Comparison of Drought Tolerance among Winter Wheat Hybrids and their Parents Using a Comprehensive Screening Method

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Abstract

Drought is known to be one of the most limiting abiotic stresses for wheat (*Triticum aestivum* L.) production, not only in the Midwest, but throughout the world. It is a complex issue and one that is difficult to screen for when breeding for new varieties. Hybrid wheat is one possible tool for breeders to use in order to make genetic gains towards better tolerance. The effectiveness of hybrid wheat as a tool to address regular periods of drought is a topic of continual discussion. The purpose of this study was to perform a comprehensive screening for drought tolerance comparing two different experimental hybrid entries to their parents. The hybrids were selected based on their good performance under drought in prior field trials. Plants were grown in PVC columns containing sensors that monitored growth media water content and matric potential. All plants were grown equally until heading. Drought treatment began 10 days post anthesis. Plants were observed until senescence/maturity. Several different agronomic characteristics were measured along with physiological traits that have previously been linked to drought tolerance. After completion of the screening, it was observed that the hybrid entries tended to fall between the two parents for a majority of the measurements. When comparing the hybrids to the parents overall, at least one parent outperformed its hybrid in every category. Parent line Parent B was one of the highest ranking genotypes for all measurements. Different drought mechanisms were observed across genotypes upon completion of the treatment. Further research is necessary to understand the hybrid response to drought when compared to pure line varieties.

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Chapter 1 - Literature Review

Agricultural drought is defined by Manivannan et al. (2008) as the lack of ample moisture required for normal plant growth and development to complete the life cycle. It has an effect on vegetative growth, reproduction, and the process of filling grain. Drought develops when crop water demand is not met by water supply (Blum, 2005). Crop breeding and production must constantly overcome many obstacles coming in the form of abiotic and biotic stresses. Though many of these stresses can prove costly, drought or water deficit is considered the single most devastating environmental stress because it causes more loss to crop productivity and is more of a major limiting factor than any other environmental stress (Boyer, 1982; Farooq et al., 2012; Lambers et al., 2008).

Though it is hard to make a sound prediction on where climate change is headed, most models show an increase in aridity in many areas of the globe (Chaves et al., 2002; Petit et al., 1999), including models that view drought becoming more severe in the future (Farooq et al., 2012).

Drought Stress and its Effects

The effects of drought can be detrimental on crop growth and development, but they are highly variable. The susceptibility of plants to water deficit changes based on the severity, the cultivar, and the crop growth stage (Anjum et al., 2011; Farooq et al., 2012). Though drought can have negative effects during all stages of growth and development, some stages can have a larger impact on economic yield. There is a widespread consensus that the reproductive growth stage is the most sensitive to water deficit (Blum, 2009). During this time, drought stress can

have a severe impact on pollen viability and anther development. Drought stress during grain filling phases is more devastating than during the vegetative period, because it can substantially decrease economic yield (Farooq et al., 2012).

The loss in economic yield due to soil water deficit comes by reducing canopy absorption of photosynthetically active radiation, making radiation use less efficient, and lowering harvest index (Earl and Davis, 2003; Farooq et al., 2012). Many plant processes are affected from the biochemical and physiological level to the whole plant level in order to cause this loss in yield.

Relative water content, leaf water potential, stomatal resistance, and rate of transpiration are important characteristics that influence plant water relations (Anjum et al., 2011; Kirkham, 2005). A significant linear relationship between stomatal resistance and reduction in yield under stress was observed in a study conducted by Golestani and Assad (1998).. Carbon dioxide assimilation rates are drastically reduced due to increased stomatal resistance, and leaf, stem, and root proliferation are all reduced (Anjum et al., 2011) Drought stress can lead to disruption of membrane structure and organelle disarray, and, when it is large enough, a loss in turgor leading to reduction of cell expansion, vegetative growth, and carbon assimilation (Sayed, 2003). Relative chlorophyll content is positively correlated with photosynthetic rate, so decreased chlorophyll content under stress has been considered a symptom of acute drought stress leading to lower primary production (Anjum et al., 2011). Photosystem II is the first complex involved in light reactions of photosynthesis. It has been observed that photosystem II is particularly sensitive to water stress (Lu and Zhang, 1999) which could lead to reduction in absorption of photosynthetically active radiation in the canopy. Another important aspect negatively affected by drought stress is crop phenology. Water stress has a strong influence on a crop's phenology by shortening the crop growth cycle (Farooq et al., 2012).

While many crops across the globe experience drought stress throughout their growing cycles, common wheat (*Triticum aestivum* L.) may be one of the most widely affected. It is a staple food crop in nearly all countries as it is very broadly adapted to the various growing environments. Throughout many wheat growing regions, limited rainfall occurs frequently during the grain fill stage (Plaut et al., 2004). The United States is no exception. Wheat is grown for food and forage in the U.S. Southern High Plains on a spectrum ranging from fully rain fed to fully irrigated (Xue et al., 2014). Most of the U.S acres are not irrigated. In most wheat growing regions, grain filling is subjected to several abiotic and biotic stresses. It generally occurs when temperature is increasing and moisture supply is decreasing (Blum, 1998). Many studies have shown the effect that drought can have on wheat from accelerating the maturity cycle to reducing relative water content resulting in an impact on photosynthetic rate. Results from a study by Siddique et al. (1999) showed that wheat exposed to drought stress had decreased leaf water potential and relative water content that led to pronounced effects on photosynthetic rate. It was also observed in a study by Praba et al. (2009) that drought reduced many yield components such as biomass, number of grains per spike, spike weight, and grain yield per spike. Grain yield reduction was 32% compared to the control in that study.

Drought can have many different effects on a plant from cellular level to the whole-plant level. This plethora of responses makes drought tolerance a complex phenomenon (Farooq et al., 2012).

Drought Tolerance

Passioura (1996) defines drought tolerance using resource economics. The crop's water supply is the resource. The most effective use of this resource is capturing as much as possible,

using it as effectively as possible when trading it to help form photo assimilate and converting as much of the assimilate as possible into harvestable form. He argues that any phenomenon not readily associated with components of water use efficiency is not likely to have an influence on yield under drought stress.

Traits explaining adaptation to drought are usually associated with plant development and structure and are constitutive rather than stress-induced (Chaves et al., 2002). A drought tolerant plant must be able to handle major vicissitudes in water supply and high evapotranspiration rates during the growing season. Mechanisms or traits related to drought tolerance may only occur during a certain period of water deficit and are usually subtle (Passioura, 1996). Responses may be altered by gene expression and cellular metabolism or possibly changes in growth and productivity. Many yield-determining processes respond to water stress (Anjum et al., 2011). Levitt (1972) defined drought tolerance in the physiological context as dehydration avoidance or tolerance. Dehydration avoidance is the ability of a plant to sustain high plant water status or cellular hydration during drought through mechanisms such as enhanced water uptake, limited water loss, and maintenance of cell hydration, while dehydration tolerance is defined as the ability to sustain or conserve plant function during a period of water deficit. When comparing the two, dehydration avoidance would be more commonplace, while dehydration tolerance as a mechanism is rare, and usually only occurs in the seed (Blum, 2005). Plants exhibiting an escape or avoidance strategy will exhibit a high degree of developmental plasticity. They may utilize the maximum available resources to enable high rates of growth and gas exchange. Another characteristic of drought tolerance is better partitioning of assimilates to developing fruits. Plants may have a higher ability to store reserves in some organs (stem and roots) and then mobilize them for fruit production (Chaves et al., 2002). This has been well documented in cereals like

wheat, maize, and barley (Gebbing et al., 1999). Whole plant traits have a major role in affecting plant dehydration avoidance under stress, and crops adapted to water limited conditions achieve that adaptation mainly by dehydration avoidance rather than tolerance (Blum, 2005). Examples of this would be adapted phenology (shortened growth cycles), maintenance of leaf turgor pressure, and storage of assimilates in the stem.

Different types of signaling are required as early warning systems so that plants can escape using the appropriate method. Signals are key players in plant resistance to stress (Chaves and Oliveira, 2004). Based upon this signaling, plants are able to make an appropriate change in their processes in order to cope with stress. An example of one of these hormones would be abscisic acid (ABA). An increase in the signaling of ABA occurs during drought stress leading to an effect in plant responses. It has been shown to promote root growth and acts as an early warning signal in response to drying of the upper roots (Blum, 2011). Blum (2011) argues that ABA sensitivity should be approached with caution; an over sensitive plant may result in premature shutdown of photosynthesis. Blum (2015) concluded that ABA can by no means be considered a drought resistance hormone because the benefit to damage ratio depends on the crop drought stress profile. This sensitivity may yield an advantage in environments prone to severe drought, while more anisohydric lines would perform relatively better under more moderate drought (Blum 2015). A wide range of mechanisms have been observed as a response to withstand drought, such as increased stomatal resistance, deeper root systems, and smaller leaves (Farooq et al., 2012). Osmotic adjustment is a major cellular drought-responsive trait that contributes to cellular dehydration avoidance and yield under stress (Blum, 2005). It results in an active accumulation of ions like K^+, Na^+, Ca^{2+}, NO_3^-, and SO_4^- or organic solutes such as free amino acids, sugars, and sugar alcohols (Moinuddin et al. 2005). Osmotic adjustment has two

major functions in plant production under drought. The first is to enable leaf turgor maintenance for the same leaf water potential, thus supporting stomatal conductance. Secondly, it improves root capacity for water uptake (Blum, 2009). All of these mechanisms play a role in allowing a plant to withstand water deficit to a certain extent. Passioura (1996) argues that the most important feature of a drought tolerant crop is its ability to time its development in relation to a variable growing season. This would fall in the lines of the dehydration avoidance strategy, and may have the most profound effect on maintaining yield. This works in some areas of the world but not in others. The complexity of plant response to drought provides a major challenge when breeding and screening for new tolerant wheat cultivars. Pair this with the amount of environmental variability and many obstacles are created for wheat breeders.

