PHENOTYPING DROUGHT TOLERANCE IN COTTON (GOSSYPIUM HIRSUTUM, L.)

A Thesis

by

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ABSTRACT

Cotton plant breeders need well-defined phenotypic parameters by which they can select drought tolerant lines as well as correlate phenotypes to allelic polymorphisms in the cotton genome. Soil-moisture availability is usually the most limiting factor for cotton crop productivity, especially in Texas. Characteristics of roots logically play an important role in determining the response of plants to limited soil moisture.

The objectives of this study were to develop and refine techniques that could be used by plant breeders to phenotype plants' drought tolerance. Approaches include using a Trimble GreenSeeker®, to identify individual and progeny rows with enhanced photosynthetic capabilities in the presence of drought, leaf canopy temperatures under drought conditions, and measurement of root parameters in growth tubes in a greenhouse. Results from these experiments were related to yield performance in field trials at three locations in 2013.

Several conclusions can be drawn from this study. First, Normalized Difference Vegetative Index and leaf temperature are rapid and reliable tools to evaluate plant health. The utility of these tools hinges upon timing of data collection, but they clearly demonstrated the propensity to differentiate phenotypic differences. Secondly, evaluation of root systems in growing tubes in a greenhouse is probably an ineffective method of characterizing drought tolerance potential since the growing conditions are radically different from what a plant would encounter in a field environment. Examining roots with this system would likely yield significant differences among plant species, but

within upland cotton, it would be difficult to determine differences among genotypes. Ultimately the best determinant of drought tolerance is performance testing in droughty conditions because it encompasses most of the contributing factors that induce drought stress and measures the cotton plant's inherent ability to recover and compensate in response to rainfall through the course of a growing season.

DEDICATION

This work is dedicated to God, my wife Tamren, my parents Joe & Bonnie Terhune, Rennie & Tammie Hensley, and the rest of my family for their encouragement and support throughout the entire process. Thank you all for everything you have helped me with in years past and years to come. I am a blessed man and love you all.

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NOMENCLATURE

U.S.	United States of America
CIL	Cotton Improvement Lab
CS	College Station, Texas
TH	Thrall, Texas
NDVI	Normalized Difference Vegetative Index
ANOVA	Analysis of Variance
USDA	United States Department of Agriculture
ESL	Extra-Long Staple
FFB	First fruiting branch
#Bolls	Number of bolls
OB/GB	Open boll/Green boll (% open)
H/N Ratio	Height to Node Ratio
DPL 491	DeltaPine 491 Monsanto, Co.
L	Location(s)
G	Genotype(s)
E	Experiment(s)
R	Rep(s)
W	Week(s)
H	Height
HVI	High Volume Instrument

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CHAPTER I

INTRODUCTION AND BACKGROUND

Cotton (*Gossypium sp.*) is produced in 17 southern U.S. states from Virginia to California. Major concentrations include areas of the Texas High and Rolling Plains, Mississippi, Arkansas and Louisiana Delta, southern Georgia, and California's San Joaquin Valley (USDA, 2012). Cotton is grown in the U.S. as an annual crop from seed planted each year, even though cotton can be, but hardly ever is, grown as a perennial in tropical climates (USDA, 2012). The foremost dominant type of cotton produced in the United States is American Upland *(Gossypium hirsutum)* (USDA, 2012). The upland type, which usually has a fiber length of 1 to 2.92 cm, accounts for about 97 percent of the annual U.S. cotton crop. American Pima or extra-long staple (ELS) *(Gossypium barbadense)* is the other U.S. cotton produced type with a fiber length of 3.25 cm (Smith and Cothren, 1999).

Cotton in the U.S. is grown with full-irrigation, partial irrigation and non-irrigated. Cultivars are needed that can endure and recover from drought so as to minimize yield loss in dryland areas and to reduce the water needed in irrigated production (Pace et al., 1999). Over six million acres of cotton are produced annually in Texas and over half of this crop is grown without irrigation in drought prone conditions (Texas A&M AgriLife, 2012). Moreover, much of the acreage described as irrigated is actually grown with soil-moisture deficits due to limited and costly irrigation water.

Cotton plant breeders need well-defined phenotypic parameters by which they can select drought tolerant lines as well as correlate phenotypes to allelic polymorphisms in the cotton genome. Azhar et al. (2009) proposed that drought-tolerant plant material in breeding programs required the presence of genetic variation in diverse environments to obtain drought-tolerance within the species. Multiple locations and different plant resource availabilities within environments allow for better simulation of drought. Water is usually the most important factor limiting crop productivity and root characteristics logically play an important role in determining the response of plants to drought. Drought stress decreases shoot growth rate, plant height, and yield, but root growth is less sensitive (Malik et al., 1979). The demand for drought tolerant genotypes will continue to expand as water resources and the funds to access them become more limited (Longenberger et al., 2006).

Previous researchers have attempted to link physiological characteristics with drought stress that could be used as indicators for phenotyping drought tolerance cotton breeding lines (Quisenberry et al., 1981; Wright and Dobrenz, 1973). Among the characteristics that have been considered include root characteristics (Ball et al., 1994; Basal et al., 2003; Cook and El-Zik, 1992; Pace et al., 1999) leaf water potential (Kaul, 1969; Quisenberry et al., 1985), and stomatal characteristics (McDaniel et al., 2000; Quisenberry et al., 1982). Responses to high temperature stress may differ under greenhouse and field conditions due to non-target environmental variation (Watson, 1952). Distinguishing differences between water deficit and high temperature tolerance of plants within greenhouse and field conditions is challenging (Watson, 1952). Also, the

assumption of which genotypes are acclimated to high temperature stress in hot growing conditions could be a limiting factor (Chen et al., 1982; Lu et al., 1994). However, such screening protocols and testing requirements could be too tedious or time consuming for a plant breeder.

