Stabilizing Naked Seed Mutants in Upland Cotton (*Gossypium hirsutum* L.)

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Abstract

Two of the best described cotton fiber mutants are the naked seed loci N_1N_1 and n_2n_2. The objective of this research was to stabilize and evaluate fiber trait data of new naked seed mutants developed from the cultivars Atlas and SC 9023 using chemical mutagenesis. In 1997, Texas High Plains upland cotton (*Gossypium hirsutum* L.) cultivars were treated with 2.45% v/v ethyl methane sulfonate (EMS) and planted in the field. M_3 seed from Atlas, Tejas, and SC 9023 mutant lines was identified with a partially fuzzless seed coat. The trait was stabilized between 2000 and 2003. Lines were evaluated for fiber quality, lint yield, and ginning efficiency in 2004, 2006, and 2007 at Lubbock, TX. In 2008, crosses were made between mutant lines and eight lines chosen for high fiber initiation, fiber quality, and lint yield. Selections in these segregating populations to stabilize the naked seed trait were made from 2010-2012. Lines exhibiting the uniform naked seed phenotype were evaluated for AFIS fiber quality, lint yield, percent lint, and fiber initiation. With continued improvement we hope to develop the elite lines necessary to commercialize this valuable trait.

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Chapter I

Introduction

Cotton, the world's most important natural textile, accounts for approximately 35% of fiber use worldwide. The United States leads the world in cotton exports but ranks third in cotton production behind China and India. The three countries combined produce two-thirds of the world's cotton annually. The cotton industry is a vital part of the U.S. economy generating ~200,000 jobs and ~$25 billion in proceeds (USDA-ERS 2012). In 2012, over 12 million acres of cotton were planted in the U.S. across 17 states with Texas accounting for ~50% of the planted acres (USDA-NASS 2012).

Cotton (*Gossypium spp.*) is a perennial shrub cultivated as an annual in the United States. Upland cotton, (*Gossypium hirsutum* L.*)* accounts for over 95% of cotton production in the U.S. The limited growing season in Texas has historically led to poor fiber quality in cultivars adapted to this region. Cotton fiber faces increasing competition with synthetic fibers which escalates demand for higher quality cottons. Consequently, the primary objectives for cotton breeders have been to simultaneously increase fiber quality and lint yield. Further progress in this endeavor may be difficult due to (1) limited germplasm resources, (2) a negative correlation between fiber quality and lint, and (3) extensive breeding (Smith and Cothren 1999).

The evolutionary history of cotton began ~10-20 million years ago. The genus *Gossypium* has since evolved into 45 diploid and 5 allotetraploid species each containing unique epidermal seed hairs. The restriction in genetic variation available

in Upland cotton (*G. hirsutum*) has resulted from multiple genetic bottlenecks. The first event was the hybridization between the A and D genome prior to domestication about 1-2 million years ago (Kohel and Lewis 1984). Two Old World diploid species, *G. arboretum* and *G. herbaceum,* and two New World tetraploid species, *G. barbadense* and *G. hirsutum,* were domesticated individually within the last 5,000 years in different regions of the world for their lint which makes excellent textile fibers (Smith and Cothren 1999). Improvement of cotton through human manipulation has produced a small elite germplasm further limiting germplasm variation. However, the result of these breeding efforts has improved fiber quality and lint yield potential of commercial cultivars, but in the process has created a very narrow genetic base that limits further enhancement (Smith and Cothren 1999).

Inducing mutations through radiation or chemical mutagenesis have been successfully used in major field crops to create genetic and phenotypic variation not previously observed. Though not widely used in cotton, incorporating the use of induced mutations into breeding programs may improve targeted characteristics more rapidly than traditional breeding techniques (Lowery 2007; Herring 2004). There are many valuable applications for genetic mutants which include increasing germplasm diversity and improving quality, yield, disease, and pest resistance (Muthussamy 2011).

Lint harvested from the fuzzy seeded cotton varieties cultivated on the High Plains has a large incidence of seed coat neps. Neps are undesirable to textile mills

since these create imperfections that detract from yarn and fabric appearance. Consequently, the presence of neps and seed coat fragments in cotton lint significantly reduce the value of cotton from West Texas. The reduction in linters (fuzz) on the seed should reduce the time and energy required for ginning while better preserving the cotton's fiber quality during processing.

The aim of this study was to develop true breeding cotton lines which contain a reduction in linters left on the seed coat after ginning. Mutants were originally selected from chemically mutated populations that we crossed with carefully selected parents to generate segregating populations. Our secondary objective was to determine if increased fiber initiation had the potential to increase lint yield in these naked seed mutant lines.

Chapter II

Literature Review

Overview

This review of the literature was conducted to identify the various cotton fiber mutants reported that control the presence or absence of lint and/or linters on the cottonseed. The effect of early fiber development, specifically cell differentiation and fiber initiation, of lint fibers and linters in cotton was also investigated.

Cotton Fibers

Cotton seed trichomes (hairs) are very long single epidermal cells that differentiate and elongate to form two types of fiber: (1) lint and (2) linters or fuzz (Smith and Cothren 1999; Wu, et al. 2006). Upland cotton is covered with both types of fibers, while most wild cotton accessions only have one type of fiber on their seeds (Smith and Cothren 1999). Lint fibers contain properties that make them ideal for use as a textile fiber. Cotton lint is now the most important textile crop in the world (Smith and Cothren 1999). Lint fibers are very long (~ 25 mm), relatively fine, spinnable, and excellent for the production of high quality textiles. Approximately 30-35% of the total seed cotton weight is attributed to lint fibers suitable for textile products (Herbert 1988). Lint fibers are the most economically important product harvested from the cotton plant. Cotton by-products produce an important source of seed oil, protein meal, and industrial cellulose (Smith and Cothren 1999). The developmental stages of

fiber elongation, secondary cell wall synthesis, and maturation, as well as the special characteristics of lint fibers have been studied extensively.

Far fewer studies have been conducted on linter development. Linters are very short (~1 mm) cylindrical fibers tightly attached to the seed coat. The morphology of linters is very similar to seed hairs present in wild *Gossypium* species short, tightly adherent, coarse, cylindrical, and have a thick secondary cell wall (Smith and Cothren 1999; Berlin 2012). Linters also maintain their circular cross sections after the capsule opens unlike lint where the central vacuole collapses into twisted ribbons that allow them to be spun into yarn (Smith and Cothren 1999). Lint development differs from that of linters beginning with later initiation at around 4-10 days post anthesis (dpa) (Smith and Cothren 1999; Tiwari and Wilkins 1995). Linters have been reported to be brown, green, or white in color (Hutchinson 1935). Fuzzy seeded cotton varieties with green linters have been referred to historically as 'green-seeded cottons' (Smith and Cothren 1999). The proportion of linters to seed is variable depending on cultivar (Hutchinson 1935; Herbert 1988). Most upland cottons have seed that is completely covered in linters, while other cultivars have very sparse linters or completely naked seeds. In other varieties it is common to see linters only on the micropylar end of the seeds distal to the funiculus; these are sometimes referred to as tufted (Herbert 1988; Hutchinson 1935). The lack of linters on naked seeds is largely due to the presence of natural or induced mutant alleles, like the N_1 allele that has been reported to delay lint initiation or n_2 allele common to commercial *Gossypium barbadense* varieties.

Linters remain attached to the seed after ginning and must be removed to allow planting with today's machinery (Wu, Machado, et al. 2006; Herbert 1988; Hutchinson 1935). Fuzzy seeded cottons must be ginned with saw gins, whereas extra-long staple cottons with fuzzless seeds are generally ginned using roller gins which preserves the lint quality of these varieties. Early settlers used a Churka gin, the first mechanical roller gin, for naked seeded cottons, but found fuzzy seeded varieties difficult to gin with the Churka gin. Fuzzy seeded cottons were consequently ginned by hand before Eli Whitney invented his cotton gin in 1794 (Bowman 2012).

