IDENTIFYING AND BREEDING DROUGHT TOLERANT COTTONS (*GOSSYPIUM* SPP.) TREATED WITH EMS-MUTANT AGENT ON THE TEXAS HIGH PLAINS

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Abstract

The semi-arid climate of the Texas High Plains often leads to periods of water deficit stress or drought, which can greatly reduce the yield and quality of cotton (*Gossypium* spp.). It is known that cultivated cotton lacks genetic diversity which is necessary when breeding for drought tolerance. Ethyl MethaneSulfonate (EMS) is chemical mutagen, which has been used to create new genetic diversity in plants. In this study, we investigated if diverse EMS treated/generated populations will have enough genetic diversity to be used for selecting breeding lines with improved drought tolerance and good fiber quality. We evaluated a diverse EMS treated panel of 13 Upland (G. *hirsutum* L.) and one G. *arboreum* L. cultivars and germplasm, advancing from M_1 to M_4 as populations. From 2014-2016, derived plant selections from these populations were assessed under different irrigation regimes to induce drought stress in different locations. Each year, experimental design was assigned based on seed availability and included original-cultivar source, two commercial cultivars as controls, and selections in a complete randomized design (CRD), augmented (ARCBD), and randomized complete block design (RCBD) RCBD with three replications, respectively. Response of drought tolerance was assessed by measuring more than 19 above and below ground agronomic traits, yield, and fiber quality. In this report, yield and fiber strength traits are presented. Preliminary analyses in 2014 revealed differences for seed cotton yield between populations and in 2015 for selected lines and controls, suggesting enough genetic diversity in these populations for yield improvement. In 2016, differences for yield between selected lines and controls was not different. However, we were able to significantly ($p \leq 0.05$) increase the fiber strength over the control under water limited conditions. We will explore the possibility of a public germplasm release for those identified superior fiber quality lines under stress conditions with good yield.

Introduction

The Texas High Plains is the number one Upland cotton (*Gossypium hirsutum* L.) producer of the USA cotton belt. However, the semi-arid climate of this region often leads to periods of water deficit stress or drought, which can greatly reduce the yield and quality of cotton. In addition, the Ogallala aquifer is being rapidly depleted; this is a major problem as 4 million bales of cotton are produced annually. McGuire, (2012) showed that over the past 65 years the aquifer has dropped by 11.9 meters. This is problematic for the producers, because 40-50 percent of producers use the aquifer for supplemental irrigation. The depletion of the aquifer has forced the breeders of the Texas High Plains to make drought tolerance their number one objective in their breeding programs. Developing drought tolerant germplasm is impeded by the impact of the environment such as temperature and water on the growth, reproduction, and quality of cotton which make breeding for drought tolerance a difficult task. In addition, this task is even more challenging because Upland cotton is known to have narrow genetic diversity which is necessary when breeding for drought tolerance.

Some methods used in cotton to increase genetic diversity are the following: the use of converted landraces, the introgression of genetic diversity from different cotton species, and the use of a mutagen such as Ethyl MethaneSulfonate (EMS). Basal et al., (2005) showed that converted land races had significantly different rooting patterns then cultivated cotton. It was shown, in a greenhouse, that these converted land races had differential excised leaf water loss when compared to the cultivated varieties of TAM94L-25 and Lankart 142. The problems with using converted land races to improve cultivated cotton is their photoperiod sensitivity, and lack of good yield and fiber quality. Vieira da Silva, (1976) showed that different species of cotton had different levels of tolerance to drought. However, the progeny from different cotton species are highly incompatible and their offspring are usually sterile. Non-cultivated species also suffer from poor yield and fiber quality. Jain et al., (1998) showed that mutagens are a useful tool if no genetic source is available; when there is tight linkage between genes; the desired trait is recessive; and it is hard to transfer the desired trait. EMS has been shown to cause point mutations in many plant species with over 1800 varieties being released for an assortment of traits. One advantage of EMS over other methods of creating genetic variability is that elite cultivars are used, which results in less time needed between the identification of tolerant lines and germplasm release. Also, it has been reported (Meredith Jr., 2000) that the traditional breeding of cotton uses a small genetic base for improvement. This low level of genetic diversity is caused by the over use of a limited number of parents to create today's cultivars. Although there have been different attempts to improve the available genetic diversity, EMS generated cotton probably is a good source of genetic diversity for breeding drought tolerance.

