

Physiological Evaluation and Root Morphological Differences associated with Novel Drought
Tolerant Corn (*Zea mays* L.) Hybrids

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“Trust in the LORD with all your heart and lean not on your own understanding; in all your ways acknowledge him, and he will make your paths straight.” Proverbs 3:5-6

Abstract

Corn hybrids designated “drought tolerant” (DT) from three commercial seed companies were evaluated in order to determine the benefit of DT technology in a mid-south environment that may receive adequate but often poorly timed rainfall. Field research was conducted in 2014 and 2015 at Milan, TN where hybrid response to stress was evaluated at two planting dates and in non-irrigated and irrigated environments and greenhouse research was conducted in Jackson, TN in 2015 to evaluate early season drought effect on plant physiology and root structure.

In 2014 and 2015, the non-irrigated trials received enough rainfall to create a high yielding environment. Since the DT hybrids were only stressed for short periods of time throughout the growing season, we were unable to evaluate growth and yield under intense stress conditions. It was determined that hybrids with the DT technology are quite competitive in high yield conditions, but may not provide a direct benefit when growers receive enough annual rainfall to produce a profitable corn crop. Early season root structure was not altered by exposure to extreme water deficit, but physiological properties were affected. Stomatal conductance, biomass, and leaf temperature were affected when corn was exposed to early season drought.

DT corn hybrids could potentially be an option for growers producing corn in humid regions that only sustain brief periods of drought throughout the growing season. However, more research under stress conditions is needed to better understand these novel technologies and how they achieve their ability to tolerate drought stress.

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Part I.
Introduction

Corn History and Production

Corn (*Zea mays* L.) belongs to the Poaceae family and is referred to as maize throughout most of the world. This crop originated in Mesoamerica and cultivation began more than 8,000 years ago (Galinat, 1988). In the sixteenth century it was distributed around the world as a food crop by Spanish and Portuguese explorers (Fageria et al., 2011). Corn has since become a staple food crop around the world. Modern corn is thought to have descended from a wild grass called teosinte (*Z. Mexicana*), but it is unclear if early selective breeding by farmers or natural selection resulted in the off-type that is produced today (Galinat, 1988; Mabberly, 1997). Corn has been produced in the Americas for hundreds of years and evidence suggests that cultivation ranged from North Dakota to Chile (Gibson and Benson, 2002).

In most years corn is the number one planted cereal crop in the United States based area, and the third most cultivated cereal crop in the world. Even though it is ranked third in hectares of production, corn produces the largest grain yield of all cereal crops worldwide (Fageria et al., 2011). Because of the crop's ability to produce a large amount of grain per area of land along with its high starch content it has become a primary source of food and feed around the world (Danforth, 2009). The United States produces approximately 32 percent of the world's corn grown for grain and silage (National Corn Growers Association 2013 Report) and there were roughly 38.6 million hectares in corn production in 2013 (USDA Forum). Corn is primarily grown in the United States for livestock feed. According to United States Department of Agriculture, 70% is used as animal feed and 20% is for industrial purposes, such as ethanol and manufacturing. As of 2013, Tennessee ranked 16th in the United States for corn production with approximately 303,000 hectares (UTCrops 2013).

Average corn yields in the U.S. have risen 118 kg per hectare from the late 1930's until the late 2000's (Tollenaar and Lee, 2011) and have doubled globally from 1961 to 2002 (Duvick, 2005). These gains can be accredited to improvements in genetics and management practices (Tollenaar and Lee, 2011). The genetic traits that are most associated with yield gains are those of increased stress tolerance (Tollenaar and Wu, 1999; Bruulsema et al., 2000; Tollenaar and Lee, 2002).

With a growing human population and shrinking land availability corn production will have to continue to increase. According to Tollenaar and Lee (2011) yield gains can be made by reducing certain abiotic stresses and increasing individual plant resource efficiency while under stress. The two greatest limiting factors affecting the productivity and efficiency of corn are nitrogen and water availability (Roth et al., 2013) with the greater of the two being water shortage (Campos et al., 2006).

Corn Growth and Development

Corn exhibits a determinate growth habit, meaning its vegetative and reproductive stages occur at different times. The vegetative stages begin with germination and emergence of the seedling and continue until the tassel is fully emerged and visible (Ritchie et al., 1993). These stages are referred to as “V” stages. The reproductive cycle begins after the tassel is completely visible and occurs in 6 progressive stages: silking, blister, milk, dough, dent and physiological maturity or “black layer” (Ritchie et al., 1993). These stages are referred to as “R” stages. At physiological maturity (R6) the kernels have reached maximum dry matter and are not affected by environmentally induced stress.

There are many different corn cultivars adapted for a wide array of climatic conditions. These cultivars can range in height from 1-8 meters and take between 60 days to 48 weeks to

reach physiological maturity (Shaw, 1988). They can be grown in tropical, sub-tropical or temperate climates; with the majority of production occurring between the 30° and 55° of longitude and elevations ranging from below sea level to 4,000m (Shaw, 1988).

Corn development is driven by heat accumulation, therefore it is not as sensitive to differences in photoperiod compared to other cash crops. Heat accumulation or heat units are expressed as growing degree units (GDUs) or growing degree-days (GDDs) and can be used to describe when biological processes in the plant will begin and end (McMaster and Wilhelm, 1997). The equation used to calculate GDUs is: $GDU = \left[\frac{T_{max} + T_{min}}{2} \right] - T_{base}$, where T_{max} and T_{min} are the daily maximum and minimum temperature, and T_{base} is the base temperature for the measured crop (McMaster and Wilhelm, 1997). For measuring GDUs in corn, the modified upper and lower daily limits are 30°C and 10°C, respectively, with the base temperature being 10°C. If daily temperatures fall outside the maximum and minimum range then the limit will be used for calculating GDUs on that day. Corn is not readily produced in areas with an average midsummer temperature of below 19°C.

Water Needs in Corn

Corn can be produced in areas that receive as low as 25 cm and up to 500 cm of annual precipitation (Shaw, 1988). However, areas that receive less than 15 cm of summer rainfall must use supplemental irrigation. Irrigation use in crop production accounts for 70% of total water use worldwide (Fischer et al., 2007), making agriculture the largest consumer of fresh water. However, only 50% of the water withdrawn actually reaches the desired crop (Fischer et al., 2007). With aquifers being drained and regulation being enforced in some areas of the United States, efficiency in water usage must continue to increase. Even though the number of irrigation systems has increased recently in the Mid-South, small and irregular shaped fields make it

difficult for center pivot or lateral irrigation systems to be installed. Tennessee has the fewest irrigated corn acres in the Mid-South because of these misshaped fields (Boyer et al., 2013). Also, the highly erodible soils that dominate the region force producers to use a no-till cropping system, eliminating the option of furrow irrigation in much of Tennessee.

Generally, the Mid-South region of the U.S. receives enough rainfall to produce a sufficient corn crop; however, that rainfall is not always timely and irrigation of the crop is still recommended (Vories et al., 2009). A corn crop's need for water varies throughout the growing season based on hybrid maturity, growth stage, and plant density. Depending on the extent and magnitude of a drought stress event, it can take multiple days for a corn crop to recover (Hsiao, 1973). Sufficient moisture is most critical from about one week prior and two weeks post R1 or silking (Bänziger et al., 2000; Tollenaar and Lee, 2011). This is not the only time the crop needs moisture, but also the time that is most crucial for adequate pollination and seed initiation. Research in grain sorghum (*Sorghum bicolor*), a more drought tolerant crop, showed two distinct types of drought stress (Rosenow et al., 1983) can impact grain development. The first type is expressed when the crop is exposed to drought stress prior to flowering. The second is when drought stress occurs during grain fill (Rosenow et al., 1983). Tolerance in sorghum cultivars to both types of stress were found, but not within the same genome.

Periodic drought often experienced in the Mid-South U.S. can greatly influence productivity, and this stress can be amplified on coarse-textured soils or if timed around early reproductive stages (Campos et al., 2004). Crop water use can be quantified by evapotranspiration (ET_c), which is the amount of water removed from the ground by soil evaporation and plant transpiration. In corn production, evaporation accounts for 20-30 percent of total ET_c and the other 70-80% comes from plant transpiration (Kranz et al., 2008). This value

can be influenced by many different factors, such as air temperature, relative humidity, solar radiation, and wind speed (Kranz et al., 2008). The size and stage of the crop will also influence the transpiration rate. A more developed corn plant will transpire faster than a younger plant because it has a more extensive root system and a greater leaf area with more stomata.

Water shortages can be alleviated through irrigation, agronomic “best management practices”, or through the use of drought tolerant corn hybrids. Some scientists believe that global warming will cause over-all rainfall distributions to shift from current areas of high corn production and likewise lead to higher temperatures, ultimately resulting in poor plant performance and lower yields in the U.S. corn belt (Campos et al., 2006).

Previous research indicates that corn yields will continue to increase as population increases until an optimal number per unit area is reached (Lang et al., 1956; Holt and Timmons, 1968; Lutz et al., 1970; Karlen and Camp, 1985; Al-Kaisi and Yin, 2003). However, as populations are increased the competition for moisture also increases. The ability of corn to tolerate increased populations is the stress component most often emphasized for increased grain yield (GY). Planting rates have increased roughly 1,000 plants per hectare per year over the past 50 years in the U.S. Corn Belt (Duvick, 2005). This has allowed yields to increase annually, but with limited advancement in individual plant gain. Variability in plant density across a given production field is detrimental to overall performance and profitability because individual plants can not completely compensate for areas with poor stands (Tokatlidis and Koutroubas, 2004).

Planting date can also influence plant growth, development, and GY through differences in soil and air temperatures and available soil moisture at critical stages during the season. Previous research shows that GY may decrease when the corn plant is exposed to cooler early-season soil temperatures that are associated with early planting (Griffith et al., 1973; Kaspar et

al., 1987; Swan et al., 1987). In no-till cropping systems, lower soil temperatures are associated with surface residue, and high surface residue is responsible for slower soil warming (Swan et al., 1987; Kaspar et al., 1990). However, the optimal planting time for best corn yields in the upper Mid-South is from late March through early April (McClure, 2009) because any impact of cool soils from early planting is less than yield impacts from heat and moisture stress during pollination and grain fill with later planting (McClure personal communication, 2015). Based on a calculation from a customized query from USDA NASS Tennessee crop progress reports for 2006 -2015 67.5 % of acreage is planted during the month of April, 18.2% during the first two weeks of May and 8.5% during the last two weeks of May (USDA NASS, 2015). Five year averages in Tennessee show a GY decrease of 12% when planting is delayed from early to late April (McClure, 2015) and approximately one bushel per day loss when delayed from May 1 to June 1 (McClure, 2009) and this reduction is due to high temperatures combined with inadequate soil moisture at critical growth stages. Because of less exposure to yield reducing agents Bruns and Abbas (2006) found that 50% of corn planted in the Mid-South has shifted from early May to early April over the past 30 years.

Natural Adaptations to Drought

Drought is the most unpredictable and limiting environmental stress that agricultural producers have to deal with. Natural selection has played a role in developing some drought tolerance in plants that complete their life cycle in low water environments. This can be seen through the development of C₃, C₄ and CAM photosynthesis in native plants. CAM plants have developed specific day and night reactions to limit the amount of time their stomata must be open during the day, which reduces the amount of water lost through transpiration. This pathway is thought to have developed during the Paleozoic era in aquatic plants (Keeley and Rundel,

2003). Corn, unlike some of the other widely cultivated cereal crops, is a C₄ plant. C₄ plants are evolved C₃ plants that have adapted to high light intensities, high temperatures, and drier climates (Gowik and Westhoff, 2011). C₄ plants utilize an adapted photosynthetic pathway that allows them to optimize growing potential and more efficiently create carbohydrates under poor growing conditions.

Some other adapted traits associated with drought tolerance include cuticularian wax composition and thickness, increased pubescence, and osmotic adjustment potential (Huang et al., 2006). Banziger et al. (2000) ranks secondary traits for drought tolerance in order of importance: increased grain yield, decreased plant bareness, decreased anthesis silking interval, delayed leaf senescence, reduced tassel size, and reduced leaf rolling (Roth et al., 2013).

Breeding for Drought-Tolerance

A renewed interest in producing more drought tolerant crops has become necessary due to volatile commodity prices, rising input costs and more frequent drought (Pingali, 2012). Removing natural selection leaves two ways to genetically generate a drought tolerant crop: selective breeding and transgenesis.

Selective or traditional plant breeding dates back more than 10,000 years when mankind switched from a hunter-gather lifestyle to an agricultural society (Acquaah, 2007). The human race has selectively bred plants and animals for a variety of different qualities; arguably the most important quality being yield and palatability. Plant breeding deliberately influences genotypes and phenotypes of plants to create a more desirable trait or traits that can be passed to offspring (Acquaah, 2007). Selective plant breeding does this through identifying a plant with desirable characteristics and crossing it with another plant of equally desirable characteristics (Acquaah, 2007). One of the most successful and noteworthy events involving the use of selective breeding

to create improved cultivars was during a period of about 30 years known as the Green revolution (Evenson and Gollin, 2003).

Another more modern technique for plant breeding is the use of transgenesis. This method can be accomplished through the use of recombinant DNA (rDNA), in which researchers can transfer genes from one organism to another (Acquaah, 2007). The first rDNA corn hybrids were made commercially available in the U.S. in 1996 (Fernandez-Cornejo and Caswell, 2006). Though non-transgenic corn is still grown in the U.S. the majority of field corn is genetically modified (Fernandez-Cornejo et al., 2014).

