# 1849 Use of Chemical Mutagenesis in Improving Upland Cotton

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**ABSTRACT**

In 2002, six cotton (*Gossypium spp*.) genotypes were treated with three differential concentrations of Na Azide and Ethyl MethaneSulfonate (EMS) as well as a distilled $H_2O$ control to determine effective dosages for chemical mutagenesis in cotton. Chemical treatment, genotype and the interaction of chemical treatment X genotype had a significant impact on germination and field emergence. Both 3% v/v EMS and 0.05% v/v Na Azide reduced seed viability to less than 50%. It also appeared that the diploid genotypes generally had a higher mortality that the tetraploid genotypes. Geneticists using mutagenesis should conduct initial studies to determine appropriate rates for specific mutagens prior to treating selected genotypes. It was also noted that use of seed lots with good germination would enhance the recovery of useful mutants.

Several mutant lines selected for the naked-tufted phenotype phenotype from the cultivars Atlas, SC 9023, and Tejas were grown in replicated plots at Lubbock, TX to determine lint yield and lint percent in both 2005 and 2006. The fibers harvested from these plots were analyzed at the Texas Tech University – International Textile Center using High Volume Instruments (HVI), Advanced Fiber Information Systems (AFIS), and standard yarn quality analyses (2005 fiber only). These trials indicate that selected naked-tufted mutants had comparable lint yields and lint percent of both the parental cultivars and commercial check cultivars. Several of the mutants also had very good HVI and AFIS fiber quality a significant reduction in seed coat neps.

## Introduction

Cotton is the world's leading textile fiber and it is grown on over six million hectares in the United States (US) (NASS, 2006). Cotton has historically been grown on more acres in this country than any other crop except for corn, wheat, and soybeans. Cotton fiber generates in exceeds of \$6 billion$yr^{-1}$, while cottonseed oil and meal add another \$500 million$yr^{-1}$ to the total US agricultural economy. More than 440,000 domestic jobs are created by cotton production and processing which has a total impact of over \$40 billion on the U.S. gross domestic product (National Cotton Council, 2005). United States cCotton exports also contribute about \$2 billion $yr^{-1}$ to our the U.S. trade balance.

United States USUnited States cotton production is under increasing competition from synthetic fibers for the manufacture of yarns and textiles. In addition, the continuing decline of the US textile industry requires that over a third of U.S. produced cotton lint must be sold

to international markets (National Cotton Council, 2005). Increasing the length, durability and strength of cotton fiber through genetic improvement should help U.S. grown cotton to compete both with synthetic fibers and in International markets.

There have been several genetic bottlenecks which severely restrict the genetic variability available in cultivated cotton. Upland cotton, *Gossypium hirsutum*, is thought to have arisen as a the result of an interspecific hybridization between an A- and D-genome diploid species about 1-2 million years ago (Wendel,1989). This single polyploidization event was the first genetic bottleneck. that constrains genetic variation available for cotton improvement. A second bottleneck was associated with occurred during the domestication of *G. hirsutum* from using only a small subset of the wild genotypes. A third bottleneck resulted from occurred when only limited sampling of only a few semi-domesticated progenitors from the Mexican-Guatemalan border which provided border were used to generate the germplasm base of our modern elite cotton gene pool. These These initial selections lines were brought northward into the U.S. and eventually into China, India, Australia and other countries (Hutchinson et al., 1947),. Finally, intensive breeding for narrowly-defined fiber quality parameters required for processing of this industrial crop has further narrowed the gene pool of commercial cotton germplasm that is commercially acceptable (May, et al.,1995)

The use of chemically-induced mutants has been highly successful in most major crops grown across the world but has only occasionally been used in improving cotton (Auld, et al., 1998). However, the relatively low level of genetic variability currently available in cotton would indicate this would be an ideal tool to increase genetic variability in this species. Mutagenesis has been shown to be an effective tool to create a wide range of phenotypic variation in both diploid and tetraploid *Gossypium* populations (Auld, et al., 2000; Larik, et al., 1983; Gaibullaev, et al., 1976; Hussein, et al., 1982 and Shattuck and Katterman, 1982). Some of these mutants such as the 'naked and tufted' trait have immediate application to cotton improvement by reducing seed coat neps as well as the energy and time required in cotton ginning. Other mutants created by the process will provide The creation of these new mutants is a powerful tools critical for both functional genetics and conventional genetic improvement studies in of cotton.