Limited rain fall or irrigation water or drought stress has been reported to negatively impact several reproductive and vegetative traits. Sharma et al., (2015) and Gerik et al., (1996) showed that some of the reproductive traits affected by drought stress are boll size and number, both of which highly influence lint yield. Gerik, et al., (1996) noticed that most of the bolls were retained at the bottom of the plant. Pace et al., (1999) and Pettigrew, (2004) showed the impact of deficit water levels on the vegetative traits of shoot and root growth. Plants that are water stressed often have a dwarfed phenotype when compared to well-watered plants. Pace et al., (1999) also showed that when cotton had been subjected to drought stress the tap-root lengths were elongated when compared to well-watered plants. However, Pettigrew, (2004) showed a reduction in the root growth of water stressed plants. The reproductive and vegetative traits negatively impacted by drought stress result in reductions of yield. It is unknown which traits are the most important targets for a breeding program.

Fiber quality is also negatively impacted by drought stress and is also important for the producers because the quality determines the end use, for examples for textiles, for household goods, etc. Many of the fiber quality traits are negatively correlated to fiber yield, so this will create another obstacle to creating high yielding germplasm with good fiber quality under drought conditions. Culp, (1979) showed that fiber strength had a correlation as high as $r = -0.93$ with lint yield. Ulloa, (2006) showed that fiber strength in an okra-leaf upland population had a relationship of $r = -0.14$ to lint percent. Ulloa also showed that the strength is fixed in early generations ($F_{2:3}$). Snowden et al., (2013) showed the impact of deficit irrigation on fiber quality parameters. They showed that fibers with higher micronaire and shorter length were created by plants under water stress conditions when compared to well-watered plants. New germplasm lines that are created with high yield under drought tolerance must also have good fiber quality. By determining which traits are the most important for increasing lint yield and fiber quality under water limited conditions; a systematic approach can be taken by a breeding program to improve cotton.

In this study, we investigated if the genetic diversity of populations derived from EMS treated cultivars and germplasm will allow us to select breeding lines for drought tolerance by assessing the plant response of more than 19 above and below ground agronomic traits, yield, and fiber quality. Herein we only present, yield and fiber strength traits as response of drought tolerance.

Materials and Methods

The EMS treated populations evaluated in this study were created by bulk harvesting 13 Upland (*Gossypium hirsutum* L.) and one *G. arboreum* L. lines from the M_1 to the M_4 generation (treated EMS populations were provided by Dr. Dick Auld). The original seed sources for the Upland populations were from Acala 1517-99, AFD Explorer, AFD Rocket, All-Tex Atlas, FM 958, GA TAMU, Paymaster Tejas, Raider 276, SC 9023, TAM 94L-25, Tamcot Sphinx TTU 0782, and TTU 0774. Even though all 14 populations were then tested for plant response with more than 19 above and below ground agronomic traits, yield, and fiber quality, here we only present seed cotton yield, seed yield, lint yield, lint percent, and fiber strength traits. These populations were tested over multiple years (2014-2016) and locations (Lubbock, TX and New Deal, TX) (Table I).

At Texas Tech University Quaker and New Deal Research locations, irrigation was applied by sub-surface drip irrigation placed approximately 25 cm below the soil surface and centered on one meter rows. Each year, two meters of buffer cotton were planted around each irrigation rate to prevent water movement within the soil from affecting the test plots of different irrigation rates. Irrigation for all years was applied once per week as needed to supplement rainfall events. In 2014, irrigation was applied once a week at 2 rates (232 mm and 347 mm annually). In 2015, there were three irrigation rates (0, 71, and 142 mm annually). In 2016, there were two irrigation rates replicated at each location (0 and 106 mm annually). The Quaker Research Farm (Lubbock, TX) was used in all three years; it has an Acuff loam soil. The New Deal Research Farm (New Deal, TX), which was only used in 2016, the soil is a Pullman clay. The higher levels of clay in this soil allow for water to be retained for longer periods of time than the Acuff loam, so the plants do not become drought stressed as quickly.