Breeding for Drought Tolerance

Now more than ever, contemporary plant breeding is under pressure to improve productivity at a rate surpassing past achievements (Blum, 2013). Development of crops for drought tolerance requires a knowledge of physiological mechanisms and genetic control of the contributing traits at different plant developmental stages (Farooq et al., 2012). The same goes for breeding wheat. Breeding has already made a significant contribution to wheat yield under drought stress. Richards et al. (2010) found that wheat yields increased by twofold to nearly 2000 kg ha^{-1} in a matter of around forty years. This was in the arid environment of Australia (Xue et al., 2014). A comprehensive exploration of the potential genetic resources, and an in-depth understanding of what makes up the traits that allow for survival in an unfriendly environment, are required (Rampino et al., 2006). Many of the improvements have been made due to increasing the harvest index. Blum (2013) believes that the route for improving yield through

harvest index in cereals is approaching an end. We continue to see headway in the development of drought-resistant cultivars, but the framework of what actually constitutes a viable choice in selection is not always clear. A perfect "ideotype" is not always well defined (Blum, 2005). Breeding for specific, suboptimal environments involves a deeper understanding of the yield determining process (Siddique et al., 1999).

Traits to select for when breeding for drought stress will depend on the level and timing of stress in the targeted area. Selecting for yield itself under stress-alleviated conditions may produce superior cultivars in not only optimal environments but also those frequently subjected to mild and moderate stress conditions (Araus et al., 2002). An ideal drought tolerant genotype would be a combination of high yield and low sensitivity to water stress. This is often the opposite of genotypes that have superior yielding capabilities (Cattivelli et al., 2008). Pantuwan et al. (2002) found that these genotypes were, in fact, often associated with a high sensitivity to water stress. When effective and successful selection for yield under stress is exercised, Blum (2005) states that a genetic shift towards a dehydration-avoidant plant type is occurring. Traits associated with dehydration avoidance include: early flowering, smaller plant, smaller leaf area, or limited tillering. Selection for certain traits such as transpiration efficiency and osmotic adjustment have been shown to improve yields under stressed conditions (Xue et al., 2014). Selecting for plants with high transpiration efficiency may be important when identifying genotypes with higher biomass or yield (Xue et al., 2014). It was found in a study conducted by Xue et al. (2014) that wheat genotypes with higher yield and biomass had higher water use efficiency under dryland conditions, while a study conducted by Morgan et al. (1986) showed that wheat plants selected for high osmotic adjustment yielded 1.5 and 1.6 times more than plants selected for low osmotic adjustment (Rekika et al., 1998). Although breeding for drought

tolerance in newer wheat cultivars may be a huge challenge, research has proved that reasonable progress has been made. Xue et al. (2014) observed that newer, drought-tolerant genotypes had higher yields under drought conditions with more seeds per spike and higher thousand kernel weight than older, less tolerant varieties. They also observed that biomass at anthesis contributed to higher yield under drought. Spike weight and number were also positively correlated to yield in drought environments. Newer cultivars also require less irrigation for high yields, which can lead to a conclusion that drought tolerance is slowly improving (Xue et al., 2014). It can be said that there is no straightforward method when breeding for drought tolerance. So many different factors can play a role when screening and making selections. A couple major points stemming from the study of Plaut et al. (2044) are that breeding for high yield will probably also provide increased drought tolerance, and that competition between vegetative organs and kernels for stored materials in the stem must be minimized.

Hybrid Wheat

Many new tools are being developed to allow for more efficient screening and breeding of elite, tolerant cultivars. High throughput phenotyping, genotypic selection, and speed breeding, to name a few, could help in the future for producing new, high-yielding wheat varieties. The breeder's "toolbox" is growing larger. Hybrid wheat is one such tool that has been explored in the past, but was never widely deployed on a commercial scale. Much work has been done on hybrid crops and the genetic basis of heterosis, but wheat is still new to the hybrid world. Hybrid cultivars are widely utilized in cereal crops like maize and rice, but, for wheat, the hybrids have yet to be widely used in commercial production (Mette et al., 2012). Hybrid wheat may hold the potential to deliver a major lift in yield and will open a wide range of new breeding

opportunities (Whitford et al., 2013). This potential is still held back by certain logistic limitations. With wheat being an autogamous species, the amount of midparent or high parent heterosis for yield is less pronounced than a species like maize (Longin et al., 2012; Mette et al., 2012). In order to be widely accepted in the Great Plains, hybrids must exhibit enhanced yield performance, and a yield stability reasonably larger than inbred cultivars across different production environments (Bruns and Peterson, 1998). According to Blum (2013), "A heterotic hybrid will most probably assimilate more than its parents over the natural range of daily change in temperature, light, and photo biological signals, notwithstanding other cues such as soil moisture, wind, or low atmospheric vapor pressure deficit which can affect leaf temperature and thus assimilation."

Capacity and cost are the major practical limitations for a more widespread use of hybrid seed. Only a few hybrid cultivars are registered for the European market, and they are based on chemical hybridizing agents (CHA's). In order for hybrid seed production to occur, there must be an efficient cross pollination between inbred lines. This challenge is overcoming the use of CHA's, which have some safety concerns, and the ability to hit the right developmental window to create male-sterile maternal plants (Mette et al., 2012). Certain environments are also more conducive to hybrid seed production.

Information regarding hybrid wheat performance is somewhat ambiguous. Leon (1994) observed that studies concerning higher yield and yield stability in hybrids have contrasting results, with some showing higher performance in the hybrids while others showing no difference between the hybrids and the pure lines (Mette et al., 2012). Mühleisen et al. (2014) used a broad base of data from multilocation field trials to re-evaluate grain yield stability in hybrid wheat when compared to inbred lines. It was observed that hybrids maintained

consistently higher yield stability than the inbred lines (Mette et al., 2012). Yield increases in modern hybrids may be due to high parent heterosis for biomass (Borghi et al., 1988). Harvest index is maintained around the mid-parent level for high yielding hybrids, and high parent heterosis for harvest index is more rare (Pickett, 1993). A hybrid line could achieve higher biomass and yields through combining yield components from their parents (Evans, 1993; Kindred and Gooding, 2005). An important observation made by Oury et al. (1995) was that the heterosis for biomass and grain yield was associated with greater assimilation post anthesis due to a greater capacity to fill grain. It can be inferred that, when crossing high parents with differing yield components (e.g., grain number and grain size), the hybrid offspring will maintain a partially dominant optimum trait from each parent (Kindred and Gooding, 2005).

A continual point of discussion concerning hybrid wheat is how it performs when exposed to abiotic and biotic stresses. How will a suboptimal environment affect the possible advantage in yield stability that hybrid wheat has to offer? The USDA Southern Regional Performance Nursery (SRPN) has tested a number of hybrid entries, and data have suggested the hybrids may have improved yield stability and response to favorable environments when grown over a broad array of production conditions (Bruns and Peterson, 1998). The important takeaway from the SRPN data is that yield stability came in response to favorable environments. Mette et al. (2012) said that hybrid wheat can also outperform inbred lines in sturdiness to abiotic and biotic stress. This statement was based upon research by Longin et al. (2013) that showed a positive mid-parent heterosis for frost tolerance as well as resistance against leaf rust, stripe rust, septoria tritici blotch, and powdery mildew. Drought is not one of the conditions that showed positive mid-parent heterosis. It has been speculated that hybrids may have lower stress susceptibility than related inbred lines, and that may contribute to the higher yield stability

observed in previous studies (Mühleisen et al., 2014). A study of an European hybrid wheat conducted by Oury et al. (1993) produced findings that may help explain how drought can affect heterosis. The site that experienced a water deficit saw a severe reduction in the grain filling period and premature senescence of the crop. This did not allow for continued grain growth in the hybrid (Kindred and Gooding, 2005).

Screening for Drought Tolerance

In order to understand, not only the ways that hybrids may tolerate stress compared to inbred lines, but also how drought tolerance can be improved upon, new methods of phenotyping and screening must be developed. It is difficult to find previous literature of an advanced screening method that involves only drought. Variability in environments usually means that several seasons are required to demonstrate the advantages of a certain cultivar (Passioura, 1996). Green (2016) developed a unique greenhouse screening method that made it possible to isolate drought stress. This allowed for an in-depth comparison between not only a drought and optimum treatment, but also a comparison between lines and hybrids. Evaluation of traits that are related to drought tolerance at physiological, cellular, and biochemical levels can help to better screen for plant response to drought (Praba et al., 2009). Looking at the physiological determinations of yield may lead to the identification of important traits related to not only higher yield but also to drought tolerance in wheat under water-limited conditions (Xue et al., 2014). Mass screening is a start to identifying effective drought-tolerant crops (Farooq et al., 2012).

