TWO APPROACHES TO EVALUATE DROUGHT TOLERANCE IN MAIZE:

SEEDLING STRESS RESPONSE AND EPICUTICULAR WAX ACCUMULATION

A Thesis

by

MEGHYN BRIANNE MEEKS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Plant Breeding

TWO APPROACHES TO EVALUATE DROUGHT TOLERANCE IN MAIZE:

SEEDLING STRESS RESPONSE AND EPICUTICULAR WAX ACCUMULATION

A Thesis

by

MEGHYN BRIANNE MEEKS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Co-Chairs of Committee,	Seth Murray
	Steve Hague
Committee Member,	Dirk Hays
Head of Department,	David D. Baltensperger

December 2010

Major Subject: Plant Breeding

ABSTRACT

Two Approaches to Evaluate Drought Tolerance in Maize:

Seedling Stress Response and Epicuticular Wax Accumulation. (December 2010)

Meghyn Brianne Meeks, B.S., Tarleton State University

Co-Chairs of Advisory Committee: Dr. Seth Murray
Dr. Steve Hague

We wanted to develop rapid and cost-effective drought tolerance screening methods for mass amounts of germplasm. In 2009 and 2010, we evaluated sixty-two maize inbred lines and their hybrid testcross progeny using seedling stress response and epicuticular wax accumulation as predictors of drought tolerance.

The seedling screening method measured germination, survival and recovery percentages after a series of drought cycles in a greenhouse environment. Eight inbred lines had significantly ($P < 0.05$) lower germination than the mean estimate, but hybrid testcrosses were not significantly different. The second-to-last day of survival cycle and the second day of recovery cycle best explained genotypic differences for inbred lines and hybrid testcrosses respectively. One inbred line performed well as both an inbred line and hybrid testcross, but poor correlation over the sample set ($R^2 = 0.0097$) was observed.

Flag leaves taken at flowering from plants under full and limited irrigation regimes were sampled for epicuticular wax. Extracted wax weight for genotypes was compared as a percentage of leaf weight (% wxlfwt) and leaf area (% wxwta). Eleven genotypes had above average %wxlfwt as both inbred lines and hybrid testcrosses. Thirteen genotypes had above average %wxwta as either inbred lines or hybrid testcrosses. Irrigation treatment was not significant ($P > 0.05$). Heritability of % wxlfwt was 0.17 (inbred lines) and 0.58 (hybrid testcrosses). Heritability of % wxwta was 0.41 (inbred lines) and 0.59 (hybrid testcrosses). Correlations (R^2) for %wxlfwt and %wxwta were 0.19 and 0.03, respectively. Heritability of grams of grain per ear and total grain yield was highest in hybrid testcrosses, with no correlation between inbreds and hybrids.

The developed seedling screening method easily allowed visible drought tolerance observations in inbred lines and hybrid testcrosses but does not seem heritable in our germplasm. Epicuticular wax weight is not an ideal primary trait to evaluate for drought tolerance, but may be a good candidate to observe as a secondary trait in relation to grain yield production in hybrids. Results from this research best supports breeder selection of hybrid germplasm using seedling drought tolerance in conjunction with epicuticular wax.

DEDICATION

To my parents

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Seth Murray, and my committee members, Dr. Steve Hague and Dr. Dirk Hays, for their mentorship and guidance throughout the course of this research. In addition, I would like to thank the many colleagues and student workers who graciously helped make this research possible.

Finally, I would like to thank my family for all their love and support and encouraging me to pursue my dreams.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

The US is the largest consumer of maize per capita in the world, ranking just above China and Brazil (Nation Master, 2005). According to the National Corn Growers Association (2010), the majority of the 2009 maize harvest was manufactured into feed for livestock and ethanol while only 9% was used for direct human consumption, planting seed and industrial uses. Greater maize production is necessary to support 1) more people, 2) economic development (more animal products wanted) and 3) energy needs. Unfortunately, environmental issues such as drought are detrimental to the maize production levels needed to support these three areas.

Although states in the Midwestern region of the US produce the most maize due to ideal climate conditions, Texas is also an important producer. In Texas, weather conditions change every year and a predicted drought is concerning. 'Drought' can describe abnormally low rainfall or soil moisture levels as well as an extended period of below normal rainfall. In addition, it can be climatic or plant based. A collaborative effort between the USDA, National Weather Service Prediction Center, National Climatic Data Center and the National Drought Mitigation Center at the University of Nebraska Lincoln brought about the development of a drought intensity index, monitoring drought from abnormally dry (D0) to exceptionally dry (D4) (US Drought Monitor, 2008). September 2007 to June 2009 was categorized as the second worst twenty-two month drought since 1895 in south central Texas (National Climatic Data Center, 2010a). During this time span, 2008 Texas agriculture losses reached an estimated $1.4 billion (High Plains/Midwest Ag Journal, 2008); while in 2009 the D4 intensity made Texas the epicenter of drought in the US (National Climatic Data Center, 2010). National losses in 2008 were an estimated $2.0 billion and more than $5.0 billion in 2009 (National Climatic Data Center, 2010b). Since national agriculture production is heavily influenced by drought and Texas agriculture seems to suffer more than other production regions, Texas should be the focus for many drought tolerance/resistance breeding

_____

This thesis follows the style of Journal of Agronomy and Crop Science.

efforts. Though major south and central crop production regions of Texas typically receive rainfall within the growing season, variability requires farmers to manage water conservatively to grow a profitable crop. Farmers depend on rainfall and supplemental irrigation for preparing soils for planting and in-season crop development.

Effects of drought are a constant threat to global food security. One solution to handling the drought water dilemma was the development of water-use efficient, drought-tolerant crops, where drought tolerant germplasm produce equivalent grain yields to susceptible germplasm under ideal conditions. Fortunately, the evolution from open-pollinated to single cross hybrids has gradually coincided with greater stress tolerance due to changes in selection protocols; however, grain yield production has not increased enough to meet current demand (Tollenaar and Wu, 1999). This is an attribute to antiquated methods of focusing on yield stability and elevating drought intensity. Since drought tolerance and grain yield are two completely different quantitative traits, new screening methods are necessary for progressive development.

Plant breeders constantly search for easily manipulated traits, breeding and screening methods to improve drought tolerance in plants. The major obstacle is the complex nature of drought tolerance and its quantitative phenotype where many factors of plant development contribute. Therefore, the ability to identify several drought tolerance genes for a single crop has been challenging. Without advances in breeding and screening techniques, the development of drought tolerant crops will be impossible.

2. REVIEW OF LITERATURE

Crop water needs are dependent on several factors. These include climate, crop species, soil properties, management inputs and grain yield goals. Unpredictable weather causes difficulty in planning field-based experiments and for production enterprises for farmers.

Crops need the most water when the climate is sunny, hot, dry, and windy, in which case evapotranspiration rates are high; and the least when the climate is cloudy, cool, and highly humid resulting in low evapotranspiration (Brouwer and Heibloem, 1986). It is estimated that grain maize needs 6.05 mm per day over the average growth period of 147-156 days in Weslaco and College Station, Texas. It is improbable for most maize production regions in Texas to receive enough timely rain that fulfills the soil-water holding capacity and evapotranspiration needs during the maize growing season. Consequently irrigation is needed to optimize crop production. Reliance on irrigation to avoid drought is a precarious solution due to depleting water supplies and the needs of urban areas.

Plant available water depends on soil properties such as texture (sand, silt, or clay), structure and depth as well as a few other properties (plant residue, crop rotation, deep profile soil moisture, and run-off). Soils higher in clay content absorb slower and retain water longer but also restrict plant roots from absorbing water readily because water molecules adhere strongly to clay particles (Davis and Wilson, 2005). Sandy soils absorb and dry quicker, but allow water to be readily available to plant roots because forces of cohesion loosely bind water to sand particles. Cycles of wetting and drying cause soil compaction which results in naturally occurring aggregates called peds. Soils with small or no peds is ideal for adequate maize root development so that roots do not have to penetrate or avert peds in search for water (Amato, 2002).

Understanding how drought conditions physiologically affect food crops is pertinent so that breeders can use this knowledge in the development of drought tolerant crops. The growth stage of a plant determines crop water needs and responses to drought. The primary criteria for plant breeding

selection is grain yield stability, but at which growth stage drought mostly afflicts grain production has long been debated by researchers.

Drought induced at different stages of plant development effects grain yield differently (Cakir, 2004). One problem with seedling drought stress is growth stunting. Hsiao and Acevedo (1974) noted that turgor pressure is critical for leaf growth. It is understandable that leaf turgor directly relates to cell enlargement, and without proper pressure, cell expansion ceases and wilting occurs. Research from Sharp and Davies (1979) supported this when they discovered leaf area development correlated with a lack of turgor pressure in drying soil. Kang et al. (2000) found maize adapted to the semi-arid loess plateau in China yielded similar to full-irrigated maize even though seedlings were water stressed. Those same stressed plants also showed signs of better adaptation to drought when water was further withheld during stem-elongation stages due to enhanced root systems that develop in response to earlier drought conditions. However, plants stressed only during stem-elongation had a significant reduction in grain yield. The re-assimilation of solutes to plant roots is a drought defense mechanism to maintain root turgor and continue root growth (Sharp and Davies, 1979). Obviously, conditioning maize seedlings to water deficits for greater root development is necessary so the negative response to drought at later growth stages is not detrimental.

Maize uses the most water during the mid-season stages of flowering and grain filling. Without sufficient water at these stages, proper synchrony or anthesis-silking interval (ASI) may be shortened or extended, thereby limiting grain production. Drought can also cause early leaf senescence during flowering, thereby reducing the leaf area index (LAI) and thus productivity (Ashghizadeh and Ehsanzadeh, 2008). According to Cakir (2004), drought stress most restricts maize development at early vegetative and reproductive stages, and irrigation withheld at both flowering and grain filling does lead to significant yield loss. Drought stress seven days after silking seems to be the most sensitive stage for grain development and final kernel number, whereas stress before silking does not affect grain yield (Grant et al., 1989). More recent findings from researchers contradict this statement. NeSmith and

Ritchie (1992) observed the greatest grain yield loss at least a week prior to anthesis in commercial hybrids planted in Michigan. Eck (1986) found that drought stress most affected grain yield during vegetative growth and grain filling, but Bruce et al. (2002) identified pollination and early grain filling as the most drought sensitive stage in elite tropical maize populations from Mexico. Although some conclusions from their research differ, Eck (1986) and Bruce et al. (2002) do agree about water needs at grain filling, for duration of kernel growth is shortened when drought prematurely stops dry matter accumulation in both embryo and endosperm; which significantly decreases embryo and endosperm final mass (Westgate, 1994).

Drought appears to affect many growth stages with influences from genotype and genotype by environment interactions. This is clear in the previously cited studies as both germplasm and environments vary greatly. These factors make it difficult and costly to measure yield loss due to stress. Therefore, the development of rapid (high-throughput) and accurate screening methods employing secondary traits as predictors of drought tolerance would be useful for genetic improvement.

Instead of focusing on when drought affects grain yield, other researchers have focused on simplifying the screening and selection of drought tolerant species and genetic variation. Singh et al. (1999) developed two drought-screening methods for cowpea (*Vigna unguiculata*), a "box screening method" and a "root box pin-board method." Cowpea drought tolerance evaluated in seedlings closely correlated with drought tolerance at the reproductive stage. Additionally, root growth positively correlated with shoot drought tolerance. However, findings from Sharp et al. (2004) do not support this statement; root growth is seemingly less sensitive to drought than shoot growth, for roots continue to reach for water lower in the soil profile plants increase the ratio of root-to-shoot growth. This is significant in that screening at an earlier stage of development, with proven positive correlation, could aid in reducing research costs incurred from evaluations at full maturity. With efficient screening methods, all of these factors could diminish to enable earlier identification of perspective drought tolerant varieties. A separate study using the "box screening method" on other major crops grown in the

semiarid tropics ranked maize as fifth between soybean (*Glycine max*) as the most drought susceptible and cowpea as the most drought tolerant (Singh and Matsui, 2002). However, Singh and Matsui (2002) observed that a sand medium was too drastic to observe variation within crops such as maize and suggest soils higher in clay content. This would allow the water binding properties of clay to stress the plants more slowly and display which genotypes adapt best to water restrictions. Since the variety of maize is unmentioned, we cannot allude to its origin nor assume sand is inappropriate for this type of study.

Longenberger et al. (2006) employed the use of Ray Leach (Canby, OR) cone-tainers™ and drought cycles for cotton. Justification for using Ray Leach cone-tainers™ is elimination of interplant competition for water resources and allowing discrete individual plant analysis. The idea of drought cycles enabled survival and recovery measurements. Variation among genotypes was discovered in drought cycle 1 but not 2 or 3, showing that additional drought cycles other than the first was unnecessary. By comparison, Longenberger et al. (2006) and Singh et al. (1999) reported interplant competition for resources is not problematic for either method, although cone-tainers™ may limit lateral root growth in some crop species.

Many target environments have drought conditions that are difficult to predict. As a result, plant-breeding methodologies to identify drought tolerant maize vary. Recurrent selection to enrich a population with favorable alleles is one breeding strategy. Edmeades et al. (1999) found selection for drought tolerant genotypes during the S_1 generation of a recurrent selection-breeding program 50% more effective than a recurrent full-sib breeding program. This suggests that genes for drought tolerance are recessive and found easier in homozygous and homogeneous inbred line populations than hybrid populations, which have been out-crossed and exhibit genetic segregation. Identification of drought tolerant genotypes in the early generations of a breeding cycle can reduce program costs by quickly eliminating inferior germplasm.

Selection only when drought occurs is another breeding strategy. Unstressed conditions do not allow the expression of drought tolerant traits and therefore accurate phenotypic selections cannot be made. Edmeades et al. (1999) recommended selection in drought stressed environments where genotype selection by grain yields have exposed superior germplasm. Bänzinger and Lafitte (1997) reiterated this idea but also stressed the importance of selection intensities heavily weighted on secondary traits in conjunction with grain yield. Secondary traits include low leaf number and short plant height (Muenchrath, 1995), more erect, angled leaves (Sangoi and Salvador, 1998), increased rooting depth and density (Ludlow and Muchow, 1990), performance with limited available nitrogen (Bänzinger et al. 2004), epicuticular wax (Ristic and Jenks, 2002), delayed leaf senescence and shortened anthesis-silking interval (Betrán et al. 2003a). Reductions in the anthesis-silking interval (ASI) and early silking were two results of selection improvement to counter initial seed losses from drought (Bruce et al. 2002).

Selection based on secondary traits has become increasingly important in drought tolerance improvement efforts. Over time, the ever-changing environment has allowed plants to evolve and produce barriers to environmental stresses. Two particular barriers are the development of cuticles and external lipids to regulate water loss. A plants' cuticle is composed of hydrophobic, biopolymer cutin and lipids (Koch et al. 2006). Lipids are waxes that can be found internally or externally. The development of external lipids, epicuticular waxes, in response to drought was a focus of this study.

Waxes have been determined as the barrier between the cuticle and environment against leaf water or solute diffusion (Schönherr, 2006). Cuticular water permeability or transpiration theoretically, positively correlates to cuticle membrane thickness (Riederer and Schreiber, 2001). Leaf thickness and epicuticular wax deposition do not increase in response to CO_2 enrichment for maize (Thomas and Harvey, 1983). Riederer and Schreiber (2001) observed an increase in water permeability of the cuticle with higher temperatures. Increased deposition of epicuticular wax on leaf surfaces in response to water stress in warm season forage crops suggests stressed plants have an enhanced ability to retain water

(Saneoka and Ogata, 1987). Therefore, this factor may be useful in predicting drought tolerance in maize.

No published data was found to determine if light absorption or reflectance indicates drought tolerance in maize. Long et al. (2003) characterized epicuticular wax as a "sunscreen" for maize in that it absorbs ultraviolet radiation. However, greater reflectance due to increased thickness of the wax layer as a response to UV light has been observed (Holmes and Keiller, 2002). Ebercon et al. (1977) found excessive epicuticular wax deposition to be an important drought avoidance mechanism in sorghum leaves due to the wax's increased reflectance of visible and near infrared radiation, decreased net radiation in the field and decreased cuticular transpiration. Ebercon et al. (1977) compared gravimetric and colorimetric methods to determine which was more efficient at finding drought resistant cultivars using epicuticular wax load. The colorimetric method was preferred because it processed samples 10 times quicker and used fewer materials than the gravimetric method. Results from both methods were similar in ranking sorghum genotypes with a correlation coefficient of 0.984. They also observed no difference in epicuticular wax accumulation between a genotype's four leaf stages, but did find differences between genotypes. This suggests that there are multiple genes associated with epicuticular wax production and drought tolerance, so there is room for genotype improvement.