Cotton plant breeders often develop new cultivars from individual plants reselected from existing commercial cultivars, or novel elite populations developed from germplasm within their own programs, and less frequently from publicly released germplasm. Thus, it is essential to either identify specific alleles for drought resistance/tolerance in adapted elite germplasm or add novel alleles from exotic sources to expand genetic and phenotypic diversity for drought avoidance or tolerance (Basal et al., 2005). Developing a new method of screening phenotypes in droughty conditions could provide the system by which breeders can make fast and efficient genetic gains within their programs. Lee (1984) surmised that wild cotton lines usually occupy regions of scarce precipitation; irrigation technologies are necessary for the effective commercial production of cotton in arid regions and thus reiterates the need for better drought tolerant cotton plants.

Objectives of this study were to develop phenotypic selection methods to determine drought tolerance using the following approaches:

1. Use of Trimble GreenSeeker™ Technologies to identify individual and progeny rows with enhanced photosynthetic capabilities in the presence of drought. This system uses the NDVI as a means of evaluating plant health.

2. Screen plant leaf temperatures throughout the growing season using an infrared temperature gauge.
3. Measure cotton taproot length, and root biomass when grown under greenhouse culture in growth tubes.
4. Determine yield and fiber quality parameters of putative drought tolerant genotypes across multiple drought prone locations.

CHAPTER II

MATERIAL AND METHODS

Genetic Material

Cotton genotypes used in this study were chosen based on potential of demonstrating drought tolerance. The breeding program of the Texas A&M Cotton Improvement Lab developed seven lines:'Tamcot-73' ((Smith et al., 2011)CV-128, PI 6622044), 07 X-26, 07 V-45, 08 WZ-83, 08 WZ-78, 02 WK-11, and 06 WE-14. Each experiment included the commercial cultivar 'DeltaPine 491' (PVP 200100159, PI 618609) as shown in Table 1.

Field Trials

Experiments were conducted in 2013 at Texas A&M AgriLife Research and Extension Farm near College Station, TX, Stiles Farm Foundation near Thrall, TX, Texas A&M AgriLife Research and Extension Center near Corpus Christi, TX, and the Texas A&M University Farm near Commerce, TX. The soil type at College Station was a Westwood silt loam (fine-silty, mixed, superactive, thermic, Udifluventic) (Viator et al., 2008), the soil type at Thrall was a Burleson clay (fine, montmorillonitic, thermic Udic Pellustert) (Biediger et al., 1992), the soil type at Corpus Christi was a Victoria or Orelia clay (fine-loamy, mixed hyperthermic Typic Ochraqualf), and the soil type at Commerce was a Houston Black (fine, montmorillonitic, thermic Udic Pellustert) (Potter et al., 1998).

Table 1. Cotton genotypes used to phenotype drought in 2013.

Genotype	Source	Pedigree	Characteristics	Area of Adaption
1. Tamcot 73	TAAR	93WB-57s/95WE-48	HY	Texas
2. DeltaPine 491	Delta & Pine Land Co.	'DP 5415'/'DP 2156'	HY	Mid-South
3. 02 WK-11	TAAR	90O-24L/'SG 125'	Okra Leaf, HY	Texas
4. 06 WE-14	TAAR	491/96WD-22//'AP9275'/96WD-22	Hairy, HY, NL	Texas
5. 07 X-26	TAAR	'Acala 1517-99'/02Y-77	Short Season	Texas
6. 07 V-45	TAAR	96W-22/02Q-42	Glabrous-HY	Texas
7. 08 WZ-83	TAAR	'FM966'/'ST474'//'FM958'/'SG125'	HY, NL	Texas
8. 08 WZ-78	TAAR	'FM966'/'FM991'	HY, NL	Texas

TAAR – Texas A&M AgriLife Research
HY – High Yielding
NL – Normal Leaf

Cotton was planted on beds using a planter equipped with 97-cm row spacing for all location studies, at a plating rate of 160,000 plants ha^{-1}. Final plant stand was between 130,000 – 140,000 plants ha^{-1}. Plots were 2 rows by 10 m long and planted between April 20th and May 2nd. All locations had four replications. Genotypes were tested in a randomized complete block design (RCBD).

Each trial was non-irrigated and insects were controlled at or below economic thresholds. Plots were maintained weed-free through the use of herbicides and hand weeding. Therefore, any differences among genotypes were overwhelmingly abiotic stress factors, primarily drought stress. One application of 22.7 kg N ha^{-1} was applied to all locations. Plots received no synthetic plant growth regulators. Due to severe drought at Corpus Christi and poor plant stands at Commerce, those locations were abandoned from the study. Weather conditions in general were typical at the other locations during the growing season and were not damaged due to late-season weather issues (Table 2).

Only two locations, College Station and Thrall, were screened for NDVI during the 2013 season. Commerce and Corpus Christi were both harvested for yield results only and not reported because of the poor growing conditions that compromised the validity of data from those locations. The College Station environment throughout the growing season received timely precipitation and had other favorable growing conditions throughout the growing season (Table 2). Thrall's environment received good early rains but went into extreme drought conditions in the month of June and received little to no precipitation during the crucial fruiting periods in July. NDVI observations were made at College Station and Thrall on the same day throughout the fruiting period.

Table 2. Average monthly temperature high and lows, and precipitation at College Station and Thrall, TX in 2013.