Improved ginning and selection of improved cotton varieties can reduce the presence of defects (neps, short fiber content, and seed coat neps) in lint somewhat. However, ginning and lint cleaning impacts lint cotton quality by reducing its staple length and changing its length distribution. Research conducted at the Fiber and Biopolymer Research Center at Texas Tech University suggests that a longer staple cotton variety in a bale does not always correlate to increased quality in processed fiber (Krifa 2006). Therefore, breeders must focus on improving cotton's processing characteristics in addition to other fiber quality parameters.

Delinting of linters is the first step in processing cottonseed for oil extraction. Oil extraction from cottonseed began in Europe during the early 1800s. American Upland cottonseed was nearly impossible to process due to the presence of linters covering upland seed. The linters absorbed much of the oil extracted decreasing production and leading to early mill failure. Delinting machinery created in 1857 by William Fee allowed crushing mills to thrive. Delinting machines are similar to saw

gins in that they consist of a series of circular saws on a revolving shaft. Theses saws project through closely spaced steel ribs and cut off the short linters. The linters are collected and pressed into bales. Abrasive delinting, machines with a physically rubbing action for linter removal, is also used in some mills. There are three classes of linters: (1) millrun, where seed is run through delinting machines once, (2) "first-cut," longer, stronger fibers from the first of two rounds of delinting, and (3) "second-cut," short linters from second series of delinting (Cottonseed and its products 2012). Although there is a market for all linter products, the cost of removal is great due to the energy needed for the process.

Linters are an important source of industrial cellulose (Ardashev 1933). There are two broad markets for cottonseed linters: chemical and non-chemical. Currently, the first class accounts for approximately seventy-five percent of total volume. First-cut linters are generally used in non-chemical applications while second-cut linters are used in chemical products. Non-chemical uses for the highest grades of first cut linters include: manufacturing absorbent cotton, medical pads, gauze, twine, wicks, and carpet yarns. A larger amount of linters undergo a "garneting" process to create felts, batting for mattresses, bedding products, and furniture cushioning (Cottonseed and its products 2012). Chemical applications of linters begin with processing to form linter pulp. Linter pulp is more expensive than competing products, but is usually preferred because of its superior quality and performance. Linter pulp is important to the chemical industry and is used in making smokeless gun powder, photographic

film, molded plastics, lacquers, fingernail polish, car engine filters, laminates, printed electrical circuit boards, and fine paper including currency notes.

Early Fiber Development

Fiber development occurs at four distinct but overlapping stages: (1) initiation, (2) elongation, (3) cell wall synthesis, and (4) maturation (Tiwari and Wilkins 1995; Romano 2011). The earliest stage of cotton fiber development begins with the differentiation of cells on the epidermis of the ovule into what will become lint fibers. The mechanisms controlling cell differentiation and the formation of fiber initials are poorly understood (Lee, et al. 2006, Liu, et al. 2012; Zhang, et al. 2007). However, only about 30% of epidermal seed trichomes develop into spinnable fiber, even though all epidermal seed cells have the same potential (Liu, et al. 2012; Taliercio, et al. 2005).

Differentiation of the epidermal cells into lint and fuzz fiber cells occurs at the time of flowering (anthesis) (Zhang, et al. 2007). Differentiation of cells begins at 16 hours pre-anthesis at the chalazal region of the ovule (proximal to the funiculus which attaches the ovule to the placenta) and moves toward the micropylar region at 10-12 hours pre-anthesis (Ramsey and Berlin 1976). Twenty four hours prior to anthesis (-1 DPA), there is a change in some epidermal cells' electron density, followed by enlargement of certain cells at sixteen hours pre-anthesis. Light and dark cell populations of epidermal cells have been observed eight hours pre-anthesis with the dark type cells differentiating into cells that will form lint fibers (Ramsey & Berlin

1976; Kohel R. J. 1984). Differentiation of epidermal cells seems to occur randomly with fiber cell initiation taking place next to cells undergoing normal mitotic division and suggests that each epidermal cell differentiates individually at anthesis (Ramsey and Berlin 1976).

Lint Fiber Initiation

Studies on the chronological development of fiber initials on the cotton ovule have been conducted, but the molecular basis of fiber initiation remains relatively unknown (Lee, et al. 2007; Smith and Cothren 1999; Stewart 1975). Scanning electron microscopy (SEM) imaging of ovules has been used effectively in most studies related to fiber initiation, but is very expensive (Smith and Cothren 1999). Transmission electron microscopy has also been used in related studies (Tiwari and Wilkins 1995).

Initiation of lint fibers begins shortly before or at anthesis (white flower stage) in upland cotton and is recognized by the random swelling of cells of the protoderm (Smith and Cothren 1999; Lee, et al. 2007; Stewart 1975). A few fiber initals arise around the sides of the ovule followed a few hours later by initials in the chalazal region of the ovule. Initials emerge outward with some lateral expansion that appears as balloon-like protrusions over the ovule (Smith and Cothren 1999; Stewart 1975; Butterworth, et al. 2009). Fiber initiation may be delayed at the micropylar end of the ovule for 2-3 days; consequently, intials will appear at various stages of development at this region (Stewart 1975, Smith and Cothren 1999). Under a microscope, initials

appear roughly twice the size of nondifferentiated epidermal cells. Fiber initial density was initially estimated at 3,300 per mm^2 of surface area (Stewart 1975).

The developmental stages of lint fiber initiation and elongation overlap. However, elongation of fiber initials is very distinct by two dpa (Stewart 1975). For the first three days of fiber development, the ultratructure is typical of diffuse growth of plant cells rather than tip growth (Tiwari and Wilkins 1995). Rapid cell elongation of single cells occurs between 2 dpa and 21 dpa, followed by secondary cell wall syntheis that begin at aproximately 16 dpa. Finally, maturation occurs when the boll opens around 50-60 dpa (Lee, et al. 2007; Li, et al. 2010; Taliercio and Boykin 2007).

Linter Initiation

The origin of linter inititaion and slow elongation is not known (Ruan, et al. 2005). However, linters initiate after lint fibers at about 5-10 dpa (Stewart, 1975; Lee, et al. 2007; Ruan, et al. 2005). Linters have been reported to initiate between 6-9 dpa in *G. arboretum*, 10-12 dpa in *G. herbaceum,* and 5-9 dpa in *G. hirsutum* (Butterworth, et al. 2009). Timing of lint fiber and linter initiation varies among genotypes but is somewhat coordinated in each ovule and among ovules with each boll (ovary) (Lee, et al. 2007). Linter initiation seems to be controlled by both environmental and genotypic components. Cultivars within a species are known to have differing quantities of lint fiber initiation and linter initiation. Variation in the number of lint and linter initials each year has been reported. Wild diploid species

have more initials than wild tetraploid species and cultivated tetraploid species contain more fiber cover than wild tetraploid cottons (Romano 2011; Butterworth, et al. 2009).

Recent studies have reported on both the number of genes and the hormones that may regulate fiber initiation. Genetic studies on the similarities of cotton seed trichomes to Arabidopsis leaf trichomes or tobacco studies have been used to explain fiber cell initiation in cotton (Shangguan, et al. 2010; Lee, et al. 2007). Several hormones are expressed differentially in fiber initiation than other stages of cotton fiber development. Abscisic acid (ABA) has been reported to inhibit fiber growth while cytokinin may increase fiber initiation but inhibit fiber growth after flowering (Lee, et al. 2007). Various pathaways involved in hormone (ABA, ethylene, and JA) biosynthesis and signal transdaction were upregulated during fiber elongation (Padmalatha, et al. 2012). There is an accumulation of indole-3-acetic acid (IAA) in cotton fiber initials and on the epidermis of the ovule at the fiber initiation stage which increased the number of lint fibers and lint percentage resulting in a >15% increase in lint yield (Zhang, et al. 2011). The hormone auxin as well as gibberellic acid and brassinosteroids have been identified as promoters of fiber cell development on *in vitro* cultured cotton ovules and *in vivo* quantification studuies that reveal a spike in auxin levels in the flower bud before initiation (Sun, et al. 2005; Lee, et al. 2007).