Under water-stressed conditions, epicuticular waxes increase (Bondada et al. 1996, Premachandra et al. 1991). Bengston et al. (1978) analyzed oat seedlings under water stress finding a decrease in cuticular transpiration amongst all varieties and an increase in epicuticular wax without finding a clear correlation between the two. Amongst other cereal grains (wheat, rice and sorghum), maize epicuticular wax is most similar in chemical composition to rice (Bianchi et al. 1979).

Epicuticular wax is a qualitative trait, specific to early vegetative stages (Lawson and Poethig, 1995). It is proven that wax concentrations decrease with increases in leaf age (McWhorter, 1993). As leaves develop, they gain greater leaf area. Leaf area has previously been used to observe the effect of herbicides on wax aggregation on leaves through measured water loss (Leavitt and Penner, 1979), and to

measure wax accumulation (O'Toole et al. 1979, McWhorter, 1993). The location for the greatest amount of wax on maize leaves may be found at the apex, although wax concentrations are uniform over entire leaf surfaces (McWhorter, 1993). Observations of maize shoot development have found epicuticular wax on juvenile tissues transitioning to adult tissues, in which the adult tissue subsequently accumulates the thickest amount (Lawson and Poethig, 1995). The difference in tissue phases can be visually rated; adult tissue appears glossy due to the absence of epicuticular wax during juvenile-adult phase transition in wild types (Lawson and Poethig, 1995). The mutant gene, *glossy15* (*gl15*), has been found to cause early maturation that affects the leaf's epidermis (Lawson and Poethig, 1995) by regulating epicuticular wax production (Lemieux, 1996). Mutant plants therefore produce adult-phase epicuticular wax in the juvenile-phase, creating a "glossy" appearance. Researchers can phenotypically identify mutants from wild types using this knowledge (Moose and Sisco, 1994). An additional gene responsible for epicuticular wax biosynthesis are the *gl2* gene, which transcribes only in juvenile leaves (Lemieux, 1996). The mutant *Teopod* genes, *Tp1 & Tp2*, have pleiotropic effects; they prolong juvenile development but do not affect late vegetative and reproductive development (Lawson and Poethig, 1995). Selection of plants that exhibit these phase-specific traits could aid in guarding against environmental threats such as pest and pathogens. Plants would undergo a phase earlier or later than normal, relied upon by pests or pathogens for their development.

Any useful trait for drought tolerance identification must be heritable. Heritability estimates of traits are dependent on the relationship of observed genetic variation to environmental variation. For drought tolerance, it is necessary to find traits less influenced by environment so that breeders can use the trait for future crop improvement in multiple environments. Unfortunately, conclusions drawn about the drought tolerance of germplasm are specific to the environment(s) in which it was tested. This is easily observed in comparing temperate and tropical germplasm and the different traits chosen to reflect drought tolerance.

Researchers from CIMMYT have identified drought tolerance germplasm in elite tropical maize families based on different secondary traits and performance at flowering under varying levels of drought stress (Bruce et al. 2002). Selected families not only performed well in tropical zones, but also temperate climates with no drastic change in grain yield (Bruce et al. 2002). Under drought stress, tropical lines exhibited higher heterosis from parental inbred lines to hybrids than under normal non-stressed conditions (Betrán et al. 2003c). Grain yield stability across locations seems to be low in temperate germplasm. This makes it difficult to ascertain an appropriate screening environment to yield equally well in multiple temperate zones. It is unpublished whether temperate germplasm would perform similar to tropical germplasm in a tropical environment.

Despite these extensive efforts with drought research, the exact timing of drought and at what growth stages limit yield production is not completely understood. The abiotic and biotic factors that vary between target environments, the germplasm and its origins often account for this. It would make the most sense to modify breeding and selection practices for genotypes adapted to specific environments, than attempting to find a specific period of the growth cycle for all species of maize.

3. SEEDLING STRESS RESPONSE

3.1 INTRODUCTION

Methods to identify and develop drought tolerant hybrid maize are important goals for researchers, as drought has intensified over the past few decades afflicting many prominent maize growing regions. In an attempt to reduce drought losses, many breeders primarily focus on grain yield stability during periods of drought stress, but little progress has occurred due to the complex nature of drought tolerance. This has led to using secondary traits to improve drought tolerance. Secondary traits are often identifiable in inbred lines and inherited by hybrid progeny. These traits include, increased rooting depth and density (Ludlow and Muchow, 1990), high leaf number and short plant height (Muenchrath, 1995), performance with limited available nitrogen (Bänzinger et al. 2004), seedling vigor (Singh et al. 1999), epicuticular wax (Ristic and Jenks, 2002), delayed leaf senescence and shortened anthesis-silking interval (Betrán et al. 2003a). Others have utilized new technology such as molecular markers associated with drought tolerance (Bruce et al. 2002). Many of these methods are too costly in terms of money and labor. In personal communication with Singh (2008), whom developed drought tolerant cowpeas germplasm through seedling screening (Singh and Matsui, 2002), he suggested a similar approach may be successful with maize. Singh et al.'s (1999) method to evaluate multiple germplasm on a small-scale seemed timely and affordable. Modifications to Singh et al. (1999) method were adopted from Longenberger et al. (2006) and made to fit the unique growth and development habits of maize. It was hypothesized that maize genotypes that survive consecutive cycles of drought stress would be drought tolerant and that tolerance would be heritable.

3.2 MATERIALS AND METHODS

The seedling screening experiment occurred in a greenhouse at the Institute for Plant Genomics and Biotechnology at Texas A&M University from January-May 2009 and during the same months in 2010. The first year of the experiment evaluated inbred lines under simulated drought conditions while the second year's experiment evaluated hybrid testcross progeny under similar irrigation regimes.

Germplasm

Germplasm was comprised of sixty-two maize inbred lines and checks (Appendix A). Plant breeders with Texas AgriLife Research at College Station and Lubbock, TX, developed fifty-five of the inbred lines with College Station sources from 2005-2007 and Lubbock sources from 2009. Six of the Texas AgriLife lines have been released (Betrán et al. 2004a, 2004b and 2004c, Llorente et al. 2004, Xu and Odvody 2004). Germplasm consisted of a wide array of grain colors and origin including white, yellow, red and blue grain types derived from tropical, Argentine and temperate origins. In 2009, each of these sixty-two inbred lines were testcrossed in a College Station, TX, field nursery to a single-cross hybrid parent (LH195 x LH287) (Monsanto Company, 1991 and 2001) of the original two inbred line checks. For eight hybrid testcrosses, a winter nursery in Weslaco, TX, was used to increase seed. In 2010, testcross progeny were evaluated as well as additional checks that included the best and worst performing inbred lines from the previous year and the hybrid parent (Appendix A).

Experimental Design

Multiple soil media were tested to determine which was the most appropriate for inducing drought. Soil moisture was difficult to control and eliminate in the Metro-Mix 300. Therefore, sand became the choice soil medium for the remainder of the study. A potting medium of river sand held in the Ray Leach 5 3/8" cone-tainers™ and trays with a cotton ball inside the base of the cone-tainer to contain the sand was found to be the most effective for controlling soil moisture in screening large amounts of germplasm. Planting depth was 2.54 cm. The inbred line trial had four planting dates (Jan 13, Feb 16, Mar 12, and Apr 17) while the hybrid testcross trial had two dates (Jan 22 and Feb 19).

Irrigation to field capacity occurred once at planting and once at the beginning of each drought cycle. The first drought cycle for the inbred line study began three days after planting (DAP) while the first drought cycle for the hybrid testcross study began five days after planting. Plastic wrap was used to ensure germination by completely covering the surface of the cone-tainers to eliminate air flow and trap moisture, and was then removed when germination peaked. The experiment was a completely randomized design with seventy entries per replication. Each genotype was represented by four seeds planted into individual cone-tainers™ grouped in a square block. There were ten replications in 2009 and eight in 2010 (Table 3.1). The first four replications in the inbred line trial were planted in potting soil. At least two replications were grown simultaneously. Because of limited seed, multiple seed per cone-tainer™ were not planted. Moreover, it was thought that multiple seed per cone-tainer would introduce inter-plant competition and defeat the purpose of using cone-tainers.

Table 3.1 Plant type, replications, planting dates and soil medium used for seedling study from January-May 2009 and during the same months in 2010

Year (Plant Type)	Replication	Planting Date	Soil Medium
2009 (Inbred)	1 & 2	13-Jan	potting mix
	3 & 4	16-Feb	potting mix
	5 & 6	12-Mar	sand
	7, 8, 9 & 10	17-Apr	sand
2010 (Hybrid)	1, 2, 3 & 4	22-Jan	sand
	5, 6, 7 & 8	19-Feb	sand

Screening Methods

Screening methods were modeled after Longenberger et al. (2006) and Singh et al. (1999) whom screened cotton and cowpea germplasm for drought tolerance. Because maize has a different growth habit from cotton and cowpea, several tests were conducted to determine which experimental

parameters, such as soil type, containers, planting depth and irrigation regimes, were best suited to induce meaningful drought stress. Germination, seedling survival and recovery percentages were obtained during the trials over nine observation days. The first drought cycle measured germination percentage and seedling survival from observation days 1-4. The second drought cycle measured seedling recovery during observation days 5-9 after re-watering.

Germination Percentage – Greenhouse Emerging seedlings were enumerated daily with a maximum of four plants per genotype in each replication. Once germination peaked for the entire replicate, this date was recorded as the first day of survival. For example, three seedlings emerged out of four seeds planted to give a germination percentage of 75%. This percentage then averaged across all replications per trial determined the average germination percentage per genotype (Eq. 3.1).

((total # of plants on the 1st day of survival) / (the # planted/genotype/replication)) * 100 (Eq. 3.1)

Germination Percentage – Field Although the sand medium used in this experiment does not represent field conditions in Texas, curiosity existed as to whether or not germination percentages observed in the greenhouse reflected germination percentages in the field. Field germination percentages were drawn from another experiment using the same germplasm grown on a Ships clay loam soil type in College Station, TX. The field trials were evaluating differences in epicuticular wax production on flag leaves at flowering under full and limited irrigation regimes. Epicuticular wax is a secondary trait to grain yield to predict drought tolerance. Twenty-five seeds were planted in fifteen-foot plots for each genotype. With the exception of checks, each genotype occurred four times. Germination percentage was calculated by equation 3.2.

(the averaged stand count of the inbred line epicuticular wax study)/ (100 seeds planted). (Eq. 3.2)

Survival & Recovery Notation of plant survival was initiated when the emergence of new plants peaked, which was approximately at the second leaf stage and visible drought stress occurred. Subjective ratings were made on a binomial scale of 'tolerant' or 'susceptible.' Wilting, curling and discoloration denoted 'susceptibility.' When the majority of the seedlings were observed susceptible, irrigation induced the

recovery cycle. Measurements for recovery occurred two days later. Recovery was measured the same as survival, where only turgid plants measured as 'tolerant.'

Statistical Analysis

The PROC MIXED procedure in SAS 9.2 was used for data analysis. BLUEs or fixed solution estimates attained from analysis of germination percentages in greenhouse and field conditions were plotted against each other for soil-type comparison.

The number of tolerant plants at the time of survival and recovery measurements gave each genotype a numeric value between 0 and 4. Each genotype's numeric value for each observation day was then divided by the recorded germination number to give percent survival and percent recovery for each day. The trials in 2009 and 2010 were analyzed separately. Analysis used genotype, planting date (plantdate), and replication within planting date (plantdate(rep)) as variance components. Fixed solution estimates for observation dates in which the most variation in inbred line and hybrid testcrosses genotypes was explained were plotted against each other for comparison. These dates also were used to determine which genotypes were significantly susceptible or tolerant.

3.3 RESULTS

Germination Percentage (Greenhouse)

The overall average for the inbred lines in sand was 83% germination (excluding the first four replications with potting soil) (Table 3.2). Eight of the sixty-two genotypes and two checks had significantly lower germination (Table 3.3). The overall average for the hybrid testcross lines was 87% germination. None of the hybrid testcross lines or checks were significantly different suggesting hybrid testcrosses had more consistent germination in comparison to inbred lines. Germination percentages in sand were consistent, thereby supporting its use as a planting medium for seedling screening experiments involving maize.

Table 3.2 Inbred line and hybrid testcross germination percentage, overall survival percentage at the end of each replication, the number of days after planting seedlings survived, the end date of each replication and mean percentages for each data parameter

	Reps[1]	1	2	3	4	5	6	7	8	Mean
Inbred	Germination %	58	80	92	88	86	92	--	--	83
	Overall Survival %	2	2	0	0	0	0	--	--	1
	Survival DAP	26	26	28	28	28	28	--	--	27
	End Date	4/5	4/5	5/13	5/13	5/13	5/13	--	--	
Hybrid	Germination %	90	88	90	91	88	82	85	81	87
	Overall Survival %	0	0	1	3	1	3	4	2	2
	Survival DAP	26	26	26	26	22	22	22	22	24
	End Date	2/17	2/17	2/17	2/17	3/17	3/17	3/17	3/17	

[1] Replications 1-4 not included for inbred lines in the table, so for replications 5-10 refer to 1-6. Replications 7 and 8 are not available as there was not an eleventh or twelfth replication in 2009.

Table 3.3 Significant inbred line genotypes, their pedigree, corresponding germination percentage and level of significance as well as the overall trial mean germination percentage

Genotype	Pedigree	Germination %	Significance
4	((CML 408/B104)x(CML 411/B104))-1-1-B-B-B-B	54	**
7	(B104/NC300)-B-1-B1-B-B-B-B	38	***
10	(B97x CML 326-B/Tx770 x A645)-2-2-B-B-B-B-B-B	63	*
12	(B104-1 x Tx714-B/B110 x FR2128-B)-12-4-B-B-B-B-B-B	45	***
18	((Tx772 x T246) x Tx772)-1-5-B-B-B-B-B-B	42	***
22	Tx772W	67	*
39	NC280-B-B-B-B-B-B	58	**
51	S2B73	67	*
	Mean	83	

Germination Percentage (Greenhouse vs. Field)

After analysis, each genotype's germination estimates (BLUEs) from the greenhouse and field experiments were plotted against each other. A small positive correlation existed among inbred lines (R^2 = 0.1205) (Fig. 3.1). Hybrid testcross field trial germination data was confounded, so it is not presented. No individual genotype stood out as germinating well in both soil types; however, there was better overall germination in the sand medium in comparison to College Station which had 31% for inbred lines. The use of plastic wrap may have elevated germination in comparison to field conditions. The results suggest that using sand for the seedling drought experiment was plausible, but may not be predictive of field germination.

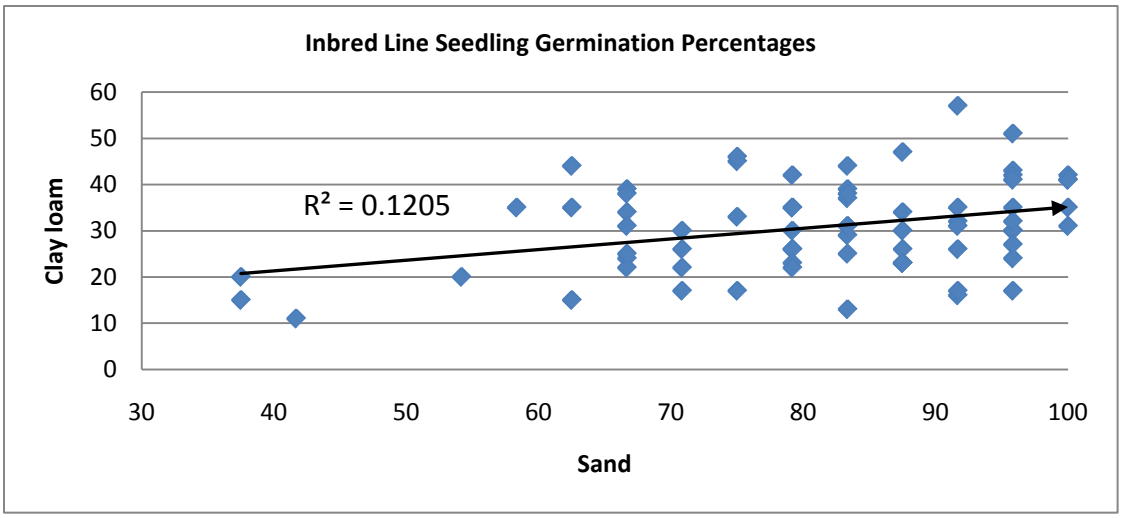


Fig. 3.1 Inbred line seedling germination percentage between sand (greenhouse) and clay loam (field), and the correlation between the two depicting the predictive power of germination in sand to germination under field conditions.