	April*		May		June		July		Aug		Sept**	
	CS	TH	CS	TH	CS	TH	CS	TH	CS	TH	CS	TH
Avg. High °F	83.3	77.5	86.3	84.9	97.2	95.7	97.6	95.2	100.8	97.4	102.4	101.0
Avg. Low °F	56.9	53.7	63.2	61.1	71.5	70.4	72.3	71.3	73.1	72.1	74.3	73.8
Avg. High °C	28.5	25.3	30.1	29.4	36.2	35.4	36.5	35.1	38.2	36.3	39.1	38.3
Avg. Low °C	13.8	12.0	17.3	16.2	22.0	21.3	22.4	21.8	22.8	22.3	23.5	23.2
Rainfall (inches)	1.95	0.0	6.84	3.22	0.78	0.0	2.59	1.7	0.29	1.03	0.01	0.0
Rainfall (mm)	49.5	0.0	173.7	81.7	19.8	0.0	65.7	43.1	7.3	26.1	0.25	0.0

* Average temperatures were recorded from April 15th.

** Average temperatures were recorded until September 15th.

At maturity, 30-boll samples were hand harvested from each plot. Bolls at both College Station and Thrall were taken from the first position of the middle fruiting branches. All the boll samples were ginned on a laboratory 20-saw gin with no lint cleaning. The fibers were sent to the Texas Tech University Fiber and Biopolymer Research Institute (FBRI), Lubbock, TX, for measurement on a High-Volume Instrument (HVI). All plots were then harvested using a mechanical one-row picker harvester and yield weights were recorded.

The GreenSeeker®, a handheld crop sensor, is an active light source optical sensor used to measure plant biomass and display as NDVI (Trimble, 2013). NDVI measures the plant biomass and vigor for each cotton genotype tested. The GreenSeeker sensor emits brief bursts of red and infrared light, and then measures the amount of light reflected back (Trimble, 2013). Green plants absorb most of the red light and reflect most of the infrared light (Trimble, 2013). Remote sensing continues to evolve as a valuable agronomic tool that provides information to breeders, scientists, consultants, and producers on the status of their crops (Hatfield et al., 2008). Observing the data used for each variety under drought conditions, NDVI, and leaf temperature values were collected to better understand each genotype's phenotypic characteristics. The screening process began when the plants were at the second true leaf stage and continued for eight consecutive weeks.

NDVI was collected between 10 AM and 2 PM for both College Station and Thrall locations. The GreenSeeker was held over the middle of each row and approximately 60-80 cm above the cotton plant. A walking speed of approximately 2.5

km/hour was maintained during all screenings (Figure 1). Screening for the entire plot at each trial location for NDVI took roughly 20 minutes.

Figure 1. Screening with the Trimble GreenSeeker™

Leaf temperature data were collected between 10 a.m. and 2 p.m. for both College Station and Thrall locations. The screening process began at the plant's second true leaf stage. Using a Fluke 62 Mini IR near-infrared thermometer, the uppermost fully developed leaf of five randomly selected individual plants in each plot were chosen for observation each week. The sampling procedures were non-destructive. The thermometer was held 20 cm from the cotton leaf. Measurements were recorded weekly for eight consecutive weeks. The five leaf temperature data points per plot were averaged together for an overall phenotypic temperature reading. Leaf temperature data

collection took approximately 20 minutes (Figure 2). Together, the NDVI and leaf temperature collection took about one hour to complete. Yield, fiber quality, and plant mapping data were ascertained from these same plots.

Figure 2. Leaf temperature collections.

Root Experiments

In an effort to determine differences among genotypes, plants were grown in growth tubes. By using these tubes, it created a controlled environment and the plants could easily be destructively sampled while keeping most of the root systems intact. Plants were grown for 40 days, which is approximately the amount of time the plant needs to begin producing reproductive structures, and coincides with alteration of source-sink dynamics in the plant. By this time the roots had extensive taproots and lateral roots, but were not fully encompassing the entire growth tube.

Each tube was 76.2 cm in length and 10.6 cm in diameter. A Westwood silt loam from Texas A&M AgriLife and Extension Farm near College Station, TX, was used to grow the plants in the tubes. Soil for growth tubes was unsieved soil. The same eight genotypes that were used in the field trials (Table 1) were tested in the root experiment. Each tube had a single plant. Tubes were watered to field capacity with approximately 75 mL per day for 40 days. The experimental design was a completely randomized design (CRD) with ten repetitions for each genotype. The tubes were placed on a wagon that could be taken into a greenhouse during precipitation events. (Figure 3) The experiment was repeated twice.

At 40 days, the soil was flushed from each tube and care was taken not to break the root systems. Taproot length was measured along with root mass including wet/dry weights and a ratio calculated between the two mass indices.

Figure 3. Root growth tubes.

Plant Mapping

Plant mapping data were collected during the 5th week of the 8-week screening trial. Only College Station and Thrall were evaluated for plant mapping data. In the middle of each row/plot, seven randomly selected plants were analyzed for height, first fruiting branch (FFB), number of nodes, number of bolls, percent open bolls versus percent green bolls, and a height to node ratio was performed. The first node immediately after the cotyledonary node was identified as the beginnings point of counting nodes. Plant heights were measure using a meter stick. FFB's were identified and bolls were counted and recorded.

Statistical Analysis

Field Trials Statistical Analysis

Analysis of variance for NDVI and leaf temperature among genotypes and locations was performed using SAS 9.3 (Cary, NC, 2010). Proc Mixed and GLM with Location, Reps, Genotype, and Weeks as fixed effects were used to determine differences among treatments. For yield, fiber, and plant mapping data analysis, Proc GLM was used. A Proc Mixed procedure was used for NDVI and leaf temperature data because the data was collected from the same plots in consecutive (non-independent and non-random) weeks with all variable sources fixed and sampling week as the repeated measure.