Physiological traits that play a role in response to drought stress and are modified by it span a wide range of vital processes, meaning that pinning down a single response pattern highly

correlated with yield under all drought environments is difficult (Cattivelli et al., 2008). Leaf chlorophyll content can be measured directly by a simple handheld device called the SPAD-meter (Pask et al., 2012). The SPAD- meter uses this content from green tissue to give an estimate of photosynthetic potential, indirectly, the effects of stress. Stomatal traits have been proposed as a selection tool for measuring drought tolerance. When used on multiple plants, they can be equally as effective as something like canopy temperature or canopy temperature depression (CTD) which is usually defined as canopy temperature minus air temperature (Bahar et al. 2011). This means a negative CTD value means a cooler canopy. CTD has been used as a selection criterion in wheat breeding in terms of heat and drought stress tolerance, and it was reported that wheat cultivars with high CTD showed a trend of higher yield under heat and drought stress (Bahar et al. 2011). They can help give an idea about gas exchange capacity or resistance to gas exchange, which ultimately leads to another estimate of photosynthetic potential under abiotic stress. A downside is that instrumentation may not be robust and stomata are extremely sensitive, making measurements highly variable (Pask et al., 2012). Due to stomatal closure, an increase in stomatal resistance would be expected under water deficit stress. The dark adapted F_v/F_m, which is a measure of the intrinsic photochemical efficiency of light harvesting in photosystem II, is one of the most easily measured traits and is commonly used in stress studies (Munns et al., 2010). Using chlorophyll fluorescence allows for the determination of the status of the photosynthetic apparatus (Pask et al., 2012). It is easier to measure than gas exchange, and Araus et al. (1998) found that it can explain some genetic variation in crop performance while also providing useful knowledge of the intricate relationships between fluorescence kinetics and photosynthesis under drought stress (Sayed, 2003). $F_v//F_m$ was used in practice to rapidly estimate the tolerance of wheat genotypes to drought in a study conducted by Havaux et al. (1988). Leaf

water potential has proven to be a reliable response variable for quantifying plant water stress (Siddique et al., 1999). Measuring leaf water potential provides an estimate of adaptation to water stress by giving not only leaf water status but also an idea of the soil water potential in the active root zone (Pask et al., 2012). Osmotic adjustment is a highly important measurement due to the fact that stomatal function is dependent on turgor, photosystem function, and adaptation to water stress (Pask et al., 2012). Water-soluble carbohydrates (WSC) of leaves or stems (culm and leaf sheath) have been considered an important physiological trait indicative of drought tolerance, because of dual functions, i.e., not only acting in osmotic regulation as the osmolyte under adverse environmental conditions, but also contributing to grain growth and development as the dominant carbon source for grain yield when active photosynthesis is inhibited by terminal drought stress during the grain fill period (Blum, 1998; Yang et al., 2007). Stem reserves can serve as an important source of carbon and are essential for adequate grain filling, especially in a stressed environment where viable light intercepting green surfaces have diminished (Blum, 1998). In wheat, genotypes associated with drought tolerance maintain more extensive root systems, and selection for high yield under moisture stress does result in a larger root system (Hurd, 1974). Morphological traits are important to measure in order to get an idea on how the plant is adapting to drought stress. They provide essential information on the crop/canopy architecture. These measurements include traits such as plant height (Pask et al., 2012).

While the literature outlines several different physiological traits that can be screened to identify drought tolerance, a recurring theme is observed that, to be effective, the traits have to be positively related to yield. Perhaps the most important screening method is yield and its components. Yield is the ultimate expression of all physiological processes. Yield components allow for the determination of yield through source/sink relationships (Pask et al., 2012). It has

been suggested that yield performance over a wide range of environments should be used as the main indicator for drought tolerance (Cattivelli et al., 2008; Voltas et al., 2005). Perhaps the only problem with this ideology is that yield is a low heritability trait making selection for it more challenging. The goal of screening for physiological traits is to have a more heritable surrogate. Ultimately though, yield has to be measured and is the quintessential trait.

Chapter 2 - Comparison of Drought Tolerance among Winter Wheat Hybrids and their Parents Using a Comprehensive Screening Method

Introduction

Lack of ample moisture required for normal plant growth and development to complete the life cycle (Manivannan et al. 2008). This is a simple definition for the complex issue of agricultural drought. It is developed when the demand for water overcomes the supply (Blum, 2005). It has detrimental effects on the vegetative, reproductive, and grain filling stages. Agricultural drought is considered to be one of the most devastating environmental stresses to crop productivity, and many climate models predict it will become more severe in the future (Farooq et al., 2012).

The effects of drought on a plant depend on the susceptibility of the plant, the severity of the deficit, the cultivar, and the crop growth stage (Anjum et al., 2011; Farooq et al., 2012). Drought stress during the reproductive stage can have an impact on pollen viability and anther development. There is a consensus that this the growth stage most susceptible to water deficit (Blum, 2009). Substantial loss in economic yield is associated with reduction of absorbed

photosynthetically active radiation, lower radiation use efficiency, and a lower harvest index (Earl and Davis, 2003). Many plant processes are affected by drought stress from the cellular level to the whole plant level. Along with these, it has a strong influence on a crop's phenology by shortening the crop growth cycle (Farooq et al., 2012).

Wheat is one of the most widely affected crops to drought stress across the globe. It is a staple food crop and has a broad growing environment. It is grown for food and forage in the U.S. Southern High Plains (Xue et al., 2014). Water deficit has an effect on the wheat grown in this region by reducing leaf water potential and relative water content. These factors lead to a lower photosynthetic rate and a shortened maturity cycle. Many yield components are reduced including biomass, grain number per spike, spike weight, and grain yield per spike (Praba et al., 2009).

A drought tolerant plant must be able to handle major vicissitudes in water supply and high evapotranspiration rates during the growing season. Many yield-determining processes respond to water stress (Anjum et al., 2011). Levitt (1972) categorized plant physiological responses as tolerance or avoidance. Dehydration avoidance would be more commonplace, while dehydration tolerance is rare (Blum, 2005). Signaling is required as an early warning system for plants to escape drought using an appropriate method. Signals are key players in plant resistance to stress (Chaves and Oliveira, 2004).

Development of crops for drought tolerance requires a knowledge of physiological mechanisms and genetic control of the contributing traits at different plant developmental stages (Farooq et al., 2012). Breeding has already made significant steps towards better drought tolerance, but a comprehensive exploration of the potential genetic resources, and an in-depth understanding of what makes up the traits that allow for survival in an unfriendly environment

are required (Rampino et al., 2006). No straightforward method exists when breeding for drought tolerance. Many factors play a role when screening and making selections. New tools are being developed to allow for more efficient progress to be made. Hybrid wheat is one such tool that has been explored in the past but never widely deployed. It may hold the potential to deliver a major lift in yield by delivering a wide range of new breeding opportunities (Whitford et al., 2013). A continual point of discussion concerning hybrid wheat is how it performs when exposed to abiotic and biotic stresses. It has been speculated that hybrids may have lower stress susceptibility than related inbred lines, leading to higher yield stability (Mühleisen et al., 2014). It is important to understand the ways that hybrids may tolerate stress compared to inbred lines, and how drought tolerance can be improved upon using new methods of phenotyping and screening. Physiological traits that play a role in response to drought stress and are modified by it span a wide range of vital processes, meaning that pinning down a single response pattern highly correlated with yield under all drought environments is difficult (Cattivelli et al., 2008). While the literature outlines several different physiological traits that can be screened to identify drought tolerance, yield has to be measured and is the quintessential trait.

This study was conducted to provide a comparison among winter wheat hybrid entries and their parents under drought stress. The main objective was to identify whether or not the hybrids would handle water deficit stress better than their respective parents. We hypothesized that the hybrids would outperform their parents when exposed to post-anthesis drought stress. Some other objectives were to gain a better understanding of certain drought tolerance mechanisms and to improve upon the advanced screening method created by Green (2016).

Materials and Methods

The plant material was received from Dow-Dupont Pioneer. Two high performing hybrid entries and their respective parents were chosen for the experiment. Each hybrid entry and its respective parents were broken into two separate groups for individual comparison. An experimental name was assigned in order to protect the pedigree information. Group 1 consisted of Hybrid 1, Parent A, and Parent B. Group 2 consisted of Hybrid 2, Parent C, and Parent D. Seeds from each of the hybrid entries and their parents were planted into a small greenhouse tray containing Profile Greens Grade growth medium (Profile Products, Buffalo Grove, IL) and placed in a growth chamber at 21^0 C with 12 hour light intervals for one to two weeks to allow for even emergence. The seedlings were then moved to a vernalization chamber kept at 4.4^0 C for six weeks. Water was added to the tray once each week and a nutrient solution, Peters Professional Hydroponic Special (5-11-26), was added to the tray at the halfway point of vernalization.

The experimental units were 153 cm-tall polyvinyl chloride (PVC) tubes with an outside diameter of 15.24 cm. Each tube was cut lengthwise and clamped back together in order to carry out root analysis later on. The base of the tubes contained a size 60 mesh that allowed for drainage of water yet permitted retention of the growth media and plant material. Seven holes were cut in an evenly spaced fashion to allow for insertion of sensors along the length of the tubes. See Green (2016) for a detailed description of the screening system.

The PVC tubes were filled with the same Profile Greens Grade growth medium used in the greenhouse trays. This growth medium is a baked porous ceramic aggregate (Adams et al., 2014). Normally used on golf courses underneath greens, it has been extensively studied as a

potential plant growth medium. Its large particle size means it has macro pores that drain at high levels of volumetric water content (VWC) (Steinberg et al., 2005). Two factors make this media well suited for this screening. When packed to its maximum bulk density of 0.68 g cm^{-3} (Steinberg et al., 2005), it drains well, and it also allows for separation of the root material. The one downside to this form of growth medium is that nutrients must be supplied during each irrigation event. The growth medium was dried until it reached a consistent gravimetric water content of approximately 0.02 g g^{-1} before it was packed to its maximum bulk density into each of the tubes. This allowed for accurate calculations assuring that each tube would have a relatively consistent bulk density. The method and equation used to fill the tubes were the same as those used by Green (2016). Four "lifts" (each "lift" had a known mass of growth medium) were used to fill each tube. Mass of the media required for each lift was calculated using the equation $M = \rho_b V(1+\Theta_g)$ where M is mass (g), V is the volume of growth medium (cm^3) in each lift, Θ_g is the gravimetric water content (g g^{-1}), and ρ_b is bulk density (g cm^{-3}). As each lift was added, the tube was tapped repeatedly to ensure uniform bulk density throughout the tube.

Once the vernalization period was complete, the PVC tubes were saturated with water and five uniform seedlings were transplanted into each of tube. There were 36 tubes in all, and this total was broken into two equal halves for treatments. Photoperiod intervals were controlled through supplemental growth lights with an intensity of 775 μM $m^{-2}s^{-1}$. For the first four weeks, plants received 12 hours of light. After that, they received 14 hours of light for two weeks. A 16-hour photoperiod was used throughout the rest of the experiment. Temperature in the greenhouse was set at 21^0 C during the day and 15.5^0 C at night.