## MATERIALS AND METHODS

**Mutation Rate Study:** In the spring of 2002, seed of six cotton lines were exposed to a distilled $H_20$ Control, three rates of Ethyl MethaneSulfonate (EMS), and three rates of Na Azide at Texas Tech University. The six lines included three tetraploid - *G. hirsutum* lines (FiberMax 958, ACALA 1517-99, and TAM 94L-25), one tetraploid - *G. barbadense* (Pima S-7) and three diploid lines – *G. arboreum* (GA-TAMU, A2-60T, and A2-120W). Four samples of 250 what? and four samples of 50 untreated cotton seed ($M_0$ generation) from each of the six lines were imbibed for 16 to 20 hours in aerated distilled water. The seed were then rinsed and placed in aerated distilled water. The EMS and Na Azide treatments were pipetted into the solution under where it is allowed to mix with the seed for two hours to produce the $M_1$ generation. The seeds were then removed and rinsed several times to remove any residual mutagen prior to hand planting the larger samples of $M_1$ seed in the field or conducting laboratory germination trial on the smaller seed samples. The remaining solutions were treated with a strong base to denature the mutagens before disposal as potentially hazardous chemicals. The four 50 seed samples of the treated $M_1$ seed were tested to determine germination 4 and 10 days after treatment in the growth chamber. Counts were made on the four 250 field planted seed samples 7, 14, and 21 days after

planting to determine the rate of emergence under field conditions. Means of all treatments were subject to analyses of variance and the means separated using a Fisher's Protected LSD at the 0.05 level of probability (SAS, 1992).

In the fall of 2002, the $M_2$ seed was harvested by removing one boll per plant. The $M_2$ generation was increased to the $M_3$ in 2004, the $M_4$ in 2005, and the $M_5$ using this same process. Because the $M_1$ generation is often highly chimeric, single plant selections were not made until the $M_3$ or later generations. Many of these populations are now being screened for fiber quality, growth habit, and physiological mutations.

**Naked and Tufted Mutant Study:** In 1996, six commercial varieties of cotton were treated with 2.45% v/v ethyl methanesulfonate. In 1999, three $M_3$ plants were identified identified from single mutant lines from the cultivars Atlas, SC 9023 and Tejas that had partially naked seed coats similar to those previously described as "naked-tufted" (Endrizzi and Ray, 1991). From 2001 to 2004, selections from the three $M_3$ naked-tufted seed coat mutants were evaluated in replicated trials at Lubbock, Texas. These trials were conducted to stabilize this trait and to evaluate the impact of the naked-tufted phenotype on lint yield, lint percent, fiber quality, and yarn spinning performance. In 2001, the initial three mutants were visually screened for the expression of naked-tufted seed coats. Fifteen individual plants exhibiting the naked-tufted phenotype were selected and grown in the field in 2002. This process was repeated in 2003 and 2004 to generated 40 individual plant selections with naked-tufted seed coats. In 2005 and 2006, these 40 selections, three parental lines and five check varieties were evaluated for expression of the naked-tufted seed coat trait, lint yield and lint percent. The fibers harvested from these plots were analyzed at the Texas Tech University – International Textile Center using High Volume Instruments (HVI), Advanced Fiber Information Systems (AFIS), and standard yarn quality analyses

## RESULTS AND DISCUSSIONS

**Mutation Rate Study:** Mutagen treatment, genotype and the interaction of mutagen treatment X genotype had a significant impact on both germination and field emergence. When measured with either laboratory germination or field emergence, the 0.0005 v/v Na Azide, 0.005 v/v Na Azide and 0.01% v/v EMS treatments did not decrease the viability of the seed (Table 1). However, the 0.05 v/v rate of EMS almost completely destroyed seed viability. The 3% v/v EMS and 0.05% v/v Na Azide treatments reduced seed viability to less than 50%. Many geneticists conducting chemical mutagenesis like to burn the population so that seed viability is reduced by approximately 50% (Auld, et al., 1998).