The similarities between genes involved in leaf trichome development in Arabidopsis and cotton may allow *A. thaliana* to be used as a model for uncovering additional genes that control fiber developemtn in cotton (Guan et. al. 2011). The overexpression of *GhMYB2* has been found to be highly expressed in *G. hirsutum*

during fiber initiation and in *A. thaliana* has activated trichome production in 4-6% of seeds (Guan, et al. 2011). FLA (Fasciclin-like arabinogalactan proteins) genes, including G*h*AGP2 and G*h*GP4, found in cotton fiber are involved in fiber developent and quality. When G*h*GP4 is suppressed, the number of fiber initals is partialy inhibited (Li, et al. 2010). A sucrose synthase gene *(SuSy*) is important to ovule development and was found to be highly expressed in initiating and elongating fibers but not in normal epidermal ovule cells. *SuSy* was found to be radically reduced in the *fl* mutant ovule where fiber initiation is extremely limited. (Shangguan, et al. 2010; Li, et al. 2010; Ruan, et al. 2005). Quantitative trait loci (QTL) have been reported for various fiber quality traits and could be used for marker assisted selection for fiber quality and fiber development. (Sun, et al. 2012) found a 50 QTL for fiber quality (strength, micronaire, elongation, uniformity, and length) by using SSRs to screen for polyorphism between the parent strains. Further progress in understanding and improving cotton fiber initiation may be through the use of expressed sequence tages (ESTs) of earlier fiber development (Shangguan, et al. 2010).

Cotton Fiber Mutants

Several cotton fiber mutants have been discovered since the early 1900s. Fiber mutants have been found in nature in many cases while in others the mutations have been induced through chemical or radiation treatments (Lee, et al. 2006, Bechere, et al. 2009; Hutchinson 1935). Fiber mutants that inhibit the growth or development of fiber initials may be useful tools for studying molecular events of early fiber

development (Wu, et al. 2006; Ruan, et al. 2005; Turley & Kloth 2002). Cotton mutants may also be used by researchers to detect and locate QTLs for lint yield, fiber development, fiber quality, and seed traits (Bechere, et al. 2012; Rong, et al. 2005).

Some fiber mutants are fuzzless and linted while others lack one or both types of cotton fibers (lint and linters). The tetraploid species in the genus *Gossypium* contains a wide variety of linter and lint fiber distribution on the seed coat (Smith and Cothren 1999). The tetraploid species in the genus *Gossypium* exhibit wide variation in fuzz and lint distribution on the seed coat (Smith and Cothren 1999). Studies have been conducted that describe and separate grades of linter coverage in a range from fuzzy to tufted to naked (Ware 1940; Ware et al. 1947; Ware et al. 1944; Bechere, et al. 2012; Hutchinson 1935). Fuzzless or naked seed from *G. barbadense and G. hirsutum* are morphologically similar (Ware, et al. 1944). Development of lint fiber in mutants has been observed to differ from normal fuzzy cottons. In some naked seed mutants, fuzz fibers form but ultimately their development is aborted, while in others few fuzz initials are observed, many of which were depressed instead of spherical in shape (Zhang, et al. 2007; Lee, et al. 2006). Significantly fewer and sometimes deformed lint fiber initials have been reported in several fiber mutants. Fiberless mutant lines MD 17 and XZ142w do not completely inhibit lint development (Turley and Vaughn 2007; Zhang, et al. 2007). Lint initiation in naked seed mutants may be restricted to the chalazal region of the ovule that will appear as a tuft on a mature seed. Lint initials increase laterally around the chalazal region as lint% increases (Turley and Vaughn, 2007).

Historically, breeding for lintless varieties to be grown strictly as an oilseed crop held the assumption that oil content might increase and that there would be less difficulty with removing lint from the seeds (Cook 1918). Naked seeded cotton relatively common prior to 1793 lost favor with breeders and producers after the invention of the cotton gin by Eli Whitney. Naked seeds have historically been associated with undesirable characteristics' such as decreased lint percent (Ware, et al. 1944). One example is the Hopi cotton obtained from the American Hopi Indians in 1901 and 1907 which features dark brown fuzzless cotton seed that produces sparse white lint between 20-30% (Lewton 1912).

Two of the best described cotton fiber mutants are the dominant N_1 gene and the recessive n_2. In both the N_1 and n_2 mutants linters initially form but later fall of the seed (Lee, et al. 2006). The dominant, but infrequently occurring, N_1 allele has a much stronger phenotypic expression than the recessive n_2 gene. The presence of four dominant N_1 alleles in tetraploid cottons results in a completely naked seed absent of all linters. This trait is expressed homogeneously in the plant. There is a severe reduction in the quantity of lint (between 0-15 percent) associated with this completely naked seed. The incorporation of the N_1 mutant into commercial cultivars has been prevented because of the unfavorable low quantity of lint (Tal and Gat 1994; Bechere, et al. 2009). It has been suggested that the variation in lint percent in N_1 lines may be due to the existence of multiple N_1 alleles in cotton or that there are other genes which may be present that interact with the N_1 allele in some manner to alter lint percent (Turley and Vaughn 2007). The N_1 phenotype has been described as producing sparse

and very short lint (Zhang, et al. 2007). The *N_1* allele was genetically mapped to chromosome 12 and may be related to but is not homeologous to *n_2* (Rong, et al. 2005; Samora, et al. 1994).

The recessive *n_2* allele occurs more often than N_1 and is expressed in many commercial varieties of *G. barbadense*. The n_2n_2 genotype does not produce a completely naked seed, since linters are retained at least at the tip in these lines. The *n_2n_2* phenotype is expressed heterogeneously and is susceptible to environmental conditions with bolls at the top of the plant containing seed with fewer fuzz fibers than bolls from the bottom of the plant (Bechere, et al. 2012; Tal and Gat, 1994). The *n_2* trait produces a substantial lint percent, usually above 30%. The lack of fuzz on these seeds makes it ideal for roller ginning. The *n_2* allele has shown a similar number of initials on the day of anthesis when compared with the control TM-1 and was dissimilar to N_1 and XinFLM phenotypes at one dpa. This suggests that fiber initiation is affected by mutants differently (Zhang, et al. 2007). The n_2 trait was initially assigned to chromosome 26 but has since been successfully mapped to chromosome 12 just outside of the N_1/*Fbl* region which is also located on chromosome 12.

Other relatively well described fiber mutants in cotton include the monogenic and dominant Ligon lintless-1 (*Li_1*) and Ligon lintless-2 (*Li_2*) (Rong, et al. 2005). These both contain extremely short fibers less than 10 mm and were termed lintless, as they contain very sparse lint (only enough to hold the seeds together) (Rong, et al. 2005; Giffee and Ligon 1929). Griffee & Ligon (1929) found that the lintless mutants experienced approximately 19% outcrossing to plants outside of the row during

progeny testing where the typical rate of outcrossing is 5-15%. The *Li_1* mutant was roughly mapped near then end on chromosome 18, while the *Li_2* mutant was also assigned to chromosome 18 with aneuploid stocks. (Rong, et al. 2005; Kohel, et al. 2002). Through EST analysis, the deficient elongation of Li_1 mutant fibers revealed several factors that contribute to fiber elongation, including the important function of HSP90 protein expression, cytoskeleton structure and related proteins, as well as calcium, and endoplasmic reticulum stress (Zhao, et al. 2010). Other known mutations include the incomplete dominant *Fbl*, a fiberless mutation that displays no lint or fuzz fibers, the recessive mutation h_a also with fiberless seed, and f_z, an epistatic recessive mutation that produces seed with fuzz fibers and no lint (Rong, et al. 2005).