Survival & Recovery

The first day of survival measurements began eleven days after planting for inbred lines while eight days after planting marked that for hybrid testcrosses. Inbred line performance analysis included and excluded the first four replications that used potting soil. Observation days 1-4 represented survival estimates (1st drought cycle) while days 5-9 represented recovery estimates (2nd drought cycle), with a slight overlap between the fourth and fifth days. First drought cycles lasted fourteen and eleven days on average respectively, but varied between replications. Second drought cycles lasted ten and nine days on average respectively, but also varied between replications. Days until susceptibility was observed decreased as the warmer months approached. Lines that exhibited the highest germination percentage, longest survival and recovery were considered drought tolerant. On the other hand, the most susceptible lines were evident. Note that replications in the inbred line trial that were planted in potting soil had a third cycle, and soil medium change removed the need for it in consecutive replications.

Inbred Line Performance (ten replications) Genotype explained the greatest percentage of variation among the sources of variation on the fourth observation day, which incidentally was the second-to-last day of survival measurements (data not provided). Planting date had an increased contribution to variation during the second drought cycle. Residual error estimates increased in percent variation explained during the first drought cycle. Genotypes were the most distinguishable in regards to drought tolerance in the second drought cycle.

Inbred Line Performance (six replications) The first four replications were in a commercial grade potting soil, so replicates evaluated in sand were analyzed separately. The first observation day coincided with the last germination measurement. Variation in genotypes was best explained on the third observation day (Fig. 3.2) or second-to-last day of survival measurements. The fifth and sixth observation day explained the greatest variation in planting date, when the greatest amount of plants would have recovered after re-watering two days before. In this analysis, experimental error accounted for the most variation. Four inbred lines were significant ($P < .05$), with above average percent survival ((Tx601 x B104-B/FR2128 x Unknown)-2-2-B-B-B-B-B ; (Ark 536-B-B-B-B-B-B); (Tx114 (B73w)-B x CML343/Tx110 x Pop24)-B-B-B-4-B-B-B-B-B ; Red Ear 5-2-4-1-4-1-B) (Fig. 3.3).

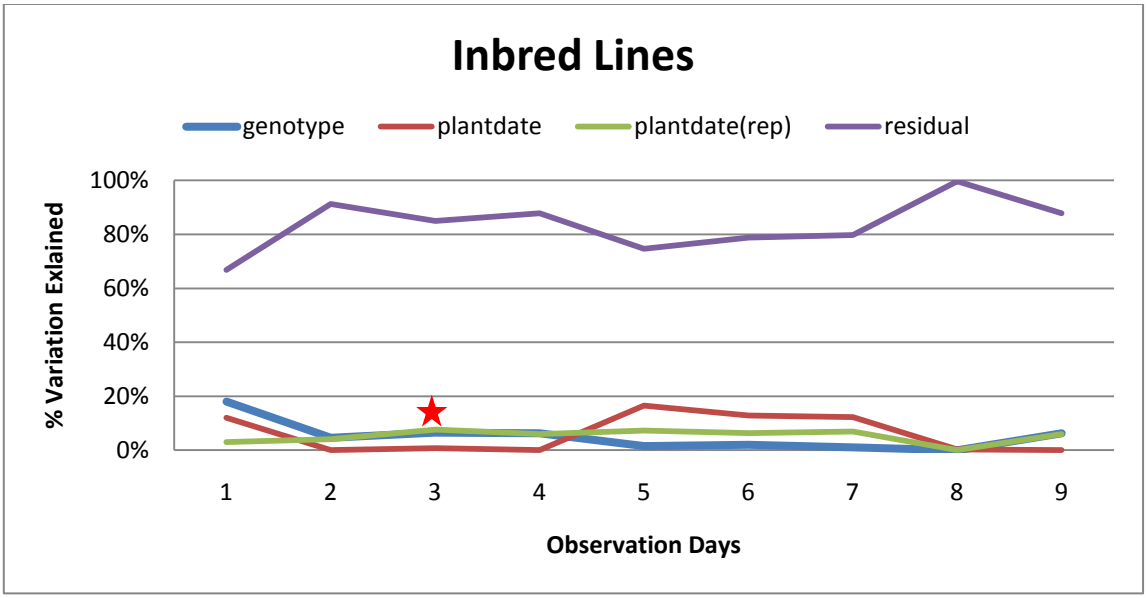


Fig. 3.2 Inbred line performance across observation days depicting percent variation explained by genotype, planting date, replication in planting date and residual error for the last 6 replications. The 3rd observation day had the highest percentage of variation among genotypes.

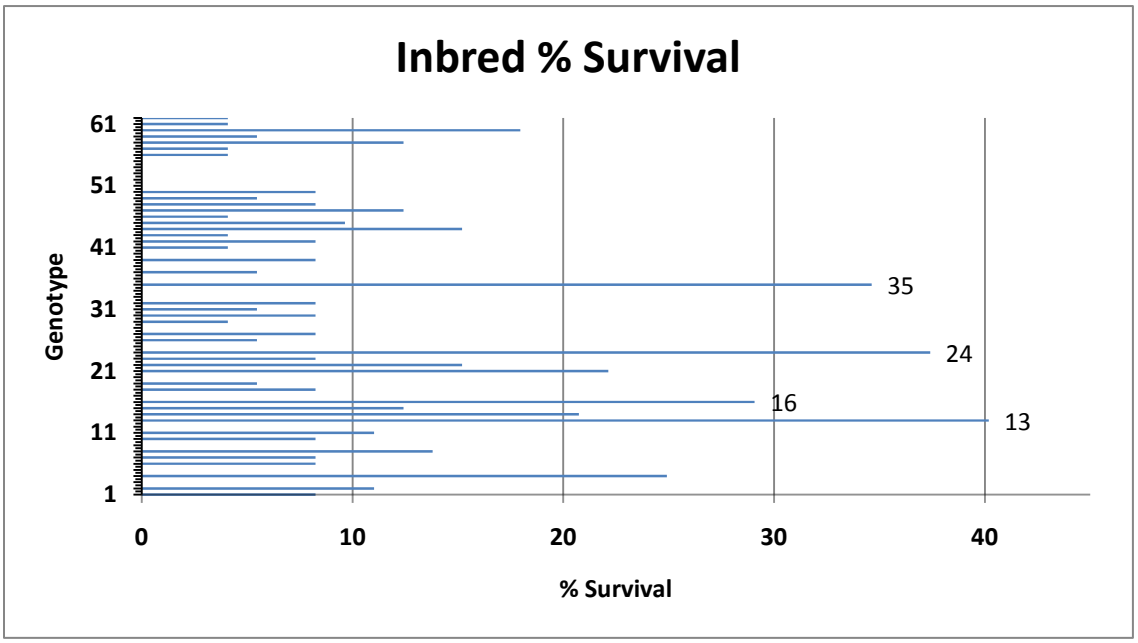


Fig. 3.3 Inbred line percent survival for each genotype on the 3rd observation day; the day which explained the greatest amount of variation for genotype. Significant genotypes are numbered to the right of their corresponding bar.

Hybrid Testcross Performance Genotype differences explained the greatest amount of variation on the sixth observation day (Fig. 3.4). Planting date had a reciprocal effect with experimental error variation (residual error). As the drought cycles progressed, planting date explained less variation while residual error increased. Rep within planting date remained relatively constant throughout both drought cycles. Fourteen hybrid testcross combinations were significant ($P < .05$), with above average percent recovery (Fig. 3.5).

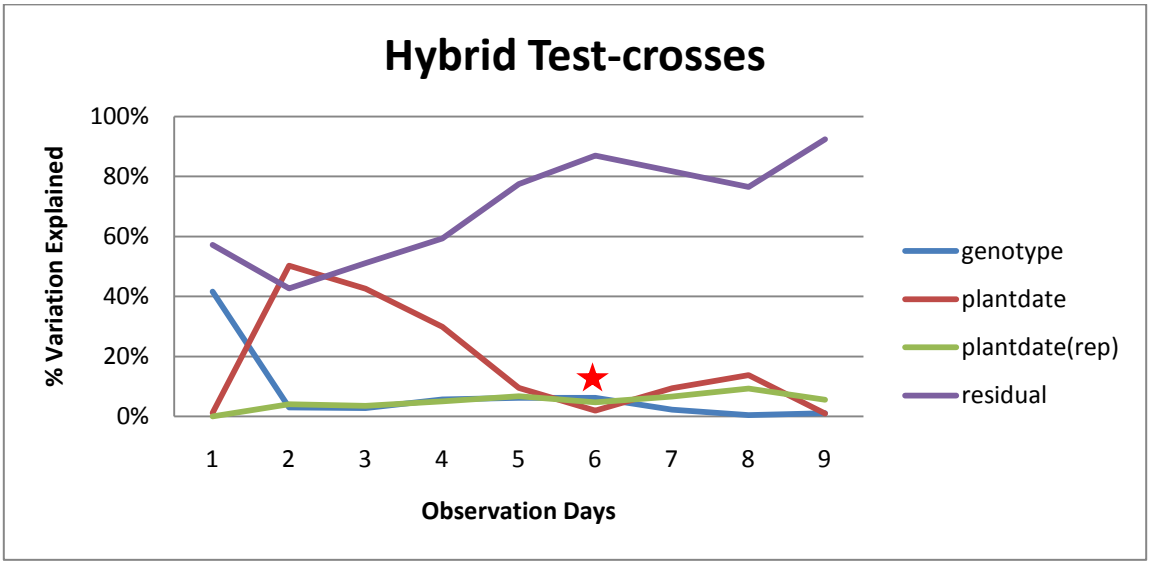


Fig. 3.4 Hybrid testcross performance across observation days depicting percent variation explained by genotype, planting date, replication in planting date and residual error. The 6th observation day had the highest percentage of variation for genotype.

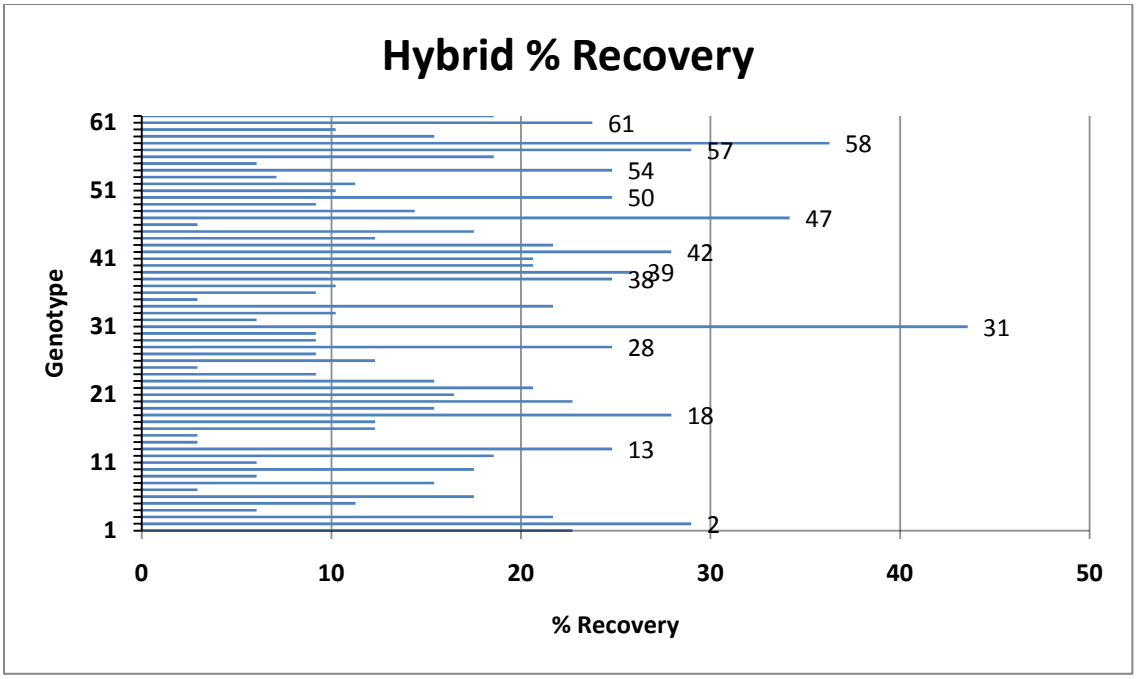


Fig. 3.5 Hybrid testcross percent recovery for each genotype on the 6th observation day; the day which explained the greatest amount of variation for genotype. Significant genotypes are numbered to the right of their corresponding bar.

Inbred-hybrid Correlation Since the first year progeny of hybrid testcrosses were products of each of the original sixty-two inbred line genotypes, we hoped drought tolerance in the inbred line trial may predict hybrid testcross performance. However, the data does not support this hypothesis. The best dates to determine differences among inbred lines (3rd observation day) and among hybrid testcrosses (6th observation day) were chosen based on those with the highest percentage of variation for genotype. Data from these days were plotted against each other using the solution estimates (BLUEs) for fixed genotype effects ($R^2 = 0.0097$) (Fig. 3.6). One of the four significant inbred lines, ((Tx601 x B104-B/FR2128 x Unknown)-2-2-B-B-B-B-B) performed well in combination as a hybrid testcross ($P < 0.05$) ((LH195/LH287) x ((Tx601 x B104-B/FR2128 x Unknown)-2-2-B-B-B-B-B)).

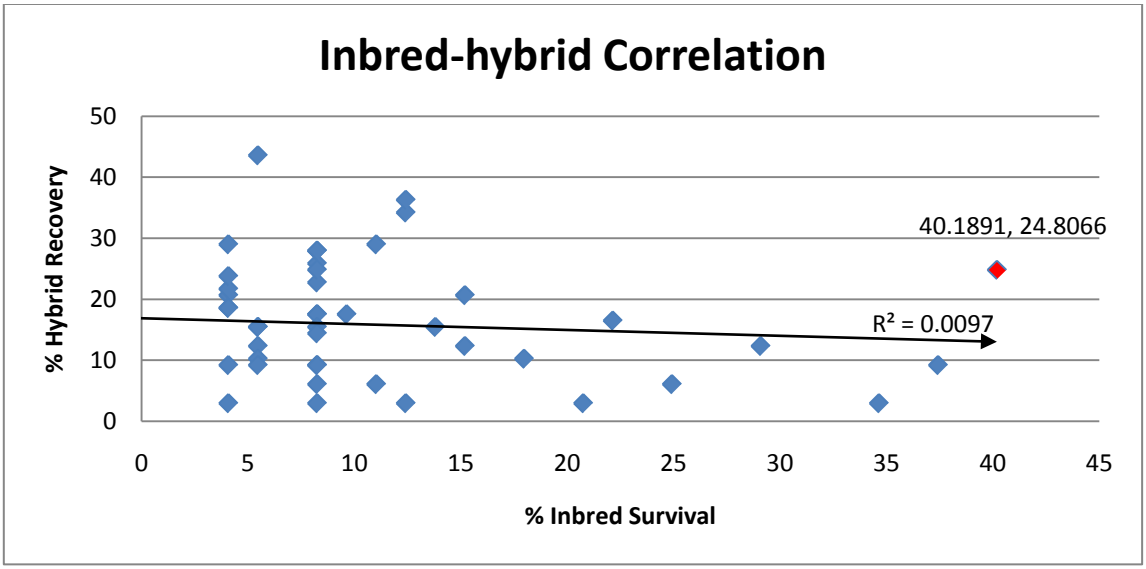


Fig. 3.6 Inbred-hybrid correlation between the 3rd and 6th observation days for inbred lines and hybrid testcrosses. The 3rd observation day for inbred lines occurred during the survival cycle, and the 6th observation day for hybrid testcrosses was during the recovery cycle. Percentages were values of estimated 'tolerant' plants on the respective date for each genotype. One genotype, performed well as both an inbred line and in hybrid testcross combination with 40% survival as an inbred line and 25% as a hybrid testcross.