Root Statistical Analysis

Analysis of variance of taproot lengths, wet and dry weights, and ratios between wet weights versus dry weights was performed using SAS 9.3 (Cary, NC, 2010) Proc GLM with Experiments, Reps, Genotypes as fixed effects to determine differences among treatments.

CHAPTER III

RESULTS AND DISCUSSION

Field Trials

NDVI

NDVI results were variable throughout the eight-week screening period. A PROC Mixed procedure found significant differences across locations in all sources measured (Table 3). Significance of NDVI for location as well as rep x location was likely a result of the mid-season drought that afflicted Thrall. NDVI genotypes were signififcantly different. Genotype x location was significantly different for NDVI and therefore each location was analyzed separately. Week and genotype x week were different likely because of genetic/physiological capabilities of plants to with stand drought prone conditions. Blum (1996) stated that plants response to drought stress are mediated by processes of responses to water deficit and associated strains such as a leaf's temperature rising.

Thrall did not have a significant genotype x week interaction (Table 4). A Proc Mixed with weeks as a repeated measure analysis was conducted. Thrall received little rain during the month of June or early fruiting stages which may have led to cotton stomata impairment. Ackerson (1980) explained that threshold leaf water potential required to initiate stomatal closure in

Table 3. ANOVA of pooled data from College Station and Thrall, Tx for NDVI in 2013.

ANOVA		
Source	**df**	**Weeks 1-8**
Loc	1	468.07**
Rep(loc)	6	13.69**
Genotype	7	2.77**
Genotype*Loc	7	4.04**
Week	7	60.50**
Genotype*Week	49	1.45*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

Table 4. ANOVA for NDVI using repeated measures results for College Station and Thrall, TX in 2013.

ANOVA			
Source	**df**	**Thrall**	**College Station**
		F-value	F-value
Rep	3	23.23*	19.07*
Genotype	7	9.24*	2.89*
Week	7	20.19*	115.17*
Genotype x Week	49	1.24	2.31*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

cotton can progressively become more negative when plants were subjected to a series of water stress cycles similar to the conditions at Thrall.

Because a significant interaction between week and genotype was not observed at Thrall, data from weeks and genotypes were pooled. Only 06 WE-14 had a (Tables 5 and 6) lesser NDVI value than other genotypes (Table 5). Not surprisingly the NDVI tended to increase each week and then in week eight significantly regressed (Table 6.) Mauney (1986) explained the rate of production of new vegetative leaves and therefore new fruiting branch sites is highly dependent on temperature, it is also sensitive to water stress. Such drought stress combined with a near-capacity boll load likely caused NDVI to drop on the eighth week as the source-sink relationship became detrimental to full vegetative growth and health.

Table 5. Thrall NDVI genotype means at Thrall, TX, in 2013.

Genotype	Means	
06 WE-14	0.672	a
02 WK-11	0.703	b
DPL 491	0.704	b
Tamcot 73	0.705	b
08 WZ-78	0.710	b
07 V-45	0.710	b
07 X-26	0.711	b
08 WZ-83	0.714	b

* Means with the same letter are not significantly different at the 0.01 level.

Table 6. NDVI values by week at Thrall, TX, in 2013. Values are the means of eight cotton genotypes.

Weeks	Means	
8	0.668	a
1	0.686	ab
2	0.702	bc
3	0.702	bc
4	0.705	bcd
5	0.713	cde
6	0.723	de
7	0.730	e

* Means with the same letter are not significantly different at the 0.01 level.

Significant genotype x week interactions were observed at the College Station site during the first three weeks. As the plants began to mature and canopies were at or near full peak coverage, differences among genotypes were not apparent. Differences among genotypes during the first three weeks of observation were likely a reflection of inherent early-season growth potential and less about true water-use efficiency since there was also adequate soil-moisture at this time.

Table 7. Genotype effects by week in 2013 at College Station, TX.

Week	Effect	df	Means	F-value
1	Genotype	7	.603	5.91**
2	Genotype	7	.613	8.34**
3	Genotype	7	.738	2.73*
4	Genotype	7	.760	0.51
5	Genotype	7	.786	0.17
6	Genotype	7	.779	0.46
7	Genotype	7	.766	0.55
8	Genotype	7	.704	1.97

* Significantly different at the 0.05 probability level.
** Significantly different at the 0.01 probability level.

Table 8. NDVI values by genotype and by week at College Station, TX in 2013.

Genotype	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8	
Tamcot 73	0.62	a	0.66	a	0.75	a	0.76	a	0.78	a	0.76	a	0.76	a	0.68	a
07 V-45	0.62	a	0.63	a	0.75	a	0.76	a	0.79	a	0.77	a	0.77	a	0.70	a
08 WZ-83	0.63	a	0.64	ab	0.76	a	0.76	a	0.79	a	0.79	a	0.76	a	0.72	a
08 WZ-78	0.64	a	0.64	ab	0.74	a	0.77	a	0.78	a	0.76	a	0.75	a	0.66	a
02 WK-11	0.60	ab	0.60	abc	0.76	a	0.78	a	0.79	a	0.78	a	0.76	a	0.70	a
06 WE-14	0.56	ab	0.56	bc	0.73	a	0.74	a	0.79	a	0.79	a	0.79	a	0.72	a
07 X-26	0.52	b	0.52	c	0.73	a	0.76	a	0.79	a	0.79	a	0.77	a	0.73	a
DPL 491	0.62	a	0.66	a	0.67	b	0.77	a	0.79	a	0.79	a	0.78	a	0.73	a
Mean	**0.60**		**0.61**		**0.74**		**0.76**		**0.79**		**0.78**		**0.77**		**0.70**	
C.V.	**10.09**		**10.51**		**4.17**		**2.49**		**1.01**		**2.28**		**2.59**		**4.56**	

* Means with the same letter are not significantly different at the 0.01 level.