Chapter III

Materials and Methods

Chemical Mutagenesis

The Texas High Plains (*Gossypium hirsutum* L.) cultivars of Atlas, Tejas, SC 9023, Sphinx, Explorer, Holland 338, and Rocket were selected for this study. In 1997, seeds were treated with 2.45% v/v ethyl methane sulfonate (EMS) at Texas Tech University in Lubbock, Texas. The M_0 generation seeds were imbibed in aerated distilled water for 16 hours and rinsed with distilled water. The seeds were then treated by pipetting EMS in aerated distilled water and mixing the solution for 2 hours producing the M_1 generation. Seeds were rinsed several times with distilled water and immediately hand planted in the field at the Quaker Research Farm at Texas Tech University. The remaining mutagen solution was denatured with a strong base before disposal as a potentially hazardous chemical.

Identification of Naked Seed Mutants

In 1998, one boll from each of the ~2,800 plants were harvested by hand to reduce the mutation load and to form the M_2 generation. Three M_3 plants from Atlas, SC9023, and Tejas with a partially naked seed coat were identified in 1999 after ginning (Figure 1.). The trait was stabilized through individual plant selections made between 2000 and 2003. Homozygous naked seed M_8 mutant lines were assessed for the influence of this trait on lint yield, lint percent, fibers/seed, fibers/mm^2, fiber quality, ginning efficiency, and yarn spinning performance with five commercial

cotton varieties (FiberMax 958 and FiberMax 989, Atlas, SC9023, and Tejas) in 2004, 2006, and 2007 at Lubbock, TX and at College Station, TX in 2007.

Crossing Naked Seed Mutants to Elite Lines

In 2008, crosses were made between three homozygous naked seed mutant lines (Atlas-ns-129-10-1-1-1, SC 9023-ns-13-2-3-1, and Tejas-ns-13-3-1-1) to fifteen elite lines (FM958, Hammer, SCM3-4, SC9023, TTU 0074,TTU 0774,TTU 0782, Holland 338, RO-409, Sphinx, Tejas-48-5-7-2-2, RO-303, RO-212, EM4-3, and Explorer). The elite line parents were chosen for high fiber quality, high lint yield potential, or high fiber initiation. Crossing was performed in the field at the Quaker Research Farm and in the greenhouse at Texas Tech University. The F_1-F_2 seed was increased in the greenhouse and selected lines were backcrossed to Hammer, Rocket, or SC9023 in 2009 in the greenhouse to form the BCF_1 generation.

Naked Seed Elite Selection

In 2010, 62 F_2 segregating lines were planted in the field at the Quaker Research Farm at Texas Tech University. Five individual plant selections from two replications of 36 lines were used to generate 360 total selections. Samples from selected lines were evaluated for lint yield, lint percent, and uniform naked seed. (Figure 1.) The lowest five bolls from selected F_3 plants were harvested to ensure overall boll maturity. Lint from 100 plants with superior naked seed was selected to undergo AFIS fiber quality analysis.

In 2011, 100 segregating F_3 lines were planted in the field with three controls (Atlas, Hammer and FM 958) to form the F_4 generation The seed cotton from three mature open bolls from three randomly selected plants in each plot were picked and ginned by hand in the field. The seeds ginned from each boll were visually estimated for uniform naked seed in the field. Only when the three bolls evaluated contained naked seed, the line was selected for further study. Five individual plant selections were taken from lines with ideal naked seed. Each five boll sample was carefully weighed and ginned by hand. The naked seed phenotype was visually evaluated for each plant (Figure 2.). Thirty-nine lines were selected for uniform naked seed and were evaluated for lint yield, lint percent, AFIS fiber quality, and Winseedle fiber initiation.

In 2012, thirty-nine F_4 lines were advanced to the F_5 generation with five controls (Atlas, FM 958, SC9023, Ballard NS (N_1N_1), and Mex. NS UA-3-3 (n_2n_2)) in May of 2012. Selected naked seed lines were crossed to low palmitic acid mutant lines in July of 2012 using the water method. Once flowering began, candles were identified in the afternoon the day before crossing and marked with flagging tape. At dawn, before the flower opened, petals at the top of the candle were removed and the flower was filled with water from a squirt bottle to sterilize the pollen. The water was carefully poured out of the flower. The flower was then emasculated manually. A one inch paper straw was placed on the stigma to prevent unintentional pollination. The flower was allowed to dry for one hour. Pollinator flowers from selected lines in the

field were used to manually pollinate the emasculated flowers. Each flower was tagged and allowed to form a boll (Table 1).

Field Design

From 1997 to present, naked seed cotton mutant lines were grown out at the Texas Tech University Quaker Research Farm. This research field is located in northwest Lubbock, Texas at an elevation of 988 meters above sea level on an Amarillo fine sandy loam/Acuff fine sandy loam soil. The field trials from 1997-2007 were replicated four times. In 2010 and 2011, selected naked seed lines were replicated but there was not enough seed for individual plant replications. The 2011 study had ten unreplicated single plants selected from ten lines, and the 2012 study had three unreplicated single plants selected from ten lines. In 2010 and 2011, a completely randomized field design was used. In 2011, a randomized design included black-eyed peas in a checkerboard pattern as a pollen block.

Throughout this study plots were planted with a commercial four row planter fitted with Almaco plot planting cones to sow ~100-150 seeds/plot. Plots were 9.14 meters long and 1.0 meter wide. Irrigation was supported with drip irrigation. The drip irrigation system had emitters spaced 30.5 cm linearly along the tape and the tape was located 25-30 cm below the surface of the bed and were. Planting occurred in late June in 2010 and May in 2011 and 2012 when a minimum soil temperature of 18.8°C was reached. Pre-irrigation was used to ensure the soil moisture profile was sufficient for seed germination. Supplementary in season irrigation was applied each year as year

depending on rainfall totals. Supplementary irrigation was applied at a rate of ~50.8 mm/week. Nitrogen fertilizer (32:0:0) was applied as urea through the drip system each year at first bloom at 78.5 kg/ha.

From 1997-present for this study, cotton was terminated using recommended rates of 1.7 kg/ha of Finish® and 34.3 kg/ha of Ginstar.® Harvest aid treatments were applied with Lee High Boy Spyder Sprayer with a CO_2 multi-boom sprayer. From 1997-2008 samples were harvested using a modified John Deere stripper and ginned using a 20-saw tabletop gin. Individual plant selections were harvested by hand picking lint from the lowest five bolls on the plant in 2010 and 2011. This was done to ensure overall maturity of bolls harvested. Additional bulk seed cotton was harvested using a John Deere stripper and ginned using a tabletop 20-saw gin.

Analysis

Fiber quality of lint from stripper harvest from 1997-2003 was analyzed at the Fiber and Biopolymer Research Institute (FBRI) at Texas Tech University using High Volume Instruments (HVI) and Advanced Fiber Information Systems (AFIS). The number of fibers/seed was measured using a 30 g sample manually ginned. The weight of the lint from 30 g was divided from the number of seeds to calculate lint/seed. Standard yarn analysis was performed to assess yarn quality. Nuclear Magnetic Resonance (NMR) Spectroscopy was used to determine indirect oil content. NMR technology measures the resonance energy absorbed by hydrogen atoms in the liquid

state of the sample. Gas Chromatography analysis was performed on samples to determine the fatty acid composition (Bechere, et al., 2009).