The laboratory seed germination of the six lots treated with just $H_20$ of seed ranged from 12.1% to 63.6%. The germination and field emergence data of the mutagenic were adjusted to a pure live seed basis to allow appropriate comparisons (Table 2). The diploid genotypes of *G. arboreum* and the single genotype of *G. barbadense* generally had a higher mortality rate when exposed to the mutagens than the *G. hirsutum* genotypes in both laboratory germination and field emergence (Tables 3 and 4). Geneticists using mutagenesis should conduct initial studies to determine appropriate rates for specific mutagens prior to treating selected genotypes. Use of seed lots with good germination and seedling vigor would enhance the recovery of useful mutants with chemical mutagenesis.

Even though this study had relatively small populations in the field, counts were made to determine the number of plants in each treatment which had chlorophyll chimeric plants. This index is often indicative of the relative effectiveness of different mutagenic treatments. It was interesting that seeds exposed to the water control and two lower rates of Na Azide

did not produce chimeric plants (Table 5). However, the highest rate of EMS (0.05% v/v) produced 16 chimeric plants despite an average rate of field emergence of 3.1%. The other 5 chimeric plants were found in seedlings exposed to the highest rate of Na Azide and the other two rates of EMS.

**Naked and Tufted Mutant Study**: The naked-tufted phenotype was correlated with a reduction in seed coat neps (r = -0.47**) and an increase in seed oil content (r = 0.68**). One of the mutants selected from SC 9023 (SC 9023-NS-57-13-3) produced yarn that provided exceptional performance for ring spinning applications. Future studies are being conducted to will be needed to determine the inheritance and potential impact of the naked-tufted phenotype on other agronomic production characteristics of upland cotton such as the energy & time required in ginning.

These mutants appear to reduce or eliminate the occurrence of fuzz or linters which are short fibers tightly attached to the seed coat in most upland cotton varieties. These mutants appear to be phenotypically similar to the "naked-tufted" mutant initially described by Endrizzi and Ray in 1991. They concluded that the $N^t_2$ marker was simple dominant gene that was allelic to $n_2$ (Endrizzi and Ray, 1991) Their studies also showed that this gene was located on the long arm of chromosome 26. Our initial interest in this phenotype was based on the hypothesis that the removal of linters during saw ginning contributed significantly to increased short fiber content in upland cotton. Consequently, genetic elimination of the linters would reduce the short fiber content while simplifying ginning, oil recovery, and delinting of cottonseed.

Turley and Kloth had earlier reported on the impact of the $N_1/n_1$, $N_2/n_2$, and the $N_3/n_3$ genes on lint percent in cotton (Turley and Kloth, 2002). They proposed that only the genotype, $n_1n_1N_2N_2N_3N_3$, would generate a normal lint percent of 40.5%. A second study by Lee, et al., reported on the gene expression of the $N_1N_1$ genotype and its impact on fuzz development (Lee, et al. 2006). Both of these studies did not include analyses of the $N^t_2$ allele or an estimate of its impact on lint percent.

Based on data collected on 2005 fiber, it appeared that a selected line with a partially naked seed coat (SC 9023-NS-57-13-3) did have reduced short fiber content, enhanced maturity, and higher quality yarn in ring spinning. However, not all partially naked or naked seed coat lines showed similar performance. This would indicate that other factors in addition to seed coat characteristic impart short fiber content and fiber maturity. Therefore, these trials are being repeated in 2006. HVI analyses did not identify this line as superior, but AFIS analyses showed that this line had very low neps/gram, low short fiber content (weight), and a very high maturity ratio. This study showed that cotton breeders need to increasing rely on AFIS analyses in late generation screening for fiber quality. Future studies will be needed to determine if the enhanced yarn characteristics of SC 9023-NS-57-13-3 are the result of the partially naked seed coat characteristic or other traits inherent in this line.

Repeated from previous paragraph

We now believe the "naked and tufted" seed mutant may be critical to the development of ELS (Extra Long Staple) stripper cotton varieties in Texas. It also appears this mutation could significantly reduce both the time and the energy required for ginning, oil extraction, and delinting of cottonseed. The superior fiber quality and improved energy efficiency obtained with the naked and tufted mutant will drive its rapid incorporation into new varieties.