Initially 07 X-26 was thought to be highly drought tolerant, especially at the seedling stage. However, in the first two weeks of observation, it had the lowest NDVI value (Table 8). This suggests that a slow initial growth rate, as reflected by NDVI, could be an indication of drought tolerance, or that 07 X-26 has an inherently slow growth rate independent of response to drought.

In the third week, DPL 491 had the lowest NDVI value. There is no obvious reason as to why this particular genotype had a rather stagnant rate of increase for NDVI among genotypes for this one week. In general, NDVI did not provide great insight into drought tolerance with this particular protocol. On the other hand, it did show promise into differentiating early-season growth, which could be of benefit in assessing seedling disease reactions or some other factor which typically affects early season growth and development.

Leaf Temperature

Leaf temperature had considerable variability throughout the trial in regards to genotypes, weeks, and locations (Table 9). Genotype x week interaction was not significant, nor was the interaction term for genotype x location. The importance of this finding is critical because this strongly suggests that it is not necessary to check plants continually throughout the growing season and that results from one location, such as College Station, would be applicable to another location, in this case Thrall, TX, in order to detect differences among genotypes for leaf temperature. Observed differences between locations was likely a result of several factors including soil-type and soil

moisture availability, but the most likely cause of the differences would be the time of day at which temperatures were taken. Plants at the Thrall trial were generally examined at 10 a.m. each week, whereas data from the College Station test were not taken until 1 p.m. During this three-hour period the ambient temperature rises considerably as well as the transpiration dynamics change during the course of daylight hours (Pallas et al., 1967). As a result, plants on average at College Station (35.24°C) were warmer than those at Thrall (32.53°C) during the data collection period.

Table 9. ANOVA of leaf temperature data pooled together from College Station and Thrall, TX with repeated measures in 2013.

ANOVA		
Source	**df**	**F-value**
Location	1	549.92*
Rep(loc)	6	11.57*
Genotype	7	2.74*
Genotype x Loc	7	0.83
Week	7	66.96*
Genotype x Week	49	1.00

* Significant at the 0.01 probability level.

Differences among weeks ranged from 30.83° C to 37.8° C (Table 10). There was a general trend of warmer leaf temperatures over time, but it's logical that changes in weather would have an effect on leaf temperatures. For instance, a cold front in the fifth week of observation dropped the ambient temperature on the day of measurement and concomitantly the leaf temperatures dropped. Likewise, as rain events occurred and

soil moisture availability improved, the leaf temperature fell accordingly because transpiration rates could increase with more improved plant water status.

Table 10. Leaf temperature means across College Station and Thrall, TX by week in 2013.

Weeks	Means	
5	30.83	a
1	32.51	b
3	32.63	b
2	32.84	b
6	34.02	c
7	34.70	c
4	35.79	d
8	37.78	e

- A lower leaf temperature is preferred -
* Means with the same letter are not significantly different at 0.01 level.

Across locations and weeks, genotype leaf temperatures ranged from 33.46° C to a high of 34.30°C (Table 11). Differences among genotypes were relatively minor, but 08 WZ-83 was warmer than 07 V-45 and 08 WZ-78. Causes of leaf temperature differences among genotypes could be the result of root systems capability of extracting soil moisture or, as Radin et al. (1994) suggested, could be due to genetically controlled differences in stomatal conductance as well as leaf area index.

There did not appear to be any relationship between leaf temperature and NDVI. Among the three lines that demonstrated differences for leaf temperature, 08 WZ-83, 07 V-45, and 08 WZ-78, none of these lines were different in terms of NDVI at any location nor in any week.

Table 11. Leaf temperature means of eight genotypes across College Station and Thrall, TX and weeks in 2013.

Genotype	°C	
08 WZ-83	34.30	a
02 WK-11	34.13	ab
Tamcot 73	33.97	ab
07 X-26	33.97	ab
DPL 491	33.86	ab
06 WE-14	33.80	ab
07 V-45	33.61	b
08 WZ-78	33.46	b

- A lower leaf temperature is preferred -

* Means with the same letter are not significantly different at 0.01 level.

Root Experiments

In terms of taproot length, results from the two experiments were different as well as the interaction between experiment and genotype (Table 12). Moreover, differences were observed between root biomass in both wet and dry state. When the ratio of wet and dry mass was analyzed, there were no differences detected for any source of variation.

Root lengths for genotypes within experiments were significantly different in both experimental runs (Table 13). Genotypes did not differ in root length, root wet weight, root dry weight, or ratio of wet to dry root weight although the genotypes did respond differently in root length across two experimental runs. These findings suggest that measuring taproot length is probably the best parameter to consider in evaluating root capability among these genotypes. It also seems logical that a cotton plant's ability to access soil moisture would be more of a function of rooting depth rather than its root breadth since cotton is grown in high intra-cotton plant densities and the physical loss of soil moisture tends to go vertical rather than horizontal.

The lack of detectable differences for root biomass ratio of wet and dry weights indicate that there were no major effects within these genotypes for extreme deviations in the formation of sclerenchyma tissue with highly lignified root systems, which would have increased root tissue density.