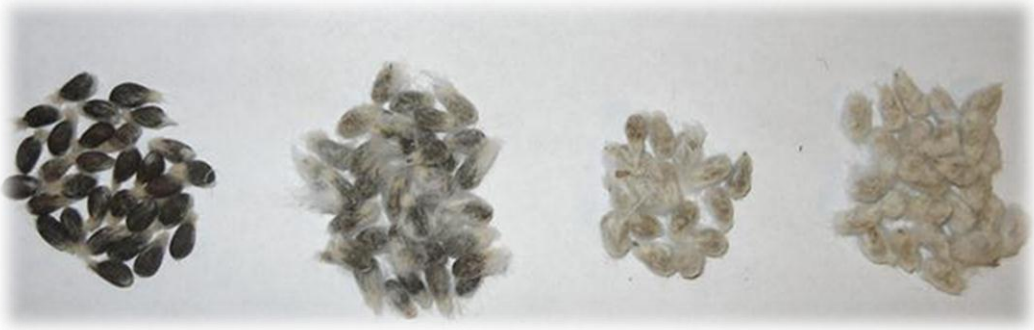
In 2010 and 2011, hand-harvested samples of five bolls each were assessed for AFIS fiber quality at the FBRI at Texas Tech University. In 2010, samples were blended prior to AFIS analysis to ensure AFIS testing was representative of the total sample. In 2011, fiber samples were not blended since samples were too small for blending. For yield analysis in 2010 and 2011, two 1 meter samples were harvested by hand from the center of each plot. These samples were initially weighed then ginned using tabletop, 20-saw gin. The lint and seed were weighed independently after ginning to determine percent lint, percent seed, and final lint yield. Lint percent was also estimated from each five boll sample hand harvested sample. Total sample weight, seed weight, and lint weight were taken separately. The lint weight was divided by the total weight to determine lint percent.

Naked seed was visually estimated on a scale of 0-100% (0 being fully fuzz and 100 being fully naked) in this study. The criterion for selection of naked seed lines was to classify the seed from each sample into one of four categories: (1) naked, (2) semi-naked, (3) semi-fuzzy, or (4) fuzzy (Figure 1). To reduce bias in the selection process, two individuals graded the seed from each plant. In 2011, naked seed was graded on a more precise grading system using a 1-16 grade scale (Figure 2). Plants with a grade of thirteen only were selected as they represented of the ideal naked seed phenotype. Three individuals each classified plants in this grading system for bias reduction.

Fiber initiation was calculated by measuring the number of fibers per seed and number of fibers per mm^2. Samples were weighed before and after hand ginning. Seed and lint were also weighed individually. Seed was then weighed after acid delinting. Seeds were counted and scanned for surface area using the Winseedle scanner. The number of fibers/seed, the number of fibers/sample, and the number of fibers/mm^2 were measure using ~100 seed from a five boll sample. Total sample weight, seed weight, and lint weight were taken separately. The product of the lint weight (g) and 1,000,000,000 (a correction factor) was divided by the product of fineness (mTex) and AFIS length by weight (mm) determined the number of fibers/sample. The number of fibers/sample was then divided by the number of seeds per sample to determine the number of fibers per seed. The number of fibers per mm^2 was determined by dividing the number of fibers/seed by the surface area.

The objective of this research was to stabilize the naked seed mutation in crosses while increasing lint yield, fiber quality, and fiber initiation. A secondary objective of this study was to analyze advanced naked seed mutant lines for lint yield, AFIS fiber quality, lint percent, and fiber initiation. The SAS 9.2 (SAS, Inc., Cary, NC) software package was used to analyze all data using the GLIMMIX procedure. Results were derived by examining the naked seed lines in this study in relation to one another and to control lines.

Figure 3.1. Four phenotypic categories of ranking linter level in naked seed mutants in 2010 (Bechere, et al., 2009).



Naked Semi-Naked Semi-Fuzzy Fuzzy

Figure 3.2. Sixteen phenotypic categories of ranking linter level in naked seed mutants in 2011 (Bechere, et al. 2012).

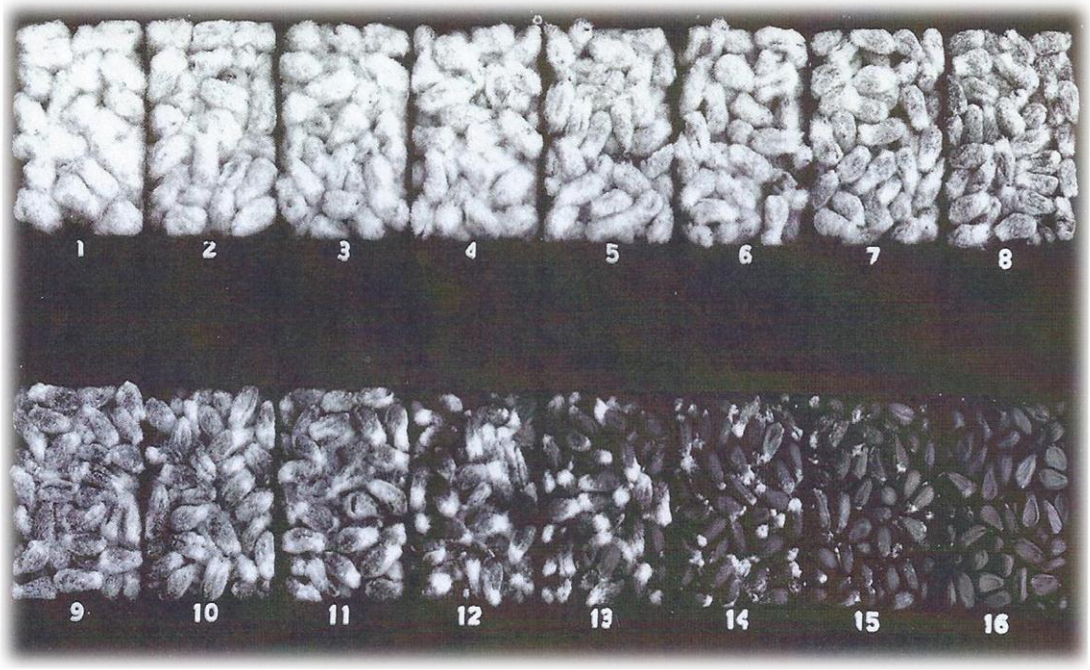


Table 3.1. Testing calendar of naked seed mutants in upland cotton (*Gossypium hirsutum* L.) from 2008-2012 at Texas Tech University in Lubbock, TX.

Year	Generation	Treatment	Discussion	Naked Seed	Percent Lint	Fiber Initials	Lint Yield	AFIS Quality
2008	F1	Cross to Elite Lines	Increase Yield & Quality					
2009	F1:F2	Greenhouse Increase		X				
	BCF1	Cross to Elite Lines						
2010	F2:F3	Field Trial	Trait Stabilization	X	X		X	X
		IPS						
2011	F3:F4	Field Trial	Trait Stabilization	X	X	X	X	X
		IPS						
2012	F4:F5	Field Trial	Trait Stabilization	X	X	X	X	
		IPS						

Chapter IV

Results and Discussion

This study on naked seed mutants in cotton was performed from 1997-2012 at Texas Tech University. Naked seed mutants were created through chemical mutagenesis using 2.45% v/v EMS in 1997. Subsequent identification and selection for the naked seed phenotype stabilized the lines over the course of seven years. Mutant lines were then crossed to select cultivars in 2008 and the lines were increased in 2009. In 2010 and 2011, individual plant selections were taken from lines derived from the crosses of select cultivars and naked seed mutants. Results reported here will be limited to the experiments performed from 2010 to 2012.

The objective of this research was to stabilize advanced lines of naked seed mutants selectively bred for increased lint yield, lint percent, fiber initiation, and fiber quality. Initial selection for the naked seed phenotype was conducted in 2010. The lines exhibiting the naked seed phenotype were then evaluated with three control lines in 2011 and five controls in 2012. In 2010 and 2011, lint yield, lint percent, and the AFIS fiber quality was determined. The AFIS fiber quality parameters evaluated were mean length (L), upper quartile length (UQL), short fiber content, nep size, neps per gram, seed coat nep (SCN) size, and SCN count per gram, fineness, and maturity ratio. In 2011, five additional indices were also evaluated: seed surface area, number of fibers per seed, number of fibers per mm^2, number of fibers per five-boll sample, number of seeds per five bolls. Data was analyzed using SAS 9.2 using the GLIMMIX

procedure. Results were derived by comparing the performance of the mutant lines with each other and the controls.

