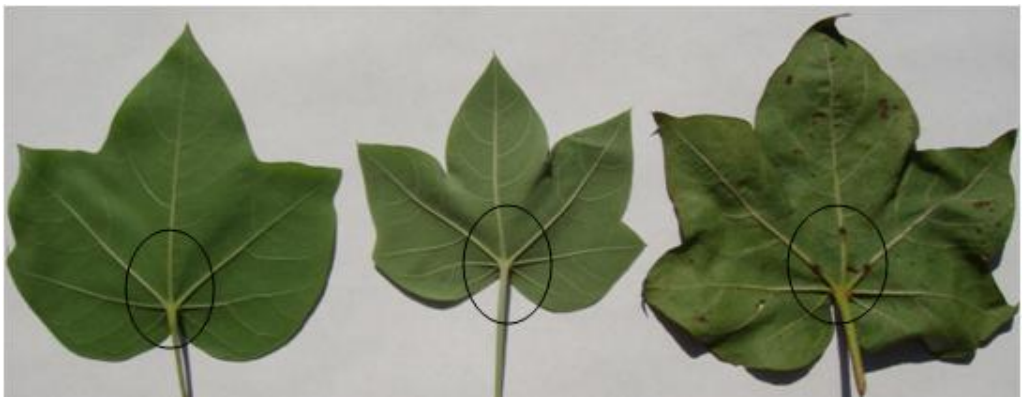

Figure 11- Comparision of leaf nectaries in *G. tomentosum, G. hirsutum* and Mutant line

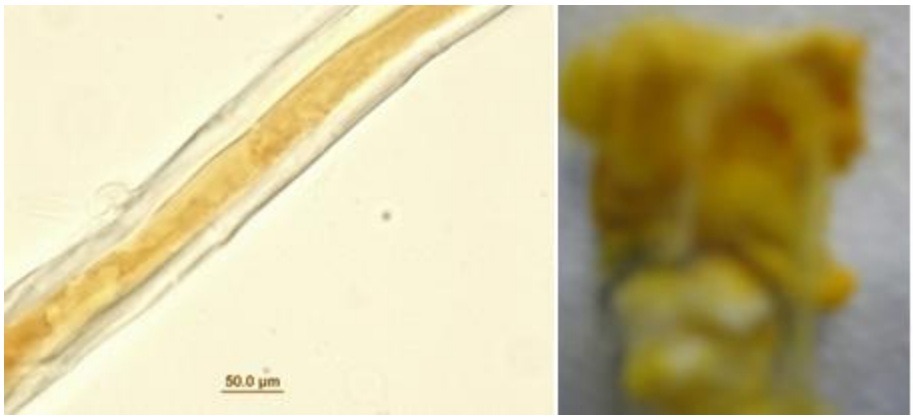

Figure 12 - Internal and external appearance of Orange color fiber from a mutant line

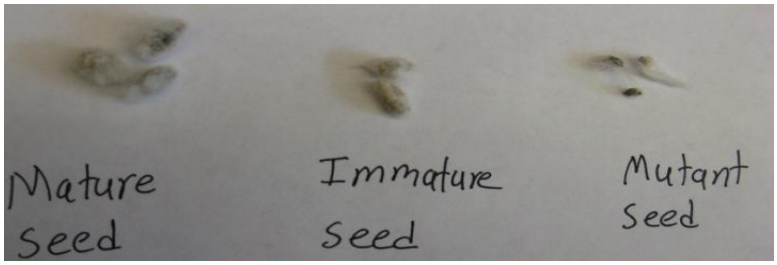

Figure 13- Comparison of seed size in mutant population

Conclusion

Phenotypic variations such as triple nectaries, plant height variation, maturity variation, trichome variation and naked seeds were relatively frequent in the population developed by EMS chemical mutagenesis

There is an opportunity for further evaluating naked seed lines for developing germplasm with no fuzz but better fiber quality.

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Chapter 5

SUMMARY

Much more variation than could be explained by non genetic factors was observed in fiber elongation, fiber strength, fiber fineness, fiber elongation and fiber length. Lesser but still useful variation was also seen in fiber uniformity and Rd value. Molecular study of such mutant populations might help to reveal new QTLs for fiber quality or perhaps contribute to finding genes involved in the complex pathway of fiber development.

Leaf and stem trichome variation was often closely associated with variation in cotton lint fiber attributes, suggesting that genes functioning in trichome development also function in fiber development. In some cases, some mutant lines showed significant increases and others decreases, in the same fiber trait, suggesting that both + and – mutant alleles for trichome and fiber qualities are present in the population. Thus, the average behavior of groups of mutants may mask variation of potential utility. For example, reduced stem trichomes were associated generally with lower fiber strength, but a few lines having lower stem trichomes had significantly higher fiber strength than the parent. It seems possible to develop smooth varieties with better fiber qualities by just using a single parent for crossing from our mutant line. More generally, this collection of mutants may offer new combinations of traits that are of value, in addition to specific mutations that may reveal previously-unknown genes.

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