Private and commercial plant breeders have begun to select and breed for specific drought tolerant characteristics in corn. Commercial seed companies have developed “drought tolerant” hybrids which may contain multiple mechanisms of tolerance to drought stress. Collectively, their technologies are referred to as ‘Optimum[®] AQUAmax[™]’ marketed by Pioneer, ‘Agrisure[®] Artesian[™]’ marketed by Syngenta and ‘Genuity[®] DroughtGard[™]’ marketed by Monsanto. Each technology is slightly different, but ‘Drought Gard’ is the only one that involves one or more genetically modified traits. Each company believes that increased yields will occur under water stressed environments, but there is contradiction surrounding how these yield gains are achieved. Also, it is not clear if there is a benefit to planting these technologies in locations that may have only sporadic dry periods during the growing season. An evaluation of commercial “drought tolerant” corn hybrids from commercial seed companies is needed in order to determine the benefit of drought stress technology in environments that may receive adequate but often poorly timed rainfall.

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Part II.

Planting Date Effect on Physiology of Novel Drought Tolerant Corn (*Zea mays L.*) Hybrids

Abstract

A field study was conducted in 2014 and 2015 at the Milan Tennessee Research and Education Center in Milan, TN to evaluate planting date (PD) effect on the physiology and yield of “drought-tolerant” (DT) hybrids from DuPont Pioneer (AQUAmax), Syngenta (Artesian) and Dekalb (DroughtGard). Each DT hybrid was compared against a comparable relative maturity (CRM) non-drought-tolerant (NDT) hybrid from the same company. Morpho-physiological measurements recorded were: Adaxial stomatal conductance (*A*), abaxial stomatal conductance (*B*), above ground biomass (ABM), plant height (PH), and leaf temperature (LT). Measurements were recorded at three growth stages: V-6, V-12, and R1. Harvest time measurements included: grain yield (GY), 500 seed weight (SW), stover weight (STW), ear weight (EW), number of rows per ear (ER), and number of kernels per row (EL).

In 2014 and 2015, the non-irrigated trials received enough rainfall to create a high yield environment. Since the DT hybrids were only stressed for short periods of time throughout the growing season, we were unable to evaluate growth under intense stress conditions, however, it was determined that hybrids with the DT technology are very competitive in high yield conditions. The hybrid with the DroughtGard technology was the only hybrid to show a significantly increased yield advantage over the CRM NDT hybrid of comparison. Harvest measurements showed higher correlations to GY with DuPont Pioneer and Syngenta, while morpho-physiological measurements more consistently had higher correlation coefficients with the Monsanto technology. *B* at the R1 growth stage showed a moderate negative correlation coefficient with DroughtGard and Artesian hybrids, suggesting that lower abaxial stomatal conductance when the plant goes reproductive correlates with higher GY. AQUAmax showed a similar trend when measured at the V-12 growth stage. Variable selection was used to predict best fit regression models. The models selected for Monsanto and DuPont Pioneer were able to

be validated for both years of data, however, only the model used for the DuPont Pioneer hybrids should be used for future predictions. The Monsanto model showed similar predictions for both years, but the prediction values were too low for reliable data. Models constructed from Syngenta's data could not be validated across both years of data suggesting that years seem to be influencing the regression relationship. Therefore, more than data is needed to find a stable predictive model that works across years.

Introduction

Corn is one of the top three crops produced in the world every year and consistently ranks first in planted area within the United States. Because of high seed production per hectare, corn consistently ranks first in total grain yield (Fageria et al., 2011). The United States accounts for roughly 32 percent of the total world corn production with 38.6 million hectares produced in 2013 (National Corn Growers Association 2013 Report). In 2012, severe widespread drought in the U.S. caused average yields to drop by more than 16 percent from the national average yield in the previous year. Average yield declined from 363.6 bushels per hectare in 2011 to 304.8 in 2012 (USDA NASS, 2013). Early in 2012 the USDA initially predicted 14.8 billion bushels of corn to be produced, but year-end reports showed only 10.8 billion actually harvested, causing a large fluctuation in grain prices.

Plant available moisture or the lack of is the most influential environmental stress that affects corn production (Cattivelli et al., 2008). The successful reproduction of a corn plant is the goal of cultivating the crop. The completion of this goal is influenced by many biotic and abiotic factors. Negative biotic influences can be controlled through the use of integrated pest management practices, such as pesticides or cultural controls (Fishel, 2009). However, the two greatest limiting abiotic factors in corn production are nitrogen availability and plant available moisture, with the most frequent limiting factor being plant available moisture (Campos et al., 2006).

Natural selection has led to water saving mechanisms being adapted by many terrestrial plant species, but breeding efforts have led plant scientists to further improve drought tolerance in agricultural crops. Modern plant breeders have begun identifying quantitative trait loci (QTL) that are associated with drought tolerance and advanced molecular genetics have allowed them to

mark and transfer desirable genes, using a transgenic approach to drought tolerance (Cattivelli et al., 2008). QTLs are polygenes or regions of the genome that are responsible for all or part of an observed phenotypic variation for a polygenic trait (Acquaah, 2007).

Specific traits that are being targeted by breeders to increase tolerance to drought are: shortened reproductive cycle to limit amount of time exposed to drought conditions, reduced leaf area to reduce area exposed to direct sun, reduced stomatal conductance to decrease transpiration rate, and deeper root system with more fibers to increase root contact with soil solution (Roth, 2013). In areas with short and or sporadic drought periods, such as the Mid-Southern U.S., past breeding efforts have focused on traits to optimize yields which may actually be detrimental in prolonged drought conditions (Roth, 2013). These traits include increased stomatal conductance with higher transpiration rates, higher leaf area, and increased “stay green” (Lopes et al., 2011).

Commercial seed companies have focused resources on reducing yield loss to drought stress by improving crop tolerance to the stress. Given corn’s highly sensitive nature to drought stress and the world’s reliance on it for food, feed and fuel, there is potentially a large market for hybrids with higher levels of drought stress tolerance. With respect to drought stress traits, both non-transgenic and transgenic hybrids are already commercially available. DuPont Pioneer and Syngenta have developed multi stress tolerant trait hybrids called Optimum[®] AQUAmax[™] and Agrisure[®] Artesian[™], respectively, using non-transgenic techniques. AQUAmax and Artesian technology were made commercially available in 2011, but in a limited number of hybrids. Monsanto, along with BASF, has developed a transgenic drought tolerant product offering called Genuity[®] DroughtGard[™] and they began a slow release of their technology across the Western Great Plains in 2013.

DuPont Pioneer focused on improving key native traits that help increase water use efficiency (WUE) in their AQUAmax technology. Fibrous root systems, vigorous ear silking, longer “stay green”, continued photosynthetic production under drought stress, and increased stomatal control to limit transpiration rates were all qualities that were selected for AQUAmax hybrids (Pioneer, 2013). AQUAmax hybrids tested in on-farm trials over 2 years yielded 8.9% and 1.9% higher in favorable and drought environments, respectively, against comparable relative maturity (CRM) conventional hybrids (Pioneer, 2013). AQUAmax hybrids appear to be more tolerant to higher populations and plant crowding. Roth et al. (2013) found that under normal growing conditions, a non-drought tolerant DuPont Pioneer 111 day relative maturity (RM) hybrid produced a higher grain yield (GY) than a AQUAmax hybrid of similar CRM at a plant density (PLD) of 79,000 plants ha⁻¹, but when PLD was increased to 109,000 plants ha⁻¹ the non-drought tolerant hybrid suffered a 100 percent greater yield decline than the drought tolerant hybrid. In a similar study conducted by Gaffney et al. (2015) AQUAmax hybrids yielded approximately 8% more total GY than CRM hybrids at higher PLD. Optimal target PLD for AQUAmax hybrids under drought stressed conditions was 49,420 to 59,304 plants ha⁻¹ (Gaffney et al., 2015). Roth et al. (2013) found that under intense drought stress, AQUAmax hybrids showed no grain yield advantage over non-drought tolerant hybrids of similar RM from the company. However, Gaffney et al. (2015) found in a three year study that AQUAmax hybrids showed a 2 to 3% GY advantage over non-drought tolerant CRM hybrids when grown under favorable conditions and a 5 to 9% GY increase when grown under drought stressed conditions.

Syngenta also used targeted corn trait selection to develop the Agrisure Artesian technology. Though limited research has been published on this technology, the seed company

promotional information claims a 17 % yield increase with Artesian hybrids grown under moderate to severe drought conditions while under favorable conditions the tested hybrids exceeded or matched the yields of the conventional hybrids (Syngenta, 2012). These gains are attributed to breeding efforts centered on improvements in germplasm characterization, genetic markers, stress phenotyping, and new gene combinations (Smieja, 2013). Becker et al. (2011) conducted field research with Artesian technology under the arid climatic conditions of the Texas High Plains and found that GY continued to increase as PD increased, when tested at 50% plant available water (PAW) in the soil profile. Highest GY was achieved at a PD of 79,000 plants ha⁻¹, but did not significantly begin to decline until a PD of 98,800 plants ha⁻¹ was reached. Artesian technology showed a negative GY response to PD above 79,000 plants ha⁻¹ when tested at the 40% PAW level in the soil profile (Becker et al., 2011). Irrigation scheduling was determined using PAW estimates gained from soil water loss equations (Piccinni et al., 2009).

Monsanto and BASF produced the first transgenic drought-tolerant hybrids. Their technology, Drought Gard, expresses a cold shock protein (CspB) derived from *Bacillus subtilis* to act as a chaperone when the plant undergoes abiotic stresses, specifically drought stress (Castiglioni et al., 2008). CspB “chaperones” corn by maintaining slowed, but otherwise normal growth during drought stressed conditions, when environmental conditions usually dictate a stop in normal metabolic functions. The cold shock protein (CSP) contains RNA-binding sequences called cold shock domains (CSDs), which gives the CSPs the chaperone effect (Horn et al., 2007). CSPs will accumulate in actively dividing meristematic tissue and inhibit cellular expansion (Nakaminami et al., 2006; Chaikam and Karlson, 2008). CSD will activate when exposed to abiotic stress, such as drought, and limit plant dry matter production (Nemali et al.,

2014). In Argentina, increased GY has been measured from a higher harvest index without an increase of plant dry matter production (Edmeades et al. 1999; Echarte et al., 2004; Tollenaar and Lee, 2006). These results were further validated by Nemali et al. (2014) when DroughtGard technology was tested against CRM non-drought tolerant hybrids and researchers found an increased GY with higher harvest index and lower plant dry matter. CspB was responsible for transiently reducing plant dry matter production, which slowed transpiration because of less leaf area exposed to environment (Nemali et al., 2014). A lower transpiration rate left higher residual soil moisture for later plant consumption and overall higher plant water use efficiency.

Previous researchers have examined different physiological effects that drought has on grain production in corn (Denmead and Shaw, 1960; Acevedo et al., 1971; Sanchez et al., 1983; NeSmith and Ritchie, 1992a; Cakir, 2004; Markelz et al., 2011) and also revealed physiological traits that improve drought tolerance (Bänziger et al., 2000; Campos et al., 2004; 2006; Blum, 2009; Lopes et al., 2011). Recently, there has been research evaluating physiological mechanisms associated with new drought-tolerant corn technology in different environments (Hao et al., 2015; Newell et al., 2015; Cooper et al., 2014; Nemali et al., 2014; Roth et al., 2013; Castiglioni et al., 2008), but no known research to evaluate all three novel technologies together in a humid region . Each drought-tolerant technology claims better WUE through different mechanisms and increased or similar GY to industry leading CRM hybrids in favorable conditions and higher GY in drought stressed environments.

This research examines physiological responses and yield of hybrids representing the three drought-tolerant technologies compared to non-drought-tolerant hybrids of CRM at multiple plant dates in irrigated and rainfed environments. Research objectives are: i) examine transpiration rates, leaf temperature, plant height and biomass accumulation at multiple growth

and reproductive stages and ii) examine and quantify plant traits [kernel number, kernel weight, and ear size] to see how they influence final GY.

Materials and Methods

An experiment was conducted over two growing seasons in 2014 and 2015 at the Milan Research and Education Center (MREC) (35.934204°, -88.729364°) in Milan, TN to assess physiological differences of commercially available drought-tolerant corn hybrids. A non-irrigated field experiment was established on a Grenada silt loam (fine-silty, mixed, active thermic Oxyaquic Fragiudalfs) in 2014 and on a Loring silt loam (fine-silty, mixed, active thermic Oxyaquic Fragiudalfs) in 2015. Both soils are considered moderately well drained and suited for corn production in Tennessee (Yin et al., 2011). Irrigated field experiments in both years were established on a Loring silt loam. Soil pH for all fields was 6.5-6.9 and the preceding crop for all sites was soybeans (*Glycine max* L.). A no-till cropping system was used for production.

A six replicate randomized complete block design was utilized for all experiments in both years. Each experiment included two planting dates and two environments: early/late and irrigated/non-irrigated. There were six hybrids planted; one drought tolerant and one non drought tolerant hybrid of CRM from each of the three companies (Table 1 and 2). In 2014, the hybrids tested were ‘Pioneer P1498YHR’, ‘Pioneer P1319’ (Pioneer Hi-Bred, Johnston, IA), ‘NK N70J 4011’, ‘NK N68-B 3111’ (Syngenta Crop Protection, Greensboro, NC), ‘Dekalb DKC 66-42’, and ‘Dekalb DKC 66-97’ (Monsanto Company, St. Louis, MO). In 2015 all hybrids were the same except Dekalb DKC 65-20’ replaced ‘Dekalb DKC 66-42’ which was not commercially available. Planting dates and physiological advancement for both years can be seen in Tables 7 and 8. All hybrids, at both plant dates and years, were planted at a depth of five cm and a

population of 79,000 seed ha⁻¹. Experimental plots consisted of four rows measuring 2.3m wide and 9m in length. Plot fertility and pest control followed University of Tennessee recommendations (Tables 3, 4, 5, and 6).