Naked Seed Segregation

Individual plants were harvested and hand ginned prior to grading for the naked seed phenotype. In 2010, 360 individual plants from 36 naked seed lines were categorized into four phenotypic categories: naked, semi-naked, semi-fuzzy, and fuzzy. Chi-square tests were done on the observed ratios of 18 lines to determine the potential types of segregation of the trait. Eight of the ten selected lines segregated at both 3:1 and 9:7 phenotypic ratios for the naked seed trait. (Table 4.1). In 2011, individual plants were graded on a 1-13 scale for naked seed. Twelve lines had a naked seed grade of 13 or higher. Ten of those were selected to undergo AFIS fiber quality analysis, fiber initiation analysis, and were grown out in 2012 (Table 4.2).

Lint Yield and Percent Lint

A hand harvested sample was collected from two one meter^2 areas in the center of each plot. The seed cotton weight of each sample was taken prior to ginning on a 20 saw table top gin. The lint and seed were weighed separately after ginning to allow the generation of the percent lint of each sample. In 2010, the naked seed mutants had a lint yield between 927 and 1426 kg/ha even though they were planted in late June which is better than any previous lint yield reported for other naked seed mutants except n^{+}_{4}. Atlas-ns-129/Hol 338-6 showed the best performance for lint yield and lint percent**.** (Table 4.3). However, its performance was only statistically better than two

lines: Atlas-ns-129/RO-409 and Atlas-ns-129//SCM3-4/SC9023 for lint yield. All of the naked seed lines contained excellent percent lint. Percent lint ranged between 35% and 41% which was exceptionally better than other described fiber mutants. The N_1N_1 has been reported to have less than 15% lint, while the n_2n_2 mutant has also been reported to have a lint percent of around 30%.

In 2011, Atlas-ns-129/Hol 338-6 had a lint yield of 922 kg/ha which was statistically superior to all other experimental lines and check cultivars (Table 4.4). The check cultivar FM958 performed better than all remaining lines except Atlas-ns 129/Tejas 48. The remaining naked seed lines had inferior lint yield to check cultivars although those differences were often not statistically significant. In 2011, every naked seed line and check cultivar performed poorly in part due to the severe drought West Texas experienced, even though these lines were all grown under full drip irrigation. Today's cotton growers expect lint yields to exceed three bales/ha, or 1681 kg/ha, in many commercial cultivars. The lint percent of naked seed lines in 2011 ranged between 38.3% and 47.3%. Atlas-ns-129/Hol 338-6 had significantly higher lint percent than all other mutant lines and check cultivars. Atlas-ns-129/Tejas was the second best performing mutant line with 39.7%.

AFIS Fiber Quality

The naked seed mutant lines developed through chemical mutagenesis generally have good fiber quality. AFIS fiber quality was measured on ten mutant lines in 2010 and on ten mutant lines and three check cultivars in 2011. In 2010, Atlas-

ns-129/Hol 338-37 had the longest fiber mean length by number and mean upper quarter length by weight (Table 4. 5). It was statistically superior to all other lines except Atlas-ns-129/Tejas 48 in both categories. Atlas-ns-129/Hol 338-37 exhibited a relatively mature and fine fiber with a maturity ratio of 0.94 and 174 mTex for fineness. Atlas-ns-129/Tejas 48 also exhibited excellent fiber length and maturity. However, the fiber was coarser than Atlas-ns 129/Hol 338-37 which measured 180 mTex. The poorest performing line was Hammer/SC 9023-ns which had very short mean fiber length, (22.60 mm), the largest number of neps (64 neps per gram), and a low fiber maturity ratio (0.89). It was significantly the lowest performing line when evaluated with AFIS fiber quality measurements.

In 2011, Overall AFIS fiber quality was decreased from the previous year except for maturity ratio with all lines except Atlas-ns-129/Tejas48a meeting the 0.90 threshold for maturity (Table 4.6). Every one of the naked seed mutant lines contained superior mean and upper quarter lengths than the three check varieties in 2011. This may have been due to the fact that naked seed samples were hand ginned while the three check cultivars were ginned with a 20-saw table top gin. The same can be said for a larger presence of neps and seed coat neps in check cultivars. Atlas-ns-129/Tejas48b had the highest mean length by number and upper quarter length by weight in 2011. It was however, not statistically better than Atlas-ns-129/RO-409-1, Atlas-ns-129/Hol338-6, or Atlas-ns-129/TTU 0782. Each of the four lines containing the highest mean length also exhibited acceptable fiber maturity ratios. Of the lines with the highest performance for lengths, Atlas-ns-129/TTU 0782 and Atlas-ns-

129/Hol 338-6 exhibited excellent fiber fineness (167 mTex and 172 mTex respectively). Atlas-ns-129/RO-409-1 and Atlas-ns-129/Tejas48b had a high level of undesirable coarse fibers and were spastically inferior to Atlas-ns-129/TTU 0782 and Atlas-ns-129/Hol 338-6.

Fiber Initiation

Fiber initiation of selected naked seed lines was measured in 2011 by measuring surface area with the WinSeedle seed scanner. Fibers/seed, fibers/sample, and fibers/mm^2 was then calculated using the AFIS mean length (mm), AFIS fineness (mTex), lint weight, and the number of seeds in the sample. Fibers/sample was derived from the product of lint weight and one billion (a correction factor) divided by the product of fineness and mean length. The number of fibers/number of fibers per seed was then calculated by dividing the number of fibers/sample by the total number of seeds in the sample. The number of fibers/mm^2 was calculated by dividing the number of fibers/sample by the surface area measured by the WinSeedle scanner.

Hammer/SC 9023-ns had significantly higher fiber initiation than any other naked seed line with 151 fibers/mm^2 and 18,600 fibers per seed (Table 4.7). That would indicate that Hammer/SC 9023-ns is a true cross because Hammer is known to exhibit exceptionally high fiber initiation. Other naked seed lines had between 120 and 98 fibers/mm^2 and between 11,000 and 9300 fibers/seed. Previously reported data indicates that fiber initiation rates approach 3,300 fibers/mm^2, however our research indicates that fiber initiation occurs at a much lower frequency than previously reported in cotton.

Table 4.1: Chi-square value of naked seed of 18 F2 lines grown in Lubbock, TX in 2010.