**LITERATURE CITED**


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**Table 1. Germination and field emergence of cotton exposed to seven mutagenic agents average over six genotypes at Lubbock, Texas in 2002.**

| Mutagen | Germination | | | Field Emergence | | |
|---|---|---|---|---|---|---|
| **Dose** | **Day 4** | **Day 10** | | **Day 7** | **Day 14** | **Day 21** |
| | - - - - - - - - - - - - - - - - - - Percent - - - - - - - - - - - - - - - - - - | | | | | |
| **Water:** | | | | | | |
| (1) Control | 100.0 a† | 100.0 a† | | 39.8 b† | 50.0 b† | 46.4 b† |
| **Na Azide:** | | | | | | |
| (5) 0.0005 v/v | 58.8 b | 78.2 c | | 42.7 b | 46.4 bc | 47.0 b |
| (6) 0.005 v/v | 47.7 c | 90.5 b | | 51.1 a | 58.5 a | 58.3 a |
| (7) 0.05 v/v | 11.0 d | 46.5 d | | 27.3 c | 46.4 bc | 48.3 b |
| **Ethyl MethaneSulfonate:** | | | | | | |
| (2) 0.01 v/v | 61.7 b | 77.3 c | | 30.5 c | 40.0 c | 40.1 b |
| (3) 0.03 v/v | 4.4 e | 25.0 e | | 9.5 d | 21.5 d | 20.8 c |
| (4) 0.05 v/v | 0.2 f | 0.4 f | | 0.1 e | 1.5 e | 1.6 d |
| **Coefficient of Variation** | 18.9% | 15.3% | | 51.1% | 36.1% | 38.8% |

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

**Table 2. Germination and field emergence of six genotypes of cotton averaged over seven mutagenic treatments at Lubbock, TX in 2002.**

| Species | Non-Adjusted | Germination | | Field Emergence | | |
|---|---|---|---|---|---|---|
| **Genotype** | **Germination** | **Day 4** | **Day 10** | **Day 7** | **Day 14** | **Day 21** |
| | - - - - - - - - - - - - - - - - - - - - - - Percent - - - - - - - - - - - - - - - - - - - - - - | | | | | |
| ***G. hirsutum*** | | | | | | |
| (4) TAMU 942-25 | 49.9 c[†] | 48.5 b[†] | 73.3 a[†] | 41.9 a[†] | 54.0 a[†] | 52.2 ab[†] |
| (5) ACALA 1517-99 | 63.6 a | 51.1 b | 67.7 b | 46.1 a | 60.4 a | 59.0 a |
| | | | | | | |
| ***G. barbadense*** | | | | | | |
| (3) Pima S-7 | 36.6 d | 43.6 c | 62.6 c | 21.2 bc | 14.6 d | 15.3 d |
| | | | | | | |
| ***G. arboreum*** | | | | | | |
| (7) *G.a* (TAMU) | 59.3 b | 58.9 a | 70.1 ab | 28.0 b | 44.9 b | 44.5 b |
| (2) $A_2$ 60-T | 14.0 e | 24.2 d | 45.2 d | 17.9 c | 22.3 c | 25.0 c |
| (1) $A_2$ 120-W | 12.1 e | 17.0 e | 39.1 e | 17.2 c | 27.5 c | 28.9 c |
| | | | | | | |
| **F Test** | | | | | | |
| Geno x Trmt | | 28.0** | 10.5** | 2.4** | 4.5** | 4.1** |
| | | | | | | |
| **Coefficient of Variation** | | 18.9% | 15.8% | 51.1% | 36.1% | 38.8% |
| | | | | | | |

[†] Means within a column not followed by the same letter differ at the 0.05 level of probability by Fisher's Protected Least Significance Test at the 0.05 level of probability.