Inbred Line Check Performance Inbred line check germination percentages in both years were similar, with the exception being from the worst performing inbred line from 2009 ((CML285/B104)-B-4-B-B-B-B-B-B-B) that estimated thirty percent better in 2010. The reason for this is unclear. In evaluating each check genotype's survival rate over the two years, every inbred line check had better survival in hybrid testcross combination than as an inbred line with the exception of ((CML285/B104)-B-4-B-B-B-B-B-B).

3.4 DISCUSSION

Plant breeders identify inbred lines with desirable alleles and attempt to accentuate the expression of those alleles in hybrid progeny. Of the four significant inbred lines identified through the seedling screening, one performed well as a hybrid testcross. Its significant performance in comparison to the mean performance as a hybrid testcross could have isolated it as seedling drought tolerant had there been a significant correlation. Excluding it from the fourteen significant hybrid testcrosses, the poor performances of the other hybrid testcrosses as inbred lines did not allude to finding any genotype performing well as both. With zero correlation, one could not suggest that seedling drought tolerance is heritable between inbred lines and hybrid testcrosses. The generation of inbred lines used for the study may have influenced the poor correlation in that drought tolerance decreases with successive levels of inbreeding (Betrán et al. 2003b). This suggests it would be best to screen hybrids for drought tolerance. As more hybrid testcross genotypes were isolated as seedling drought tolerant than as inbred lines, screening only hybrids may be more conservative but in turn over-estimate significance.

Great amounts of variation were expected among inbred lines and hybrid testcrosses. Finding inbred lines that performed well was expected as all of them had been inbred for several generations. Inbreeding increases allelic homozygosity and population homogeneity within each inbred line, thereby making it easier to distinguish inbred line genotypes from one another as their response to drought stress should be uniform. The single-cross hybrid parent should have been mostly genetically heterozygous, as its two parents were from heavily inbred, commercial inbred line populations before making the three-way cross with each of the sixty-two inbred lines. Once crossed, assuming each of the sixty-two inbred line parents were homozygous at most loci and hybrid parents were heterozygous, first year progeny would segregate genetically into fifty-percent heterozygous and homozygous. Therefore, at least fifty-percent variation existed before making observations of hybrid testcrosses. This variation most likely contributed to the experimental error as the seedling stress response varied within genotypes. Having more replications would have offset this factor, and given more statistical power.

Although this variation existed, seedling drought tolerance between inbred lines and hybrid testcrosses distinguished few statistically significant genotypes.

There are a few aspects about the materials and methods in this experiment where more control may have reduced experimental error. The first is the use of a greenhouse. Although some greenhouses have ways of controlling temperature, the microenvironments of a greenhouse will cause within experiment variability. Depending on placement, replications may receive different amounts of sunlight or shading which effects soil moisture and the ability to induce a balanced drought effect. To avoid this problem, growth chambers may be more appropriate, because light and heat intensity are controllable. The second aspect is the timing of the experiment. Being able to measure more than one replication simultaneously is critical to the developed method, but due to having only one researcher, the number of replications planted at one time was limited. Normally, farmers plant their fields by March 1 in College Station when soil temperatures are warming up. When the greenhouse experiment began in January, inside temperatures were warmer than outside but the sunlight that is normally available at planting on March 1 did not occur. Therefore, planting replications earlier than March 1 may have caused a delay in germination and drought response. Greater differences in genotypes may have appeared if multiple replications were planted at a time when farmers normally plant.

Most small-scale drought experiments use some form of controlled irrigation or moisture monitoring system. As this method did not employ either of the two, it is arguable that some plants may have received more or less water than others, increasing the experimental error. Simply watering to an observable field capacity is subjective to the researcher and not a discrete scientific measurement. Field capacity is also dependent on soil type. The only alternative measurement would have been weighing individual cone-tainers™ at the same time as condition measurements to monitor daily water loss. However, this would have drastically extended measurement time and defeated the purpose of a rapid screening assay.

That planting date had an increasing contribution to percent variation explained in the inbred line experiment (ten replications) is understandable since temperatures increased throughout the duration of the study, allowing greater visual differences among genotypes as the sand dried out, and plant response to drought stress changed from the first drought cycle. In addition, the increase in residual error was likely because of the subjective nature of evaluating individual plants and the difficulty in differentiating tolerant from susceptible plants early on in the development of the screening assay. Differences were difficult to detect in the first four replications with potting soil because the plants would overgrow and soil moisture was difficult to deplete in a timely manner. The first drought cycle for the first four replications were longer than the remaining replications, so by the second cycle the seedlings had already exhausted themselves trying to find water and outgrown their cone-tainers™ to the point where re-watering would not allow them to recover and those that did were very obvious. This explains the reduction in the residual error and finding significant differences between genotypes in the second drought cycle. The sand medium solved the problem of managing moisture status and plant size. The exchange between planting date and experimental error in the hybrid testcross seedling screening experiment was most likely due to too few surviving plants giving us less power for analysis. High experimental error for both years was due to the inability to include more replications or seed per genotype.

3.5 SUMMARY AND CONCLUSIONS

Summary

This screening method isolated a few significant genotypes as either good performing inbred lines or hybrid testcrosses. In comparison to inbred lines, hybrid testcrosses had better germination in both field and greenhouse experiments. The best observation day to discern drought tolerance among inbred lines was the third day or second-to-last day of survival (approximately thirteen days after planting). The sixth day or first day of recovery (approximately eighteen days after planting) best discriminated drought tolerance among hybrid testcross progeny. One genotype was significant as both an inbred line and hybrid testcross for seedling drought tolerance. However, as the correlation between the two trials was insignificant, we conclude that inbred line seedling drought tolerance does not predict hybrid testcross performance so this one genotypic correlation might be coincidental. This trait may be highly dependent on the parentage of the testcross, where single-cross hybridization may not present as much observable variation.

Conclusions

Drought tolerant plants need to sustain life through several periods of drought, but at the same time produce sufficient grain at season's end. The materials and methods used for the seedling

screening provided cost and time effect results. Growing plants in sand allowed quick drainage while providing enough moisture for seedling development. It also effectively induced visual drought effects as cycles progressed. Though moisture and irrigation were not directly measured, individual plantings eliminated interplant competition for water resources.

Although this research does not suggest seedling drought tolerance to be transmitted from inbreds to hybrids, obvious differences in response to drought stress exist among genotypes. Maize breeders may use this method as a replicated screening procedure to expedite single-cross hybrid selection or confirm its response to field drought stress in the off-season before planting yield trials. This experiment is not intended to be long-term, but extending this procedure to observe response to drought over an entire life cycle may provide insight into the relationship of drought at each stage of growth beginning at the seedling stage.

4. EPICUTICULAR WAX ACCUMULATION

4.1 INTRODUCTION

The US is the largest consumer of maize (*Zea mays* L.) per capita in the world, ranking just above China and Brazil, totaling 207,020 thousand metric tons in 2004 (Nation Master, 2005). Needless to say, it is an important staple crop for many who depend on it to feed livestock, humans and the production of other consumer products such as ethanol. Two factors affect the need for greater maize production: 1) growing populations and 2) fuel consumption. Maize accounts for a large portion of American's diet as it is used to make thousands of food products (Center for Crops Utilization Research, 2008). Until scientists find an alternative source for fuel, we continue to depend heavily on maize production for ethanol. By 2006, the United States depended on 20% of its corn harvest for ethanol production (Green Car Congress, 2007). One of the tribulations involved with needing to produce more maize to meet our demand is drought.

Drought conditions are a major concern for Texas maize producers as weather is unpredictable between years. From September 2007 to June 2009, drought plagued South Central Texas, ranking as the second worst twenty-two month period since 1895 (National Climatic Data Center, 2010a). During this time span, 2008 Texas agriculture losses reached an estimated $1.4 billion (High Plains/Midwest Ag Journal, 2008). The annual variability of rainfall in major South and Central Texas crop production regions directly forces farmers to manage water conservatively to grow a profitable crop. The development of water-use efficient, "tolerant" crops suggests one solution to handling the drought water dilemma. Plant breeders have been searching for appropriate traits, breeding and screening methods to develop drought tolerant crops. Methods of development have varied. Many breeders have focused on yield stability under times of water stress, with little progress due to the complex nature of this trait. Consequently, this has lead to a search for secondary traits, which are often identifiable in inbred lines and inherited to good yielding hybrids, to improve drought tolerance. As fresh water

resources are quickly being depleted, we wanted to develop a fast and cost-effective method to screen for drought tolerance in maize using epicuticular wax accumulation as a predictor.

Epicuticular wax is a layer of lipids that exists outside of a leaf's cuticle layer. It has been hypothesized that a thicker layer should correlate with drought tolerance due to less transpiration and water-use efficiency (Bengston et al. 1978). Mondal and Hays (2007) have shown that this wax layer thickens as temperatures rise; a possible form of heat tolerance in wheat (*Triticum* spp.) Maize grows during spring and summer months when the chance of rainfall diminishes, thereby experiencing more extreme drought conditions and higher temperatures than wheat. However, as a C_4 plant this may be less important for maize, as it is more adapted to higher daytime temperatures. During flowering and grain filling are the critical times when drought conditions most affect grain yield (Eck, 1986, Bruce et al., 2002, Cakir, 2004). After pollination, leaves assimilate water and nutrients to ear shoots for grain development. Removing the flag leaf at a time of stress and extracting the epicuticular wax could aid in determining if a genotype is drought tolerant. The flag leaf differs from other leaves because it emerges simultaneously with the panicle and the last leaf to emerge. Accordingly, the young age of the leaf means that it has not been exposed to elements in the environment that remove epicuticular waxes such as acid rain (Baker and Hunt, 1985), wind (Hall and Jones, 1961), insects and abrasion with other leaves. Each of these factors supports the flag leaf's ability to reflect drought stress on panicle emergence and the panicle's ability to produce sufficient pollen. Comparison between plants with and without a flag leaf may also provide insight into its role in grain development.

4.2 MATERIALS AND METHODS

This study was conducted at the Texas AgriLife Experiment Stations in Weslaco and College Station, TX, during May and June 2009 and again in 2010. Research conducted the first year included inbred lines under full and limited irrigation while the second year's experiment evaluated hybrid testcrosses under similar irrigation regimes.

Germplasm

Germplasm was comprised of sixty-two maize inbred lines and two commercial checks (Appendix A). Texas AgriLife researchers from College Station and Lubbock developed fifty-five of the inbred lines at College Station between 2005 and 2007 and Lubbock from 2009. Six of the Texas AgriLife lines have been released (Betrán et al. 2004a, 2004b and 2004c, Llorente et al. 2004, Xu and Odvody 2004). Lines consisted of a wide array of colors and origin including white, yellow, red and blue, tropical, Argentine and temperate populations. In 2009, each of these sixty-two inbred lines were testcrossed in a College Station, TX field nursery to a single-cross hybrid parent (LH195 x LH287) (Monsanto Company, 1991 and 2001) of the original two inbred line checks. An off-season nursery in Weslaco, TX, was used to increase seed. In 2010, we evaluated first year progeny of these testcrosses as well as additional checks that included the best and worst performing inbred line from the previous year and the hybrid parent of the testcross. Inbred line checks were replicated in isolation to evaluate performance across years.

Experimental Design

Fields were prepared in a split-block design with seventy entries, two irrigation treatments (blocks) and two replications in both locations (See Appendix E). Plots had twenty-five seed for every five meters. Irrigation treatments were full and limited irrigation. Irrigation treatments in full irrigation blocks occurred slightly more than required both years, while the limited irrigation blocks were irrigated when necessary, except at flowering to induce stress. Soil moisture sensors installed in each irrigation treatment monitored irrigation and rainfall events to corroborate drought stress. Soil types differed between locations with a Raymondville clay loam in Weslaco and a Ships clay loam in College Station.

Three random, non-consecutive plants were sampled from each plot. These plants were harvested separately from the remaining plants in each plot. All ears were hand harvested. Additional measurements included plant and ear height, flowering and silking time, ear length, grain weight per ear and total grain yield, wax and fresh leaf weight, and flag leaf area.

Extractions

Modifications to Mondal and Hays (2007) leaf collection and extraction techniques were made to accommodate for larger flag leaf area and wax sample preservation (Mondal, personal communication 2008). Bewick et al. (1993) and Mayeux et al. (1981) developed similar protocols for whole leaf samples. Twenty-milliliter chloroform resistant scintillation vials corresponded to plot number and location by label. Eight-ounce jars were weighed and calibrated to an equivalent weight on a scale calibrated to measure 1^{-4}g. Leaf samples were carefully removed from plants with scissors, stored in gallon size plastic bags, and placed in a cooler to slow biological processes and preserve wax. Whilst collecting leaves, gentle handling and packaging retained the integrity of samples to minimize damage or loss of the wax layer. Extraction preparation involved laying leaves from each plot on top of each other, rounding them backwards against the natural curl and placing them into one of the eight-ounce jars. Fresh leaf weights were recorded and jars were passed on to receive twenty-five milliliters of HPLC grade chloroform by pipette. After covering with a Teflon coated lid, the jars were shaken for 15 seconds and the leaves were removed with tweezers. Leaves were then photographed for leaf area analysis and discarded. Wax is soluble in chloroform and requires little processing time once the chloroform has evaporated. The amount of time that the sample is in solution has often been dependent on subject matter, and whether or not the intent is to remove internal waxes as well. Times have varied between 2 (O'Toole et al. 1979), 15 (Mayeux et al. 1981) and 30 (McWhorter et al. 1990, Koch et al. 2006) seconds for whole leaf samples. The remaining solution was poured into a vial via a glass funnel and covered with a Teflon coated cap. Note that the vials had a twenty-milliliter volume and received twenty-five milliliters of chloroform. Not all of the residual five milliliters makes it to the vial due to evaporation and leaf absorption. Samples

were evaporated under a fume hood. Final wax weight was measured to 1^{-4}g. Wax weight, leaf weight and average flag leaf area were measurements used to calculate wax weight by leaf weight (% wxlfwt) and wax weight by leaf area (% wxwta). Analyzing them separately helped explain the results from %wxlfwt and %wxwta analysis. Adobe Photoshop was used to obtain an average leaf area (cm^2) for each genotype (Jarou, 2009).

Additional Traits

Additional traits included standard notes such as plant and ear height, 50% flowering and silking and total grain yield per plot. Plant height is the number of centimeters from ground to tip of tassel. Ear height is the number of centimeters from ground to node of the uppermost ear. Flowering time was measured as the number of days after planting when at least 50% of the plants have full panicle expansion and anthers almost begin shedding pollen. Silking was measured as the number of days after planting when at least 50% of the plants expose one inch of silks. Total grain yield analysis compared grain from sampled and remaining(control) plants in each plot. Grain yield for full and limited irrigated plots were compared within and across locations by genotype.

Other measurements taken were average ear length and average grain weight per ear. For ear length, three random ears from each sample were selected and measured for plot average. Ear lengths compared by genotype under full and limited irrigation treatments. Grain weight per ear was used to compare the effect of flag leaf removal on grain yield, which was based on ear number in each sample as the average grams produced per ear from sample and control plants. Grain weight per ear and total grain yield were adjusted based on % kernel moisture in 2010.

Soil Moisture Monitoring

Five Decagon ECH_2O EC-5 soil moisture sensors were installed at each testing site each year. One sensor was placed in each replication by irrigation treatment as well as a control sensor between full and limited irrigation blocks (See Appendix E). These sensors measured volumetric water content (m^3/m^3) at a root zone depth of twelve inches. Measurements were taken every ten minutes throughout

flowering and grain filling stages in 2009. Because of the minimal change in ten minute intervals, measurements were taken every hour in 2010. Sensors were not installed prior to flowering to avoid damage from field traffic. Sensors used as controls only measured variation as influenced by rain events as border plants were not irrigated. Volumetric water content (m^3/m^3) was averaged among irrigation treatment sensors by location, and plotted against the control sensor.