Soil moisture sensors (Spectrum Technologies, Inc., Aurora, IL) were inserted to depths of 15 and 61 cm in the non-irrigated and irrigated studies only in 2015 due to equipment/budget constraints. Sensors were placed in one location of the irrigated and non-irrigated fields near the second plant date of DuPont Pioneer P1498 Optimum AQUAmax. Due to the small field area used for testing, there was minimal topographical or soil variation.

Total above-ground plant biomass was determined at the V-6, V-12 and R-1 growth stage by destructively sampling five plants from equally spaced intervals in non-harvest rows both years. At the V-6 and R-1 stage, plants were sampled from the first non-harvest row of the plot. At the V-12 stage, samples were collected in the last non-harvest row of each plot. Each sample was labeled and dried for 72 hours at 60°C using large walk-in plant dryers. Dry weight was then measured with a high precision professional lab scale.

Uniformly colored surveying flags marked five randomly selected plants in the first harvest row of each plot that were used for non-destructive measurements. Plant height, collar count, and leaf temperature were taken at the V-6, V-12, and R-1 growth stages on the same five plants per plot at each sampling date. Similar methods were used both years.

Height was measured at the V-6 growth stage using a standard wood meter stick, and V-12 and R-1 measurements were taken using a fiberglass telescoping measuring rod. Plant heights were measured from the ground to the break in the youngest collared leaf, if no tassel was present. If the tassel was present, height measurements were taken from the ground to the top of the tassel. Growth stage was determined by counting the number of unrolled leaves

with a fully expanded collar. ULINE (Uline Corp., Pleasant Prairie, WI) fluorescent red flagging tape was loosely tied around three plants throughout each plot between the 5th and 6th leaves to more accurately count leaves in more mature corn later in the season. Leaf temperature was recorded on the youngest collared leaf using a Raytek ST Pro Plus noncontact, infrared digital thermometer (Raytek Corp., Santa Cruz, CA).

Transpiration was quantified via stomatal conductance for both years. Data were collected at V-6, V-12, and R-1 growth stages using a Decagon SC-1 Leaf Porometer (Decagon Devices, Pullman, WA). Stomatal conductance was measured on the abaxial and adaxial surfaces of two plants in the first harvest row of each plot. The youngest collared leaf was selected in vegetative plants while the ear leaf was used during the reproductive stage. Data were collected from the same two plants per plot for the duration of the test.

At R-6, five plants per plot were hand removed at evenly spaced intervals from non-harvest rows for ear and stalk measurements. The ears and stover were separated and oven dried at 60°C until a constant weight was reached. Dry ear and stalk samples were weighed on a high precision professional lab scale. Kernel number (KN) was determined by counting number of rows around (ER) and number of kernels down each ear (EL). The two center plot rows were harvested with a small plot combine and weights were corrected to 155 g kg⁻¹ standard moisture to calculate GY. A seed sample collected at harvest from each plot was used to calculate 500 seed weights both years.

Data were subjected to analysis of variance using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC). Hybrid, plant date, and environment (irrigated and non-irrigated) were treated as fixed main effects; replication and year were treated as random effects. Means were separated using Fishers Protected LSD at a significance level of 0.05. Data were

also subjected to correlation analysis and regression modeling using PROC CORR and PROC GLIMMIX procedure in SAS 9.4. Pearson correlation coefficients and P values for each comparison were calculated and reported. Akaike's Information Criterion (AIC) and Mallow's CP were used in a variable selection process to fit best model.

Results and Discussion

For this experiment, we assumed each company had distinctly different drought-tolerant trait packages in their hybrids, therefore, DT hybrids are only compared to a NDT hybrid of similar CRM within each company. All hybrid, environment, plant date, hybrid x environment, hybrid x plant date, plant date x environment, and plant date x hybrid x environment interactions for GY and its components and other morpho-physiological measurements were analyzed by company. Data were analyzed by year for regression and pooled across years for mean separation and correlation (Blouin, 2011). Correlation coefficients were considered to be weak if <0.3 , moderate if $0.3-0.7$, and strong if >0.7 .

Grain yield and other Physiological measurements

GY for all hybrids was higher in 2014 than 2015. This increase is probably due to slightly warmer spring temperatures and more timely rainfall events in 2014 (Figure 1). Figure 2 shows shallow and deep soil water levels in the non-irrigated site for the 2015 growing season. Corn was only moisture stressed at 15 cm in late July to early August and moisture stressed at 61 cm beginning at around July 20 to July 24 and from July 31 to around August 21. The first PD hybrids were near R-5 and the second PD hybrids were near R-2 to R-3 when sporadic water deficiencies were recorded later in the season.

DuPont Pioneer Optimum AQUAmax and similar CRM hybrid (Table 1 and 2).

Treatment effects of measured parameters are reported in Table 10. Environment significantly affected A during later stages, B during early stages and LT very early only. Plant height was generally not affected. There were no significant hybrid, plant date x hybrid, plant date x environment, or plant date x hybrid x environment effects on GY (Table 11) so only plant date effects will be discussed. When DT and NDT hybrids were grouped together the early plant date yielded 224.1 (bu/A) and the late plant date yielded 234.5 (bu/A) (Table 12). These differences were significantly different ($p < 0.01$). The reduction in yield from the early plant date across both years of data is thought to be associated with cooler early season temperatures and high soil moisture levels. Imholte and Carter (2002) saw similar results when planting in to cold wet soil early in the growing season. Plant date significantly affected A early, LT early and ABM during later stages (Table 10). A significant response was seen in B at all growth stage measurements based on planting date analysis, with the later measurement dates (V-12 and R-1) showing lower rates of transpiration (Table 10). Plant date significantly affected STW and EW which probably impacted grain yield (Table 10).

Syngenta Agrisure Artesian and similar CRM hybrid (Table 1 and 2).

Hybrid significantly affected plant height at later stages. Syngenta hybrids were most affected by environment of the three companies. A was significantly affected by environment very early, LT and PH at all stages and ABM at later stages (Table 10). There was no significant hybrid, plant date x hybrid, plant date x environment, or plant date x hybrid x environment effect on GY (Table 11) so plant date effects will be focused on. DT and NDT hybrids were grouped together and the early plant date yielded less than the later date. The early plant date had an average yield of 219.0 (bu/A) while the late plant date had an average yield of 236.0 (bu/A) (Table 12). These

differences were significantly different ($p < 0.05$). The yield difference across both years is again attributed to differences in soil moisture and temperature. Significant differences ($p < 0.05$) in *B* at all stages of measurement and ABM at the later dates (V-12 and R-1) were seen when analyzed by plant date (Table 10). Plant date significantly affected yield possibly from differences in STW, EW and EL (Table 10).

Monsanto Genuity DroughtGard and similar CRM hybrid (Table 1 and 2).

Hybrid significantly affected ABM mid-season and PH at later stages (Table 10). Environment effect was significant for *A* at later stages, *B* very early, LT during early stages and PH very early (Table 10). There were no significant environment, hybrid x environment, or plant date x hybrid x environment effects on GY (Table 11) so hybrid, plant date, and plant date x environment effects will be discussed. Hybrid interactions were considered to be significant when pooled across both years ($p < 0.05$). NDT hybrid had an average yield of 227.2 (bu/A) which was significant lower than DT₂, but not DT₁ (Table 13). DT₁ yielded 235.3 (bu/A) which was statistically no different from DT₂ with an average yield of 236.9 (bu/A) (Table 13).

Previous research showed similar results, with DroughtGard hybrids slightly out yielding a NDT hybrid of similar CRM (Newell et al., 2015). NDT, DT₁, and DT₂ were grouped together by plant date and showed a significant difference ($p < 0.01$), with the earlier plant date yielding less than the later. Average yield for the earlier plant date was 227.0 (bu/A), while the later date was 239.2 (bu/A) (Table 13). The plant date x environment also showed a significant response ($p < 0.05$). The later plant date under irrigated conditions yielded highest (245.0 bu/A) compared to all other planting dates and environments: early/irrigated, early/non irrigated, and late/non irrigated. Those yields were 226.1, 228.0 and 233.4 (bu/A), respectively, and are not significantly different from one another (Table 13). Significant differences were noted for *B* in

all analysis, except when compared by hybrid (Table 10). ABM means also separated in all analysis, except when compared by environment (Table 10). Plant date affected grain yield and this was likely due to differences in SW, STW, EW and EL (Table 10).

Regression and correlation of GY and morpho-physiological measurements

Pearson correlation coefficients were calculated and reported for each company (Tables 14, 15, and 16) to determine if individual plant or harvest measurements were correlated to GY. Multiple regression best fit models were picked using AIC and Mallow's CP values. Models that could not be validated across both years were run individually and different models were chosen for best prediction. Harvest and morpho-physiological values chosen for analysis were: grain yield (GY), 500 seed weight (SW), stover weight (STW), ear weight (EW), number of kernel rows per ear (ER), number of kernels per row (EL), above ground biomass at V-6, V-12, and R-1 ($ABM_{V-6, V-12, \text{ or } R-1}$), adaxial stomatal conductance at V-6, V-12, and R-1 ($A_{V-6, V-12, \text{ or } R-1}$), abaxial stomatal conductance ($B_{V-6, V-12, \text{ or } R-1}$), leaf temperature at V-6, V-12, and R-1 ($LT_{V-6, V-12, \text{ or } R-1}$), and plant height V-6, V-12, and R-1 ($PH_{V-6, V-12, \text{ or } R-1}$). Attention is given and reported to variables showing to have moderate to strong correlations (0.30 and above) with GY.

DuPont Pioneer Optimum AQUAmax and similar CRM hybrid (Table 1 and 2).

As might be expected, harvest measurements showed more consistent and higher correlation coefficients with GY than the morpho-physiological traits. SW, STW, and EW all showed moderate positive correlations at significant levels: 0.51, 0.54, and 0.59 respectively ($p < 0.05$) (Table 14). All stages of A , $B_{V-6, V-12}$, LT_{V-12} , and PH_{V-6} showed weak or no correlation and will not be discussed. $ABM_{V-6, R-1}$, B_{R-1} , LT_{V-6} , and $PH_{V-12, R-1}$ showed significant moderate correlations to GY ($p < 0.05$): 0.61, 0.65, -0.38, 0.38, 0.49, 0.57, respectively (Table 14). Roth et al. (2013) found similar results, when experimenting with DT hybrids, in that biomass, kernel

number, and grain weight was highly correlated with GY. Variable selection was used to identify regression models for predicting GY from 2014. Of the 21 variables tested, one adequate model with under 7 variables was found, containing SW, EL, $ABM_{V-6, R-1}$, A_{V-12} , and B_{R-1} . This model explained 47% of GY differences, had a Mallow's CP value < 7 , and an AIC within 2 units of the smallest AIC model. The chosen model was validated on 2015 data, and results showed predictions of GY with this model at 50%, indicating the relationships found could be used to predict new data with some confidence. When data was pooled together over both years it could be used to predict data with an accuracy of 67%, suggesting that this model could be used to predict GY in the future.

Syngenta Agrisure Artesian and similar CRM hybrid (Table 1 and 2).

Again, harvest measurements showed more moderate correlation than the morpho-physiological measurements. SW, STW, EW, and EL all resulted in moderate positive correlation coefficients: 0.63, 0.55, 0.61, and 0.44, respectively ($p < 0.05$) (Table 15). B_{R-1} showed a negative moderate correlation ($p < 0.05$), while LT_{V-6} showed a moderate positive correlation to GY ($p < 0.05$), -0.49 and 0.53, respectively (Table 15). ER, $ABM_{V-12, R-1}$, A (at all stages), $B_{V-6, V-12}$, LT_{V_{12}, R_1} , and PH (at all stages) showed a weak correlation and may not be useful for future studies (Table 15). Variable selection was again used to identify regression models to predict GY using data from 2014. Of the 21 variables tested one adequate model with under 5 variables was found, containing SW, EW, ER, and B_{V-12} . This model explained 45% of GY differences, had a Mallow's CP value < 5 , and an AIC within 2 units of the smallest AIC model. The chosen model was then used to validate 2015 data, resulting in only 9% of GY differences being explained and ultimately being disregarded. A new model using EL, $ABM_{V-6, R-1}$ was found using variable selection. This model explained 46% of GY differences in 2015, but when validated with data

from 2014 resulted in only 13% of GY differences being predicted. Therefore years seem to be influencing the regression relationship and more data will be needed to produce a stable model to predict GY across years. A proposed model for future prediction was found using variable selection on both years of data pooled together and it uses EW, ABM_{V-6} , B_{R-1} , and LT_{V-6} . This model explained 62% of GY differences, had a Mallows' CP value < 5 , and an AIC within 2 units of the smallest AIC model.

Monsanto Genuity DroughtGard and similar CRM hybrid (Table 1 and 2).