**Table 3. Germination after 10 days of six genotypes of cotton exposed to seven mutagenic treatments in Lubbock, TX in 2002.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Mutagen** | **TAMU** | **ACALA** | **Pima** | **TAMU** | **$A_2$** | **$A_2$** | |
| **Dose** | **946-25** | **1517-99** | **S-7** | **GA** | **60-T** | **120-W** | |
| | - - - - - - - - - - - - - - - - - - - - - - Percent - - - - - - - - - - - - - - - - - - - - - - | | | | | | |
| **Water** | | | | | | | |
| (1) Control | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | |
| | | | | | | | |
| **Na Azide** | | | | | | | |
| (5) 0.0005 v/v | 93.3 | 93.0 | 85.3 | 98.8 | 44.0 | 43.8 | |
| (6) 0.005 v/v | 95.5 | 93.0 | 91.8 | 100.0 | 79.0 | 83.5 | |
| (7) 0.05 v/v | 52.3 | 52.0 | 52.0 | 73.8 | 19.5 | 22.5 | |
| | | | | | | | |
| **Ethyl MethaneSulfonate** | | | | | | | |
| (2) 0.01 v/v | 97.0 | 90.5 | 97.5 | 91.5 | 63.0 | 24.3 | |
| (3) 0.03 v/v | 75.3 | 38.3 | 11.8 | 24.5 | 0.0 | 0.0 | |
| (4) 0.05 v/v | 0.0 | 0.0 | 0.0 | 2.3 | 0.0 | 0.0 | |
| | | | | | | | |
| **LSD (0.05) = 5.2%** | | | | | | | |
| | | | | | | | |

**Table 4. Field emergence after 21 days of six genotypes of cotton exposed to seven mutagenic treatments in Lubbock, TX in 2002.**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Mutagen** | **TAMU** | **ACALA** | **Pima** | **TAMU** | **$A_2$** | **$A_2$** |
| **Dose** | **946-25** | **1517-99** | **S-7** | **GA** | **60-T** | **120-W** |
| | - - - - - - - - - - - - - - - - - - Percent - - - - - - - - - - - - - - - - - - - - - - |
| **Water** | | | | | | |
| (1) Control | 63.0 | 62.8 | 28.0 | 51.5 | 40.3 | 32.8 |
| | | | | | | |
| **Na Azide** | | | | | | |
| (5) 0.0005 v/v | 66.5 | 81.5 | 20.0 | 58.5 | 30.5 | 25.3 |
| (6) 0.005 v/v | 67.3 | 84.0 | 14.8 | 53.8 | 54.0 | 76.3 |
| (7) 0.05 v/v | 59.0 | 73.8 | 19.3 | 65.5 | 25.3 | 46.8 |
| | | | | | | |
| **Ethyl MethaneSulfonate** | | | | | | |
| (2) 0.01 v/v | 60.0 | 79.3 | 5.0 | 69.0 | 18.3 | 9.0 |
| (3) 0.03 v/v | 49.0 | 31.3 | 11.3 | 13.3 | 7.3 | 12.5 |
| (4) 0.05 v/v | 0.3 | 0.3 | 9.0 | 0.3 | 0.0 | 0.0 |
| | | | | | | |
| **LSD (0.05) = 7.6%** | | | | | | |
| | | | | | | |

**Table 5. Total chlorophyll chimeric plants observed in six genotypes of cotton exposed to seven mutagenic treatments in Lubbock, TX in 2002.**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Mutagen** | **TAMU** | **ACALA** | **Pima** | **TAMU** | **$A_2$** | **$A_2$** | |
| **Dose** | **946-25** | **1517-99** | **S-7** | **GA** | **60-T** | **120-W** | |
| | - - - - - - - - - - - - - - - Total Seedlings - - - - - - - - - - - - - - - - - - - | | | | | | |
| **Water** | | | | | | | |
| (1) Control | 0 | 0 | 0 | 0 | 0 | 0 | |
| | | | | | | | |
| **Na Azide** | | | | | | | |
| (5) 0.0005 v/v | 0 | 0 | 0 | 0 | 0 | 0 | |
| (6) 0.005 v/v | 0 | 0 | 0 | 0 | 0 | 0 | |
| (7) 0.05 v/v | 0 | 1 | 1 | 0 | 0 | 0 | |
| | | | | | | | |
| **Ethyl MethaneSulfonate** | | | | | | | |
| (2) 0.01 v/v | 0 | 0 | 0 | 1 | 0 | 0 | |
| (3) 0.03 v/v | 0 | 0 | 1 | 0 | 1 | 0 | |
| (4) 0.05 v/v | 0 | 0 | 4 | 3 | 6 | 3 | |
| | | | | | | | |

Naked Seed | Partially Naked Seed | Partially Fuzzy Seed | Fuzzy Seed

**Figure 1. Seed of four cotton lines demonstrating differential expression of the "naked and tufted" phenotype after saw ginning.**