Pedigree	Total Naked	Naked Plants	X^2	X^2	X^2
	-n-	-n-	-3:1-	-9:7-	-13:3-
SC 9023-ns/Tejas 48	**20**	**16**	**0.07 (0.61)**	**2.01 (0.03)**	**0.00 (0.89)**
Atlas-ns-129/TTU 0782	**20**	**15**	**0.00 (0.61)**	**1.25 (0.08)**	**0.10 (0.36)**
Atlas-ns-129/RO-409	**20**	**14**	**0.07 (0.11)**	**0.67 (0.13)**	**0.31 (0.08)**
Atlas-ns-129/Hol 338-6	**20**	**14**	**0.07 (0.11)**	**0.67 (0.13)**	**0.31 (0.08)**
Atlas-ns-129/Hol 338-37	**20**	**13**	**0.27 (0.06)**	**0.27 (0.12)**	**0.65 (0.01)**
Atlas-ns-129//SCM3-4/SC 9023	**20**	**13**	**0.27 (0.06)**	**0.27 (0.12)**	**0.65 (0.01)**
SC 9023-ns/Tejas 48	**20**	**12**	**0.60 (0.30)**	**0.05 (0.30)**	**1.11 (0.10)**
Atlas-ns-129/Sphinx 4-2//FM958	**20**	**12**	**0.60 (0.30)**	**0.05 (0.30)**	**1.11 (0.10)**
Atlas-ns-129/RO-409	20	12	0.60 (0.30)	0.05 (0.30)	1.11 (0.10)
SC 9023-ns-57-13-3-1-2/SC 9023	20	10	1.67 (Reject)	0.14 (0.06)	2.40 (Reject)
Atlas-ns-129/Tejas 48	**20**	**10**	**1.67 (**Reject**)**	**0.14 (0.06)**	**2.40 (**Reject**)**
Hammer/SC 9023-ns	**20**	**10**	**1.67 (**Reject**)**	**0.14 (0.06)**	**2.40 (**Reject**)**
Atlas-ns-129/Tejas 48	20	8	3.27 (Reject)	0.94 (0.14)	4.19 (Reject)
SC 9023-ns-57-13-3-1-2/SC 9023//FM958	20	7	4.27 (Reject)	1.61 (0.05)	5.27 (Reject)
SC 9023-ns-57-13-3-1-2/SC 9023//FM958	20	6	5.40 (Reject)	2.45 (0.01)	6.47 (Reject)
SC 9023-ns-57-13-3-1-2/SC 9023	20	4	8.07 (Reject)	4.67 (Reject)	9.24 (Reject)
Tejas-ns-28-13-3-1-1/TTU 0782	20	3	9.60 (Reject)	6.05 (Reject)	10.80 (Reject)
Hammer/SC 9023-ns	20	2	11.27 (Reject)	7.61 (Reject)	12.50 (Reject)
Average	**20**	**13**	**2.74**	**1.61**	**3.39**

Table 4.2. Average and range of linter grade from five individual space plants selected from 39 F3 naked seed lines at Lubbock, TX in 2011 using a standard index (Bechere, et al., 2012). Lines shown in bold were advanced to 2012.

Pedigree	Grade	Min	Max	Standard Deviation
	-n-	-n-	-n-	
Atlas-ns-129/Tejas 48-2	**13**	**13**	**13**	**0.00**
Atlas-ns-129/RO-409-1	**13**	**12**	**13**	**0.45**
Atlas-ns-129/Hol 338-6	**13**	**12**	**13**	**0.45**
SC 9023-ns/Tejas 48a	**13**	**12**	**13**	**0.50**
Atlas-ns-129/TTU 0782	**13**	**12**	**13**	**0.58**
Atlas-ns-129/RO-409-2	**13**	**12**	**13**	**0.55**
SC 9023-ns/Tejas 48b	**13**	**12**	**13**	**0.55**
Hammer/SC 9023-ns	**13**	**12**	**13**	**0.55**
Atlas-ns-129/Tejas 48-1	**13**	**11**	**13**	**0.89**
Atlas-ns-129/RO-409-3	**13**	**12**	**13**	**0.58**
Atlas-ns-129/Tejas 48	13	12	13	0.55
Atlas-ns-129/TTU 0782	13	12	13	0.55
Atlas-ns-129//SCM3-4/SC 9023	12	12	13	0.55
Atlas-ns-129/Hol 338-6	12	12	13	0.55
Atlas-ns-129//SCM3-4/SC 9023	12	11	13	0.89

Table 4.2. continued.

Pedigree	Grade	Min	Max	Standard Deviation
	-n-	-n-	-n-	
Atlas-ns-129//SCM3-4/SC 9023	12	12	13	0.55
SC 9023-ns/Tejas 48	12	12	13	0.45
Atlas-ns-129/Sphinx 4-2//FM958	12	11	13	0.96
Atlas-ns-129/Hol 338-6	12	11	13	1.00
Atlas-ns-129/Hol 338-37	12	11	13	0.71
Atlas-ns-129/Hol 338-6	12	11	13	0.71
Atlas-ns-129/Tejas 48	12	11	13	0.82
SC 9023-ns/Tejas 48	12	12	12	0.00
Atlas-ns-129/Hol 338-6	12	10	13	1.10
Atlas-ns-129/Sphinx 4-2//FM958	12	11	13	0.84
Atlas-ns-129/Hol 338-6	12	9	13	1.64
Atlas-ns-129/Tejas 48	12	10	13	1.10
SC 9023-ns/Tejas 48	12	10	13	1.30
Atlas-ns-129/ro-409-3-1-1	12	10	12	1.00
Atlas-ns-129/Hol 338-37	11	10	13	0.58

Table 4.2. continued.

Pedigree	Grade	Min	Max	Standard Deviation
	-n-	-n-	-n-	
Atlas-ns-129/Tejas 48	11	10	13	1.30
Hammer/SC 9023-NS-13-2-3-1	11	9	12	1.41
Atlas-ns-129/Hol 338-37	11	10	12	1.00
Atlas-ns-129/RO-409	11	7	12	2.24
SC 9023-ns/Tejas 48	11	8	13	2.22
Atlas-ns-129//SCM3-4/SC 9023	11	8	13	1.92
Atlas-ns-129/RO-409	11	10	12	1.10
SC 9023-ns/Tejas 48	11	8	12	1.89
Hammer/SC 9023-ns	10	8	12	1.79
Average	**12**	**11**	**13**	**0.51**

Table 4.2 Lint yield and average percent lint of 10 F_2 lines selected for naked seed grown at Lubbock, TX in 2010.

Pedigree	**Lint Yield**	**Percent Lint**
	-kg/ha-	-% cotton-
Atlas-ns-129/Hol 338-6	1462 a†	0.41 a†
Atlas-ns-129/Hol 338-37	1320 ab	0.35 bc
Hammer/SC 9023-ns	1284 ab	0.37 bc
SC 9023-ns/Tejas 48a	1255 ab	0.38 ab
Atlas-ns-129/TTU 0782	1220 ab	0.39 ab
SC 9023-ns/Tejas 48b	1113 ab	0.37 bc
Atlas-ns-129/Tejas 48	1048 ab	0.38 ab
Atlas-ns-129/Spinx4-2//FM958	- -	0.39 ab
Atlas-ns-129/RO-409	1020 b	0.38 ab
Atlas-ns-129//SCM3-4/SC 9023	927 b	0.39 ab
CV %	20.2%	14.4%

† Means not followed by the same letter differ at the 0.05 level of probability by Fisher's Protected Least Significant Difference Test.

Table 4.3. Lint yield and percent lint of ten naked seed lines and three check cultivars grown at Lubbock, TX in 2011.

Line	Lint Yield	Percent Lint
	-kg/ha-	-%-
Atlas-ns-129/Holland338-6	922 a†	47.3 a†
FM 958	822 ab	42.5 b
Atlas	779 abc	40.1 bc
Atlas-ns-129/Tejas 48	739 abcd	39.7 bc
Hammer	678 bcde	47.6 a
Atlas-ns-129/RO-409	669 cde	41.9 b
Atlas-ns-129//SCM 3-4/SC 9023	653 cde	41.1 bc
Atlas-ns-129/Hol 338-37	650 cde	38.4 c
Atlas-ns-129/Sphinx4-2//FM958	639 def	41.0 bc
Atlas-ns-129/TTU 0782-1-5-2	619 ef	40.7 bc
SC9023-ns-57/Tejas48b	549 efg	39.3 bc
Hammer/SC9023-ns	535 gf	41.8 b
SC9023-ns-57/Tejas48a	492 g	40.5 bc
CV%	20.8%	7.2%

† Means not followed by the same letter differ at the 0.05 level of probability by Fisher's Protected Least Significant Difference Test.

Table 4.4. Advanced Fiber Information Systems (AFIS) fiber quality analysis of selected lines for mean length b number, upper quarter length by weight, fineness, maturity ratio, seed coat neps, and neps averages of ten F_2 lines at Lubbock, TX in 2010.