For data collection, five individual plants were hand harvested from each plot, in 2014 and 2015 to evaluate yield and fiber strength traits. In 2016, one square meter was hand-harvested from each plot to extrapolate yield. In 2015 and 2016, 25 bolls were hand-harvested, from each plot, to evaluate the fiber quality. For all years and locations, the fiber quality traits of micronaire, length, uniformity, strength, elongation, and leaf trash were evaluated by high volume instrument (HVI) at the Texas Tech Fiber and Biopolymer Research Institute (FBRI) (Lubbock, TX).

Different experimental designs were used due to the differences in the number of individuals being tested and the availability of seed (Table I). In 2014, M_4 seeds from the 14 populations were tested in a complete randomized design within each irrigation rate. The seeds were planted, 10 seeds per meter, in single rows 229 meters long (2,290 individual plants). In 2015, M_5 seeds from the previous year's selections, four non-mutated sister lines and two commercial controls (FiberMax 958 and Phytogen 72) were tested in an augmented randomized complete block design within each irrigation rate at Lubbock, TX. The augmented design had four control blocks with the non-mutated sister lines and two commercial controls being replicated two times within each block. The non-mutated lines and two controls were occasionally moved from their randomized location to insure proper evaluation of changes in soil properties and irrigation non-uniformity. This design was selected as Lin and Poushinsky, (1983) reported that randomly distributing checks may not provide the adjustment needed to account for soil variation. The seeds of the individual plants were divided into three groups, each of these groups were planted in 4.5 meter plots at a rate of 10 seeds per meter within each water rate in the aforementioned augmented designs. In 2016, the test lines were evaluated at two locations (New Deal, TX and Lubbock, TX) under two irrigation rates) in a randomized complete block design with three replications within each irrigation rate block to evaluate the genotype by environmental performance of the selected drought tolerant lines. The seeds were planted at a rate of 10 seeds per meter with a plot length of 4.5 meters per replication. By using these experimental designs, we were able to test multiple agronomic and fiber quality traits for the efficacy in use for selecting key agronomic traits of interest to our breeding program.

Table I. Experimental design and evaluation method of EMS populations at Lubbock and New Deal, TX

Year	Experimental Design (within each water rate)	Irrigation Rates	Evaluation	Location
2014	CRD	2	T-test between selected groups within a population	Lubbock, TX
2015	ARCBD	3	Mixed models ANOVA with controls as fixed effects and test subjects as random	Lubbock, TX
2016	RCBD	2	ANOVA with Dunnett option (FM 958 as control)	Lubbock, TX New Deal, TX

Data Analysis

In 2014, the differences between good line-performers and bad line-performers for each population were determined using Independent Samples T Test in SPSS (IBM Corp., 2013). In 2015, the data was separated into two groups due to the different species evaluated. The differences within each species were determined using Proc Mixed (SAS Institute, 2013) with the non-mutated sister lines and commercial controls evaluated as fixed effects and the test lines as random effects. In 2016, the differences were determined using Proc GLM with the Dunnett option (SAS Institute, 2013).

Results and Discussion

In 2014, based on phenotypic evaluations, three *G. hirsutum* L. and one *G. arboreum* L. populations were selected from the original 14 EMS treated populations for further evaluation. The seed source for these three *G. hirsutum* L. populations were Raider 276, TTU 0774, and Tamcot Sphinx. Divergent selection was performed on these four populations; resulting in 25 good and 25 poor line-performers for a total of 200 selections (Fig. I). These selections were further evaluated for seed yield, lint yield, percent and fiber strength. Some of the selections were not carried forward due to low seed or lint levels which prevented further testing. It was hoped that by the end of the season, these traits will help us to identify or distinguish drought tolerant and susceptible lines.

In 2015, the divergent selections (line-performers) from the previous year, excluding those without enough seed, were again evaluated throughout the growing season by measuring five random plants. At the end of the season 25 random bolls were harvested from each plot for fiber quality evaluation by HVI at the FBRI. A reselection was performed at the end of the growing season based on phenotypic evaluations of the field plots (Fig. I). This resulted in 39 families from the four selected populations, four non-mutated sister lines, and two controls to evaluate in 2016.