SW, STW and EW were the only harvest measurements that showed moderate positive correlation coefficients towards GY ($p < 0.05$): 0.63, 0.54, and 0.55, respectively (Table 16). There were a higher number of morpho-physiological measurements with moderate correlation coefficients when testing the Monsanto DT technology than DuPont Pioneer or Syngenta's proprietary DT technology. Morpho-physiological measurements with moderate positive correlations ($p < 0.05$) were $ABM_{V6, R1}$, LT_{V6} , and $PH_{V12, R1}$: 0.52, 0.55, 0.50 0.36, and 0.42, respectively (Table 16). Measurements with moderate negative correlations ($p < 0.05$) were B_{R-1} , PH_{V-6} , and LT_{R-1} : -0.43, -0.33, and -0.30, respectively (Table 16). EL, ER, ABM_{V-6} , A (at all stages), $B_{V-6, V-12}$, and LT_{V-12} showed a weak correlation and may not be needed in future studies (Table 16). Variable selection was again used to identify regression models to predict GY using data from 2014. Of the 21 variables tested only one adequate model with under 5 variables was found, containing SW, B_{V-6} , LT_{R-1} , and PH_{R-1} . This model explained only 37% of GY differences, had a Mallows' CP value < 5 , and AIC within 2 units of the smallest model. The chosen model was validated on 2015 data, resulting again in only 34% of GY explained. This model could consistently be used to describe some of the GY differences, but should not be used

to predict new data. More data will be needed to produce a stable model to predict GY across years.

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Part III.

**Early Season Physiological Mechanisms and Root Morphological Differences Associated
with Drought Tolerant Corn in the Greenhouse**

Abstract

A greenhouse study was conducted in 2015 at the West Tennessee Research and Education Center in Jackson, TN to evaluate effect of early season drought stress on leaf transpiration, plant growth and root morphology of “drought-tolerant” (DT) hybrids from Pioneer (AQUAmax), Syngenta (Artesian) and Dekalb (DroughtGard). Each DT hybrid was compared against a comparable relative maturity (CRM) non-drought-tolerant (NDT) hybrid from the same company. Adaxial stomatal conductance (*A*), abaxial stomatal conductance (*B*), above ground biomass (ABM), below ground biomass (BBM), total root length (RL), total root surface area (RSA), average root diameter (RD), and total root volume (RV) were measured when plants reached V6 growth stage. Leaf temperature (LT) was measured at growth stage V4, V5 and V6. Response to drought stress was variable within companies and no consistent trends were established. Early season drought stress reduced *A*, ABM and increased LT at V6 for all hybrids. Early season drought stress reduced *B* in two of three Dekalb hybrids. There were no significant AQUAmax or DroughtGard hybrid x environment interactions for plant or root measurements. The AQUAmax DT hybrid had lower *a*, reduced RSA, RD and RV compared to the NDT comparison when analyzed across environments. The DroughtGard DT hybrids performed similar to the NDT hybrid with respect to plant and root measurements when analyzed across environments. An Artesian DT hybrid had greater *A* and ABM when analyzed across environments with significantly greater root diameter than the NDT hybrid at both environments. Under an early drought stress environment, commercially designated DT hybrids were not consistently superior to NDT hybrids with respect to plant and root development.

Introduction

Corn production in the United States is a vital part of global grain production and is essential for international food security. From 2008-2010, the U.S. produced 39% of global corn, and this 39% accounted for 49% of total exports worldwide (Boyer et al., 2013). In 2012 the U.S. experienced drought conditions across 80% of agricultural land used for corn production and as a result yields were reduced by 21% (Claeys and Inzé, 2013). The dramatic yield reduction caused grain prices to increase by 53%, when compared to the previous 5 years of non-drought corn production (Boyer et al., 2013). Because corn is consistently one of the top three crops produced worldwide and is the most cultivated crop in the U.S. it plays a vital role in maintaining global food security (Danforth, 2009). Drought is expected to increase in severity and continue to impact and potentially limit corn production (Petit et al., 1999).

Breeding strategies for drought tolerance have often focused on above ground aspects of the corn plant such as: reduced leaf area, reduced stomatal conductance, reduced transpiration rates, shorter flowering stages to limit time exposed to drought stress (Bänziger et al., 2000; Campos et al., 2004; 2006; Blum, 2009; Lopes et al., 2011). Blum (2009) argued that biomass production and grain yield are directly correlated with transpiration rates therefore breeding strategies that target high water use efficiency by reducing transpiration rates will ultimately produce lower yielding varieties with less tolerance to drought stress. His answer to this problem was to focus breeding strategies on the efficient use of water via increased root area and depth. Increased root depth gives corn the ability to reach moisture buried deeper in the soil profile and increased root area gives it the ability to access more pockets of stored moisture (Hund et al., 2008). Root depths of native vegetation in arid climates are consistently deeper than those in humid regions (Schenk and Jackson, 2012).

When managing mobile inputs such as water, increased root density in upper rooting zones is less crucial for drought tolerance than root distributions in lower zones, which are more saturated with moisture (Bänziger et al., 1997). A more extensive, deep root system has a larger effect on the aboveground portions of the plant when water becomes limiting (Bänziger et al., 1997). Some drought-tolerant cultivars of sorghum have been selected for a deeper rooting system (Ludlow et al., 1990; Santamaria et al., 1990) and higher water use efficiency (Wright and Smith, 1983). When screening for drought tolerance in corn, Bolaños et al. (1993) found a decrease of 33% in root biomass in the dry upper 50 cm of soil after 8 cycles of selection.

Recently, researchers have evaluated some of the physiological mechanisms that increase stress tolerance in commercially available drought-tolerant hybrids (Hao et al., 2015; Newell et al., 2015; Cooper et al., 2014; Nemali et al., 2014; Roth et al., 2013; Castiglioni et al., 2008). DT hybrids with AQUAmax technology were found to use no more water than conventional hybrids while attaining higher grain yield and water use efficiency (Hao et al., 2015) and DroughtGard hybrids produced less leaf area when exposed to drought stress (Nemali et al., 2014). However, there has been no published research identifying key morphological differences exhibited by the root systems of drought-tolerant corn hybrids when grown under early season drought stressed conditions.

This research examines the physiological responses of commercially designated drought-tolerant hybrids and non-drought-tolerant hybrids of comparable relative maturity (CRM) at an early vegetative stage when intensely managed in a greenhouse environment under well-watered (WW) and water-stressed (WS) conditions. It also examines differences in root morphology at an early vegetative stage when grown under WW and WS conditions. Research goals are to i) identify plant traits [reduced transpiration, leaf temperature and plant biomass] associated with

early season drought tolerance, and to ii) quantify root morphology of drought-tolerant hybrids [total length, area and root length diameter] in WW and WS conditions compared against CRM hybrids.

Material and Methods

A greenhouse experiment was conducted at the West Tennessee Research and Education Center (WTREC) in Jackson, TN (35.632227°, -88.857739°) in 2015 to assess differences in root morphology and early season physiological characteristics of market ready drought-tolerant corn hybrids.

Seven corn hybrids were used in this experiment; one drought-tolerant and one non drought-tolerant entry of similar CRM from both Pioneer and Syngenta, and two drought-tolerant and one non drought-tolerant hybrid of similar CRM from Monsanto (Table 17). The hybrids selected for evaluation were ‘Pioneer P1498 Aquamax’, ‘Pioneer P1319’, ‘NK N70J 4011Artesian’, ‘NK N68-B 3111’, ‘Dekalb DKC 66-42 DroughtGard’, and ‘Dekalb DKC 65-20 DroughtGard’ and ‘Dekalb DKC 66-97’. All hybrids were grown in the greenhouse to the V-6 growth stage in PVC pipe containers (0.101 m top and bottom diameter and 0.508 m tall) lined with a 0.4 mm plastic sleeve that was sealed at the bottom and filled with calcined clay (Turface Quickdry; Profile Products LLC, Buffalo Grove, IL). The porous ceramic growth medium chosen has a high cation exchange capacity (30 meq/100g) and has been used for greenhouse corn production in previous research (Eddy and Hahn, 2010).

A four replicate split-plot design was used in this experiment with hybrid as the main plot and environment: well-watered (WW) and water stressed (WS) as the sub-plot. Experimental plots consisted of two containers and one hybrid. Each container was filled with three kg of calcined clay followed by 500 ml of tap water. Fertilizer solution consisted of 1.5 g of 20-20-20

(N-P- K) Peters general purpose fertilizer with minors (0.05 Mg, 0.0125 B, 0.0125 Cu, 0.05 Fe, 0.025 Mn, 0.005 Mo, and 0.025 Zn), 1.8 g calcium nitrate, 1.0 g of magnesium sulfate, and 0.5 g of potassium nitrate mixed with 1,500 ml of tap water. Solution was then added to growing medium and allowed to diffuse throughout container. The amount of tap water added before fertilizer solution was added was adjusted so that a total of 100% and 80% of field capacity was obtained at planting for WW and WS, respectively. One seed per container was sown at a depth of five cm and thirty ml of tap water was added to close the furrow and firm seed bed after planting. Planting date for all hybrids was June 12, 2015.

WW treatments were maintained at 100% field capacity throughout the course of the experiment (Table 18). Drought stress was imposed by not watering WS treatments any more after planting and allowing containers to dry out over the next three weeks (Table 19). Weights were recorded at planting for two random containers in each replication which were then used to calculate declining percent soil water content in WS containers (Table 19) and estimate water amounts needed to maintain WW containers at 100% field capacity (Table 18). WW containers were watered every three days to maintain close to 100% field capacity (Table 18). WS containers reached moderate drought stress (approximately 50% soil water content) by about 8 days after planting (Table 19) when corn was at V-2 stage.

Greenhouse environment was controlled with VersiSTEP Integrated Control System (Wadsworth Control Systems Inc, Arvada, CO). Day and night air temperatures were maintained between 26-30° C and the relative humidity was 70%. No supplemental lighting was used to alter day and night cycles

Vegetative growth stage was determined by counting the number of unrolled leaves with a fully expanded collar, similar to Hanway (1963). Leaf temperature was recorded at V-4, V-5,

and V-6 growth stage by measuring the youngest fully-expanded leaf with a Raytek ST ProPlus noncontact, infrared digital thermometer (Raytek Corp., Santa Cruz, CA).

Transpiration was measured via stomatal conductance. Data were collected prior to plant harvest on the sixth fully-expanded leaf using a Decagon SC-1 Leaf Porometer (Decagon Devices, Pullman, WA). Stomatal conductance was measured on the abaxial and adaxial surfaces of each plant. Above ground plant material was collected at the six leaf stage, dried in an oven at 60° C until a constant weight was reached and then weighed. At this time, plastic sleeves were removed from containers and split so that all below ground root material could be collected for analysis. To separate root structures from substrate, samples were washed gently over a 50 mm sieve with tap water.

Root samples from each treatment were separated into 17 cm segments and suspended in 2 cm of water in transparent trays. Root samples were then imaged with a flatbed scanner equipped with a top light (Epson Perfection V700 Photo, Epson America, Inc., USA). Root length, surface area, diameter, and volume were calculated using the image processing software WinRhizo Pro 2013a (Regent Instruments, Québec, Canada). After root image analysis was completed, below ground plant matter was oven dried at 60° C until a constant weight was reached and weights were recorded.

Data were analyzed using the PROC GLIMMIX procedure of SAS 9.4 (SAS Institute; Cary, NC). Hybrid and environment were considered to be fixed effects and replication was considered to be a random effect. Means were separated using Fisher's Protected LSD procedure at the 0.05 level of significance.

Results and Discussion

For this experiment, we assumed each company had distinctly different drought-tolerant trait packages in their hybrids, therefore, DT hybrids are compared to a NDT CRM within each company. All hybrid, environment, and hybrid x environment interactions for plant traits and root measurements were analyzed by company.

Plant traits associated with drought stress

Physiological traits selected for statistical analysis were: adaxial stomatal conductance (*A*), abaxial stomatal conductance (*B*), above ground biomass (ABM), below ground biomass (BBM), and leaf temperature (LT). LT was recorded at V-4, V-5, and V-6 growth stage, while all other measurements were taken at the six leaf stage. For each company, environment significantly reduced ABM (Table 20), indicating we were successful in creating early season moisture stress.

DuPont Pioneer Optimum AQUAmax and a similar CRM hybrid (Table 17).

There was no significant hybrid x environment effect on *A*, *B*, ABM, BBM or LT for DT or NDT Pioneer hybrids under WW or WS conditions (Table 21) so only main effects are discussed. WS treatments had significantly lower abaxial and adaxial stomatal conductance (63.3 and 33.9 less mmol H₂O m⁻²s⁻¹ respectively), reduced above ground plant size and increased V-6 LT for both hybrids. However, both the Pioneer DT and NDT hybrids responded similarly to this early season drought stress. *A* was lower for the AQUAmax hybrid compared against the CRM hybrid: 37.8 versus 62.3 mmol H₂O m⁻² s⁻¹, respectively (Table 21). This trend continued in both WW or WS environments, 81.7 and 18.4 mmol H₂O m⁻² s⁻¹, respectively.

Lower gas exchange rates have been associated with reduced water potential in previous studies (Lawlor and Fock, 1978; Ackerson 1983; Schussler and Westgate, 1991; Zinselmeier et al., 1995; Kang et al., 2000; Cruz de Carvalho et al., 2011; Markelz et al., 2011; Roth et al., 2013).

This may indicate the AQUAmax hybrid loses less water through its leaves in different soil moisture environments, but this did not result in larger plants or roots compared to the NDT hybrid in an early stress environment.

Syngenta Agrisure Artesian and similar CRM hybrid (Table 17).

There was a significant ($P < 0.05$) hybrid x environment interaction for A and ABM in a well-watered environment but no significant hybrid x environment A, ABM, BBM and LT effects under drought stress (Table 22). With adequate water, the DT (drought-tolerant) hybrid showed a higher stomatal conductance rate than the NDT (non-drought-tolerant) hybrid, 42.8 and 29.6 mmol H₂O m⁻² s⁻¹, respectively (Table 22), which would suggest higher transpiration rates.