The experimental design for the screening was a split-plot design. One factor was the drought treatment and the other was the optimum treatment. It was a completely randomized

design within each treatment factor, and each entry was replicated three times per treatment. Pairing for physiological measurements across treatments was based upon similar maturity dates. Both treatments were handled the same until heading. Water and equal amounts of fertilizer were applied daily based upon sensor data. The entire experiment was replicated twice. The first replication ran from December 2016 to March of 2017, and the second went from March 2017 to June 2017.

Three different types of sensors were used to monitor soil water status. The first type was the EC-5 volumetric water content sensor (Decagon Devices, Pullman, WA). All tubes contained four of these at evenly spaced intervals in order to model and maintain the water content. Next was the MPS-6 sensor (Decagon Devices), which is able to measure matric potentials from -9 to -100,000 kPa. Three of these sensors were evenly spaced on each tube of the drought treatment. Each MPS-6 had a ceramic plate that was coated with a fine silicate powder in order to improve hydraulic conductivity between the sensor and the growth medium. To pair with the MPS-6 sensors, three mini-column tensiometers (Soil Measurement Systems,7090 N Oracle Rd, Tucson, AZ) were evenly spaced on the optimum treatment at equal depths. Tensiometers were special ordered with a 16 cm barrel. They were paired with a pressure transducer (Honeywell, 2080 Arlingate Lane Columbus, OH) that has an effective range down to -34 kPa. This was an adequate measure for the optimum treatment.

Sensors were wired into seven AM16-32B multiplexers (Campbell Scientific, Logan, UT). Each multiplexer allowed for up to 48 EC-5 sensors and up to 16 tensiometers. The multiplexers were contained in a "Data Acquisition Cabinet". This cabinet kept the data acquisition system free from dust and moisture. Multiplexers communicated sensor readings to two CR1000 dataloggers (Campbell Scientific). Dataloggers stored readings four times a day

from each of the sensors and stored them on the control program Loggernet (Campbell Scientific). This sensor information was also used to control the automatic watering system.

The watering system utilized information from the EC-5 volumetric water content sensors in order to maintain a consistent moisture level throughout the experiment and eliminate the need for manual watering. Readings were checked from the top depth sensor in each tube. Each tube was assigned an individual 12V solenoid valve that could be run automatically or overridden once the treatment was initiated. Plastic tubing led from the solenoid valve down to an emitter placed right above the surface of the Profile substrate. The program used scheduled watering scans at 8:00 am and 8:00 pm. If the volumetric water content reading from the top EC-5 sensor fell below the 38% threshold, the watering system was triggered to water for five minutes. This threshold was imposed in order to maintain a well-watered condition throughout the day because the growth medium drains quickly, and plants rapidly used water. It was determined by measurement that during the five minute period, the emitters supplied 189 mL of water/nutrient solution. Fertilizer used was Peters 5-11-26 professional hydroponic nutrient solution (Hummert International, 1415 N.W. Moundview Drive Topeka, KS). This was supplemented with calcium nitrate. The nutrient solution was mixed based upon labeled rates and added to a 15-gallon tank full of reverse osmosis water supplied in the greenhouse. The tank was connected to a pump that hooked into the main irrigation system manifold.

Up until treatment initiation, all tubes were well-watered based upon the EC-5 data. Because the Profile was so well-drained, it was decided to impose a period of mild drought stress for 10 days post-flowering. This would allow for certain drought tolerance mechanisms, such as osmotic adjustment, to develop without being exposed to a sudden severe stress and to mimic more closely the way drought develops under field conditions. During this mild stress period,

matric potential data were taken from the top depth MPS-6 sensor. Low water levels were applied (about 1-2 minutes) in order to maintain the matric potential of the soil in the given tubes at around -5 bars. Once the 10 days of mild drought stress were complete, water was completely shut off. A toggle switch on the irrigation control panel allowed for an override of the program controlling the individual solenoid valves. The optimum treatment continued to receive water based upon the 38% volumetric water content threshold until at least three of the plants in each tube had primary tillers reach physiological maturity as determined by a having a yellow peduncle.

Plant measurements were initiated once the 10 day mild stress period had ended. The tenth day of the mild stress period was considered to be moisture treatment day 0. Three different categories of measurements were taken: physiological, agronomic, and root. Physiological measurements were taken on only primary tillers throughout the experiment. Measurements were taken every other day for the drought treatment, and every four days for the optimum treatment. They were taken continually until either physiological maturity or stressed leaves no longer allowed for an accurate measurement. A SPAD-meter (Spectrum Technologies, 3600 Thayer Court, Aurora, IL) was used to measure chlorophyll index. These measurements were taken from three separate flag leaves and on three portions of the flag leaf: the base, the middle, and the tip leading to an average of nine measurements per tube. Stomatal resistance was taken with an SC-1 leaf porometer (Decagon Devices). Two to five plants were measured per tube based on flag leaf variability. If two of the stomatal resistance measurements fell within 100 s^{-1} m^{-1} of each other, the measurements were concluded for that tube. Each measurement was taken at the base of the leaf on the adaxial surface. Leaf water potential was measured using a Model 1000 pressure bomb (PMS Instrument, Corvallis, OR), and determined by the pressure at which water

visibly extruded from the xylem tissue. Two separate leaf water potential readings were taken. The first set at visible lower canopy stress (chlorosis, wilting, lower leaf senescence). Three F-1 leaves, which are produced directly below the flag leaf, were measured from the drought treatment and the optimum treatment. The next set of measurements was taken when visible flag leaf stress (wilting, leaf curling, leaf tip necrosis) occurred. Three flag leaves were measured and then immediately rehydrated for two hours in double distilled water and placed in a freezer at -20° C until osmolality measurements could be taken. At both the point of lower canopy stress and flag leaf stress, soil water potential values were recorded. Osmolality was taken with a Vapro vapor pressure osmometer model number 5600 (Wescor, Logan, UT). Leaf tissue samples were removed from the freezer and allowed to thaw. Tissue from each of the three flag leaves was placed into 1.5 mL microtubules and ground up. Paper disks were saturated with leaf sap and placed in the osmometer to obtain osmolality readings. Osmotic potential was calculated from Kirkham (2005, p. 308). From the osmotic potential values, osmotic adjustment was calculated by finding the difference between drought stress and optimum treatments.

Certain agronomic traits were measured during the treatment cycle. Days to lower leaf stress were calculated by subtracting the treatment initiation date from the date that visible lower canopy stress occurred. Days to flag leaf stress were calculated similarly, with treatment initiation date being subtracted from visible signs of flag leaf stress. Grain fill duration was calculated by subtracting heading date from the point at which each tube either senesced or reached physiological maturity. The reason for the use of physiological maturity along with senescence while calculating grain fill duration was that certain genotypes in the drought treatment reached physiological maturity while leaves were still healthy enough to get viable physiological measurements. The rest of the agronomic traits were measured following

completion of the experiment. Plant height was taken from the base of the plants to the tip of the spike, not including awns and was averaged over five primary tillers. Plants were harvested at the base and placed in a dryer at 50° C for two days. Once drying was complete, total aboveground biomass was measured for all entries. Spikes were then harvested, counted, and weighed in order to get spike count and harvest weight. Harvest weight was recorded as the mass of all the spikes from an individual tube. Thousand kernel weight was calculated by counting out 100 seeds from each entry, getting a mass measurement, and multiplying that by 10. Once thousand kernel weight was measured, total seed count was the last agronomic trait to be measured. Total seed count was acquired using an Old Mill 9000 seed counter (Old Mill Company, Savage Industrial Center, Savage, MD)

Root characteristics were the last to be measured. Once plants had been harvested from the tubes, each tube was laid out horizontally on a greenhouse bench and split open. Root depth was measured as the bottom of the main root mass. It was measured this way due to the fact that some of the root systems had "runners" or single root strands that would follow a seam in the tube down to the bottom. Once depth had been calculated, root material was carefully removed from the profile and cleaned of any major foreign material. Roots were cleaned, washed, and placed in a dryer at 50° C for two days. Once the drying process was completed, roots were removed and mass of the root structure was taken for each entry. A method utilizing Archimedes principle developed by Green (2016) was used to calculate the root volume via water displacement.

Initial statistical analysis of the data showed a significant experiment effect; therefore, the experiments were analyzed separately as a split plot. The comparison across treatments was analyzed as a completely randomized design. The observed experimental effect

was likely due to different greenhouse conditions. Temperature varied, but Experiment Two had a higher average temperature than Experiment One. Agronomic and root characteristics were compared using simple contrasts, and they were analyzed separately from physiological data due to the fact that the physiological data were taken over several days. Genotype and treatment were both treated as fixed effects. Treatment day was labeled as a random effect for the physiological analysis. Data were analyzed for both experiments using SAS version 9.4. Each hybrid and its respective parents were separated for comparison. Type, the identification as either a hybrid or a parent, and group, the identification of one hybrid parent combination, were both nested within all genotypes. Proc Glimmix analysis of variance within groups, between parents and hybrids as a whole, across all genotypes, and across both treatments were completed. For physiological leaf traits, a Proc Glimmix procedure was used to compare the slopes across all genotypes on each of the treatment days. This analysis also covered within group, parents and hybrids as a whole, all genotypes, and across treatments for all three replications in each experiment. Stomatal resistance was only analyzed for Experiment One due to instrument issues during the second experiment. All statistical analyses were completed at α levels of 0.1, 0.05, and 0.01.

Results

Treatment analysis was run for every trait measured to ensure there were differences between the drought and optimum treatment. All graphs use standard error bars to show variance. Table 1 summarizes the significant responses for the treatment effect for all agronomic and root characteristics along with leaf water potential.