Both locations during the 2009 field season had drought conditions. In 2010, abundant rainfall provided sufficient irrigation limiting furrow irrigation needs. Due to the extremity of the 2009 drought, the limited irrigation blocks were irrigated simultaneously with irrigated blocks to ensure plant survival. Irrigation was withheld from limited blocks only once during flowering because environmental stress was heavy and already induced, so withholding water only ensured stress. The limited irrigation block in College Station for the year 2010 did not receive any supplemental irrigation from April to August while the full-irrigated block received two extra treatments.

Statistical Analysis

SAS 9.2 PROC MIXED analyzed wax weight as a percentage of fresh leaf weight and average flag leaf area for inbred lines and hybrid testcrosses. Sources of variations evaluated in the models included genotype, location, irrigation block, replication (location*block), genotype x location, genotype x block,

and genotype x location x block and flag leaf removal for grain yield. Interactions that were not significant at a p-value < 0.05 level were removed from random and fixed models until all sources of variation were significant, but main effects were retained. Irrigation treatment was not significant for most tests, but was necessary to leave in the model because replication was nested within irrigation treatment. Year was nested within location because we could not combine data from both years with different types of plants. Fixed model analysis kept replication (location*block) as a random variable. Variance component and broad-sense heritability estimates (H^2) were taken from random analysis, while fixed analysis inferred estimates of differences in genotype means (BLUEs). Inbred-hybrid correlations were drawn from BLUEs. Pearson's correlation coefficients were also computed between all measurements from hybrid analysis (Appendix D). Significant sources of variation were either $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

4.3 RESULTS

Percent Wax Weight by Leaf Weight (%wxlfwt)

In 2009, location, replication (location x block), and genotype x location were all highly significant ($P < 0.001$) sources of variation in the random model (Table 4.1). Location explained 63% of the observed variation. Heritability was low ($H^2 = 0.17$) (Table 4.2). In the fixed model, genotype was also highly significant. In 2010, no environmental interactions accounted for observed variation. All sources of variation with the exception of irrigation treatment were significant ($P < 0.05$) or highly significant ($P < 0.001$). Most variation was due to experimental error followed by location (23%), genotype (16%) and replication (location x block) (14%). Heritability was moderate ($H^2 = 0.58$). Poor correlation existed between inbred lines and their hybrid testcrosses ($R^2 = 0.19$) (Table 4.2). The best and worst inbred line checks, performed similarly in 2010 as they produced more wax by leaf weight than in 2009 or in hybrid testcross combination. The worst inbred line as a hybrid testcross produced more % wxlfwt than the best inbred line.

Table 4.1 Sources of variation explaining a percentage of observed variation and their significance between inbred lines and hybrid testcross progeny for % wxlfwt and % wxwta

		% Explaining Observed Variation and Significance								
		Genotype	**Location**	**Block**	**Replication**	**GxL**	**GxB**	**GxLxB**	**Residual**	**Flag**
%Wxlfwt	**Inbred**	2^{ns}	63^{***}	0^{ns}	3^{***}	7^{***}	--	--	25	--
	Hybrid	16^{***}	23^{*}	0^{ns}	14^{***}	--	--	--	47	--
%Wxwta	**Inbred**	11^{***}	25^{**}	1^{ns}	1^{*}	--	--	--	61	--
	Hybrid	16^{***}	25^{*}	0^{ns}	15^{***}	--	--	--	44	--

* Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level

Table 4.2 Broad-sense heritability estimates and inbred-hybrid correlations for % wxlfwt and % wxwta

	Broad-sense Heritability (H^2)		Inbred-Hybrid
Trait	**Inbred**	**Hybrid**	**Correlation (R^2)**
%Wxlfwt	0.17	0.58	0.19
%Wxwta	0.41	0.59	0.03

Percent Wax Weight by Leaf Area (% wxwta)

In 2009, genotype, location, and replication (location x block) were all significant sources of variation in the random model (Table 4.1). Experimental error accounted for 61% of the observed variation but location explained 25%. Heritability was moderate (H^2 = 0.41) (Table 4.2). In the fixed model, irrigation treatment was the only non-significant source of variation. In 2010, no environmental interaction was significant. Genotype, location and replication (location x block) were significant. Most observed variation was experimental error (44%), but location explained 25% followed by genotype (16%) and replication (location x block) (15%). Heritability was moderate (H^2 = 0.59). Poor correlation (R^2 = 0.03) existed between inbred lines and hybrid testcrosses (Table 4.2).

Grain Weight Per Ear and Total Grain Yield

Grain yield was analyzed by two methods 1) comparing average grain yield per ear from sampled plants to the control plants in each plot for genotypes and 2) total grain yield by genotype. Genotype, location, irrigation block, replication (location x block), sampled plants (flag-leaf-removed plants), and all the representative interactions among them were used to predict grain yield variation.

Grain Weight Per Ear In 2009, flag leaf removal and irrigation treatment were insignificant at determining differences in grain weight produced on ears (Table 4.3). However, all other sources of variation were significant. Location explained 23% of the variation (Table 4.4). Heritability was moderate at H^2 = 0.34 (Table 4.5). All interactions and other sources of variation except sampled plants and irrigation treatment were significant in the fixed model. In 2010, all interactions, location and irrigation

treatment were insignificant, but genotype, flag leaf removal and replication (location*block) were highly significant ($P < 0.001$). Genotype explained 14% of variation while flag leaf removal only explained 4%. Flag leaf removal increased hybrid grain weight per ear by 8.5 grams. Heritability was high ($H^2 = 0.64$). A poor correlation existed between estimates for 2009 and 2010 ($R^2 = 0.05$) (Table 4.5).

Table 4.3 Sources of variation significance between inbred lines and hybrid testcrosses for grain weight per ear and total grain yield

		Source of Variation Significance							
Trait	**Plant Type**	**Genotype**	**Location**	**Block**	**Replication**	**GxL**	**GxB**	**GxLxB**	**Flag**
Grain Weight per Ear	**inbred**	***	*	ns	***	***	**	*	ns
	hybrid	***	Ns	ns	***	ns	ns	ns	***
Total Grain Yield	**inbred**	***	Ns	ns	***	***	***	***	ns
	hybrid	***	***	ns	***	***	ns	***	ns

* Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level

Table 4.4 Sources of variation explaining a percentage of observed variation between inbred lines and hybrid testcross progeny for grain weight per ear and total grain yield

		% Explaining Observed Variation								
Trait	**Plant Type**	**Genotype**	**Location**	**Block**	**Replication**	**GxL**	**GxB**	**GxLxB**	**Residual**	**Flag**
Grain Weight per Ear	**Inbred**	6	23	0	11	--	--	6	54	--
	Hybrid	14	3	5	8	--	--	--	67	4
Total Grain Yield	**Inbred**	29	0	0	16	19	--	11	25	--
	Hybrid	21	30	2	1	8	--	13	27	--

Table 4.5. Broad-sense heritability estimates and inbred-hybrid correlations for grain weight per ear and total grain yield

	Broad-sense Heritability (H^2)		Inbred-Hybrid Correlation (R^2)
Trait	**Inbred**	**Hybrid**	
Grain Weight per Ear	0.34	0.64	0.05
Total Grain Yield	0.46	0.71	0.00

Total Grain Yield In 2009, all sources of variation were significant except location, irrigation treatment and flag leaf removal (Table 4.3). Both location and irrigation treatment were not significant for random and fixed models. All interactions were highly significant in the fixed model. Genotype explained 29% of the observed variation while heritability was moderate (H^2 = 0.46) (Table 4.4 and 4.5). In 2010, irrigation block, genotype*block and flag leaf removal were not significant sources of variation. Genotype only explained 21% of variation while location explained 30%. Heritability was high (H^2 = 0.71). No correlation was evident between 2009 and 2010 (R^2 = 0.00) (Table 4.5).

Additional Traits

Genotype was highly significant for plant height, ear height and ear length (Table 4.6). For plant height and ear height, location was only significant for inbred lines, and no environmental interactions were significant. Inbred line heritability was low but hybrid testcross heritability was high (Table 4.7). Inbred – hybrid correlations were extremely low for both plant height (R^2 = 0.02) and ear height (R^2 = 0.00). For ear length, location, irrigation treatment and replication (location x block) were not significant for inbred lines as they were for hybrid testcrosses, and genotype x location was significant for inbred lines. Ear length had the highest heritability for inbred lines (H^2 = 0.54). Average flag leaf area was the highest heritable trait for hybrid testcrosses (H^2 = 0.74). With the exception of wax weight, hybrid testcrosses had the greatest heritability for all measurements. Despite these heritability estimates, most traits were uncorrelated between inbred lines and hybrid testcrosses. Flag leaf weight (R^2 = 0.46) and average flag leaf area (R^2 = 0.40) had the highest correlations. Five genotypes were significant for flag

Table 4.6 Sources of variation and their significance between inbred lines and hybrid testcrosses for additional traits

		Source of Variation Significance							
Trait	**Plant Type**	**Genotype**	**Location**	**Block**	**Replication**	**GxL**	**GxB**	**GxLxB**	**Flag**
Plant Height	**inbred**	***	*	ns	***	ns	ns	ns	ns
	hybrid	***	ns	ns	***	ns	ns	ns	ns
Ear Height	**inbred**	***	**	ns	**	ns	ns	ns	ns
	hybrid	***	ns	ns	***	ns	ns	ns	ns
Ear Length	**inbred**	***	ns	ns	ns	***	ns	ns	ns
	hybrid	***	***	*	*	ns	ns	ns	ns
Grain Weight per Ear	**inbred**	***	*	ns	***	***	**	*	ns
	hybrid	***	ns	ns	***	ns	ns	ns	***
Total Grain Yield	**inbred**	***	ns	ns	***	***	***	***	ns
	hybrid	***	***	ns	***	***	ns	***	ns
Wax Weight	**inbred**	**	**	ns	***	ns	ns	**	ns
	hybrid	*	ns	ns	***	*	ns	ns	ns
Flag Leaf Weight	**inbred**	*	***	ns	***	*	ns	ns	ns
	hybrid	***	ns	ns	***	ns	ns	ns	ns
Flag Leaf Area	**inbred**	**	ns	ns	***	***	ns	ns	ns
	hybrid	***	ns	ns	***	ns	ns	ns	ns

* Significant at 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level

Table 4.7 Broad-sense heritability estimates and inbred-hybrid correlations for additional traits

	Broad-sense Heritability (H^2)		**Inbred-Hybrid**
Trait	**Inbred**	**Hybrid**	**Correlation (R^2)**
Plant Height	0.45	0.72	0.02
Ear Height	0.37	0.68	0.00
Ear Length	0.54	0.64	0.16
Wax Weight	0.30	0.26	0.01
Flag Leaf Weight	0.33	0.71	0.46
Flag Leaf Area	0.41	0.74	0.40

Table 4.8 Sources of variation explaining a percentage of observed variation and their significance between inbred lines and hybrid testcross progeny for additional traits

		% Explaining Observed Variation								
Trait	**Plant Type**	**Genotype**	**Location**	**Block**	**Replication**	**GxL**	**GxB**	**GxLxB**	**Residual**	**Flag**
Plant Height	**Inbred**	13	17	2	6	--	--	--	62	--
	Hybrid	18	20	0	33	--	--	--	28	--
Ear Height	**Inbred**	9	26	0	3	--	--	--	61	--
	Hybrid	29	0	0	17	--	--	--	54	--
Ear Length	**Inbred**	26	0	0	1	16	--	--	57	--
	Hybrid	22	22	5	1	--	--	--	50	--
Wax Weight	**Inbred**	6	29	0	4	--	--	10	51	--
	Hybrid	4	6	0	44	5	--	--	40	--
Flag Leaf Weight	**Inbred**	0	96	0	0	0	--	--	3	--
	Hybrid	33	3	0	10	--	--	--	54	--
Flag Leaf Area	**Inbred**	15	5	0	4	14	--	--	62	--
	Hybrid	36	6	0	6	--	--	--	52	--

leaf weight as both inbred lines and hybrid testcrosses, where genotype 25 was consistently heavier than the mean and genotypes 1, 41, 53 and 61 were consistently lighter (See Appendix A). Four genotypes were significant for average flag leaf area as both inbred line and hybrid testcross, where genotypes 8, 25, 32 and 46 were larger than average (See Appendix A). Incidentally, all four genotypes also had smaller leaf areas as hybrid testcrosses than as inbred lines. Flowering time correlated with plant height, ear number, ear length, grain weight per ear and silking time (See Appendix D). Silking time correlated with grain weight per ear, total grain yield, flag leaf weight, %wxlfwt and %wxwta. Each source of variation explaining observed variation for each trait is presented in Table 4.8.

Soil Moisture Monitoring

Irrigation and rainfall events were successfully detected both years. In 2009, sensor results contrasted with expectations (Appendix B). Full irrigated blocks measured lower soil moisture levels during grain filling than limited irrigated blocks that had water withheld during flowering. In 2010, sensors reported original expected trends in moisture content; fully irrigated blocks had higher moisture content than limited irrigation blocks. Agronomic and precipitation data is in Appendix C.

4.4 DISCUSSION

Analysis of %wxlfwt revealed this trait may be better observed in hybrids. Inbred line germplasm did not show any significant differences among genotypes, but hybrid testcross progeny were significantly different. In addition, heritability was much higher for %wxlfwt in hybrid testcrosses. For breeding, not being able to observe differences in wax production in inbred lines makes selection for hybridization more difficult and reliant upon the breeding values of each inbred line parent. The poorest inbred line check's outstanding wax production as a hybrid testcross confirms the difficulty in selecting for this drought tolerance trait from inbred lines.

%wxwta analysis presented a different case. Genotypes were significantly different as both inbred lines and hybrid testcrosses. However, most observed variation for %wxwta in both inbred lines and hybrid testcrosses was calculated as experimental error. Heritability was moderate for both plant types. Unfortunately, a poor correlation between inbred line and hybrid testcross wax production based on leaf area makes heritability calculated by parent-offspring regression difficult. The assumption is that the wax production genes from the hybrid parent of the testcross are dominant and reduce the influence of each of the sixty-two inbred lines on wax production in testcross progeny. Although best and worst inbred line checks performed better in 2010 than in 2009 when drought stress was more intense, the difference between the inbred line checks and hybrid testcross progeny performance is not significant. Therefore, the hybrid parent's effect on wax production may be minimal or less effective than each inbred line genotype, contrary to our previous assumption. These results conflict with our hypothesis that drought stress would induce wax production, and that this trait is heritable from parent to offspring.

In comparing %wxlfwt to %wxwta, %wxwta was a better measurement because heritability was the greatest for both inbred lines and hybrid testcrosses. This may be due to the discrete nature of flag leaf area, whereas fresh leaf weight is dependent on leaf area and water content. Although leaf area can be reduced in response to drought stress (Blum, 1996), leaf water content seems to be more variable by genotype possibly due to thicker wax per smaller surface units and possible lower rates of transpiration

in comparison to non-stressed plants. In this study, the genotype x environment interaction significantly influenced both leaf area and weight in 2009, when under extreme drought stress. However, in 2010, growing conditions had little effect on leaf weight or area. Hybrids in general are assumed to have larger leaves and therefore, an inherent greater fresh leaf weight; but this does not necessarily mean the thickness of the wax layer or content is less per surface unit. The average flag leaf area for hybrid testcrosses was 10 cm^2 less than inbred lines, but the proportion of average wax weight to average flag leaf area was almost two times greater per cm^2. % Wxlfwt comparison was four times greater for hybrid testcrosses, not because of leaf area, but because of adequate soil moisture throughout most of the growing season in 2010. Therefore, % wxwta was a more reliable measurement for comparing genotypes for drought tolerance than % wxlfwt

When comparing grain yield produced on individual ears between plants with the flag leaf removed and control plants, there was no significant effect on inbred lines. However, there was a small effect on hybrid testcrosses, where plants without the flag leaf during grain filling had approximately 8.5 grams more at harvest. For total grain yield, all significant inbred lines performed better than the mean while significant hybrid testcrosses performed worse than the mean, leading to a null correlation between the two. According to Cakir (2004), drought induced stress at flowering significantly reduces grain yield per ear in hybrid testcrosses. Therefore, even without flag leaf removal, drought stress should have reduced grain yield. Genotype explained the greatest amount of variation observed in the total grain yield analysis with moderate to high heritability of inbred lines and hybrid testcrosses.