Pedigree	Length (n)	Upper Quarter Length (w)	Fineness	Seed Coat Neps	Neps	Maturity Ratio
	-mm-	-mm-	-mTex-	-Cnt/g-	-Cnt/g-	
Atlas-ns-129/Hol 338-37	26.3 a†	33.9 a†	174 def†	12 ab†	51 bc†	0.94 bc†
Atlas-ns-129/Tejas 48	25.9 a	34.3 a	188 bc	6 cd	40 cde	0.95 ab
Atlas-ns-129/RO-409	25.0 b	31.2 cde	190 b	7 cd	36 e	0.94 ab
Atlas-ns-129/TTU 0782	24.9 bc	32.1 bc	189 b	6 cd	37 e	0.96 a
SC 9023-ns/Tejas 48a	24.6 bc	32.0 bc	169 f	9 bc	51 b	0.92 d
SC 9023-ns/Tejas 48b	24.4 bc	31.6 cd	179 cde	8 bcd	57 ab	0.89 e
Atlas-ns-129/Hol 338-6	24.1 cd	32.5 b	181 bcd	8 bcd	48 bcd	0.91 d
Atlas-ns-129/Sphinx4-2//FM958	23.4 de	30.8 de	168 f	5 d	40 cde	0.92 cd
Atlas-ns-129//SCM3-4/SC 9023	22.9 ef	29.4 f	207 a	5 cd	38 ed	0.95 ab
Hammer/SC 9023-ns	22.6 f	30.3 fe	170 ef	13 a	64 a	0.89 e
CV %	4.2%	3.5%	5.7%	55.5%	26.0%	2.1%

† Means not followed by the same letter differ at the 0.05 level of probability by Fisher's Protected Least Significant Difference Test.

Table 4.5. Advanced Fiber Information Systems (AFIS) fiber quality analysis of selected lines for mean length by number, upper quarter length by weight, fineness, maturity ratio, seed coat neps, and neps average of F_3 lines at Lubbock, TX in 2011.

Pedigree	Length (n)	Upper Quarter Length (w)	Fineness	Seed Coat Neps	Neps	Maturity Ratio
	-mm-	-mm-	-mTex-	-Cnt/g-	-Cnt/g-	
Atlas-ns-129/Tejas48b	25.0 a†	30.4 ab†	188 abc†	2 d†	65 d†	0.98 a†
Atlas-ns-129/RO-409-1	24.6 ab	29.6 bcd	189 ab	1 d	62 d	0.99 a
Atlas-ns-129/Hol338-6	24.4 abc	30.7 a	177 abcd	3 cd	82 bcd	0.95 abc
Atlas-ns-129/0782-1-5-2	23.9 abc	29.9 abc	172 de	3 cd	71 cd	0.96 abc
SC 9023-ns/Tejas48a	23.6 bcd	29.3 bcde	167 def	3 cd	123 bc	0.91 cde
Atlas-ns-129/RO-409-2	23.5 bcd	28.0 fg	192 a	2 d	58 d	0.99 a
SC 9023-ns/Tejas48b	23.5 bcd	29.0 cdef	173 cde	1 d	101 bcd	0.93 bcde

Table 4.6. continued

Pedigree	Length (n)	Upper Quarter Length (w)	Fineness	Seed Coat Neps	Seed Coat Neps	Maturity Ratio
	-mm-	-mm-	-mTex-	-Cnt/g-	-Cnt/g-	
Atlas-ns-129/RO-409-3	23.4 bcd	28.3 ef	178 abcd	3 cd	62 d	0.95 abcd
Hammer/SC 9023-ns	23.1 cd	28.6 def	154 fg	3 cd	77 cd	0.90 e
Atlas-ns-129/Tejas 48a	22.5 d	28.6 def	145 g	1 d	107 bcd	0.89 e
FM 958	20.8 e	28.3 f	180 abcd	5 bc	131 b	0.96 ab
Atlas	20.0 ef	27.3 g	175 bcd	7 b	199 a	0.93 bcde
Hammer	19.4 f	26.4 h	162 ef	15 a	181 a	0.91 de
CV %	3.5%	2.3%	5.7%	36.2%	29.2%	2.9%

† Means not followed by the same letter differ at the 0.05 level of probability by Fisher's Protected Least Significant Difference Test.

Table 4.7. Fiber initiation of ten naked seed lines grown at Lubbock, TX in 2011.

Pedigree	**Fibers/mm^2**	**Fibers/Seed**	**Fibers/Sample**	**Surface Area**
	-n-	-thousands-	-millions-	-mm-
Hammer/SC 9023-ns	151 a†	18.6 a†	1.85 a†	123a†
Atlas-ns/Hol 338-6	120 b	11.0 b	1.44 abcd	92 ef
SC 9023-ns/Tejas 48b	117 b	11.0 b	1.45 abcd	94 def
SC 9023-ns/Tejas 48a	117 b	11.8 b	1.69 ab	101bcde
Atlas-ns-129/RO-409-1	116 bc	11.9 b	1.45 abcd	103 bcd
Atlas-ns-129/TTU 0782	108 bcd	11.9 b	1.32 bcd	111 b
Atlas-ns-129/RO-409-2	107 bcd	11.2 b	1.18 d	105 bc
Atlas-ns-129/RO-409-3	105 bcd	11.1 b	1.63 abc	106 b
Atlas-ns-129/Tejas 48-1	100 c	8.7 c	1.28 bcd	87 f
Atlas-ns-129/Tejas 48-2	98 d	9.3 c	1.24 cd	95 cdef
CV %	9.0%	7.9%	17.2%	5.7%

† Means not followed by the same letter differ at the 0.05 level of probability by Fisher's Protected Least Significant Difference Test.

Chapter 5

Conclusion

The primary objective of this study was to stabilize a naked seed mutation in Upland cotton mutants created through chemical mutagenesis and increase fiber initiation, lint yield, and percent lint of selected lines. Over the course of this study, from 2010-2012 the naked seed phenotype was made more stable. Lint yield, turnout, and fiber properties of these mutants were shown to be are superior to other known naked seed mutants and comparable to control cultivars with fuzzy seed.

Phenotypic segregation in the majority of the F_2 populations at a perceived 3:1 phenotypic ratio leads us to believe the naked seed mutant were a dominant trait controlled by at least one allele. The chi-square analysis of segregation also fit a 9:7 phenotypic ratio for most populations so there may be additional alleles controlling this trait at a separate locus (Table 4.1). Additional allele testing is needed to properly evaluate these new naked seed mutants. The naked seed phenotype was a very stable after three cycles of selection (Table 4.4). In 2011, the ten selected lines had an average naked seed grade of 13 with a standard deviation ranging from 0.00 and 0.89. The standard deviation of the unselected lines ranged from 0.45 to 2.24 with an average naked seed grade of 12 indicating possible segregation.

The naked seed mutants created through chemical mutagenesis had a lint yield which approached 2-3 bales per acre (1121 to 1681 kg/ha) and a turnout in excess of 37% in 2010 despite being planted in late June (Table 4.2). Despite the excessive

drought of 2011, yields ranged from 1 to almost 2 bales per acre with turnout ranging from 38.4 to 47.3%. (Table 4.5). The lint yield and turnout of the lines we selected was superior to any previously described naked seed mutant except n^{+}_{4} (Bechere, et al. 2009). Also naked seed mutant lines had excellent AFIS length, a minimal number of seed coat neps, very few neps, acceptable fineness, and high maturity ratios.

The naked seed mutants created through chemical mutagenesis show great potential for improving cotton quality on the Texas High Plains. Seed cotton from the naked seed mutant lines could be ginned with roller gins and delinting requirements could be reduced. These mutants have an acceptable yield potential, excellent turnout, and superior fiber quality properties. In the future we will perform experiments to determine if these naked seed mutants are allelic to other known fiber mutants N_1N_1, n_2n_2, or $n^{+}_{4}n^{+}_{4}$. With continued improvement we may be able to commercialize this valuable trait in upland cotton in the future.

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