Table 1: Significant responses for the treatment effect for all agronomic and root characteristics along with leaf water potential within each experiment. *p<0.1, **p<0.05, ***p<0.01, NS- not significant

	Treatment	
Measured Trait	**Exp. 1**	**Exp. 2**
Grain Fill Duration	***	***
Plant Height	NS	NS
Aboveground Biomass	***	***
Spike Count	NS	NS
Harvest Weight	**	NS
1000 Kernel Weight	**	**
Total Seed Count	NS	NS
F-1 Leaf Water Potential	***	***
Flag Leaf Water Potential	***	***
Root Depth	NS	NS
Root Mass	***	*
Root Volume	***	NS

Table 2 includes the overall grand means for the optimum and drought treatments while including the level of significance for each.

Table 2: Significant responses across several measured traits for the treatment effect with the grand mean for each treatment. *p<0.1, **p<0.05, ***p<0.01, NS- not significant

Measured Trait	Drought Mean	Optimum Mean	Level of Significance
Grain Fill Duration	27.5	31	***
Plant Height	63	64	NS
Aboveground Biomass	17.6	21.1	***
Spike Count	9.6	10	NS
Harvest Weight	10	12.1	**
1000 Kernel Weight	29.7	28	**
Total Seed Count	258	252.8	NS
F-1 Leaf Water Potential	-16.6	-9.5	***
Flag Leaf Potential	-20.1	-9.3	***
Root Depth	87	81.3	NS
Root Mass	7.1	3.7	***
Root Volume	14.6	9	***

Table 3 summarizes responses for agronomic characteristics, root traits, and leaf water potential across all comparisons (Parent vs. Hybrid overall, within group, all genotypes).

Table 3: Significant and non-significant responses for all agronomic characteristics, root traits, and leaf water potential. Data are divided by experiment, then by treatment for all comparisons. *p<0.1, **p<0.05, ***p<0.01, NS- not significant

	Parent vs Hybrid				Within Group				All Genotype			
	Exp 1		Exp 2		Exp 1		Exp 2		Exp 1		Exp 2	
	Dr.	Op.	Dr.	Op.	Dr.	Op.	Dr.	Op.	Dr.	Op.	Dr.	Op.
Grain Fill Duration	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Days to Lower Leaf Stress	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Days to Flag Leaf Stress	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Plant Height	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Aboveground Biomass	**	**	NS	NS	NS	NS	NS	NS	**	***	NS	**
Spike Count	NS	**	NS	NS	NS	**	NS	NS	NS	**	NS	NS
Harvest Weight	NS	*	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
1000 Kernel Weight	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Total Seed Count	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F-1 Leaf Water Potential	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Flag Leaf Water Potential	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Root Depth	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Root Mass	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Root Volume	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS

Grainfill Duration

For grain fill duration in Experiment One, there was a significant difference between treatments (Table 2) with the optimum treatment mean being approximately three days longer than the drought treatment. No significant differences were found between the overall grand mean and within group analysis of hybrids and their parents (Table 3). In the across genotype analysis, the only significant difference was between the longest grain fill duration (Parent C at 31.3 days) and the shortest duration (Parent D at 23.7 days) during the drought treatment (Fig. 1). The relative mean when compared to the paired optimum treatment for Parent C was 94%. For

Experiment Two, significant differences were seen between treatments (Table 1). No other significant differences were seen when comparing hybrids vs. parents and across all genotypes.

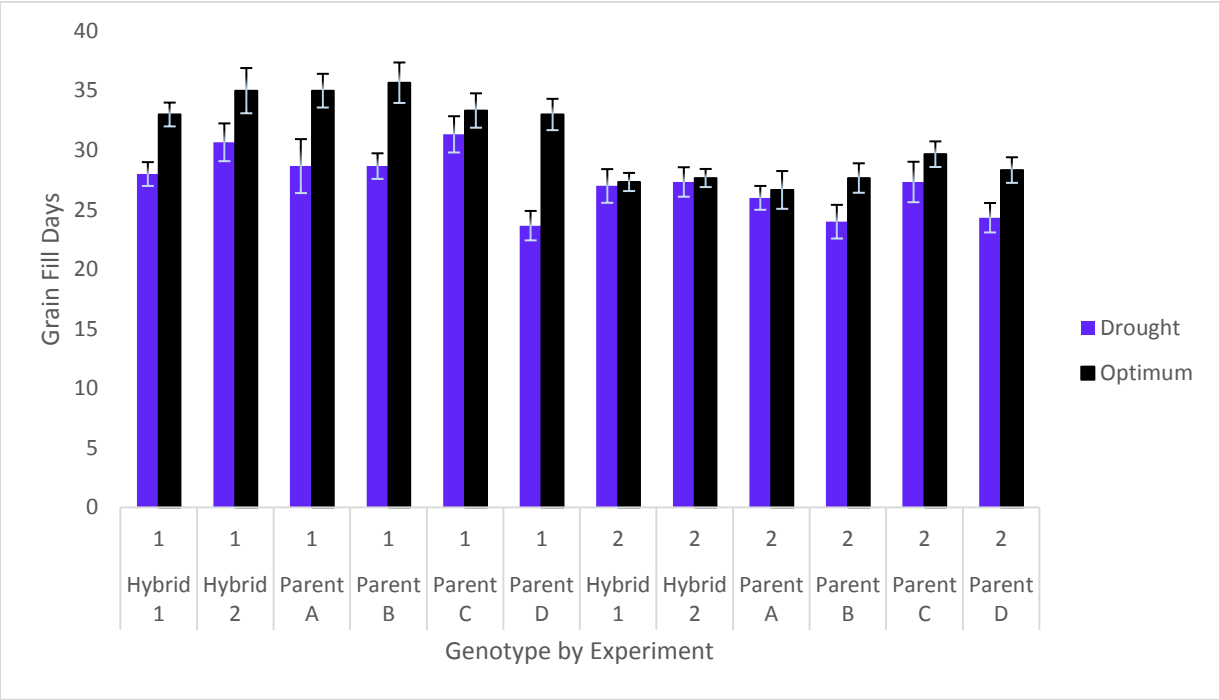

Figure 1: Grain fill duration across all genotypes separated by experiment and treatment.

Days to Lower Canopy Stress

Analysis of days to lower canopy stress for both experiments produced no significantly different results across any of the comparisons (Table 3). Though non-significant, Parent C showed the longest period of time to exhibit signs of lower canopy stress for both experiments (13.7 and 12.7 days for Experiments One and Two, respectively), while Parent D was observed to have the shortest time (7.7 and 9.3 days for Experiments One and Two, respectively).

Days to Flag Leaf Stress

Results for days to flag leaf stress in both experiments were similar to those for days to lower canopy stress. No significant differences between hybrids and parents, and no significant

differences across all genotypes were observed (Table 3). Another point of comparison was that, once again, Parent C performed the highest (18 and 16 days for Experiments One and Two, respectively), while Parent D was again the lowest (12 and 11.7 days for Experiments One and Two, respectively).

Aboveground Biomass

It was observed that there was a significant difference in aboveground biomass between treatments across both experiments (Table 2). The mean for the optimum treatment was approximately three grams higher than the drought, i.e., 21.1g compared to 17.6 g. In Experiment One, when comparing the overall mean between parents and hybrids, the parents produced a significantly higher biomass than that of the hybrids (21.1 g vs. 17.6 g) in the drought treatment (Table 3). No significant differences were seen, though, after performing a within group analysis. Across all genotypes for the drought treatment, Parent B had the highest mean biomass at 22.9 g, which was significantly greater than only the lowest mean biomass which was that of Hybrid 2 (Fig. 2). The same comparison for the optimum treatment showed that the biomass for Parent B (34.8 g) was significantly greater than the three lowest genotypes Parent A (17.1g), Hybrid 2 (21.4g), and Hybrid 1 (24.3g) (Fig. 2). Biomass was significantly greater in Experiment One than in Experiment Two. In the second experiment, significant differences were seen between treatments, but significant differences among genotypes were only observed for the optimum treatment. Parent B had an above ground biomass of 27.43 g in Experiment Two, which was significantly greater than the lowest two genotypes (Parent A and Parent D). Parent D went from being one of the highest producers of biomass in the first experiment, to the lowest producer for both treatments in the second.

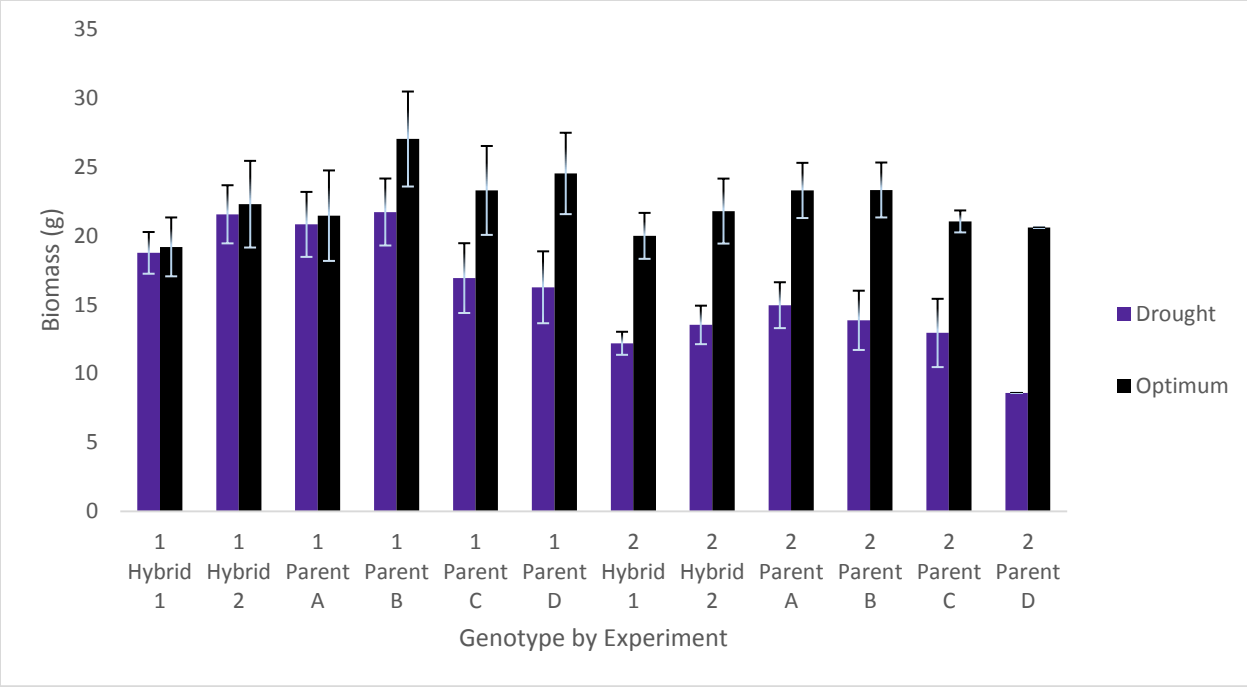

Figure 2: Aboveground biomass across all genotypes for both treatments and experiments

Leaf Water Potential

No significant differences were seen between experiments for F-1 leaf water potential during lower canopy stress. A significant difference was observed between treatments. The mean F-1 leaf water potential for the drought treatment was -16.6 bars, while the mean for the optimum was -9.5 bars. No significant differences were observed for any of the other comparisons (type, within group, across all genotypes) (Table 3). It was observed that, even though non-significant, Parent D samples produced the least negative water potential for the drought treatment across both experiments. In contrast, Parent B, one of the highest biomass producers, tended to have the most negative F-1 leaf water potentials for both experiments, at around -20 bars (Appendix A, Fig 35.