Additionally, the DT hybrid showed a higher accumulation of above ground biomass (ABM), 6.4 g (Table 22) under well-watered conditions but ABM accumulation similar to the NDT hybrid under drought stress. Water stress reduced A, ABM and increased LT at V-4 and V-6 but effects were not significantly different for hybrids (Table 22). Dry matter accumulation has been shown to drive nutrient uptake and nutrient assimilation to the kernel (Raymond et al., 2009; Setiyono et al., 2010; Ning et al., 2012; Ciampitti et al., 2013). Higher A for the DT hybrid with adequate water probably is the result of more rapid plant growth and explains the increased ABM.

However, we were not able to detect physiological differences in the DT hybrid that would indicate there was an advantage during early season moisture stress over the NDT hybrid.

Monsanto Genuity DroughtGard and similar CRM hybrid (Table 17).

There was no significant hybrid x environment interaction for any measurement and no significant differences in data collected for hybrids across moisture environments (Table 23).

Significant responses were apparent when the Monsanto hybrids were combined and tested by environment (WW and WS (Table 23)). Environment decreased A and ABM, and increased LT

at V-4 and V-6. Abaxial conductance was lower in WS than WW environment, 23.5 and 70.3 mmol H₂O m⁻² s⁻¹, respectively. ABM also decreased significantly in the WS environment when compared to the WW conditions, 3.8 g to 9.4 g (Table 23). LT significantly increased in the WS environments when measured at both V-4 and V-6, but not at V-5 and this was similar for both DT and NDT hybrids. Water stress has been shown to adversely affect the physiological functions of DT hybrids, but on a smaller scale than NDT hybrids (Castiglioni et al., 2008; Roth et al., 2013; Nemali et al., 2014). Cold shock proteins (CSPs) found in DroughtGard hybrids are responsible for transiently reducing biomass so that transpiration can be reduced; lower transpiration rates conserves plant available moisture in the soil for later consumption (Nemali et al., 2014). Our work shows with WS a trend towards lower A and B with slightly greater ABM in DT hybrids, but results were not significantly different.

Root morphology

Measurements selected for statistical analysis were: root length (RL), total surface area (RSA), average root diameter (RD), and total root volume (RV). All measurements were recorded at the six leaf stage. There were no hybrid x environment interactions for RL, RSA, RD or RV for Pioneer, Syngenta or Monsanto hybrids (Table 24) therefore only main effects will be discussed.

Pioneer Optimum AQUAmax and similar CRM hybrid (Table 17).

Water stress significantly decreased RL, RSA and RV across hybrids. Across environments the DT hybrid showed significantly lower RSA, RD, and RV than the NDT hybrid. RSA for the DT hybrid was 655 cm² versus 825.2 cm² for the NDT hybrid (Table 25). RD was averaged over the entire root structure and found to be 0.61 mm for the NDT hybrid and 0.56 for the DT hybrid. The DT hybrid showed a 24% reduction in RV when compared to the NDT hybrid (Table 25). Eghball and Maranville (1993) showed similar results for greater root length and overall root

mass under WW greenhouse conditions. The DT hybrid produced less surface root mass under water stress and did not appear to have a root morphology advantage over the NDT hybrid.

Syngenta Agrisure Artesian and similar CRM hybrid (Table 17).

Environment significantly reduced RL, RSA and RV across both hybrids ($P < 0.05$). The DT hybrid produced greater ($P < 0.01$) RD under both WS and WW environments. The NDT hybrid measured RD of 0.52 mm and the DT hybrid was 0.60 mm (Table 26). Hybrids grown under WW conditions had consistently larger root systems than hybrids produced under WS conditions. RL, RSA, and RV measurements were 5391.1 and 4217.6 cm, 920.8 and 703.9 cm⁻², 12.8 and 9.6 cm⁻³, respective to WW and WS conditions. Again, these results re-enforce the findings that Eghball and Maranville (1993) concluded. The only root morphology advantage the DT hybrid may have is its ability to produce a consistent root diameter under different soil moisture environments.

Monsanto Genuity DroughtGard and similar CRM hybrid (Table 17).

The Monsanto hybrids tested showed no significant hybrid effects or hybrid x environment interaction ($P > 0.05$), but did show significant differences in RL, RSA, and RV when hybrids were grouped together and analyzed by environment. Hybrids grown under WW conditions consistently had larger root systems than hybrids produced under WS conditions. RL, RSA, and RV measurements were 6687.9 and 4265.4 cm, 1155.3 and 736.6 cm⁻², 16.1 and 10.3 cm⁻³, respective to WW and WS conditions (Table 27). Both DroughtGard hybrids trended towards longer RL and greater RSA and RV under water stress, however results were not significantly different from the NDT hybrid.

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Part IV.
Conclusions

The overall objective of this research was to evaluate novel drought-tolerant (DT) corn hybrids for a humid region that receives adequate rainfall in “normal” corn growing seasons. The first part of this research evaluated planting date effects on the physiological mechanisms that help the hybrid to show better tolerance to drought. This part of the research was conducted under field conditions. The second part of this research evaluated early season physiological mechanisms and root morphological differences associated with DT hybrids in a greenhouse setting.

Part II.

Grain yield and other harvest measurement means

Based on the analyses of DuPont Pioneer, Syngenta or Monsanto hybrids, planting date consistently influenced GY but not in a way we would expect. In both years the late plant date had higher yields than the early plant date. This response is likely due to environmental differences and water availability. 2014 was a more advantageous year for corn production in Tennessee than in 2015 and is recognized in GY differences. Even though 2015 received adequate rainfall to produce above average corn yields, it was not as abundant or timely as the precipitation received during the 2014 growing season. The DroughtGard hybrids were the only DT technology that showed hybrid differences. The DT₁ hybrid (Table 1 and 2) showed a significantly higher yield than the NDT hybrid of CRM that it was compared against. More data is needed with prolonged periods of drought during the growing season to better understand if these DT technologies tested will be beneficial for producers in the Mid-South or in production areas that usually receive enough precipitation to achieve economically profitable corn yields. Syngenta claims in promotional information that their technology can substantially increase yields during periods of drought and exceed or match yields of conventional hybrids under

favorable conditions. This research proves that statement – we had essentially no stress in the first year and only limited stress in the second and the DT hybrids performed relatively the same as their conventional counterparts. ANOVA values for all other measurements are represented in Table 10. Of all 22 variables recorded and analyzed no consistent trend could be established.

Regression and correlation of GY and morpho-physiological measurements

Hybrids from DuPont Pioneer and Syngenta showed more harvest measurements such as SW, STW, EW, ER, and EL to be positively correlated with GY than morpho-physiological measurements, which should be expected. Monsanto hybrids showed the inverse with more morpho-physiological measurements being moderately correlated than harvest time measurements. However, all hybrids showed SW, STW, and EW to be moderately positively correlated to GY. *B* showed a more consistent negative correlation than any other variable analyzed. Previous research has shown and is readily accepted that a reduction in transpiration through stomatal conductance will reduce water usage by the plant. It is the consensus view of the companies that produce the DT technology that this process will conserve soil moisture for later consumption by the plant and ultimately translate in to increased yields and decreased drought stress. More data is needed for Syngenta and Monsanto to confidently predict GY through multiple regression models. However, the model chosen to predict GY for DuPont Pioneer hybrids was validated across both growing years and could be used for future prediction.

The complex nature of drought stress is a possible explanation of why the two studies had different results. The timing, intensity and duration of drought can greatly influence GY in corn production and though both studies were replicated over multiple years, environmental factors were different in each. Maximum and minimum daily temperature along with precipitation amounts can be seen in Figure 1. During both years of the study the research site received

adequate rainfall to produce a “high yield” environment. Soil moisture levels throughout the 2015 growing season can be seen represented at two depths of 15 and 61 cm (Figure 2). Upper limits of table show field capacity for native soil and lower limit represents drought stress for crop. During the 2015 growing season all hybrids at both plant dates were exposed to drought for a short amount of time late in the season.

Part III.

Plant traits associated with drought stress

Based on the analyses of DuPont Pioneer, Syngenta or Monsanto hybrids, water stress consistently lowered A and ABM and increased LT at V-6. This response is likely due to reduced plant available water in the soil profile. This response has been noted in previous research and it is readily accepted that stomatal conductance will decrease when corn is exposed to drought conditions. The AQUAmax hybrid was the only DT technology that showed a significantly lower adaxial transpiration rate than the NDT hybrid of CRM that it was compared against. Artesian DT had higher A and ABM when water was abundant but similar responses to drought as the NDT. DroughtGard hybrids responded similar to the NDT with respect to plant growth under drought stress. LT, BBM, and B were not consistent variables for predicting drought stress tolerance. Significant responses to these quantifiable traits were not repeatedly seen in the statistical analysis.

Root morphology

All hybrids showed significant differences in RL, RSA, and RV when analyzed by environment. WW conditions regularly had larger measurements for all three of these traits. Hybrids with the AQUAmax technology showed no differences in performance when subjected to drought conditions than the CRM hybrid. Hybrids that had the Artesian technology showed a higher root diameter than the CRM hybrid it was compared against, but showed no advantage in any other

recorded responses. Hybrids with the transgenic DroughtGard trait showed no advantage over the CRM hybrid of comparison.

Drought is highly complex in nature and can be affected by a multitude of variables such as: day length, relative humidity, day and night temperature, and timing relative to corn development. With these environmental factors controlled by manipulating greenhouse parameters, this research was able to accurately test each DT technology and evaluate its performance. Future research should focus on evaluating this novel technology under sporadic periods of drought at different and more critical stages of corn development, such as ear development and reproductive stages.

Appendix
Tables & Figures

Table 1. Experiment hybrids and corresponding technology for field evaluation (2014).

Hybrid	Description	Relative Maturity
Pioneer P1498YHR Optimum AQUAmax ^a	Drought-Tolerant	114 days
Pioneer P1319 ^a	Non-Drought-Tolerant	113 days
NK N70J 4011 Agrisure Artesian ^b	Drought-Tolerant	112 days
NK N68-B 3111 ^b	Non-Drought-Tolerant	111 days
Dekalb DKC 66-42 DroughtGard ^c	Drought-Tolerant	116 days
Dekalb DKC 66-97 ^c	Non-Drought-Tolerant	116 days

^a Pioneer Hi-Bred, Box 1000, Johnston, IA 50131.

^b Syngenta Crop Protection, Inc. Box 183000, Greensboro, NC 27419.

^c Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

Table 2. Experiment hybrids and corresponding technology for field evaluation (2015).

Hybrid	Description	Relative Maturity
Pioneer P1498YHR Optimum AQUAmax ^a	Drought-Tolerant	114 days
Pioneer P1319 ^a	Non-Drought-Tolerant	113 days
NK N70J 4011 Agrisure Artesian ^b	Drought-Tolerant	112 days
NK N68-B 3111 ^b	Non-Drought-Tolerant	111 days
Dekalb DKC 65-20 DroughtGard ^c	Drought-Tolerant	115 days
Dekalb DKC 66-97 ^c	Non-Drought-Tolerant	116 days

^a Pioneer Hi-Bred, Box 1000, Johnston, IA 50131.

^b Syngenta Crop Protection, Inc. Box 183000, Greensboro, NC 27419.

^c Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

Table 3. Pesticide applications for 2014 early plant date.

Date	Formulation	Product Name	Rate	Crop Timing
03/24/2014	Glyphosate	Roundup Power Max ^{®b}	2240 g ha ⁻¹	Burndown
03/24/2014	Dicamba	Sterling Blue ^{®g}	840 g ha ⁻¹	Burndown
03/24/2014	Flumioxazin	Valor ^{® SXh}	104 g ha ⁻¹	Burndown
04/17/2014	Atrazine	Atrazine 4L ⁱ	1540 g ha ⁻¹	Pre ^a
04/17/2014	Glyphosate	Roundup Power Max ^{®b}	1540 g ha ⁻¹	Pre ^a
04/17/2014	Dimethylcyclopropane carboxylate	Mustang Max ^j	140 g ha ⁻¹	Post ^a
05/12/2014	Glyphosate	Roundup Power Max ^{®b}	1540 g ha ⁻¹	Post ^a
05/12/2014	Atrazine	Atrazine 4L ⁱ	3360 g ha ⁻¹	Post ^a
05/27/2014	Mesotrione	Callisto ^{®e}	210 g ha ⁻¹	Post ^a
05/27/2014	S-Metolachlor	DuPont [™] Cinch ^{®f}	1470 g ha ⁻¹	Post ^a
05/27/2014	Glyphosate	Roundup Power Max ^{®b}	1540 g ha ⁻¹	Post ^a
07/03/2014	Azoxystrobin plus Propiconazole	Quilt Xcel ^g	735 g ha ⁻¹	Post ^a
07/03/2014	Dimethylcyclopropane carboxylate	Mustang Max ^j	210 g ha ⁻¹	Post ^a

^a Abbreviations: Post, postemergence; Pre, preemergence

^b Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

^c Bayer CropScience LP, Box 12014, Research Triangle Park, NC 27709.

^d BASF Corporation, Box 13528, Research Triangle Park, NC 27709.

^e Syngenta Crop Protection, Inc. Box 183000, Greensboro, NC 27419.

^f DuPont de Nemours and Company, 1007 Market Street, Wilmington, DE 19898.

^g Winfield Solutions LLC. Subsidiary Land O' Lakes, Inc. 4001 Lexington Ave, Arden Hills, MN 55126.

^h Valent U.S.A. Corporation. Box 8025, Walnut Creek, CA 94596.

ⁱ Loveland Products, Inc. Box 1286, Greeley, CO 80632.

^j FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

Table 4. Pesticide applications for 2014 late plant date.