In 2016, the selected families from the previous year, were again evaluated throughout the growing. At the end of the year, 25 bolls were harvested from each plot to evaluate fiber quality by HVI. One square meter was harvested from each plot to extrapolate yield. Herein, we present agronomic traits (2014), end of season seed cotton yield (2015), and fiber strength (2016).

Selection Method

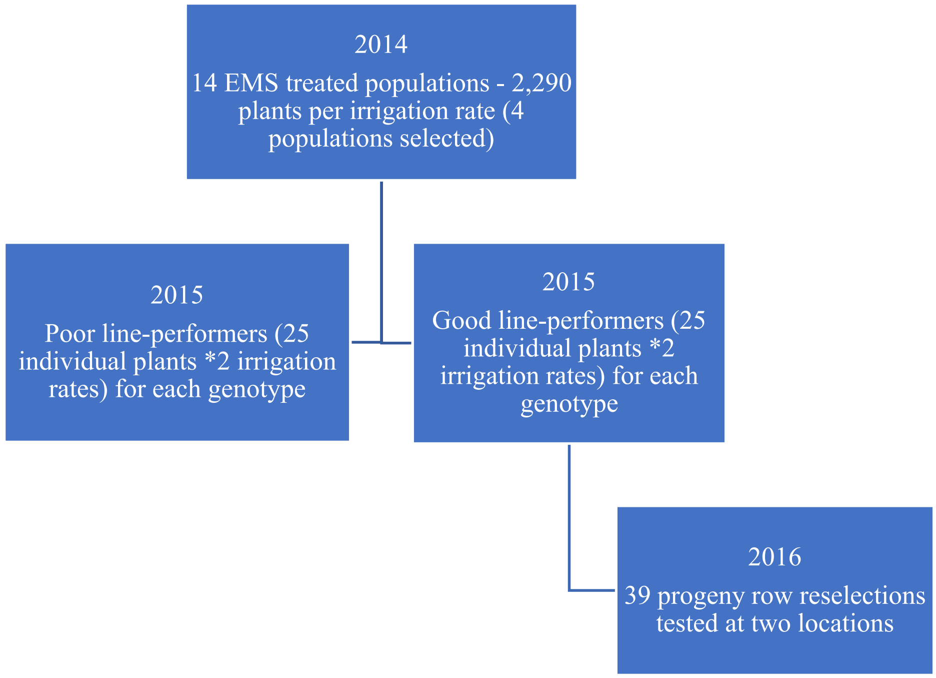

Figure I. Selection process of EMS treated populations during a three-year study (2014-2016).

In 2014, 200 divergent selections from the selected four populations were evaluated by using independent t-tests. There were significant differences for the traits evaluated between the good and poor line- performers for each population (Tables II-V). *G. hirsutum* and *G. arboreum* populations were slightly different in their levels of physiological response to stress and level of significance for lint percent. However, the seed cotton yield, seed yield, and lint yield were highly significant ($p \leq 0.0001$) for all populations.

Table II. 2014 Student's t-test of good performers and bad performers of population one – *G. arboreum*

Dependent Variable	**F-Statistic**	**Significance**
Seed Cotton Yield	151	***
Seed Yield	144	***
Lint Yield	138	***
Lint Percent	19	***

Traits within columns followed by * are significantly different at the 0.01≤ P ≤0.05, ** 0.001≤ P ≤0.01, *** P ≤ 0.0001

Table III. 2014 Student's t-test of good performers and bad performers of population two – *G. hirsutum* – Raider 276

Dependent Variable	**F-Statistic**	**Significance**
Seed Cotton Yield	114	***
Seed Yield	111	***
Lint Yield	115	***
Lint Percent	6	**

Traits within columns followed by * are significantly different at the 0.01≤ P ≤0.05, ** 0.001≤ P ≤0.01, *** P ≤ 0.0001

Table IV. 2014 Student's t-test of good performers and bad performers of population three – *G. hirsutum* – TTU 0774