The difference was even greater between treatments for leaf water potential during flag leaf stress for both experiments. The mean for the optimum treatment (-9.3 bars) resembled that

of the one taken during lower canopy stress, while the mean for the drought treatment was about -20 bars. As seen in the water potential for lower canopy stress, no other significant differences were observed between parents and hybrids or across genotypes. Two hybrid entries fell between their parents for flag leaf water potential. Though no significant difference was seen between experiments for the overall mean, there was rank change across genotypes. For example, Parent D went from the most negative water potential (-25.8 bars) in Experiment One to one of the least negative in Experiment Two (-15 bars) (Appendix A, Fig. 36). This was observed for several of the traits.

Soil Water Potential

Soil water potential at the top root zone was taken at the point of lower canopy stress and flag leaf stress. Significant variability occurred among tubes, making the experimental error high. Soil water potential values for the drought treatment at the point of lower canopy stress ranged from -5 to -70 bars, while soil water potential values at flag leaf stress ranged from -6 to -76 bars. Loss of data due to system error did not allow for viable conclusions from the soil water potential data among genotypes.

Plant Height

Plants in Experiment One were significantly taller at 72.5 cm than in Experiment Two, where the mean height was 56.8 cm. No other significant differences occurred (Table 3). It was observed that Parent B, though non-significant, was the tallest across genotypes for both experiments (Appendix A, Fig 33). This parent was also one of the greater biomass producers.

Spike Number

No significant difference was observed between treatments for spike number (Fig. 3). Experiment One showed a significantly higher count than Experiment Two. When comparing the

overall mean for parents and the hybrids, the parents produced an average of approximately 2 more spikes per tube (10.9) than the hybrids (8.4) (Table 3). This significant difference was not observed within the group analysis. Across genotypes for Experiment One, there were no significant differences in the drought treatment, while, for the optimum, Parent B produced significantly more spikes (20) than the three lowest genotypes (Fig. 3). No significant differences across genotypes were observed for Experiment Two in either treatment. Parent B had one of the highest spike counts for both treatments.

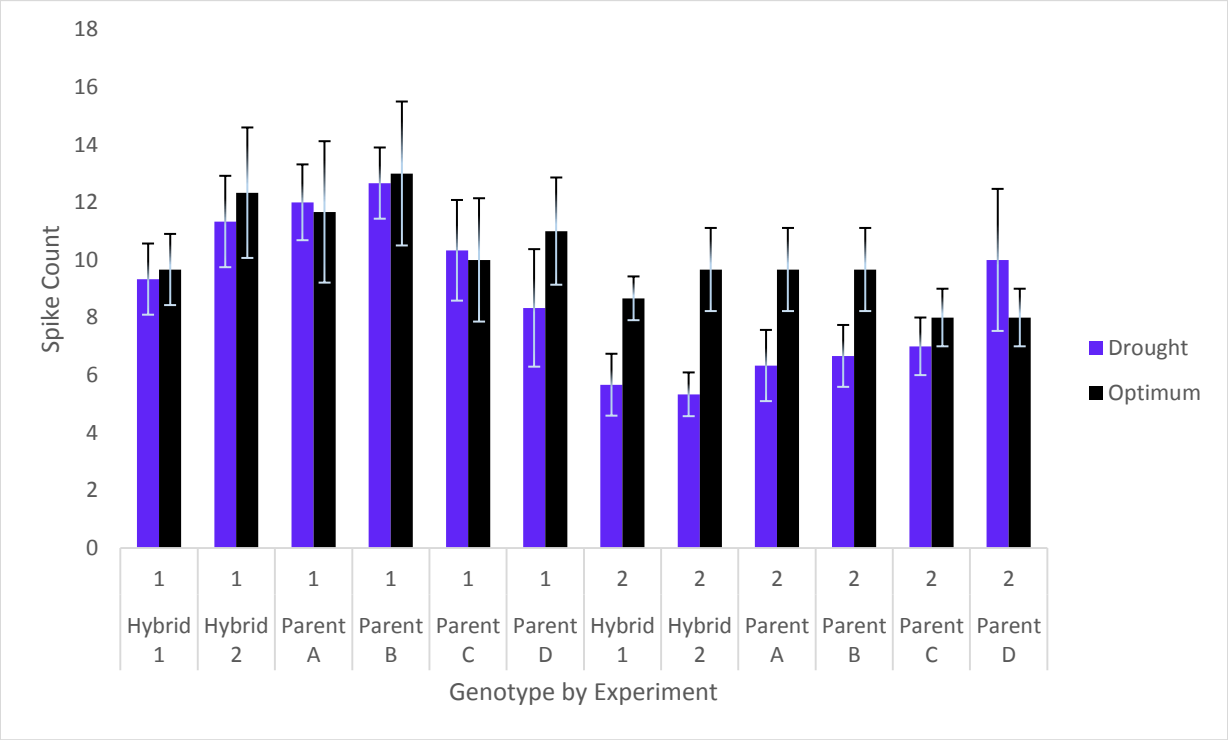

Figure 3: Genotype by experiment analysis for spike count across all genotypes.

Harvest Weight

Harvest weight (mass of all the spikes) analysis showed significant differences between experiments with the mean of Experiment One being five grams higher than that of Experiment Two (14.8 vs 9.6, respectively) (Appendix A, Fig 34). Significant differences were also seen

between treatments with the mean of the optimum treatment being consistently approximately two grams higher for both experiments. Although parents were significantly better than the hybrids when looking at the overall mean (Table 3), within group analysis showed that the only time both parents were significantly greater than the hybrid genotypes was for group two in Experiment One. No significant differences were observed in Experiment One for the drought treatment, while Parent B produced a significantly higher harvest weight (20.1 g) than the three lowest entries for the optimum treatment. Experiment Two yielded no significant results, though Parent B was ranked at the top for both treatments.

Thousand Kernel Weight

Grand means for thousand kernel weight were significantly different between experiments and across treatments (Tables 1 and 2). The mean for Experiment One was 31.5 g compared to 26.1 g in Experiment Two. The optimum treatment produced, on average, 1.5 more grams of seed per entry than the drought treatment. No significant differences were observed for the overall mean between hybrids and parents (Table 3), or within their respective groups. No significant differences were observed across any of the genotypes, but Parent B was ranked at the top for all except the drought treatment in Experiment Two.

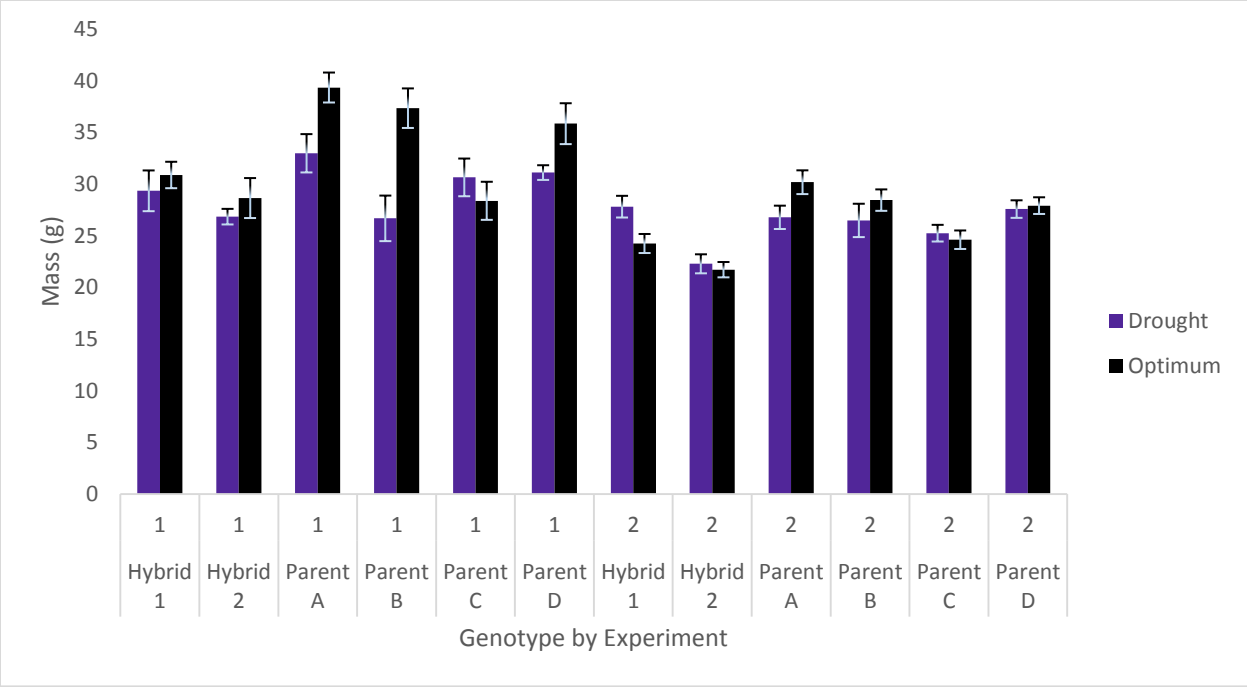

Figure 4: Genotype by experiment analysis of thousand kernel weight across all genotypes.