Date	Formulation	Product Name	Rate	Crop Timing
03/24/2014	Glyphosate	Roundup Power Max® ^b	2240 g ha ⁻¹	Burndown
03/24/2014	Dicamba	Sterling Blue® ^c	840 g ha ⁻¹	Burndown
03/24/2014	Flumioxazin	Valor® SX ^d	104 g ha ⁻¹	Burndown
05/07/2014	S-metolachlor plus Atrazine	DuPont™ Cinch® ATZ Lite ^e	2590 g ha ⁻¹	Pre ^a
05/07/2014	Atrazine	Atrazine 4L ^f	490 g ha ⁻¹	Pre ^a
06/02/2014	Atrazine	Atrazine 4L ^f	3360 g ha ⁻¹	Post ^a
07/03/2014	Azoxystrobin plus Propiconazole	Quilt Xcel ^g	735 g ha ⁻¹	Post ^a
07/03/2014	Dimethylcyclopropane carboxylate	Mustang Max ^h	210 g ha ⁻¹	Post ^a

^a Abbreviations: Post, postemergence; Pre, preemergence

^b Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

^c Winfield Solutions LLC. Subsidiary Land O' Lakes, Inc. 4001 Lexington Ave, Arden Hills, MN 55126.

^d Valent U.S.A. Corporation. Box 8025, Walnut Creek, CA 94596.

^e DuPont de Nemours and Company, 1007 Market Street, Wilmington, DE 19898.

^f Loveland Products, Inc. Box 1286, Greeley, CO 80632.

^g Syngenta Crop Protection, Inc. Box 183000, Greensboro, NC 27419.

^h FMC Corporation, 1735 Market Street, Philadelphia, PA 19103.

Table 5. Pesticide applications for 2015 early plant date.

Date	Formulation	Product Name	Rate	Crop Timing
03/27/2015	Glyphosate	Roundup Power Max® ^b	2240 g ha ⁻¹	Burndown
03/27/2015	Dicamba	Sterling Blue® ^f	840 g ha ⁻¹	Burndown
03/27/2015	Flumioxazin	Valor® SX ^d	104 g ha ⁻¹	Burndown
04/11/2015	Paraquat	Gramoxone® SL 2.0 ^c	700 g ha ⁻¹	Burndown
04/11/2015	Atrazine	Atrazine 4L ^e	1540 g ha ⁻¹	Pre ^a
04/11/2015	Glyphosate	Roundup Power Max® ^b	1540 g ha ⁻¹	Pre ^a
04/11/2015	S-Metolachlor	Dual® II Magnum® ^c	1070 g ha ⁻¹	Pre ^a
04/11/2015	Lambda-cyhalothrin	Warrior® II ^c	27 g ha ⁻¹	Pre ^a
05/12/2015	Glyphosate	Roundup Power Max® ^b	1540 g ha ⁻¹	Post ^a
05/12/2015	Atrazine	Atrazine 4L ^e	3360 g ha ⁻¹	Post ^a
05/22/2015	Glyphosate, S-Metolachlor, Mesotrione	Halex GT® ^c	1170 g ha ⁻¹ 1170 g ha ⁻¹ 117 g ha ⁻¹	Post ^a

^a Abbreviations: Post, postemergence; Pre, preemergence

^b Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

^c Syngenta Crop Protection, Inc. Box 183000, Greensboro, NC 27419.

^d Valent U.S.A. Corporation. Box 8025, Walnut Creek, CA 94596.

^e Loveland Products, Inc. Box 1286, Greeley, CO 80632.

^f Winfield Solutions LLC. Subsidiary Land O' Lakes, Inc. 4001 Lexington Ave, Arden Hills, MN 55126.

Table 6. Pesticide applications for 2015 late plant date.

Date	Formulation	Product Name	Rate	Crop Timing
03/27/2015	Glyphosate	Roundup Power Max® ^b	2240 g ha ⁻¹	Burndown
03/27/2015	Dicamba	Sterling Blue® ^f	840 g ha ⁻¹	Burndown
03/27/2015	Flumioxazin	Valor® SX ^d	104 g ha ⁻¹	Burndown
5/14/2015	Paraquat	Gramoxone® SL 2.0 ^c	700 g ha ⁻¹	Burndown
05/14/2015	S-metolachlor	Dual® II Magnum® ^c	1070 g ha ⁻¹	Pre ^a
05/14/2015	Atrazine	Atrazine 4L ^e	490 g ha ⁻¹	Pre ^a
05/14/2015	Dimethylcyclopropane carboxylate	Mustang Max ^d	210 g ha ⁻¹	Pre ^a
06/04/2015	Atrazine	Atrazine 4L ^e	3360 g ha ⁻¹	Post ^a
06/04/2015	Glyphosate	Roundup Power Max® ^b	1540 g ha ⁻¹	Post ^a
06/12/2015	Glyphosate, S-Metolachlor, Mesotrione	Halex GT® ^c	1170 g ha ⁻¹ 1170 g ha ⁻¹ 117 g ha ⁻¹	Post ^a

^a Abbreviations: Post, postemergence; Pre, preemergence

^b Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

^c Syngenta Crop Protection, Inc. Box 183000, Greensboro, NC 27419.

^d Valent U.S.A. Corporation. Box 8025, Walnut Creek, CA 94596.

^e Loveland Products, Inc. Box 1286, Greeley, CO 80632.

^f Winfield Solutions LLC. Subsidiary Land O' Lakes, Inc. 4001 Lexington Ave, Arden Hills, MN 55126.

Table 7. Crop progression for field evaluation (2014).

Phenological data	2014 Early Plant Date Non-Irrigated	2014 Early Plant Date Irrigated	2014 Late Plant Date Non-Irrigated	2014 Late Plant Date Irrigated
Plant date	4/11/2014	4/11/2014	5/6/2014	5/6/2014
V-6 stage	5/23/2014	5/23/2014	6/4/2014	6/4/2014
V-12 Stage	6/17/2014	6/17/2014	6/23/2014	6/23/2014
Silking Date (R-1)	6/30/2014	6/30/2014	7/7/2014	7/7/2014
Physiological maturity (R-6)	8/15/2014	8/15/2014	8/27/2014	8/27/2014
Harvest	9/22/2014	9/22/2014	9/23/2014	9/23/2014

For each plant date values are averaged across hybrids.

Table 8. Crop progression for field evaluation (2015).

Phenological data	2015 Early Plant Date Non-Irrigated	2015 Early Plant Date Irrigated	2015 Late Plant Date Non-Irrigated	2015 Late Plant Date Irrigated
Plant date	4/12/2015	4/12/2015	5/13/2015	5/13/2015
V-6 stage	5/22/2015	5/22/2015	6/10/2015	6/10/2015
V-12 Stage	6/15/2015	6/15/2015	6/30/2015	6/30/2015
Silking Date (R-1)	6/25/2015	6/25/2015	7/16/2015	7/16/2015
Physiological maturity (R-6)	8/7/2015	8/7/2015	8/24/2015	8/24/2015
Harvest	9/8/2015	9/8/2015	9/21/2015	9/21/2015

For each planting date values are averaged across hybrids.

Table 9. 2014/2015 Rainfall (Non-irrigated) & Rainfall + Irrigation (Irrigated) from planting to maturity for field evaluation.

Weeks after planting	Early Plant Date Non-Irrigated		Early Plant Date Irrigated		Late Plant Date Non-Irrigated		Late Plant Date Irrigated	
	2014	2015	2014	2015	2014	2015	2014	2015
0	5.4	4.7	5.4	4.7	1.0	16.6	1.0	16.6
1	0	2.8	0	2.8	5.4	4.2	5.4	4.2
2	5.5	0	5.5	0	0	5.8	0	5.8
3	0	0.7	0	1.7	1.5	1.3	1.5	1.3
4	5.7	14.2	5.7	14.2	15.7	0	15.7	2.1
5	0.7	3.5	0.7	3.5	3.2	6.0	3.2	7.0
6	0.1	8.2	0.1	8.2	0.8	7.7	2.2	7.7
7	4.1	0.6	4.1	0.6	3.0	4.3	4.6	4.3
8	15.9	1.3	15.9	1.3	0.4	0.6	2.5	2.7
9	0.3	5.7	1.4	8.9	0.8	0.6	2.9	3.4
10	0.3	7.8	3.1	7.8	4.0	3.0	6.1	5.1
11	3.2	4.4	3.9	4.4	0	0	2.1	1.0
12	0.8	0.7	4.9	1.7	0	2.3	3.1	2.8
13	3.9	0.6	2.2	3.4	6.2	0	6.2	1.0
14	0.2	3.0	2.1	5.1	2.2	3.5	2.2	3.5
15	0.1	0	2.1	1.0	2.6	6.3	2.6	6.3
16	6.2	1.5	6.2	3.0	2.1	0	2.1	0
17	3.1	0.8	3.1	1.9	---	2.8	---	2.8
18	1.7	3.4	1.7	3.4	---	0	---	0

Rainfall and Irrigation totals for later plant date were not recorded for the final two weeks of the 2014 growing season. All values represented in cm per week.

Table 10. ANOVA for harvest and physiological measurements.

DuPont Pioneer												
Variable	A V-6/V-12/R-1	B V-6/V-12/R-1	LT V-6/V-12/R-1	ABM V-6/V-12/R-1	PH V-6/V12/R-1	GY	SW	GW	STW	EW	ER	EL
Hyb	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/*	ns	**	ns	ns	ns	**	ns
Environ	ns/**/**	***/**/ns	***//ns/ns	ns/ns/ns	ns/ns/ns	ns	ns	ns	ns	ns	ns	ns
Hyb*Environ	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns	ns	ns	ns	ns	ns	ns
PD	**/**/ns	*/**/**	***//***//ns	ns//***//***	ns/ns/ns	**	ns	ns	***	***	ns	ns
PD*Hyb	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns	ns	ns	ns	ns	ns	**
PD*Environ	ns/ns/ns	ns/ns/ns	ns//***//ns	ns/ns/ns	ns/ns/ns	ns	ns	ns	ns	ns	ns	ns
PD*Hyb*Environ	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/*	ns	*	ns	ns	ns	**	ns
Syngenta												
Hyb	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns//**/**	ns	*	ns	ns	ns	***	ns
Environ	**/ns/ns	***///**	**//*/	ns//*/	*/**/*	ns	**	*	ns	***	ns	ns
Hyb*Environ	**/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns	ns	ns	ns	ns	ns	**
PD	ns/ns/**	ns//*/	***//***//**	ns//***//***	ns/ns/*	***	ns	ns	***	***	ns	**
PD*Hyb	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/*	ns	*	ns	ns	ns	ns	*
PD*Environ	ns/ns/ns	ns/ns/ns	ns//**//ns	ns//*/ns	*/**//ns	ns	ns	ns	ns	*	ns	ns
PD*Hyb*Environ	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns	ns	ns	ns	ns	ns	ns
Monsanto												
Hyb	ns/ns/ns	ns/ns/ns	ns/ns/ns	ns//**//ns	ns//*/	*	ns	ns	ns	ns	ns	ns
Environ	ns//*/	***//ns/ns	**//*/ns	ns/ns/ns	**//ns/ns	ns	***	ns	ns	***	ns	ns
Hyb*Environ	ns/ns/ns	ns//**//ns	ns//**//ns	ns//**//ns	ns/ns/ns	ns	ns	ns	ns	ns	ns	ns
PD	ns/ns/ns	ns//*/	**//***//ns	ns//***//***	ns/ns/ns	**	***	ns	***	***	ns	**
PD*Hyb	ns/ns/*	ns/ns/**	***//ns/*	***//**//ns	***//ns/ns	ns	ns	ns	***	ns	ns	*
PD*Environ	ns/ns/ns	ns/ns/*	ns//***//ns	**//***//**	ns//***//ns	ns	ns	ns	ns	**	ns	ns
PD*Hyb*Environ	ns/ns/**	ns//**//ns	ns//*/ns	ns//**//ns	ns/ns/*	ns	ns	ns	ns	ns	ns	ns

Hyb = hybrid (NDT and DT), NDT = non-drought-tolerant, DT = drought-tolerant; Environ = environment (irrigated and non-irrigated); PD = planting date (early & late); ns = not significant, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Data pooled across 2014 & 2015. Subscript numbers and letters indicate plant growth stage at the time of measurement. adaxial stomatal conductance (A), abaxial stomatal conductance (B), leaf temperature (LT), above ground biomass (ABM), plant height (PH), grain yield (GY), 500 seed weight (SW), grain weight (GW), stover weight (STW), ear weight (EW), rows per ear (ER), and kernels per row (EL).

Table 11. ANOVA for grain yield.

Fixed effects	Monsanto	DuPont Pioneer	Syngenta
Hyb	*	ns	ns
Environ	ns	ns	ns
Hyb*Environ	ns	ns	ns
PD	**	**	*
PD*Hybrid	ns	ns	ns
PD*Environ	*	ns	ns
PD*Hybrid*Environ	ns	ns	ns

Hyb = hybrid (NDT and DT), NDT = non-drought-tolerant, DT = drought-tolerant; Environ = environment (irrigated and non-irrigated); PD = planting date (early & late); ns = not significant, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Data pooled across 2014 & 2015.

Table 12. Grain yield (bu/a) for DuPont Pioneer and Syngenta.

Fixed Effects		Pioneer	Syngenta
Hyb.		ns	ns
PD	early	224.1 B	219.0 B
	late	234.5 A	236.0 A
PD*Hyb		ns	ns
PD*Environ		ns	ns
PD*Hyb*Environ		ns	ns

Hyb = hybrid (NDT and DT), NDT = non-drought-tolerant, DT = drought-tolerant; Environ = environment (irrigated and non-irrigated); PD = planting date (early & late); ns = not significant Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Data pooled across 2014 & 2015.