Dependent Variable	F-Statistic	Significance
Seed Cotton Yield	191	***
Seed Yield	199	***
Lint Yield	166	***
Lint Percent	9	**

Traits within columns followed by * are significantly different at the 0.01≤ P ≤0.05, ** 0.001≤ P ≤0.01, *** P ≤ 0.0001

Table V. 2014 Student's t-test of good performers and bad performers of population four – *G. hirsutum* – Tamcot Sphinx

Dependent Variable	F-Statistic	Significance
Seed Cotton Yield	191	***
Seed Yield	199	***
Lint Yield	166	***
Lint Percent	9	**

Traits within columns followed by * are significantly different at the 0.01≤ P ≤0.05, ** 0.001≤ P ≤0.01, *** P ≤ 0.0001

In 2015, under the high irrigation rate, the control average for the trait of seed cotton yield was 200 grams of seed cotton yield for 25 bolls. There were 12 lines from the three *G. hirsutum* populations that performed better than the control average that ranged from 227 for T84 to 239 grams for T59 (Fig. II). In 2015, under the low irrigation rate the control average was 163 grams of seed cotton yield for 25 bolls. There were six lines that performed better than the control average ranged from 184 for T70 to 225 grams for T148 (Fig. III). Of these six lines, two of them T22 and T28 were significantly better than the control in both irrigation rates.

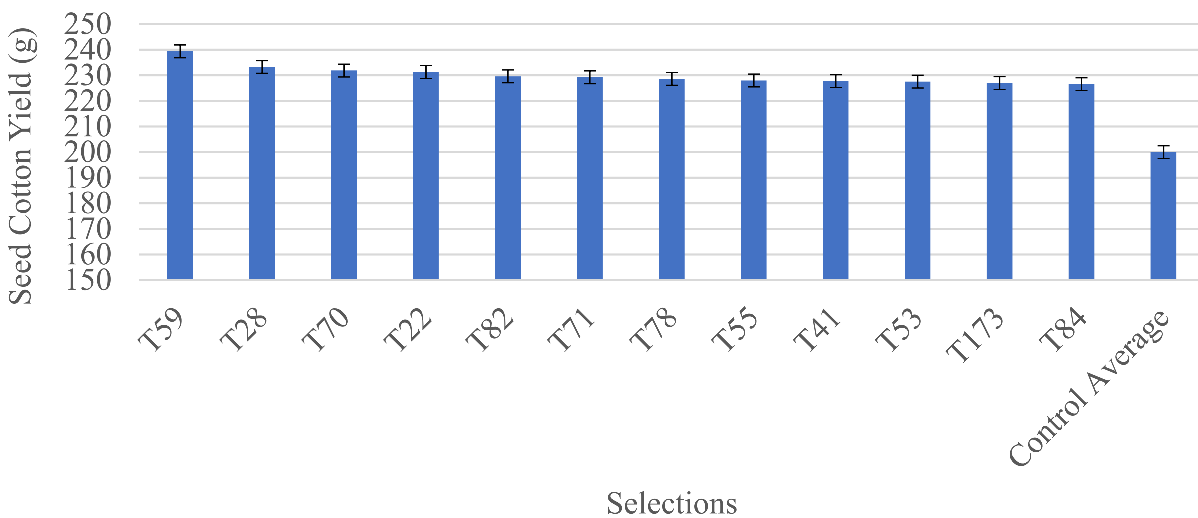

Figure II. 2015 Seed cotton yield of lines significantly ($p \leq 0.05$) better than the control average under the high irrigation rate. Error bars represent least significant differences.