Total Seed Count

The trend between experiments continued for total seed count. Experiment One produced a significantly higher seed count than Experiment Two (299.1 vs 211.7) (Fig 5). No significant differences were observed between treatments. Treatment did not influence seed set, which was a goal of the timing of the stress. Parents also produced a significantly greater amount of seed than the hybrids (Table 3), with approximately 50 more seeds per entry on average. Within group analysis showed no significant difference. Though non-significant, both parents performed better than the hybrids for both groups. No significant differences were found across all genotypes for either of the experiments or treatments.

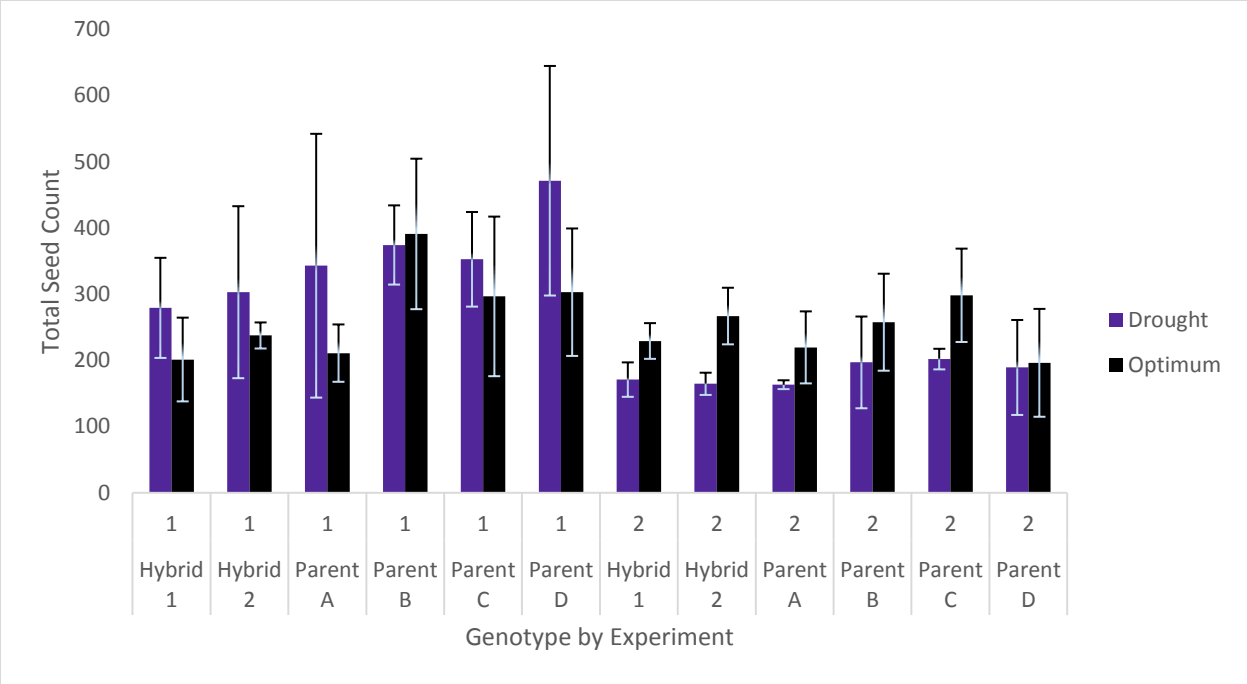

Figure 5: Genotype by experiment analysis for total seed count across all genotypes.

Parent C was one of the lower yielders for traits like harvest weight and thousand kernel weight, but it produced one of the highest seed outputs for both experiments and treatments. This will be a point of speculation later on in the discussion. Table 3 separates the analysis for agronomic characteristics into each separate experiment and treatment. The comparisons of parent/hybrid overall, parent/hybrid within group, and across all genotypes are shown.

Root Depth

Experiment Two had an average root depth of 92.5 cm, which was significantly greater than that of Experiment One where the average was 75.9 cm. No other significantly different results were observed for the rest of the comparisons (Table 3). While non-significant, the drought treatment mean depth was 87 cm while the optimum mean was 81.3 cm. The greatest mean root depth for Experiment One was 100 cm (Parent A), and the minimum mean depth was

64.3 (Parent B). For Experiment Two, the maximum mean depth was in the optimum treatment (112 cm). Parent D had the shallowest mean depth at 77.3 cm.

Root Mass

Root mass followed the trend of above ground biomass in that mass was significantly greater for the first experiment. Root mass in Experiment One averaged approximately four grams more than that in Experiment Two (Fig.6). The drought treatment was observed to have a root mass significantly greater than that of the optimum treatment (7.1 g vs 3.7 g). No other significant differences were observed for any of the comparisons (Table 3). When subjected to drought, though non-significant, Parent A had one of the greatest root masses for both experiments (13.6 and 3.6 g for Experiments One and Two, respectively).

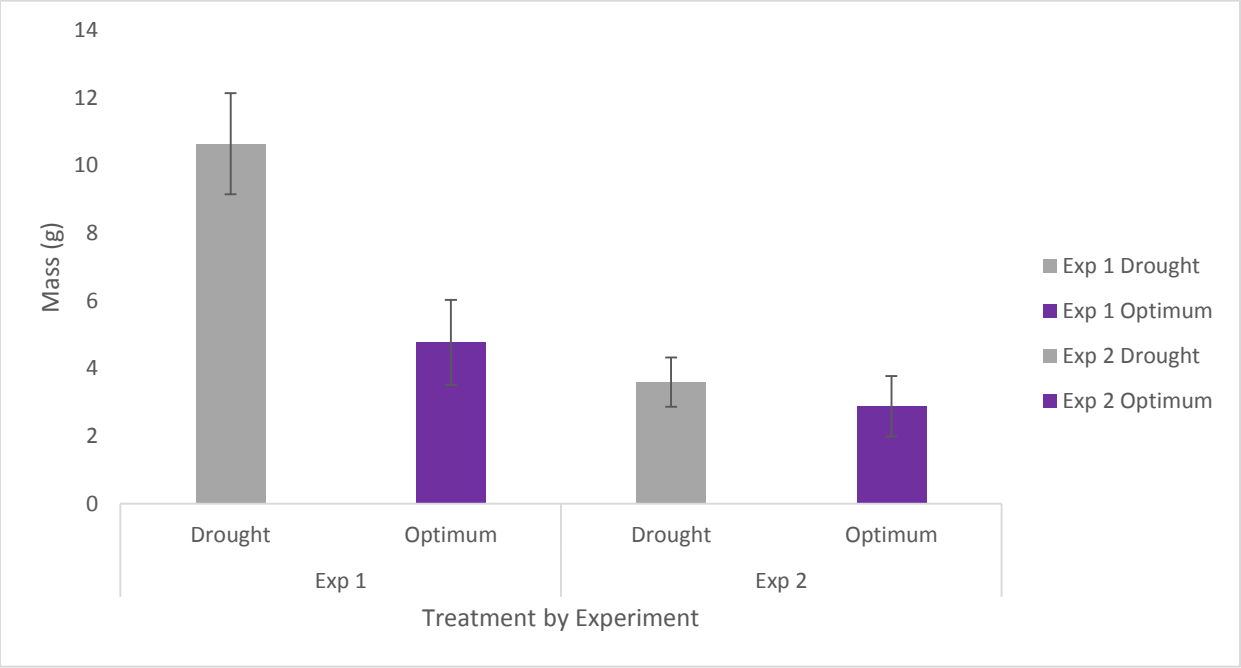

Figure 6: Root mass analysis between treatments for both experiments.

Root Volume

Root volume analysis showed that Experiment One had a mean root volume of 16.2 cm^3. This was over a twofold difference when compared to Experiment Two, which had a mean of 7.4 cm^3. The drought treatment once again had a significantly greater root volume than the optimum treatment (14.6 cm^3 vs 9.0 cm^3 for the drought and optimum treatments, respectively). No significant parent vs. hybrid differences were observed (Table 3). When comparing root volume across all genotypes, Parent D had a significantly greater volume (32.3 cm^3) than the lowest entry Hybrid 2 (15.3 cm^3). This was also the maximum root volume for both experiments and treatments. Though Parent D ranked high in root volume for the first experiment, it ranked last for both treatments in the second (5.8 cm^3 and 3.6 cm^3 for the drought and optimum treatments, respectively).

Physiological data (SPAD, stomatal resistance, and variable fluorescence) were analyzed based on treatment effects and differences in slope for all three measurements.

Chlorophyll Index

A type III test of fixed effects for chlorophyll index showed that the following were significant at an α of 0.05: Treatment, Day, Day*Treatment, Day*Experiment, Day*Treatment*Experiment. No significant differences were observed across all of the genotypes. Significant differences were observed between experiments from treatment day four onward. Increased temperature in the greenhouse during the second experiment is a possible confounding factor that would explain the decreased levels of chlorophyll seen in Experiment Two.