Flag leaf removal seemed to not affect yield in inbred lines, but contrastingly increased grain yields in hybrid testcrosses. While this has not been reported in maize, removal of flag leaves in wheat appears to detract grain yield (Verma et al. 2004). Since this was not the primary objective for this study, there was not an optimally balanced sample with and without flag leaves, but this does present a possible new area of maize physiology for investigation. To address this type of research question, an experiment could be designed in which plots are split with the sub-plot being flag-leaf removal versus a

control. For example, ten sample plants from one plot compared to ten plants in the corresponding plot only harvesting the upper ear. Having a consistent number of ears to contribute to grain yield for each plot, would have improved the experimental design for grain yield and ear length, but harvesting all ears was necessary to obtain ear length averages, as some plots did not have three ears. Regardless, this finding was intriguing and further experimentation may corroborate it.

Replication (location x block) was highly significant in this experiment. This was a surprising aspect about the grain yield analysis given that location significance was inconsistent and irrigation treatment was overall not significant for grain yield. One possible explanation can be that field characteristics may have varied from the front to the back of the field at College Station, causing some location effect, and irrigation treatments were arranged in a way to account for field variation.

Additional traits were analyzed to determine if irrigation treatment had any effect. Shortened plant height, low ear height and smaller ears are indicative phenotypic stunting traits from drought. Flowering and silking time also were measured, which typically are used to compare differences in grain yield. If no additional traits measured a significant difference in irrigation treatments, then the experiment may be best conducted within an environment where climate and soil moisture may be better controlled, such as a greenhouse. However, it would be difficult to make greenhouse accommodations for this large of an experiment. It is highly unlikely that every genotype is drought tolerant, yet it is more likely to find one tolerant amongst many susceptible genotypes.

Heritability for plant height, ear height and length was lower for inbred lines than hybrid testcrosses. There also was little correlation between inbred lines and hybrid testcrosses for either trait. Suggesting that tall or short height genes from inbred lines would have little effect on the height of its hybrid testcross progeny. In this case, the hybrid parent had dominant genes for plant and ear height. On the other hand, ear lengths typically are shorter in inbred lines and longer in hybrids. Ear length can be an important component of grain yield. The consistent moderate heritability for ear length suggests it would be a better trait to measure for drought studies than plant or ear height.

Flowering and silking time measurements were taken to observe variability and genetic effects on the other measured traits. Flowering and silking times correlate with many other secondary traits. Alleles which coordinate flowering and silking time may also affect plant height, ear length, grain filling, epicuticular wax production and leaf area.

To improve the precision of this study, one methodological change could be sampling flag leaves from genotypes that flower at the same time, rather than sampling all plots of an irrigation treatment on the same day because changes in wax production are associated with leaf stage development (Kerstetter and Poethig, 1998). Sampling plants that flower later than the rest of the plants for an irrigation treatment may change differences in wax production because some leaves may not have developed as much epicuticular wax. This also may reduce pollen contamination from early flowering samples taken when the majority of the plots had flowered. However, sampling in this way would have been more difficult and it would not be a high-throughput method. Additionally, the sampling environment would change with this method, for instance, if some cultivars were measured on a day with a rain event; they might have lower wax yield due to abrasion loss.

Another limitation existed with extraction techniques. Sample contamination due to pollen and insects was not initially taken into account. In 2009, there was considerable aphid (*Rhopalosiphum maidis* (Fitch)) infestation at both locations, and pollen had begun shedding by the time of sampling. Due to these two factors, aphid exoskeletons and pollen contributed to wax weights. The use of a filter paper, as done by Leavitt and Penner (1979), may have mitigated the contamination and reduced experimental error. However, filtering would have been resource intensive. It was decided to remain consistent with the extraction techniques throughout the remainder of the experiment. While this contamination was less visible in 2010, sampling of flag leaves was modified so that the majority of aphids and pollen that accumulate at the base of leaves could be eliminated. This was done by cutting samples above heavily contaminated areas and before pollen began flowing. Since %wxlfwt and %wxwta

is relative to leaf size, this did not affect the relationship of these measurements but may have changed the average flag leaf area by genotype thereby reducing average flag leaf weight.

Benefits of using soil moisture sensors include having physical moisture readings throughout plant development, knowing soil moisture content at the time of sampling to corroborate drought stress timing with flowering, and obtaining an understanding of soil properties in regards to soil moisture retention. The greatest limitation in these environments was the inability to cause a significant change in soil moisture, even though at sampling time, the limited irrigated blocks showed lower moisture content. Without this effect, we could not predict differences among genotypes based on drought stress. In addition, the limited irrigation regime had higher volumetric water content at the end of the season. This suggests these plants senesced early or developed deeper roots. However, this does not explain insignificant irrigation treatment effects in 2010, where rainfall was ample and supplemental irrigation was rarely necessary. This scenario emphasizes the difficulty in screening for drought in a field environment where there is little control over soil moisture.

4.5 SUMMARY AND CONCLUSIONS

Summary

Measurement of flag leaf epicuticular wax in relationship to flag leaf area was more heritable than measurement of wax as a proportion of flag leaf weight. Using both measurements, epicuticular wax was found to significantly differ among genotypes; however, it only explained a small amount of observed variation. Without a definitive drought effect, genotypes cannot be classified as drought tolerant. Irrigation treatments had no effect on percent wax accumulation based on leaf weight or area. Differences in soil moisture content within environments did not corroborate a lack of significance. Location seemed to explain the greatest amount of variation for % wxlfwt and % wxwta other than experimental error. Fresh flag leaf weight and flag leaf area had two of the highest heritability estimates for hybrid testcrosses. Although, grain weight per ear between sampled and control plants was not the primary trait to observe for this experiment, higher grain yield in sampled hybrid testcross plants was surprising and further analysis could determine if flag leaf removal at flowering is beneficial for hybrid grain production.

Conclusions

Overall, the developed extraction method successfully detected differences in epicuticular wax content among inbred lines and hybrid testcross germplasm was successful. However, not finding significant differences in wax production based on drought stress, leads us to conclude that either

epicuticular wax development is not a drought stress response or conducting this drought experiment under field conditions was inappropriate. It is imperative for future drought studies to measure soil moisture content in field environments. Location and replicate were influential over wax production in relation to fresh leaf weight and leaf area, while block (irrigation treatment) was not effective at inhibiting or promoting wax production. Although some genotypes consistently had higher levels of epicuticular wax as inbred lines and hybrid testcrosses, the poor correlation from one to the other does not suggest epicuticular wax is heritable based on fresh leaf weight or flag leaf area. However, moderate heritability for most of the measured traits does suggest drought tolerance should be sought from hybrid testcrosses. For breeding, the only limitations are identifying parents to make the testcross and selecting appropriate traits to measure. Epicuticular wax is not an ideal secondary trait to evaluate for drought tolerance because heritability for wax weight is low. On the other hand, epicuticular wax may be a good candidate to observe in relation to grain yield production in hybrids.

5. CONCLUSIONS

Climate conditions are rapidly changing and one of the effects is an increased level of drought intensity in areas heavily dependent on maize production. Therefore, drought tolerance is a physiological trait necessary for maize breeders to evaluate. Methods to investigate and observe this trait have varied. Most scientists agree that drought tolerant germplasm needs to sustain life through several periods of drought, but at the same time produce sufficient grain at season's end.

The experiment involving seedling screening provided cost and time effect results. Even though seedlings did not exhibit heritability of drought tolerance from inbred lines to hybrid testcrosses, hybrid testcrosses exhibited much better response to drought stress. Therefore, it would be useful in selecting drought tolerant single-cross hybrids before planting yield trials.

The epicuticular wax extraction experiment successfully detected differences in wax content among inbred lines and hybrid testcross germplasm. However, conducting this experiment under field conditions was ultimately inappropriate, as significant differences in wax production based on drought stress were not found. This led us to question if epicuticular wax accumulation is actually a drought stress response. Based on our results, epicuticular wax is not an exemplary secondary trait to use for primary trait evaluation of drought tolerance. On the other hand, epicuticular wax may be a good candidate to observe in relation to grain yield production in hybrids.

REFERENCES

Amato, M., and J. T. Ritchie, 2002: Spatial distribution of roots and water uptake of maize (*Zea mays* L.) as affected by soil structure. Crop Sci. 42, 773-780.

Ashghizadeh, H.R., and P. Ehsanzadeh, 2008: Maize performance under drought: decreased photosynthetic area vs. decreased efficiency of PSII. In: J. F. Allen, E. Gantt, J. H. Golbeck and B. Osmond (eds.), Photosynthesis. Energy from the Sun. Proceedings of the 14th International Conference on Photosynthesis, pp. 1445-1449. Glasgow, Scotland.

Baker, E., and G. Hunt, 1985: Erosion of waxes from leaf surfaces by simulated rain. New Phytol. 102, 161-173.

Bänziger, M., and H. R. Lafitte, 1997: Efficiency of secondary traits for improving maize for low-nitrogen target environments. Crop Sci. 37, 1110–1117.

Bänziger, M., P. S. Setimela, D. Hodson, and B. Vivek, 2004: Breeding for improved drought tolerance in maize adapted to southern Africa. Agr. Water Manage. 80, 212–224.

Bengtson, C., S. Larsson, and C. Liljenberg, 1978: Effects of water stress on cuticular transpiration rate and amount and composition of epicuticular wax in seedlings of six oat varieties. Physiol. Plant. 44, 319-324.

Betrán, F. J., D. Beck, M. Bänziger, and G. O. Edmeades, 2003a: Secondary traits in parental inbreds and hybrids under stress and non-stress environments in tropical maize. Field Crops Res. 83, 51-65.

Betrán, F. J., D. Beck, M. Bänziger, and G. O. Edmeades, 2003b: Genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. Crop Sci. 43, 807–817.

Betrán, F. J., J. M. Ribaut, D. Beck, and D. Gonzalez de León, 2003c: Genetic diversity, specific combining ability, and heterosis in tropical maize under stress and nonstress environments. Crop Sci. 43, 797–806.

Betrán, F., A. Bockholt, F. Fojt, and K. Mayfield, 2004a: Registration of Tx732 maize germplasm line. Crop Sci. 44, 1028-1029.

Betrán, F., A. Bockholt, F. Fojt, and K. Mayfield, 2004b: Registration of Tx770 maize germplasm line. Crop Sci. 44, 1029-1030.

Betrán, F., A. Bockholt, F. Fojt, K. Mayfield, and D. Pietsch. 2004c. Registration of Tx714 maize germplasm line. Crop Sci. 44: 1028-1028.

Bewick, T., D. Shilling, and R. Querns, 1993: Evaluation of epicuticular wax removal from whole leaves with chloroform. Weed Technol. 7, 706-716.

Bianchi, G., E. Lupotto, and S. Russok, 1979: Composition of epicuticular wax of rice, *Oryza sativa*. Experientia 35, 1417-1540.

Blum, A., 1996: Crop responses to drought and the interpretation of adaptation. Plant Growth Regul. 20, 135-148.

Bondada, B. R., D. M. Oosterhuis, J. B. Murphy, and K. S. Kim, 1996: Effect of water stress on the epicuticular wax composition and ultrastructure of cotton (*Gossypium hirsutum* L.) leaf, bract, and boll. Environ. Exp. Bot. 36, 61–69.

Brouwer, C., and M. Heibloem, 1986: Crop water needs. In: Irrigation water management: irrigation water needs. pp. 8-18. FAO, Rome, Italy.

Bruce, W.B., G. O. Edmeades, and T. C. Barker, 2002: Molecular and physiological approaches to maize improvement for drought tolerance. J. Exp. Bot. 53, 13-25.

Cakir, R., 2004: Effect of water stress at different development stages on vegetative and reproductive growth of corn. Field Crops Res. 89, 1-16.

Center for Crops Utilization Research, 2008: Corn uses poster. Iowa State Univ., Ames, IA. Available at: http://www.ncga.com (accessed 15 October 2010).

Davis, J. G., and C. R. Wilson, 2005: Choosing a soil amendment. Colorado State University Extension, Fort Collins, CO. Available at: http://www.ext.colostate.edu/pubs/garden/07235.html (accessed 15 October 2010).

Ebercon, A., A. Blum, and W. R. Jordan, 1977: A rapid colorimetric method for epicuticular wax content of sorghum leaves. Crop Sci. 7, 179-180.

Eck, H. V., 1986: Effects of water deficits on yield, yield components, and water use efficiency of irrigated corn. Agron. J. 78, 1035–1040.

Edmeades, G. O., J. Bolaños, S. C. Chapman, H. R. Lafitte, and M. Bänziger, 1999: Selection improves drought tolerance in tropical maize populations: I. Gains in biomass, grain yield, and harvest index. Crop Sci. 39, 1306–1315.

Grant, R. F, B. S. Jackson, J. R. Kiniry, and G. F. Arkin, 1989: Water deficit timing effects on yield components in maize. Agron. J. 81, 61-65.

Green Car Congress, 2007: USDA: Booming US ethanol production could require additional 1 billion bushels of corn in 2007-08. Available at: http://www.greencarcongress.com/2007/01/booming_us_etha (accessed 15 October 2010).

Hall, D., and R. Jones, 1961: Physiological significance of surface wax on leaves. Nature 191, 95-96.

High Plains/Midwest Ag Journal, 2008: Texas drought losses estimated at $1.4 billion. Available at: http://www.hpj.com/archives/2008/sep08/sep15/2008Texasdroughtlossesestim.cfm (accessed 15 October 2010).

Holmes, M. G., and D. R. Keiller, 2002: Effects of pubescence and waxes on the reflectance of leaves in the ultraviolet and photosynthetic wavebands: a comparison of a range of species. Plant Cell Environ. 25, 85-93.

Hsiao, T. and E. Acevedo, 1974: Plant responses to water deficits, water-use efficiency, and drought resistance. Agric. Meteorol. 14, 59-84.

Jarou, Z., 2009: Measuring leaf area with Adobe Photoshop CS3. ChlorFilms Videos. Available at: http://www.chlorofilms.org/index.php/crpVideo/display/videoid/46 (accessed 15 October 2010).

Kang, S., W. Shi, and J. Zhang, 2000: An improved water-use efficiency for maize grown under regulated deficit irrigation. Field Crops Res. 67, 207-214.

Kerstetter, R., and R. Poethig, 1998: The specification of leaf identity during shoot development. Annu. Rev. Cell Dev. Biol. 14, 373–98.

Koch, K., W. Barthlott, S. Koch, A. Hommes, K. Wandelt, W. Mamdouh, S. De-Feyter, and P. Broekmann, 2006: Structural analysis of wheat wax (*Triticum aestivum*): from the molecular level to three-dimensional crystals. Planta 223, 258–270.

Lawson, E., and R. Poethig, 1995: Shoot development in plants: time for a change. Genetics 11, 263-268.

Leavitt, J., and D. Penner, 1979: Prevention of EPTC: induced epicuticular wax aggregation on corn (*Zea mays* L.) with R-25788. Weed Sci. 27, 47-50.

Lemieux, B., 1996: Molecular genetics of epicuticular wax biosynthesis. Plant Sci. 1, 312–318.

Llorente, C., F. Betrán, A. Bockholt, and F. Fojt, 2004: Registration of Tx772 maize. Crop Sci. 44, 1036–1037.

Long, L. M., H. P. Patel, W. C. Cory, and A. E. Stapleton, 2003: The maize epicuticular wax layer provides UV protection. Funct. Plant Biol. 30, 75–81.

Longenberger, P. S., C. W. Smith, P. S. Thaxton, and B. L. McMichael, 2006: Development of a screening method for drought tolerance in cotton seedlings. Crop Sci. 46, 2104-2110.

Ludlow, M. M., and R. C. Muchow, 1990: A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43, 107–153.

Mayeux, H., W. Jordan, R. Meyer, and S. Meola, 1981: Epicuticular wax on goldenweed (*Isocoma* spp.) leaves: variation with species and season. Weed Sci. 29, 389-393.

McWhorter, C.G., 1993: Epicuticular wax on johnsongrass (*Sorghum halepense*) leaves. Weed Sci. 41, 475-842.

McWhorter, C. G., R. N. Paul, and W. L. Barrentine, 1990: Morphology, development and recrystallization of epicuticular waxes of Johnsongrass (*Sorghum halepense*). Weed Sci. 38, 22-33.