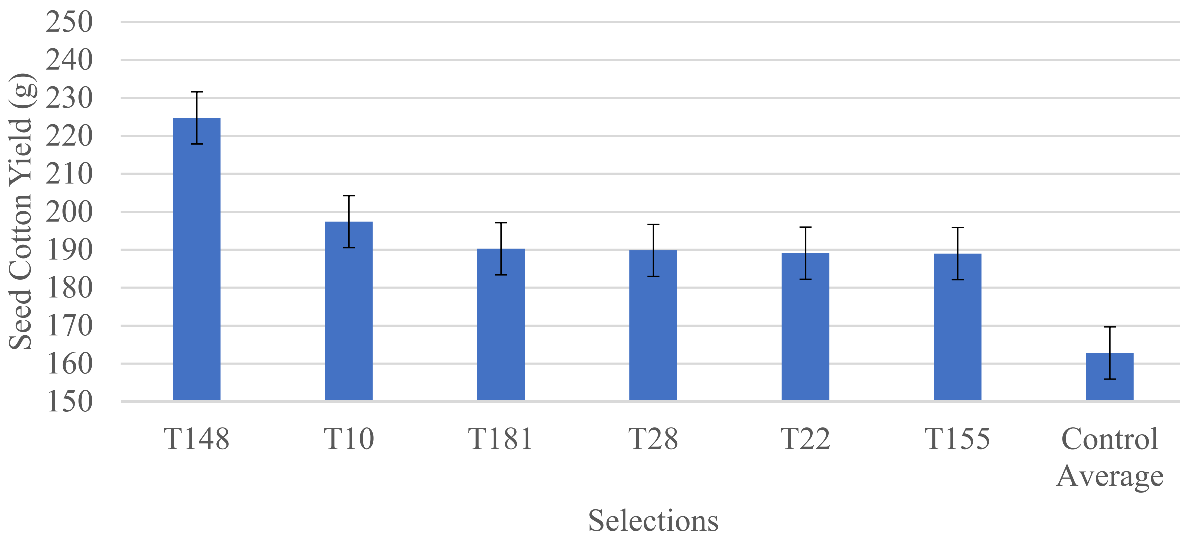

Figure III. 2015 Seed cotton yield of lines significantly ($p \leq 0.05$) better than the control average under the low irrigation rate. Error bars represent least significant differences.

In 2016, seed cotton yield, at each location and within each irrigation rate was not different than the control, FM 958 (data not shown). One reason for this outcome is the use of more stringent experimental design (RCBD) with replications. The augmented design that was used for the reselection may be flawed due to its conservative nature of committing type I errors, because it lowers the chance of discarding false negatives (type II errors). Sahagun-Castellanos, 1985 suggested that the success of selection from augmented designs were highly influenced by the traits that were evaluated.

In 2016 at Lubbock location, there were seven lines that had higher strength than the control (FM 958). The strength ranged from 37 for T65 to 39.5 grams per tex for T91 (Fig. IV). At New Deal, there were five lines that had better strength than the control (FM 958). The strength ranged from 39 for T51 to 42 grams per tex for T82 (Fig. V). Five selections had significantly ($p \leq 0.05$) higher strength than the control at both locations. As previously reported (Snowden et al., 2013) the limited irrigation resulted in increased micronaire when compared to the high irrigation rate, suggesting poorer quality fiber being produced in limited irrigation environments. This study agreed with previous studies where the higher irrigation rate had better fiber quality then the lower irrigation rate.

After evaluating the EMS treated populations over multiple irrigation rates and locations; lines with similar yield comparable to the control and better fiber quality were identified. These lines will provide new sources of genetic variability when breeding for drought tolerance.