Table 13. Grain yield (bu/a) for Monsanto.

Fixed Effects			Monsanto
Hyb		NDT	227.2 B
		DT ₁	235.3 AB
		DT ₂	236.9 A
PD		early	227.0 B
		late	239.2 A
PD * Hyb			ns
PD * Environ	early	irrigated	226.1 B
	early	non irrigated	228.0 B
	late	irrigated	245.0 A
	late	non irrigated	233.4 B
PD * Hyb * Environ			ns

Hyb = hybrid (NDT and DT), NDT = non-drought-tolerant, DT = drought-tolerant; Environ = environment (irrigated and non-irrigated); PD = planting date (early & late); ns = not significant Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

Monsanto: DT¹ = Dekalb DKC 65-20, DT² = Dekalb DKC 66-42. Data pooled across 2014 & 2015.

Table 14. DuPont Pioneer Pearson correlation coefficients for morpho-physiological parameters in 2014 & 2015.

	GY	SW	STW	EW	EL	ABM V-6	ABM V-12	ABM R-1	A _{V-12}	A _{R-1}	B _{V-6}	B _{V-12}	B _{R-1}	LT V-6	PH V-12	LT R-1	PH R-1
GY	1.00	0.51	0.54	0.59	0.29	0.61	0.26	0.65	-0.08	-0.10	0.03	0.03	-0.38	0.38	0.49	-0.06	0.57
SW		1.00	0.22	0.28	0.01	0.20	0.30	0.40	-0.08	0.07	0.09	0.02	-0.28	0.43	0.25	-0.04	0.48
STW			1.00	0.80	0.23	0.66	0.11	0.64	0.01	-0.30	-0.04	0.07	-0.25	0.32	0.33	-0.04	0.37
EW				1.00	0.36	0.57	0.05	0.57	0.09	-0.26	0.05	0.17	-0.22	0.30	0.39	-0.13	0.43
EL					1.00	0.04	0.00	0.23	0.15	0.00	0.03	0.12	-0.12	0.23	0.22	-0.06	0.30
ABM V6						1.00	0.41	0.54	0.13	-0.20	0.15	0.24	-0.12	0.09	0.55	0.05	0.45
ABM V12							1.00	0.24	0.10	0.00	0.12	0.14	-0.13	0.14	0.37	0.32	0.43
ABM R1								1.00	0.02	0.04	-0.01	0.00	-0.37	0.49	0.41	-0.06	0.51
A _{V12}									1.00	0.18	0.32	0.72	0.22	0.12	0.01	-0.05	0.06
A _{R1}										1.00	0.36	0.04	0.10	0.43	-0.15	0.02	-0.09
B _{V6}											1.00	0.41	0.18	0.14	0.01	0.01	-0.08
B _{V12}												1.00	0.19	0.07	0.07	-0.08	0.15
B _{R1}													1.00	-0.30	-0.30	-0.03	-0.37
LT _{V6}														1.00	0.04	0.03	0.32
PH _{V12}															1.00	-0.04	0.66
LT _{R1}																1.00	-0.02
PH _{R1}																	1.00

Numbers represented in bold font are moderately to highly correlated at $p < 0.05$. Subscript numbers and letters indicate plant growth stage at the time of measurement. Grain yield (GY), 500 seed weight (SW), stover weight (STW), ear weight (EW), ear length (EL), above ground biomass (ABM), adaxial stomatal conductance (A), abaxial stomatal conductance (B), leaf temperature (LT), plant height (PH).

Table 15. Syngenta Pearson correlation coefficients for morpho-physiological parameters in 2014 & 2015.

	GY	SW	STW	EW	ER	EL	ABM _{V-6}	B _{V-6}	B _{V-12}	B _{R-1}	LT _{V-6}	PH _{V-6}	PH _{V-12}	LT _{R-1}	PH _{R-1}
GY	1.00	0.63	0.55	0.61	-0.06	0.44	0.54	0.04	-0.07	-0.49	0.53	-0.11	0.20	-0.09	0.15
SW		1.00	0.30	0.42	-0.37	-0.09	0.32	0.17	0.18	-0.09	0.21	-0.07	-0.12	0.11	-0.12
STW			1.00	0.81	0.03	0.24	0.65	-0.16	0.01	-0.25	0.25	0.11	0.17	0.02	0.19
EW				1.00	0.06	0.34	0.53	0.11	0.01	-0.26	0.41	-0.04	0.12	-0.01	0.03
ER					1.00	-0.06	-0.19	0.01	-0.03	0.00	0.04	-0.11	0.01	-0.04	0.12
EL						1.00	0.17	0.07	-0.25	-0.43	0.40	-0.03	0.33	-0.13	0.13
ABM_{V6}							1.00	-0.05	0.12	-0.15	0.01	0.27	0.32	0.08	0.07
B_{V6}								1.00	0.12	0.12	0.19	-0.20	-0.17	0.20	-0.09
B_{V12}									1.00	0.15	0.06	-0.21	-0.20	0.34	-0.17
B_{R1}										1.00	-0.44	0.20	-0.10	0.20	-0.08
LT_{V6}											1.00	-0.53	-0.16	-0.20	0.22
PH_{V6}												1.00	0.34	-0.09	0.06
PH_{V12}													1.00	-0.03	0.42
LT_{R1}														1.00	-0.19
PH_{R1}															1.00

Numbers represented in bold font are moderately to highly correlated at $p < 0.05$. Subscript numbers and letters indicate plant growth stage at the time of measurement. Grain yield (GY), 500 seed weight (SW), stover weight (STW), ear weight (EW), ear length (EL), above ground biomass (ABM), abaxial stomatal conductance (B), leaf temperature (LT), plant height (PH).

Table 16. Monsanto Pearson correlation coefficients for morpho-physiological parameters in 2014 & 2015.

	GY	SW	STW	EW	ABM_{V-6}	ABM_{R-1}	B_{V-12}	B_{R-1}	LT_{V-6}	PH_{V-6}	LT_{V-12}	PH_{V-12}	LT_{R-1}	PH_{R-1}
GY	1.00	0.63	0.54	0.55	0.52	0.55	0.01	-0.43	0.50	-0.33	-0.08	0.36	-0.30	0.42
SW		1.00	0.27	0.41	0.28	0.42	-0.03	-0.38	0.50	-0.36	-0.17	0.17	-0.27	0.21
STW			1.00	0.80	0.69	0.52	0.18	-0.28	0.27	0.04	-0.22	0.26	-0.12	0.24
EW				1.00	0.61	0.58	0.20	-0.32	0.34	-0.10	-0.36	0.08	-0.14	0.10
ABM_{V6}					1.00	0.44	0.25	-0.15	-0.08	0.11	-0.01	0.40	-0.07	0.25
ABM_{R1}						1.00	0.07	-0.44	0.45	-0.22	-0.29	0.25	-0.16	0.26
B_{V12}							1.00	0.00	-0.02	-0.16	0.11	0.12	0.18	-0.03
B_{R1}								1.00	-0.52	0.33	0.20	-0.31	0.25	-0.29
LT_{V6}									1.00	-0.53	-0.32	0.04	-0.31	0.21
PH_{V6}										1.00	-0.05	-0.02	0.20	0.01
LT_{V12}											1.00	-0.08	0.27	-0.06
PH_{V12}												1.00	-0.22	0.54
LT_{R1}													1.00	-0.37
PH_{R1}														1.00

Numbers represented in bold font are moderately to highly correlated at $p < 0.05$. Subscript numbers and letters indicate plant growth stage at the time of measurement. Grain yield (GY), 500 seed weight (SW), stover weight (STW), ear weight (EW), above ground biomass (ABM), abaxial stomatal conductance (B), leaf temperature (LT), plant height (PH).

Table 17. Experiment hybrids and corresponding technology of greenhouse research (2015).

Hybrid	Description	Relative Maturity
Pioneer P1498YHR Optimum Aquamax ^a	Drought Tolerant	114 days
Pioneer P1319 ^a	Non Drought Tolerant	113 days
NK N70J 4011 Agrisure Artesian ^b	Drought Tolerant	112 days
NK N68-B 3111 ^b	Non Drought Tolerant	111 days
Dekalb DKC 65-20 Drought Gard ^c	Drought Tolerant	115 days
Dekalb DKC 66-97 ^c	Non Drought Tolerant	116 days
Dekalb DKC 66-42 Drought Gard ^c	Drought Tolerant	116 days

^a Pioneer Hi-Bred, Box 1000, Johnston, IA 50131.

^b Syngenta Crop Protection, Inc. Box 183000, Greensboro, NC 27419.

^c Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63137.

Table 18. WW greenhouse container weights (g) and water amounts (ml) added during experiment.

Plot #	Date						
	6/12/2015	6/15/2015	6/18/2015	6/22/2015	6/24/2015	6/26/2015	6/29/2015
103	6455	6040	6205	6075	6030	6150	5885
203	6450	6105	6295	6125	6190	6345	6195
303	6440	6075	6255	6190	6100	6205	5945
401	6435	6055	6225	6060	6035	6155	5840
Average	6445	6069	6245	6113	6089	6214	5966
mL added to each container	Planting (Field capacity)	376	200	333	356	231	Harvest

Table 19. WS greenhouse container weights (g) and soil water concentration (%) during experiment.

Plot #	Date													
	6/12/2015		6/15/2015		6/18/2015		6/22/2015		6/24/2015		6/26/2015		6/29/2015	
	(g)	SWC	(g)	SWC	(g)	SWC	(g)	SWC	(g)	SWC	(g)	SWC	(g)	SWC
103	6025	67%	5885	62%	5530	50%	5275	42%	5165	38%	5105	36%	5035	34%
203	6040	67%	5750	57%	5585	51.5%	5365	44%	5250	40%	5180	38%	5090	35%
303	6070	67%	5725	55%	5625	52%	5410	45%	5300	41%	5235	39%	5155	36%
401	6065	67%	5740	56%	5625	52%	5425	45%	5325	42%	5255	40%	5165	37%
Average	6050	67%	5775	57.5%	5591	51.4%	5369	44%	5260	40.3%	5194	38.3%	5111	35.5%

Table 20. ANOVA for physiological mechanisms of greenhouse research.

DuPont Pioneer					
Variable	<i>A</i>	<i>B</i>	<i>ABM</i>	<i>BBM</i>	<i>LT</i> (V-4/V-5/V-6)
Hyb	*	ns	ns	ns	ns / ns / ns
Environ	***	*	***	ns	ns / ns / **
Hyb x Environ	ns	ns	ns	ns	ns / ns / ns
Syngenta					
Variable	<i>A</i>	<i>B</i>	<i>ABM</i>	<i>BBM</i>	<i>LT</i> (V-4/V-5/V-6)
Hyb	*	ns	**	ns	ns / ns / ns
Environ	***	ns	***	ns	* / ns / **
Hyb x Environ	*	ns	*	ns	ns / ns / ns
Monsanto					
Variable	<i>A</i>	<i>B</i>	<i>ABM</i>	<i>BBM</i>	<i>LT</i> (V-4/V-5/V-6)
Hyb	ns	ns	ns	ns	ns / ns / ns
Environ	*	ns	***	ns	* / ns / ***
Hyb x Environ	ns	ns	ns	ns	ns / ns / ns

Hyb = hybrid (NDT and DT), NDT = non-drought-tolerant, DT = drought-tolerant; Environ = environment (WW and WS); ns = not significant, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Adaxial leaf transpiration (*A*) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), abaxial leaf transpiration (*B*) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), above ground biomass (*ABM*) (g), below ground biomass (*BBM*) (g), leaf temperature (*LT*) ($^{\circ}\text{F}$).

Table 21. Least significant differences for physiological mechanisms of DuPont Pioneer hybrids in greenhouse research.

Hyb						
Fixed Effects		<i>A</i>	<i>B</i>	<i>ABM</i>	<i>BBM</i>	<i>LT</i> (V-4/V-5/V-6)
Environ	Hyb					
	NDT	62.3 a	29.0 a	5.8 a	2.2 a	86.6 a / 88.6 a / 81.1 a
	DT	37.8 b	26.1 a	6.1 a	1.8 a	86.8 a / 87.5 a / 80.3 a
Environ						
		<i>A</i>	<i>B</i>	<i>ABM</i>	<i>BBM</i>	<i>LT</i> (V-4/V-5/V-6)
WW		81.7 a	44.5 a	8.7 a	2.2 a	86.4 a / 87.8 a / 79.6 b
WS		18.4 b	10.6 b	3.1 b	1.9 a	87.0 a / 88.3 a / 81.8 a
Hyb x Environ						
		<i>A</i>	<i>B</i>	<i>ABM</i>	<i>BBM</i>	<i>LT</i> (V-4/V-5/V-6)
WW	NDT	104.9 a	46.6 a	8.4 a	2.4 a	85.6 a / 87.9 a / 79.8 b
WS	NDT	19.7 c	11.5 b	3.1 b	2.0 a	87.6 a / 89.4 a / 82.4 a
WW	DT	58.5 b	42.4 ab	9.0 a	1.9 a	87.2 a / 87.8 a / 79.5 b
WS	DT	17.0 c	9.8 b	3.1 b	1.8 a	86.5 a / 87.2 a / 81.2 ab

Hyb = hybrid, Environ = environment (WW and WS); WW = Well-watered, WS = Water stressed; NDT = Non-drought-tolerant, DT = drought-tolerant
Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Adaxial leaf transpiration (*A*) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$),
abaxial leaf transpiration (*B*) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), above ground biomass (*ABM*) (g), below ground biomass (*BBM*) (g), leaf temperature (*LT*) ($^{\circ}\text{F}$).

Table 22. Least significant differences for physiological mechanisms of Syngenta hybrids in greenhouse research.