Table 12. ANOVA of root characteristics from cotton plants grown in tubes across experiments in 2013.

		Mean Square						
Source	**df**	**Length (cm)**	**df**	**Wet weight (g)**	**df**	**Dry weight (g)**	**df**	**Ratio wet/dry**
Exp.	1	3524**	1	92.2**	1	6.0**	1	12.5
Genotype	7	220**	7	2.2	7	0.2	7	1.4
Exp.*Genotype	7	498**	7	2.6	7	0.3	7	1.6
Error	95	46	115	2.6	111	0.2	111	1.6

* Significant at 0.05 probability level.
** Significant at 0.01 probability level.

Table 13. ANOVA of root characteristics from cotton plants grown in tubes by experiment in 2013.

		Mean Squares							
	Source	**df**	**Length (cm)**	**df**	**Wet. weight (g)**	**df**	**Dry weight (g)**	**df**	**Ratio wet/ dry**
Exp. #1	Genotype	7	103.7**	7	2.98	7	0.20	7	1.69
	Error	60	26.8	65	2.94	65	0.17	65	1.90
Exp. #2	Genotype	7	614.6**	7	1.73	7	0.23	7	1.38
	Error	35	78.4	50	2.19	31	0.20	31	1.06

* Significant at the 0.05 levels.

In the first experiment, 07 V-45 had a root length significantly longer than Tamcot 73, 06 WE-14, and DPL 491 (Table 14). In the second experiment, Tamcot 73 and 06 WE-14 were longer than DPL 491, 08 WZ-83, 07 X-26, and 08 WZ-78 (Table 15). The most likely cause of the differences between the experimental runs was the ambient temperature. The second experiment was conducted when average daily high temperatures were 5-10° C warmer than when the first experiment was conducted. The elevated temperatures undoubtedly increase evapo-transpiration rates, which cause more severe drought stress on the plants in the second experiment. Interestingly, the ranks among genotypes changed substantially, and overall lengths of the roots in the second experiment were about 30% longer. This suggests a differential effect for root development depending on drought stress.

Table 14. Experiment 1. Means of root parameters of cotton genotypes grown in tubes.

Genotype	Taproot Mean Length (cm)		Wet Wt. (g)		Dry Wt. (g)		Ratio wet/dry	
07 V-45	43.7	a	4.93	a	1.22	a	3.70	a
08 WZ-78	38.6	ab	4.37	a	0.95	a	3.41	a
07 X-26	38.4	ab	6.07	a	1.43	a	4.64	a
08 WZ-83	38.4	ab	5.60	a	1.32	a	4.28	a
02 WK-11L	37.8	ab	5.82	a	1.28	a	4.54	a
Tamcot 73	34.1	bc	5.13	a	1.16	a	3.96	a
06 WE-14	33.8	c	5.02	a	1.09	a	3.92	a
DPL 491	33.6	c	4.84	a	1.14	a	3.80	a
Mean	**37.2**		**5.23**		**1.2**		**4.0**	
C.V.	**13.7**		**32.6**		**34.7**		**33.4**	

* Means with the same letter are not significantly different at 0.01 level.

Table 15. Experiment 2. Means of root parameters of cotton genotypes grown in tubes.

Genotype	Taproot Mean Length (cm)		Wet Wt. (g)		Dry Wt. (g)		Ratio wet/dry	
Tamcot 73	69.0	a	4.02	a	0.86	a	3.85	a
06 WE-14	61.1	a	4.34	a	0.79	a	3.84	a
02 WK-11L	53.9	ab	3.81	a	0.81	a	3.53	a
07 V-45	50.9	ab	2.92	a	0.42	a	1.45	a
DPL 491	44.4	b	3.21	a	0.43	a	2.90	a
08 WZ-83	42.5	bc	3.01	a	0.55	a	3.25	a
07 X-26	40.4	bc	3.30	a	0.85	a	2.91	a
08 WZ-78	32.7	c	3.60	a	1.03	a	3.87	a
Mean	**49.1**		**3.5**		**0.71**		**3.3**	
C.V.	**19.2**		**43.1**		**60.9**		**32.0**	

* Means with the same letter are not significantly different at 0.01 level.

The greenhouse study observing root systems in a growth tube might not be a good estimation of a taproot in a natural environment. Ben-Porath and Baker (1990) found that cotton taproots that were restricted to tubes or smaller pot sizes will flower earlier as well a flower faster. This indicates that the cotton plants overall physiological nature can be misleading when grown in a restricted plant environment such as a tube. Thus, drought tolerance in cotton plants maybe easily misidentified when grown in artificial environments.

Yield Performance Trials

Lint, Yield, and Fiber Quality

Lint yield, lint percent, fiber length and strength were significantly different between locations (Table 16 & 17). There were no location x genotype interaction differences for lint yield, lint percent, micronaire, nor length uniformity effects. Genotypes were different for lint percent, fiber length, strength, length uniformity, and elongation. These findings suggest that fiber traits, lint yield, and lint percent were more of a function of growing environment rather than genotype in 2013 at College Station and Thrall.

Table 16. Lint yield and lint percent ANOVA of pooled data from College Station and Thrall, TX in 2013.

ANOVA				
		Mean Squares		
Source	df	Lint (kg/ha)	df	Lint %
Location	1	24,835,674**	1	0.00190**
Rep(loc)	6	33,900	6	0.00017
Genotype	7	3,594	7	0.00131**
Loc*Genotype	7	9,647	7	0.00019
Error	34	6,590	42	0.00016

* Significant at the 0.05 probability level.
** Significant at the 0.001 probability level.