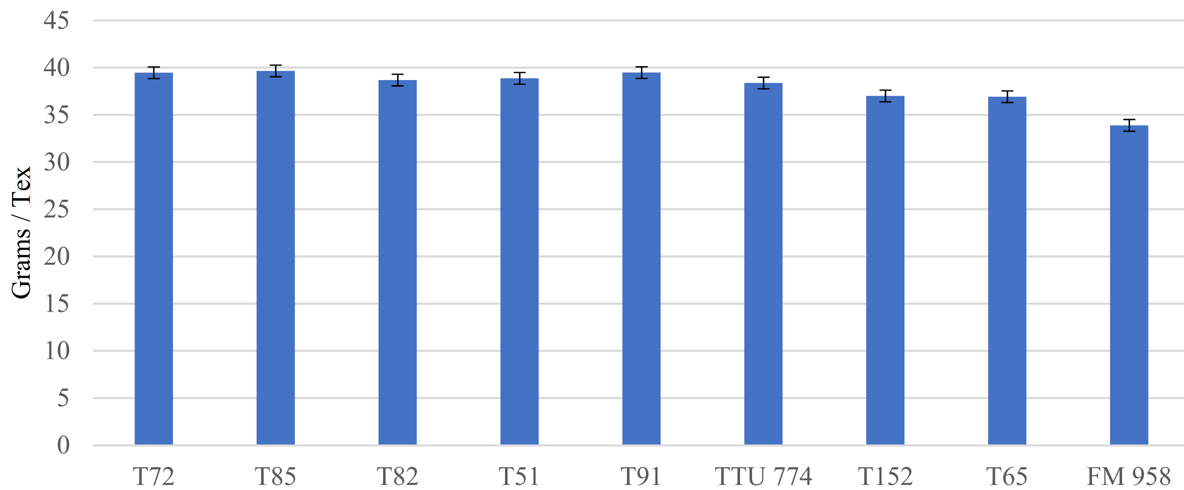

Figure IV. 2016 Strength of lines significantly ($p \leq 0.05$) better than the control FM 958 at Lubbock (Dunnett's test).

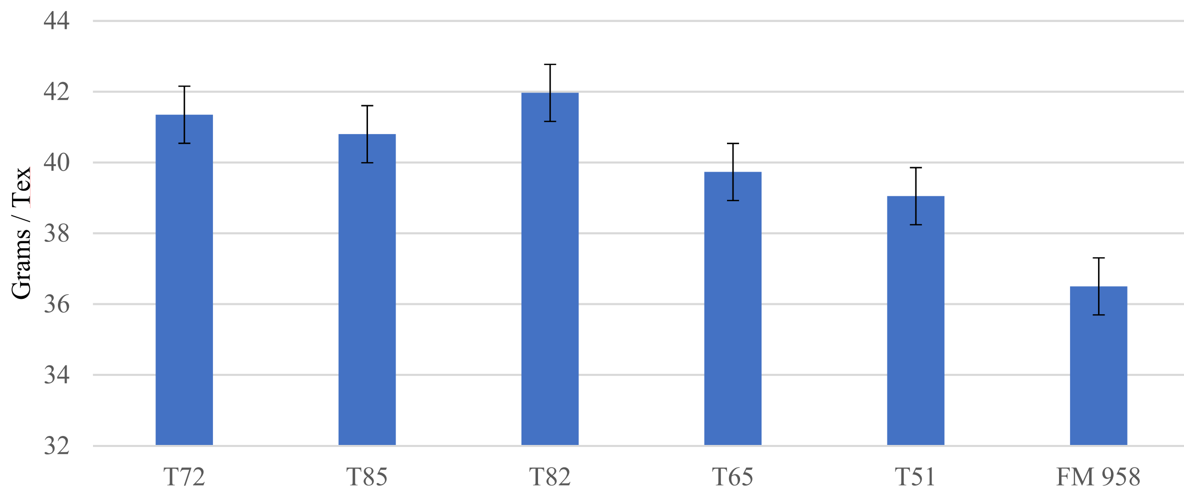

Figure V. 2016 Strength of lines significantly ($p \leq 0.05$) better than the control FM 958 at New Deal (Dunnett's test).

Summary

The high number of episodic droughts and declining availability of irrigation water in the High Plains, TX has led to the necessity to identify genetic resources to combat cotton yield and fiber losses. The evaluation of EMS treated lines used in this study indicated that EMS-created genetic diversity may be limited for selecting superior drought tolerance genotype-lines in these treated populations. However, this limited diversity may have been influenced by the selection method or original cultivars used in this study. Although our selection approach did not provide us with superior yielding breeding lines under limited irrigation or drought, we were able to select superior lines with fiber strength over the control under water limited conditions. There are several lines with superior fiber quality and good yield such as T82 for possible germplasm release. We were able to maintain lint yield while increasing fiber strength. This suggests that EMS overall is useful for increasing the fiber quality of these lines selected for drought tolerance. This study increases our knowledge of EMS used as a tool for improving fiber quality expanding previous studies in upland cotton (Vining, 2012; Brown et al., 2013). We will explore the possibility of a public germplasm release for those identified superior fiber quality lines under stress conditions with good yield.

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