Mondal, S., and D. Hays, 2007: Leaf epictuticular wax improves heat tolerance in wheat. ASA-CSSA-SSSA 2007 International Annual Meetings. 4-8 November 2007. New Orleans, LA.

Moose, S. P., and P. H. Sisco, 1994: Glossy15 controls the epidermal juvenile-to-adult transition in maize. Plant Cell 6, 1343-1355.

Monsanto Company, 1991: Inbred corn line LH195. United States patent #5059745.

Monsanto Company, 2001: Inbred corn line LH287: United States patent #6281414.

Muenchrath, D. A., 1995: Morphological and physiological characterization of a desert adapted traditional Native American maize (*Zea mays* L.) cultivar. PhD dissertation, Iowa State University, Ames, Iowa.

Nation Master, 2005: Corn consumption by country. Available at: http://www.nationmaster.com/graph/agr_gra_cor_con-agriculture-grains-corn-consumption&b_map=1 (accessed 15 October 2010).

National Climatic Data Center, 2010a: Annual drought summary 2009. NCDC, Asheville, NC. Available at: http://www.ncdc.noaa.gov/sotc/index.php (accessed 15 October 2010).

National Climatic Data Center, 2010b: Billion dollar U.S. weather disasters. NCDC, Asheville, NC. Available at: http://www.ncdc.noaa.gov/oa/reports/billionz.html (accessed 15 October 2010).

National Corn Growers Association, 2010: U.S. corn growers meet the need for food and fuel. Available at: http://www.ncga.com (accessed 15 October 2010).

NeSmith, D. S., and J. T. Ritchie, 1992: Short and long-term responses of maize to a pre-anthesis soil water deficit. Agron. J. 84, 107–113.

O'Toole J. C., R. T. Cruz, and J. N. Seiber, 1979: Epicuticular wax and cuticular resistance in rice. Physiol. Plant. 47, 239-244.

Premachandra, G. S., H. Saneoka, M. Kanaya, and S. Ogata, 1991: Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize. J. Exp. Bot. 42, 167–171.

Riederer, M., and L. Schreiber, 2001: Protecting against water loss: Analysis of the barrier properties of plant cuticles. J. Exp. Bot. 52, 2023–2032.

Ristic, Z., and M. A. Jenks, 2002: Leaf cuticle and water loss in maize lines differing in dehydration avoidance. J. Plant Physiol. 159, 645-651.

Saneoka, H., and S. Ogata, 1987: Relationship between water use efficiency and cuticular wax deposition in warm season forage crops grown under water deficit conditions. Soil Sci. Plant Nutr. 33, 439-448.

Sangoi, L., and R. J. Salvador, 1998: Maize susceptibility to drought at flowering: a new approach to overcome the problem. Ciência Rural (Santa Maria) 28, 699-706.

Schönherr, J., 2006: Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. J. Exp. Bot. 57, 2471–2491.

Sharp, R. E., and W. J. Davies, 1979: Solute regulation and growth by roots and shoots of water-stressed maize plants. Planta 147, 43-49.

Sharp, R. E., V. Poroyko, L. G. Heijlek, W. G. Spollen, G. K. Springer, H. J. Bohnert, and H. T. Nguyen, 2004: Root growth maintenance during water deficits: physiology to functional genomics. J. Exp. Bot. 55, 2343–2351.

Singh, B. B., Y. Mai-Kodomi, and T. Terao, 1999: A simple screening method for drought tolerance in cowpea. Indian J. Genet. 59, 211–220.

Singh, B. B., and T. Matsui, 2002: Cowpea varieties for drought tolerance. In: C. A. Fatokun, S. A. Tarawali, B. B. Singh, P. M. Kormawa and M. Tamo (eds.), Proceedings of the 3rd World Cowpea Conference, pp. 287–300. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Thomas, J. F., and C. N. Harvey, 1983: Leaf anatomy of four species grown under continuous CO_2 enrichment. Botanical Gazette 144, 303-309.

Tollenaar, M., and J. Wu, 1999: Yield improvement in temperate maize is attributable to greater stress tolerance. Crop Sci. 39, 1597–1604.

US Drought Monitor, 2008: Explanation of the US drought monitor. Available at: http://www.drought.unl.edu/dm/classify.htm (accessed 15 October 2010).

Verma, V., M. Foulkes, A. Worland, R. Sylvester-Bradley, P. Caligari, and J. Snape, 2004: Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135, 255–263.

Westgate, M. E., 1994: Water status and development of the maize endosperm and embryo during drought. Crop Sci. 34, 76-78.

Xu, W., and G. Odvody, 2004. Tx204 and Tx205 Inbred lines. Germplasm Enhancement of Maize (GEM) 2004 Annual Report. Available at: http://www.public.iastate.edu/~usda-gem/GEM_Annual_Reports/GEM_AR_04.htm (accessed 22 October 2010).

APPENDICES

APPENDIX A

TX#	Genotype[1]	Pedigree	Color	Temperate	Southern	Exotic	Notes
Tx715	1	B104/CML285-B-2-B-B-B-B-B	Y	X		X	Yield, TX Adapted, TBR [2]
Tx716	2	Tx770/CML288-B-3-B-B-B-B-B	Y		X	X	Yield, TX Adapted, TBR
Tx717	3	(CML 408/B104)-B-2-1-B-B-B-B	Y	X		X	Yield, TX Adapted, TBR
Tx718	4	((CML 408/B104)x(CML 411/B104))-1-1-B-B-B-B	Y	X		X	Yield, TX Adapted, TBR
Tx719	5	((CML 325/B104)x(CML285/B104))-2-2-B-B-B-B	Y	X		X	Yield, TX Adapted, TBR
Tx720	6	((B104/NC300)x(CML 415/B104))-4-1-B-B-B-B	Y	X	X	X	Yield, TX Adapted, TBR
Tx721	7	(B104/NC300)-B-1-B1-B-B-B-B	Y	X	X		Yield, TX Adapted, TBR
Tx722	8	(CML285/B104)-B-4-B-B-B-B-B-B	Y	X		X	Yield, TX Adapted, TBR
Tx723	9	(CML288/NC300)-B-9-B1-B-B-B-B-B	Y	X	X		Yield, TX Adapted, TBR
Tx724	10	(B97x CML 326-B/Tx770 x A645)-2-2-B-B-B-B-B-B	Y	X	X	X	Yield, TX Adapted, TBR
Tx725	11	(NC300 x Tx714-B/B104-1/CML343)-2-1-B-B-B-B-B-B	Y	X	X	X	Yield, TX Adapted, TBR
Tx726	12	(B104-1 x Tx714-B/B110 x FR2128-B)-12-4-B-B-B-B-B-B	Y	X	X		Yield, TX Adapted, TBR
Tx727	13	(Tx601 x B104-B/FR2128 x Unknown)-2-2-B-B-B-B-B	Y	X	X		Yield, TX Adapted, TBR
Tx728	14	(B104-1 x Tx714-B-B)-1-5-B-B-B-B-B-B	Y	X	X		Yield, TX Adapted, TBR
Tx729	15	BS13(S)C8-11-1-B-B-B-B-B-B-B	Y	X			Yield, TX Adapted, TBR
Tx737	16	Ark 536-B-B-B-B-B-B	Y		X		Yield, TX Adapted, TBR
Tx735	17	((Tx772 x Tx745) x Tx745)-3-3-B-B-B-B-B-B	Y		X		Yield, TX Adapted, TBR
Tx736	18	((Tx772 x T246) x Tx772)-1-5-B-B-B-B-B-B	Y		X		Yield, TX Adapted, TBR
Tx738	19	AR16021:S08a02 Derived line (energy dense)-B-B-B-1-B-B-B-B-B	Y		X		Yield, TX Adapted, TBR
Tx812	20	(P69Qc3HC107-1-1#-4-2#-4-B-B-1-4-B-B-B-B-B X CML 193)-B-B-2-B-B-B-B-1	Y			X	QPM, Yield; TX Adapted
Tx813	21	Pop. 69 Templado Amarillo QPM-B-B-B2-12-B-B-B-B-B	Y			X	QPM, Yield; TX Adapted
Tx772W	22	Tx772W	W		X		Yield, TX Adapted, TBR
Tx117	23	CML78 x CML269-B-B-B-B-B	W		X		Yield, TX Adapted, TBR
Tx120	24	(Tx114 (B73w)-B x CML343/Tx110 x Pop24)-B-B-B-4-B-B-B-B-B	W		X		Yield, TX Adapted, TBR
Tx121	25	CML311-B/CI66-B/Tx114 (B73w)-B xCML343)-B-B-B-1-B-B-B-B-B	W		X		Yield, TX Adapted, TBR
Tx122	26	CML311-B/CI66-B/Tx114 (B73w)-B xCML343)-B-B-B-2-B-B-B-B-B	W		X		Yield, TX Adapted, TBR
Tx131	27	CML269/TX130-B-B-B-5-1-B-B-B-B-B-B	W		X		Yield, TX Adapted, TBR
Tx132	28	CML269/TX130-B-B-B-1-3-B-B-B-B-B	W		X		Yield, TX Adapted, TBR
Tx133	29	CML269/TX114-B-B-B-1-1-B-B-B-B-B	W		X		Yield, TX Adapted, TBR
Tx903	30	(Lfy2361-B/Tx114 (B73w)-B Dark blue-B)Tx114/Lfy2304-B-B-B-1-3-B-B-B-3-B	B			X	TX Adapted, TBR
Tx904	31	Ethiopia12-B-3-3-B-B2-B1-2-B	B			X	TX Adapted, TBR
	32	BLUE	B			X	TX Adapted, TBR

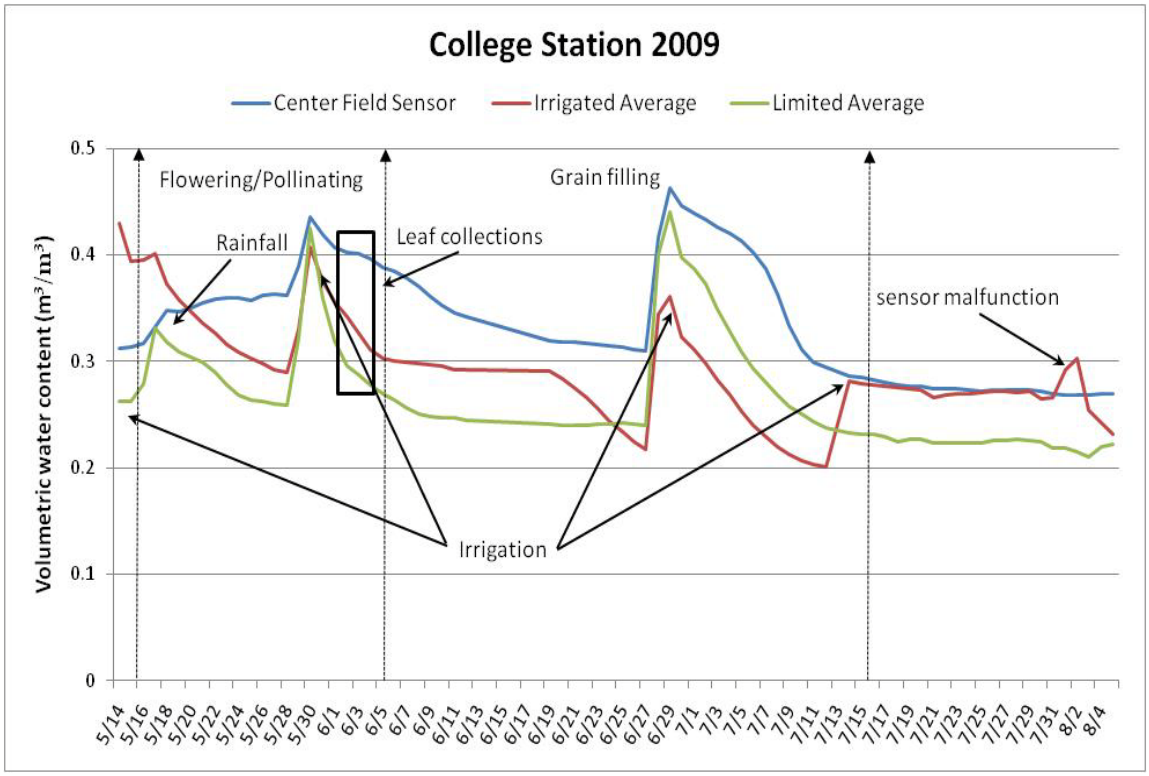
TX#	Genotype[1]	Pedigree	Color	Temperate	Southern	Exotic	Notes
	33	BLUE	B			X	TX Adapted, TBR
	34	Red Ear 2-2-2-1-1-2-B	R			X	TX Adapted, TBR
	35	Red Ear 5-2-4-1-4-1-B	R			X	TX Adapted, TBR
	36	B104-1-B-B-B-B-B-B-B-B-B		X			
	37	Ark 536-B-B-B-B-B-B			X		
	38	CML325-B-B-B-B-B-B-B-B-B				X	
	39	NC280-B-B-B-B-B-B			X		
	40	DKB830:S19(entry 96)-B-B-B-9-B-B-B-B-B-B-B		X			
	41	EX66: DK888 N11 bulk selfs-B-B-B-B-B-B-B-B		X			
	42	B 73-B-B-B-B-B-B-B-B	Y	X			
Tx772	43	Tx772-B-B-B-B-B	Y		X		Released in 2004
Tx714	44	Tx714-B-B-B-B-B-B-B	Y		X		Released in 2004
Tx732	45	Tx732-B-B-B-B-B-B-B			X		Released in 2004
	46	Tx6252-B-B-B-B-B-B-B			X		
	47	Tx760-B-B-B-B-B-B-B			X		
Tx770	48	Tx770-B-B-B-B-B-B			X		Released in 2004
	49	Tx771-B-B-B-B-B-B-B			X		
	50	FR2128-B-B-B-B-B-B-B			X		
		Wenwei Xu Lines					
	51	S2B73	Y				
	52	C3A632-1A	LYD				
	53	C2A554-4	LYSD				
	54	S2B73BC	Y				
	55	AR03056:N0902-1	Y				
	56	C3S1B73-1	YSD				
	57	C3S1B73-3-3	YD				
	58	C3S1B73-3-1	YD				
	59	C3W64A-1	YF				
	60	B5C2	LYF				
Tx204	61	Tx204	YD				Released
Tx205	62	Tx205	YD				Released
		2009 Checks					
	63	LH195	Y				
	64	LH195	Y				
	65	LH195	Y				
	66	LH195	Y				
	67	LH287	Y				

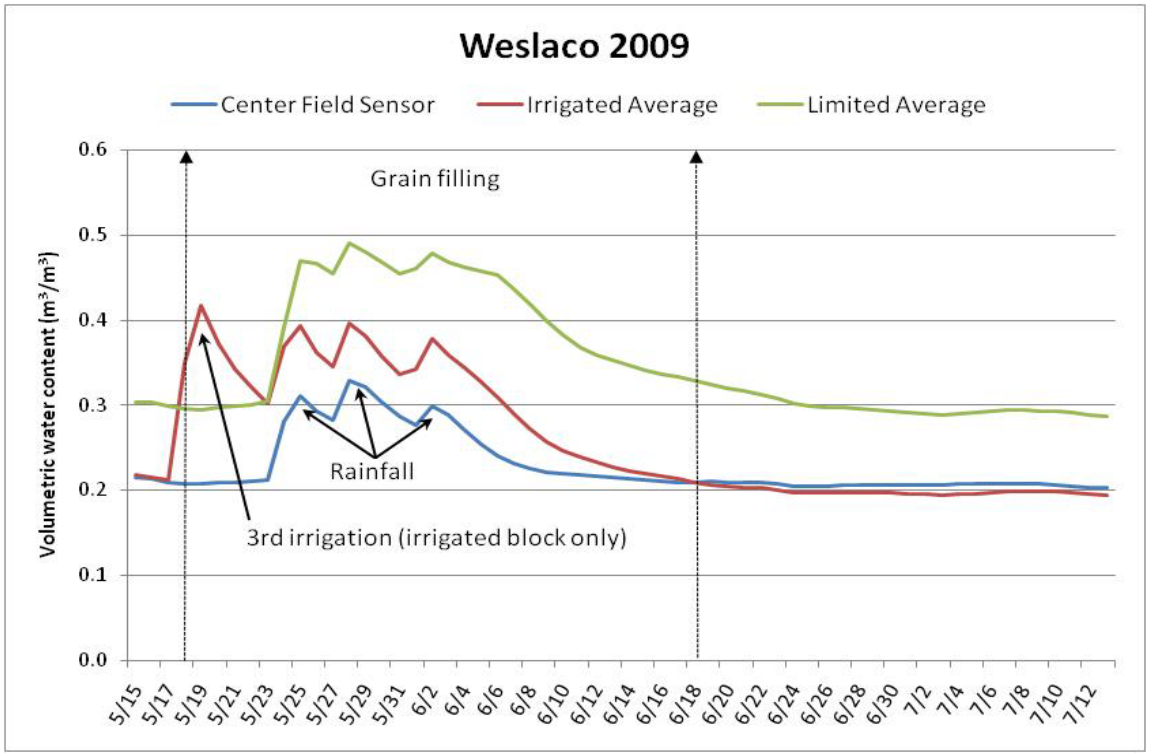
TX#	Genotype[1]	Pedigree	Color	Temperate	Southern	Exotic	Notes
	68	LH287	Y				
	69	LH287	Y				
	70	LH287	Y				
		2010 Checks					
	63	P31G66	Y				
	64	BH9440W	Y				
	65	LH195/LH287	Y				
	66	LH195/LH287	Y				
	67	LH195/LH287	Y				
	68	LH195/LH287	Y				
	69	(CML285/B104)-B-4-B-B-B-B-B-B	Y				
	70	Red	R				