Table 17. ANOVA of fiber traits of eight cotton genotypes with data pooled together from College Station and Thrall, TX in 2013.

ANOVA

		Mean Squares				
Source	df	Length	Strength	Micronaire	Length Uniformity	Elongation
Location	1	0.0044**	11.99**	0.191	2.68	0.12
Rep(loc)	6	0.0004	1.47	0.091	0.89	0.07
Genotype	7	0.0076**	24.77**	0.119	5.20**	2.14**
Loc*Genotype	7	0.0005	0.25	0.082	1.50	0.15
Error	42	0.0010	1.25	0.079	1.01	0.12

* Significant at the 0.05 probability level.
** Significant at the 0.001 probability level.

For lint percent among genotypes, most were tightly clustered near 39%, except for 07 X-26, which was lower than all other genotypes at 36.3% (Table 18). 07 X-26 also had the longest fiber length at 29.9 mm, the strongest fiber at 344 kN m kg^{-1} , and the best length uniformity. It is common to see negative associations between high-quality fiber and low lint percent. Overall, the rankings among genotypes did not change based on location for most fiber traits.

Having a cultivar with inherently high-quality fiber traits is important to producers, especially those who grow completely rain fed crops. Large discounts can be incurred when cotton is severely drought stressed and the resulting fiber is low quality. Not only will the producer harvest less lint, the value of that lint can be substantially less than that from non-drought stressed plants. Even if drought conditions result in fiber length 10% shorter and weaker, discounts can be avoided because the initial genetic capacity, or starting point, was so high.

Table 18. Lint percent and fiber traits of eight genotypes at College Station and Thrall, TX in 2013.

Genotype	Lint% (%)	Length (mm)	Strength kN m kg^{-1}	Mic. (units)	Uniformity (%)	Elongation (%)
07 X-26	36.3	29.9	344	4.0	84.1	5.6
Tamcot 73	38.0	27.9	317	4.3	83.1	6.1
06 WE-14	39.9	27.6	290	4.2	82.1	6.0
08 WZ-78	40.4	27.9	291	4.1	81.6	5.3
02 WK-11L	38.8	27.9	302	4.2	83.0	6.9
08 WZ-83	39.4	28.1	308	4.4	82.8	5.4
Delta Pine 491	38.6	28.7	302	4.2	82.4	5.4
07 V-45	39.7	27.1	303	4.2	81.9	6.1
Mean	38.9	2.81	306	4.2	82.6	5.8
LSD (K=100)	1.2	1.1	18.4	n.s.	1.0	0.3
C.V. %	1.9	1.9	1.8	2.7	2.0	1.8

Paterson et al. (2003) explained that cotton is particularly sensitive to genotype by environment interactions associated with soil moisture availability. Findings suggest that a complex trait such as fiber quality may also be sensitive to drought conditions, presumably in conjunction with the development of genotypes that also contain genes with slow concentrative adjustments in productivity under drought conditions (Saranga et al., 2001). Finally, Zarco-Tejada et al. (2005) shared that vegetative structure and canopy chlorophyll concentration could provide complementary information about within-field yield variability using NDVI.

Plant Mapping

Plant mapping is a valuable assessment that enables researchers to determine vegetative and fruiting patterns. Drought or high temperature environments are sometimes associated with cotton sterility and boll retention problems (Reddy et al., 1992). Such effects can be detected with plant mapping.

At College Station, the field quality was on a gradient in terms of soil quality and therefore significant differences among replications were observed for many plant mapping traits (Table 19). The only parameter that was different among genotypes was plant height. At Thrall, there were no differences among genotypes for plant mapping traits except for the ratio of open boll to green bolls, which is a measure of a crop maturity. Perhaps if more plants were mapped per plot, more differences could have been detected with the larger sample size.

The coefficients of variation (CV %) were mostly favorable according to (Gomez and Gomez, 1984) with a CV% of less than 20% suggesting reliability. Total bolls, and open boll green boll (OB/GB) ratings had high CV's at College Station (Table 20) while the total number of bolls had a high CV at Thrall (Table 21).

There was a 26.5% difference between the tallest genotype, 06 WE-14, and the shortest genotype, Tamcot 73 at College Station (Table 20). These results are typical of how these particular lines tend to grow across environments. It is doubtful that plant height had a meaningful effect on yield, fiber quality, or even NDVI. At Thrall, the earliest maturing genotypes were 02 WK-11L, Tamcot 73, and 08 WZ-78 based on OB/GB (Table 21). 02 WK-11L was the only genotype with okra leaves and Tamcot 73 generally has one of the earliest maturity habit in this group of genotypes regardless of the growing environment.

Table 19. ANOVA of cotton plant mapping at College Station and Thrall, TX in 2013.

College Station, TX							
Source	**df**	**FFB**	**Nodes**	**Height**	**#Bolls**	**OB/GB**	**H/N Ratio**
Rep	3	0.96**	3.16*	115.4*	6.46	0.01	1.02
Genotype	7	0.04	0.31	81.5*	5.70	0.09	0.56
Error	21	0.10	1.06	30.8	3.96	0.03	0.41
Thrall, TX							
Source	**df**	**FFB**	**Nodes**	**Height**	**#Bolls**	**OB/GB**	**H/N Ratio**
Rep	3	0.27	0.92	48.8	1.77	0.04*	0.34
Genotype	7	0.12	1.49	51.3	0.57	0.06**	0.27
Error	21	0.09	1.42	34.0	2.94	0.01	0.19

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

Table 20. Plant mapping of eight cotton genotypes at College Station, TX in 2013.