Hyb						
Fixed Effects		<i>A</i>	<i>B</i>	ABM	BBM	LT (V-4/V-5/V-6)
Environ	Hyb					
	NDT	29.6 b	13.5 a	5.1 b	2.1 a	87.2 a / 88.9 a / 81.0 a
	DT	42.8 a	22.7 a	6.4 a	2.3 a	86.8 a / 87.8 a / 80.7 a
Environ						
		<i>A</i>	<i>B</i>	ABM	BBM	LT (V-4/V-5/V-6)
WW		53.1 a	21.1 a	7.7 a	1.9 a	86.4 b / 88.3 a / 79.5 b
WS		19.3 b	15.1 a	3.9 b	2.4 a	87.6 a / 88.4 a / 82.2 a
Hyb x Environ						
		<i>A</i>	<i>B</i>	ABM	BBM	LT (V-4/V-5/V-6)
WW	NDT	39.5 b	15.5 a	6.5 b	1.6 b	86.9 ab / 89.0 a / 79.6 b
WS	NDT	19.7 c	11.6 a	3.6 c	2.6 a	87.6 ab / 88.8 a / 82.3 a
WW	DT	66.6 a	26.7 a	8.8 a	2.2 ab	85.9 b / 87.6 a / 79.4 b
WS	DT	18.9 c	18.6 a	4.1 c	2.3 ab	87.7 a / 88.0 a / 82.1 a

Hyb = hybrid, Environ = environment (WW and WS); WW = Well-watered, WS = Water stressed; NDT = Non-drought-tolerant, DT = drought-tolerant
Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Adaxial leaf transpiration (*A*) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$),
abaxial leaf transpiration (*B*) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), above ground biomass (*ABM*) (g), below ground biomass (*BBM*) (g), leaf temperature (*LT*) ($^{\circ}\text{F}$).

Table 23. Least significant differences for physiological mechanisms of Monsanto hybrids in greenhouse research.

Hyb						
Fixed Effects		A	B	ABM	BBM	LT (V-4/V-5/V-6)
Environ	Hyb					
	NDT	54.3 a	45.0 a	6.5 a	2.2 a	86.3 a / 88.0 a / 80.7 a
	DT ¹	39.2 a	18.8 a	7.4 a	2.8 a	85.7 a / 88.3 a / 81.1 a
	DT ²	47.1 a	30.2 a	5.9 a	2.1 a	86.0 a / 88.2 a / 80.6 a

Environ						
		A	B	ABM	BBM	LT (V-4/V-5/V-6)
WW		70.3 a	49.2 a	9.4 a	2.3 a	85.5 b / 88.0 a / 79.5 b
WS		23.5 b	13.6 b	3.8 b	2.4 a	86.5 a / 88.2 a / 82.1 a

Hyb x Environ						
		A	B	ABM	BBM	LT (V-4/V-5/V-6)
WW	NDT	79.4 a	81.2 a	9.9 a	2.5 a	85.5 b / 88.7 a / 79.3 b
WS	NDT	29.2 bc	8.9 b	3.1 b	1.9 a	87.2 a / 87.3 a / 82.1 a
WW	DT ¹	64.4 ab	29.9 ab	10.5 a	2.8 a	85.0 b / 87.4 a / 79.9 b
WS	DT ¹	14.0 c	7.7 b	4.2 b	2.7 a	86.3 ab / 89.2 a / 82.3 a
WW	DT ²	67.0 ab	36.3 ab	7.7 a	1.7 a	86.1 ab / 88.1 a / 79.3 b
WS	DT ²	27.2 bc	24.1 ab	4.1 b	2.4 a	86.0 ab / 88.2 a / 82.0 a

Hyb = hybrid, Environ = environment (WW and WS); WW = Well-watered, WS = Water stressed; NDT = Non-drought-tolerant, DT = drought-tolerant. Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. DT ¹ = Dekalb DKC 65-20, DT ² = Dekalb DKC 66-42. Adaxial leaf transpiration (A) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), abaxial leaf transpiration (B) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), above ground biomass (ABM) (g), below ground biomass (BBM) (g), leaf temperature (LT) ($^{\circ}\text{F}$).

Table 24. Statistical analysis for greenhouse root morphology.

DuPont Pioneer				
Variable	RL	RSA	RD	RV
Hyb	ns	*	*	*
Environ	*	***	ns	***
Hyb x Environ	ns	ns	ns	ns
Syngenta				
Hyb	ns	ns	**	ns
Environ	*	*	ns	*
Hyb x Environ	ns	ns	ns	ns
Monsanto				
Hyb	ns	ns	ns	ns
Environ	***	***	ns	***
Hyb x Environ	ns	ns	ns	ns

Hyb = hybrid (NDT and DT), NDT = non-drought-tolerant, DT = drought-tolerant; Environ = environment (WW and WS); ns = not significant, * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. Total root length (RL) (cm), total root surface area (RSA) (cm²), average root diameter (RD) (mm), and total root volume (RV) (cm³).

Table 25. Least significant differences for AQUAmax technology in greenhouse root morphology research.

Hyb					
Fixed Effects		RL	RSA	RD	RV
Environ	Hyb				
	NDT	4136.4 a	825.2 a	0.61 a	12.1 a
	DT	3534.5 a	655.0 b	0.56 b	9.2 b
Environ					
WW		4604.0 a	942.8 a	0.59 a	13.6 a
WS		3066.3 b	537.7 b	0.58 a	7.6 b
Hyb x Environ					
WW	NDT	4863.0 a	1063.1 a	0.62 a	15.9 a
WS	NDT	3410.0 ab	587.3 c	0.60 ab	8.2 bc
WW	DT	4346.3 ab	821.9 b	0.55 b	11.3 b
WS	DT	2722.8 b	488.2 c	0.56 ab	7.1 c

Hyb = hybrid, Environ = environment (WW and WS); WW = Well-watered, WS = Water stressed; NDT = Non-drought-tolerant, DT = drought-tolerant
Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Total root length (RL) (cm), total root surface area (RSA) (cm²), average root diameter (RD) (mm), and total root volume (RV) (cm³).

Table 26. Least significant differences for Artesian technology in greenhouse root morphology research.

Hyb					
Fixed Effects		RL	RSA	RD	RV
Environ	Hyb				
	NDT	5064.3 a	802.1 a	0.52 b	10.4 a
	DT	4544.4 a	822.5 a	0.60 a	12.0 a
Environ					
WW		5391.1 a	920.8 a	0.56 a	12.8 a
WS		4217.6 b	703.9 b	0.55 a	9.6 b
Hyb x Environ					
WW	NDT	5227.3 a	837.7 ab	0.52 b	10.9 ab
WS	NDT	4901.3 ab	766.5 ab	0.51 b	9.8 b
WW	DT	5554.9 a	1003.7 a	0.59 a	14.6 a
WS	DT	3534.0 b	641.3 b	0.60 a	9.4 b

Hyb = hybrid, Environ = environment (WW and WS); WW = Well-watered, WS = Water stressed; NDT = Non-drought-tolerant, DT = drought-tolerant
Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Total root length (RL) (cm), total root surface area (RSA) (cm²), average root diameter (RD) (mm), and total root volume (RV) (cm³).

Table 27. Least significant differences for DroughtGard technology in greenhouse root morphology research.

Hyb					
Fixed Effects		RL	RSA	RD	RV
Environ	Hyb				
	NDT	5541.9 a	952.0 a	0.56 a	13.2 a
	DT ¹	5662.0 a	993.5 a	0.58 a	14.1 a
	DT ²	5226.0 a	892.4 a	0.57 a	12.4 a
Environ					
WW		6687.9 a	1155.3 a	0.56 a	16.1 a
WS		4265.4 b	736.6 b	0.58 a	10.3 b
Hyb x Environ					
WW	NDT	7204.9 a	1257.3 a	0.57 a	18.2 a
WS	NDT	3878.9 c	628.6 d	0.55 a	8.2 d
WW	DT ¹	6901.9 ab	1189.5 ab	0.56 a	16.5 ab
WS	DT ¹	4422.1 c	797.5 cd	0.60 a	11.6 cd
WW	DT ²	5956.8 b	1001.2 bc	0.56 a	13.7 bc
WS	DT ²	4495.2 c	783.6 cd	0.58 a	11.2 cd

Hyb = hybrid, Environ = environment (WW and WS); WW = Well-watered, WS = Water stressed; NDT = Non-drought-tolerant, DT = drought-tolerant
Means followed by same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. DT¹ = Dekalb DKC 65-20, DT² = Dekalb DKC 66-42.
Total root length (RL) (cm), total root surface area (RSA) (cm²), average root diameter (RD) (mm), and total root volume (RV) (cm³).



Figure 1. Weather conditions for 2014 & 2015 (maximum, minimum air temperature, and precipitation).

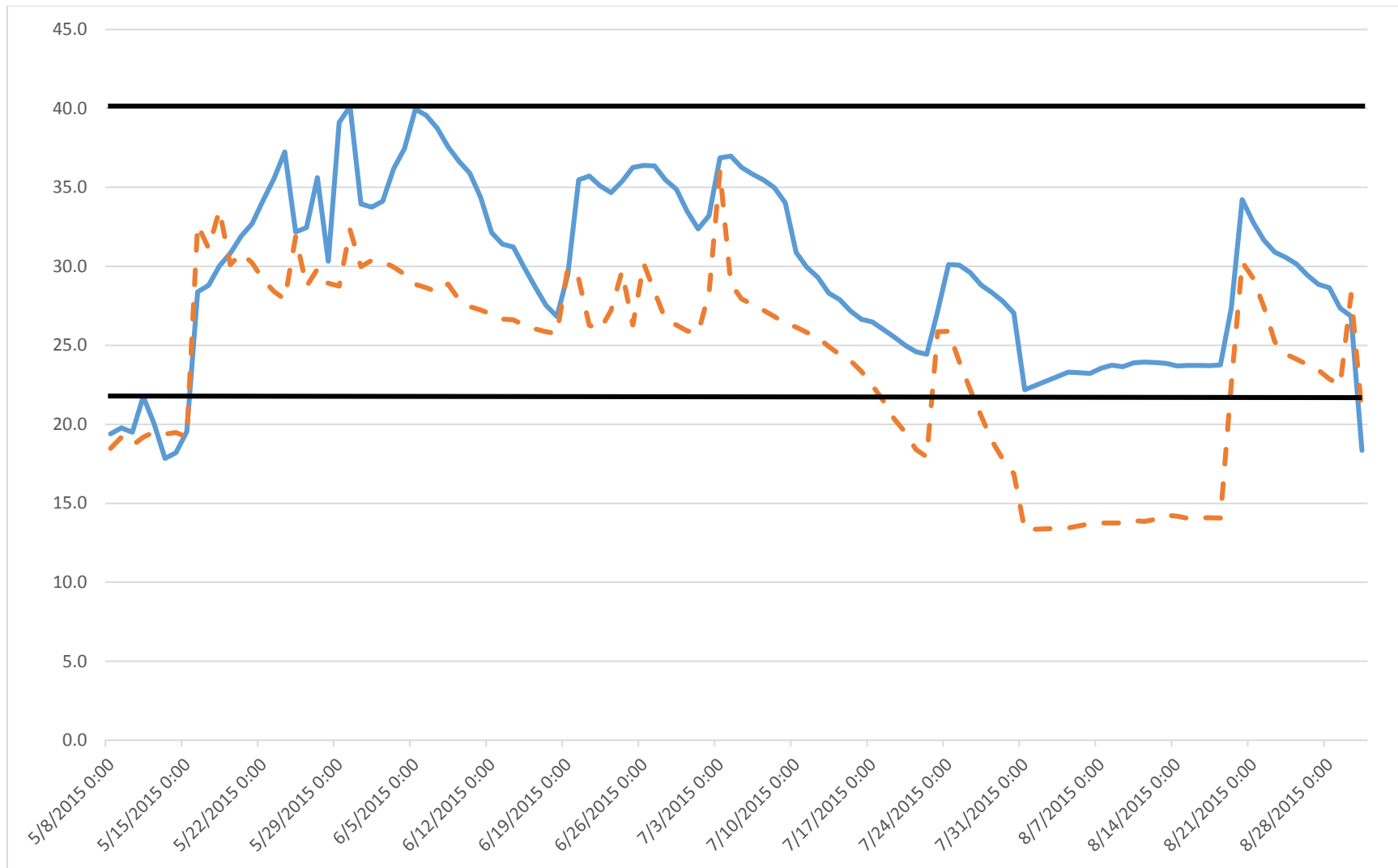


Figure 2. Volumetric Water Content (%VWC) of soil (2015) in non-irrigated field. Solid line represents %VWC of soil at a depth of 15 cm. Dashed line represents %VWC of soil at a depth of 61 cm. Upper most horizontal line represents field capacity of soil, lower horizontal line represents when crop was stressed

Vita

Austin Scott was born March 27, 1989 in Jackson, TN, the youngest child of Kyle Scott of Hickory Valley, TN and Tina Vickers of Bartlett, TN. He graduated from Bolivar Central High School in May of 2007, after which he joined the Marine Corps. Austin attended the University of Tennessee at Martin and received a Bachelor of Science degree in Agriculture with a concentration in Crop and Soil Management in May of 2014. After graduation, he accepted a position at the University of Tennessee as a Graduate Research Assistant in the Plant Science Department with Dr. Angela McClure, UT Extension Corn and Soybean Specialist. He was awarded a Master of Science degree in Plant Science with a concentration in Crop Science in December 2015. Upon graduation, Austin accepted an industry position with a nationally recognized seed company as the Field Agronomist for Tennessee and Kentucky.