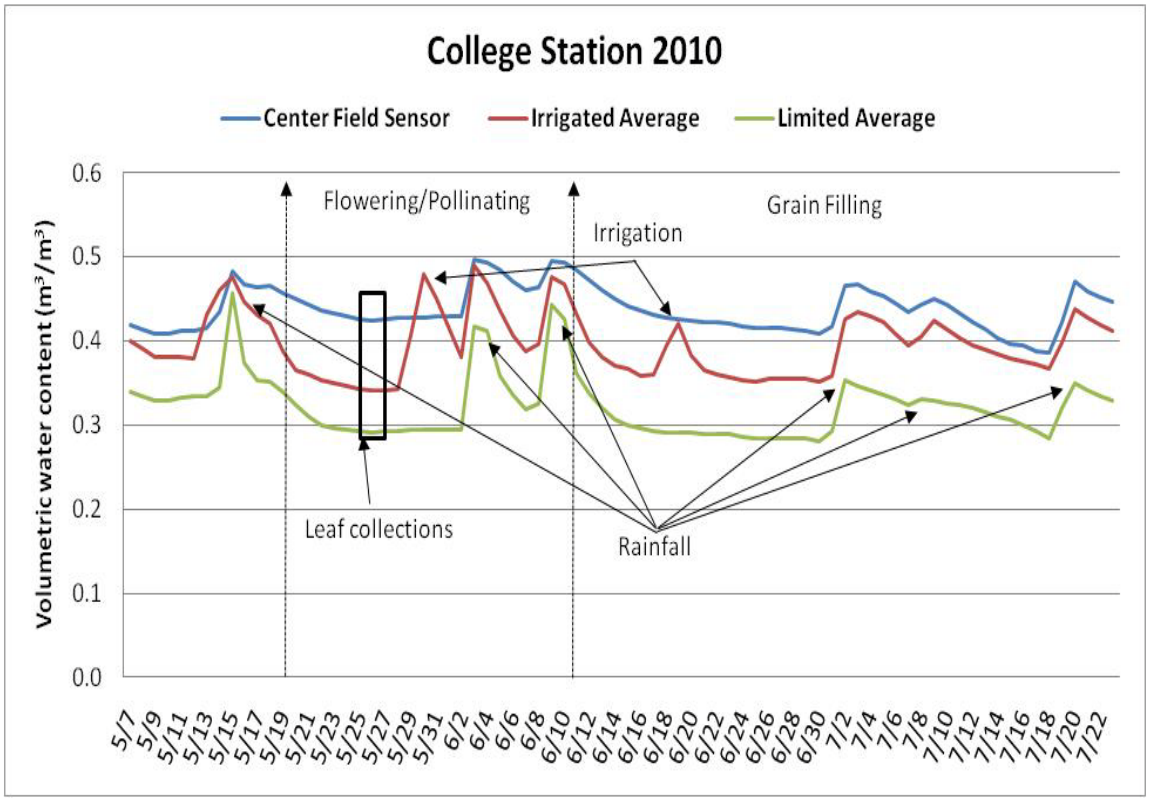
[1] Genotypes 1-62 were crossed to a hybrid parent (LH195/LH287) that were screened in 2010

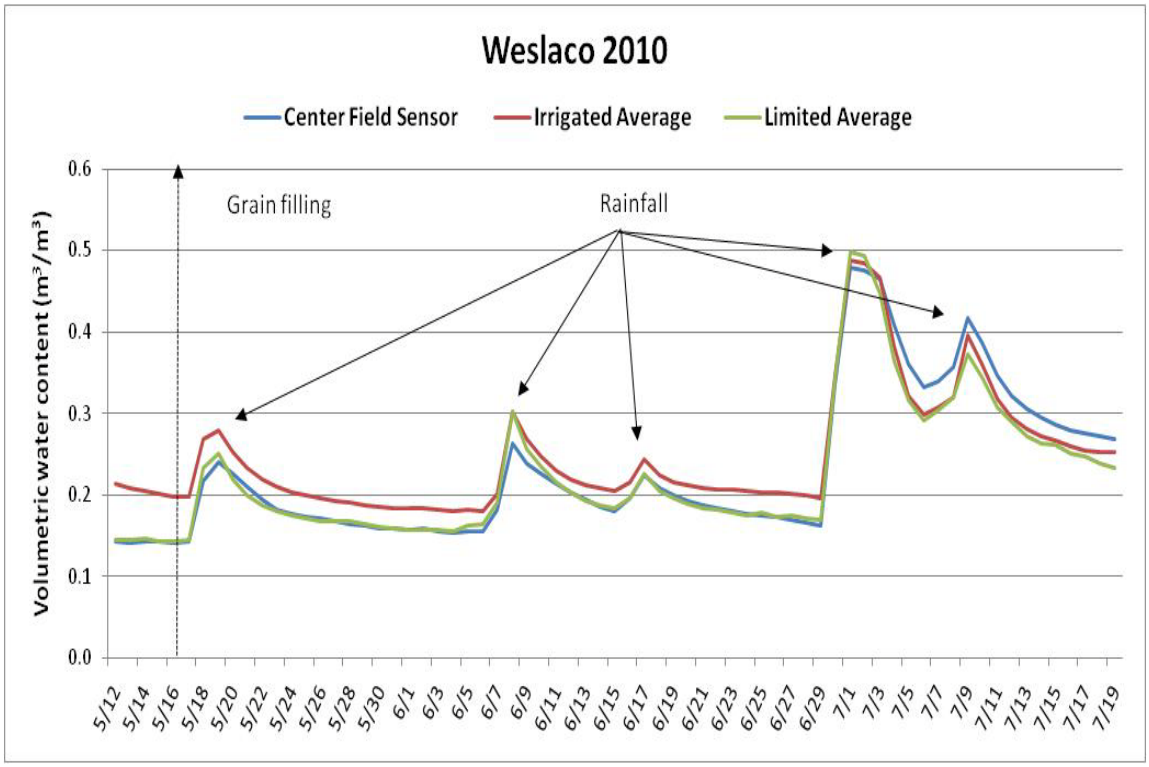
[2] "TBR" - Elite lines to be released by Texas A&M / Texas AgriLife Research

APPENDIX B


College Station 2009
Center Field Sensor
Irrigated Average
Limited Average
Volumetric water content (m³/m³)
Flowering/Pollinating
Rainfall
Leaf collections
Grain filling
sensor malfunction
Irrigation


Weslaco 2009
Center Field Sensor
Irrigated Average
Limited Average
Volumetric water content (m³/m³)
Grain filling
Rainfall
3rd irrigation (irrigated block only)


College Station 2010
Center Field Sensor
Irrigated Average
Limited Average
Volumetric water content (m³/m³)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Flowering/Pollinating
Irrigation
Grain Filling
Leaf collections
Rainfall
5/7
5/9
5/11
5/13
5/15
5/17
5/19
5/21
5/23
5/25
5/27
5/29
5/31
6/2
6/4
6/6
6/8
6/10
6/12
6/14
6/16
6/18
6/20
6/22
6/24
6/26
6/28
6/30
7/2
7/4
7/6
7/8
7/10
7/12
7/14
7/16
7/18
7/20
7/22


Weslaco 2010
Center Field Sensor
Irrigated Average
Limited Average
Volumetric water content (m³/m³)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Grain filling
Rainfall
5/12
5/14
5/16
5/18
5/20
5/22
5/24
5/26
5/28
5/30
6/1
6/3
6/5
6/7
6/9
6/11
6/13
6/15
6/17
6/19
6/21
6/23
6/25
6/27
6/29
7/1
7/3
7/5
7/7
7/9
7/11
7/13
7/15
7/17
7/19

APPENDIX C

Agronomic Data for Soil Moisture Sensors					Precipitation Data for Soil Moisture Sensors								
	College Station		Weslaco			College Station				Weslaco			
	2009	2010	2009	2010		2009	in	2010	in	2009	in	2010	in
Planting date	3/6	3/6	2/17	2/18		5/16	1.32	5/15	0.84	5/24	1.43	5/18	1.49
Sensor installation	5/14	5/7	5/15	5/12		7/30	0.35	6/3	0.82	5/28	0.84	6/7	1.39
Sensor removal	8/5	7/23	7/13	7/19		7/31	0.66	6/9	4.27	6/1	0.66	6/8	0.41
Irrigation date (3")	2/13	5/30	2/23	3/31				7/1	0.16			6/16	0.46
	5/14	6/19	3/31	5/7				7/8	0.02			6/29	0.25
	5/29		5/18					7/19	NA			6/30	7.29
	6/28											7/1	1.21
	7/14											7/7	0.79
												7/8	1.08
Flag leaf collections	June 1-4	May 25-26	May 9-10	May 11-12								7/9	0.84
Soil type	Ships clay loam		Raymondville clay loam		**Total**		2.33		6.11		2.93		15.2

APPENDIX D

Pearson's correlations for hybrid testcross analysis																
	Pop	Height	Earheight	Ear#	Earwt	Avgcobwt	Earlength	Grain/ear	Totalyield	Wxwt	Lfwt	Avglfarea	%wxlfwt	%wxwta	Flowering	Silking
Pop		ns	***	***	***	ns	***	ns	***	***	***	**	***	***	*	ns
Height	ns		***	ns	***	ns	ns	***	***	ns	***	***	***	***	*	ns
Earheight	***	***		***	***	ns	*	***	***	*	*	***	ns	ns	ns	ns
Ear#	***	ns	***		***	ns	***	ns	***	***	***	**	***	***	*	ns
Earwt	***	***	***	***		***	***	***	***	ns	ns	ns	ns	ns	ns	ns
Avgcobwt	ns	ns	ns	ns	***		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Earlength	***	ns	*	***	***	ns		***	***	*	ns	ns	*	*	*	ns
Grain/ear	ns	***	***	ns	***	ns	***		***	ns	*	***	*	**	**	**
Totalyield	***	***	***	***	***	ns	***	***		***	ns	ns	***	*	ns	**
Wxwt	***	ns	*	***	ns	ns	*	ns	***		***	***	***	***	ns	ns
Lfwt	***	***	*	***	ns	ns	ns	*	ns	***		***	***	***	ns	*
Avglfarea	**	***	***	**	ns	ns	ns	***	ns	***	***		***	***	ns	ns
%wxlfwt	***	***	ns	***	ns	ns	*	*	***	***	***	***		***	ns	**
%wxwta	***	***	ns	***	ns	ns	*	**	*	***	***	***	***		ns	*
Flowering	*	*	ns	*	ns	ns	*	**	ns	ns	ns	ns	ns	Ns		***
Silking	ns	ns	ns	ns	ns	ns	ns	**	**	ns	*	ns	**	*	***	

APPENDIX E

Limited Irrigated														Full Irrigated							
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
X	X	70	69	68	67	66	X	X	X	X	X	X	70	69	68	67	66	X	X		
X	X	61	62	63	64	65	X	X	X	X	X	X	61	62	63	64	65	X	X		
X	X	60	59	58	57	56	X	X	X	X	X	X	60	59	58	57	56	X	X		
X	X	51	52	53	54	55	X	X	X	X	X	X	51	52	53	54	55	X	X		
X	X	50	49	48	47	46	X	X	X	X	X	X	50	49	48	47	46	X	X		
X	X	41	42	43	44	45	X	X	X	X	X	X	41	42	43	44	45	X	X		
X	X	40	39	38	37	36	X	X	X	X	X	X	40	39	38	37	36	X	X	Rep 1	
X	X	31	32	33	34	35	X	X	X	X	X	X	31	32	33	34	35	X	X		
X	X	30	29	28	27	26	X	X	X	X	X	X	30	29	28	27	26	X	X		
X	X	21	22	23	24	25	X	X	X	X	X	X	21	22	23	24	25	X	X		
X	X	20	19	18	17	16	X	X	X	X	X	X	20	19	18	17	16	X	X		
X	X	11	**12**	**13**	14	15	X	X	X	X	X	X	**11**	**12**	13	14	15	X	X		
X	X	10	9	8	7	6	X	X	X	X	X	X	10	9	8	7	6	X	X		
X	X	301	2	3	4	5	X	X	X	X	X	X	401	2	3	4	5	X	X		
X	X	70	69	68	67	66	X	X	X	X	X	X	70	69	68	67	66	X	X		
X	X	61	62	**63**	**64**	65	X	X	**X**	**X**	X	X	61	**62**	**63**	64	65	X	X		
X	X	60	59	58	57	56	X	X	X	X	X	X	60	59	58	57	56	X	X		
X	X	51	52	53	54	55	X	X	X	X	X	X	51	52	53	54	55	X	X		
X	X	50	49	48	47	46	X	X	X	X	X	X	50	49	48	47	46	X	X		
X	X	41	42	43	44	45	X	X	X	X	X	X	41	42	43	44	45	X	X		
X	X	40	39	38	37	36	X	X	X	X	X	X	40	39	38	37	36	X	X	Rep 2	
X	X	31	32	33	34	35	X	X	X	X	X	X	31	32	33	34	35	X	X		
X	X	30	29	28	27	26	X	X	X	X	X	X	30	29	28	27	26	X	X		
X	X	21	22	23	24	25	X	X	X	X	X	X	21	22	23	24	25	X	X		
X	X	20	19	18	17	16	X	X	X	X	X	X	20	19	18	17	16	X	X		
X	X	11	12	13	14	15	X	X	X	X	X	X	11	12	13	14	15	X	X		
X	X	10	9	8	7	6	X	X	X	X	X	X	10	9	8	7	6	X	X		
X	X	201	2	3	4	5	X	X	X	X	X	X	101	2	3	4	5	X	X		
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

[1] An “X” represents border plots

[2] Soil moisture sensors were installed between plots with bold-typed, larger text

VITA

Meghyn Brianne Meeks was raised in Hurst, Texas where she graduated from Lawrence D. Bell High School in 2004. She earned her Bachelor of Science degree from Tarleton State University in 2008, graduating Magna Cum Laude. Her major was horticulture landscape management, with special interest on golf course management, and a minor in business.

Desiring a future in breeding grasses, she attended graduate school for a Master of Science degree at Texas A&M University. While there, she completed two and a half years of research in the Quantitative Genetics and Maize Breeding program under Dr. Seth Murray. Upon completion of this research, Meghyn began her doctorate degree in plant breeding under Dr. Ambika Chandra, Assistant Professor of Turfgrass Breeding and Molecular Genetics for Texas AgriLife Research. She can be contacted via email or through the Department of Soil and Crop Sciences at Texas A&M University.

Email: m.stalcup@hotmail.com

Address: Department of Soil and Crop Sciences, 370 Olsen Boulevard, 2474 TAMU, College Station, TX 77843-2474, Phone (979) 845 – 3041, Email: soilcrop@tamu.edu