College Station, Texas						
Genotype	**FFB (#)**	**Nodes (#)**	**Height (cm)**	**Bolls (#)**	**OB/GB %**	**H/N Ratio**
Delta Pine 491	4.5	14.5	66.9	7.5	13.0	4.7
Tamcot 73	4.6	15.0	53.9	9.4	46.0	3.6
07 X-26	4.8	15.1	61.6	10.1	15.0	4.1
07 V-45	4.7	14.9	62.5	6.7	30.7	4.2
08 WZ-83	4.8	14.7	60.7	7.0	40.2	4.1
08 WZ-78	4.6	14.5	59.7	7.6	47.2	4.1
02 WK-11L	4.6	15.0	64.9	8.2	54.5	4.4
06 WE-14	4.6	15.2	68.2	8.9	33.0	4.5
Mean	4.6	14.9	62.3	8.2	35.0	4.3
LSD (0.05)	NS	NS	9.6	NS	NS	NS
C.V. %	8.9	6.9	8.9	24.4	49.7	8.0

Table 21. Plant mapping of eight cotton genotypes at Thrall, TX in 2013.

Thrall, Texas						
Genotype	**FFB (#)**	**Nodes (#)**	**Height (cm)**	**Bolls (#)**	**OB/GB %**	**H/N Ratio**
Delta Pine 491	4.6	13.1	53.2	4.8	51.0	4.1
Tamcot 73	4.8	13.3	48.9	4.4	81.0	3.6
07 X-26	4.5	13.1	54.7	4.5	57.0	4.2
07 V-45	4.7	14.2	59.6	4.2	54.3	4.2
08 WZ-83	4.5	12.5	50.2	3.9	67.0	4.1
08 WZ-78	4.3	12.4	52.2	4.5	74.5	4.2
02 WK-11L	4.8	12.8	52.7	3.6	85.3	4.1
06 WE-14	4.4	12.3	49.9	4.2	65.8	4.1
Mean	4.5	12.9	52.5	4.2	67.0	4.1
LSD (0.05)	NS	NS	NS	NS	15.0	NS
C.V. %	8.6	9.2	11.1	40.4	16.2	8.0

CHAPTER IV

CONCLUSION

The objectives were to evaluate root systems in growth tubes, as well as NDVI and leaf temperatures in field plots to determine potential means of drought tolerance as well as measure lint yield, fiber quality, and plant maps from the same plots. It was thought that such information would benefit cotton breeders by allowing them to more efficiently phenotype drought tolerance. Results from the field trials and growth tubes provided several valuable insights into the design and execution of such protocols for high-throughput phenotyping.

First, a single NDVI measure once per week can only measure macro-vegetative growth and to a lesser extent green color, which is an indication of photosynthetic activity. Cotton has the ability to regulate fruit load, which is the primary photosynthetic sink, in response to growing conditions in order to maintain vegetative health. Climatological factors and boll load can be affected in variable environments (Ehlig and LeMert, 1973). This is an evolutionary strategy since cotton is botanically a perennial species. As such, drought stress would likely first affect tissue within the fruiting structure, and the propensity of the plant to add fruiting structures before it would alter vegetative growth. NDVI does not have the capability to account for this type of strategy to minimize stress tolerance. However, if NDVI were to be taken twice per day, once in the morning and again in mid-afternoon when wilting is most likely to occur, then NDVI

likely could detect differences in plants' abilities to maintain leaf turgor pressure, which is a good indicator of drought tolerance.

In addition, NDVI appears to have utility in other cotton research areas. Since differences among genotypes were found early in the growing season, it would seem logical that NDVI would be a useful tool to evaluate seedling disease effects, early-season insect pest management, and starter fertilizer regimes. Also, irrigation scheduling capability could be derived from remote sensing technology. Gonzalez-Dugo and Mateos (2008) suggested that remote sensing techniques could be used to measure crop evapotranspiration on a large scale basis. Likewise, it also seems likely that NDVI could assist in quantifying efficacy of defoliation treatments.

Secondly, differences among genotypes for leaf temperature were relatively stable across locations and weeks within a growing season. Therefore, it would be a prudent use of resources to only take this type of measurement at a few locations a few times per growing season since the genotype rankings do not appear to change across time or location. Leaf temperature was found to be profoundly affected by soil-moisture status and ambient temperature. Consequently, leaf temperature information could be integrated into irrigation scheduling models, and equipment which would in-turn make more efficient use of water resources for producers.

Finally, root measurements in growth tubes did not relate to any of the drought tolerant characterizations observed from the field trials. In addition, there were dramatic differences in response to genotypes from the first to second experiment, which was likely in response to temperature. Further investigations are needed to determine

potential epigenetic effects related to soil temperature, transpiration rates, and daylength effects on taproot lengths.

In retrospect, we probably should have had a tighter control over plant spacing within the plots, the most recent engineered mechanical planters allow growers to have great control over seed placement. Intra-plant competition could be a large source of experimental error introduced into studies. In regards to testing roots in growth tubes, the inherent variability of individual genotypes to determine the degree of phenotypic variation probably should have been measured. Since roots have not been under direct selection pressure in cotton breeding programs, there are likely latent differences within populations for root length. Each genotype should have had a minimal of 100 individual plants grown in growth tubes to determine the inherent phenotypic variation before comparisons were made among genotypes in replicated trial.

For breeding programs, NDVI technology, leaf temperature assessment, and root analysis in a controlled environment are promising screening procedures to determine drought tolerance if external sources of variation can be mitigated. Moreover, all of these methodologies can improve other agronomic studies and be implemented as tools to help cotton growers be more efficient with their resources.

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