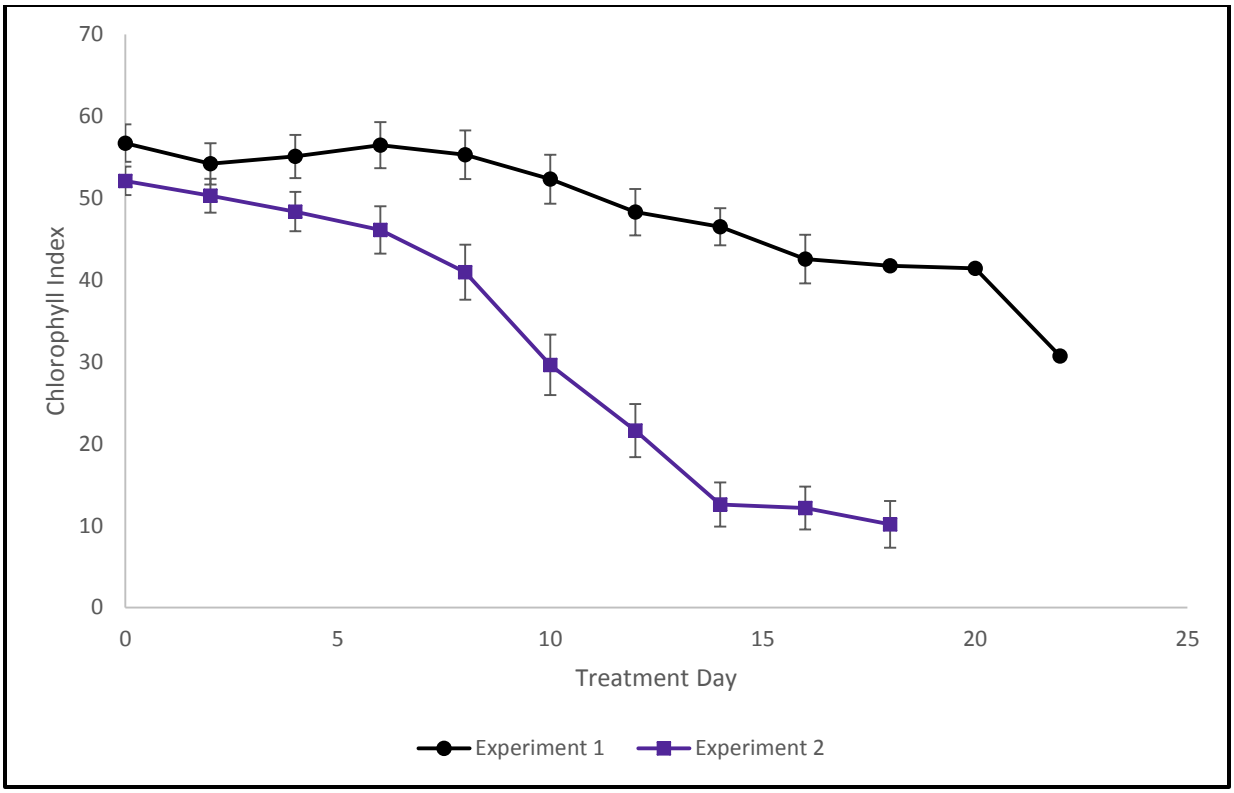

Figure 7: Chlorophyll index for Experiments One and Two.

The optimum treatment had a significantly higher chlorophyll index than the drought treatment following treatment day four as well (Fig 8). By the time of completion, the grand mean for the optimum treatment was approximately 40 units greater than the drought, which would be expected.

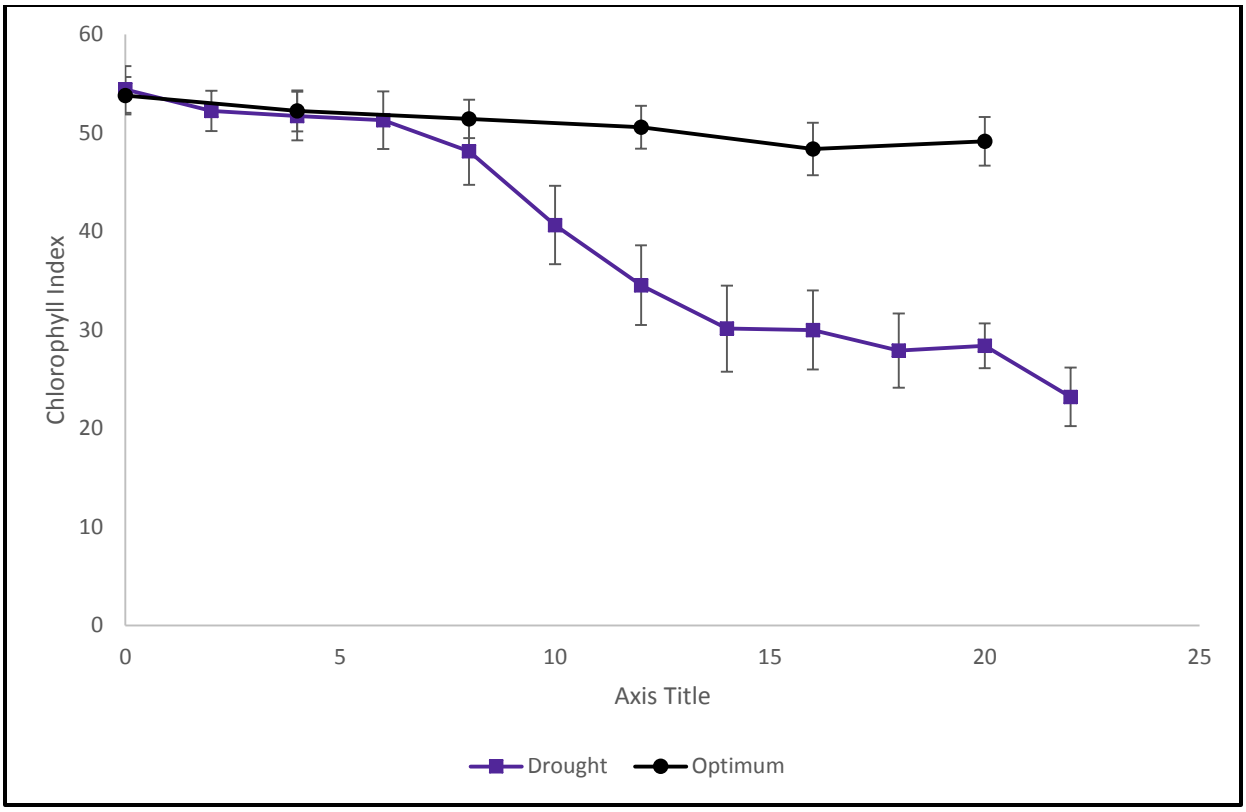

Figure 8: Chlorophyll index for the drought and optimum treatments

While there were no significant differences observed across genotypes, a within group analysis for both experiments in the drought treatment revealed some small differences. In Experiment One statistical and visual analysis of group two (Hybrid 2, Parent C, and Parent D) showed some small significant differences between the hybrid and its parents towards the completion of the treatment. Parent D was also observed to have significantly lower chlorophyll index values and a shorter grain fill period (Fig. 9).

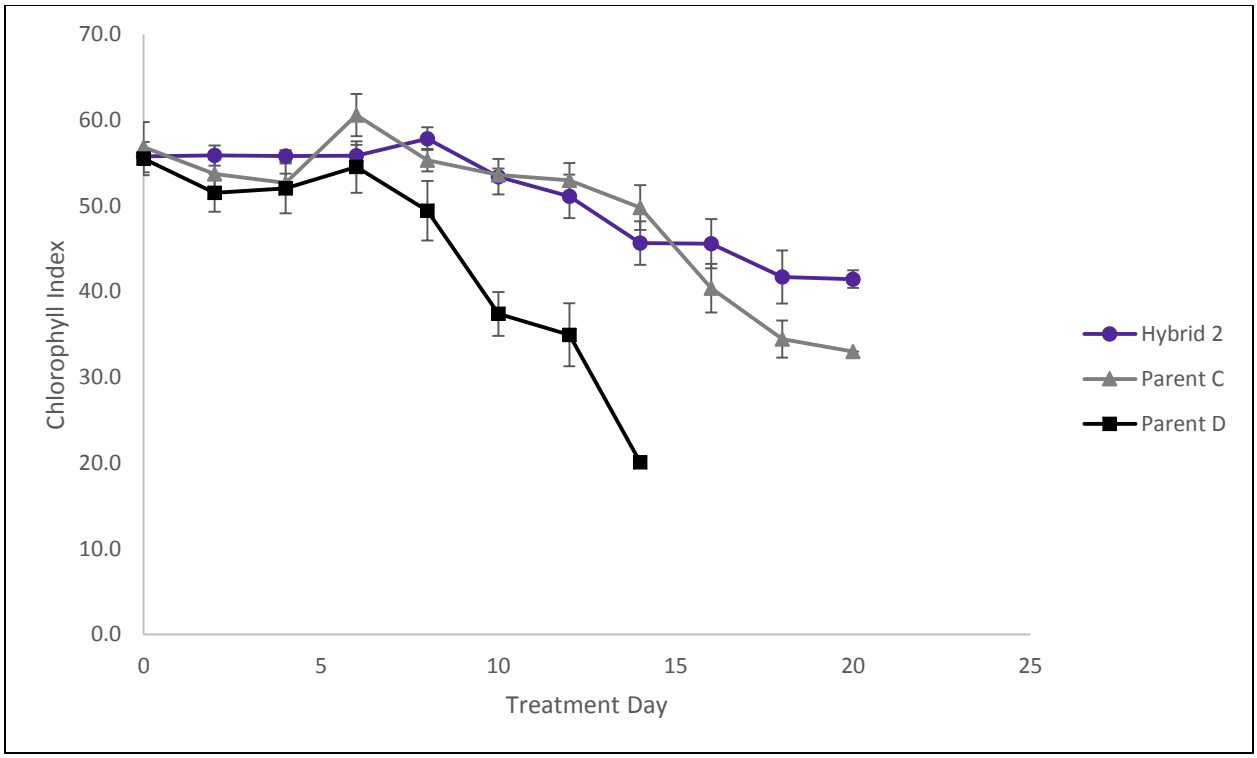

Figure 9: Analysis for Experiment One within group two for chlorophyll index; values are for the parents and the hybrid

Stomatal Resistance

In Experiment One, the same fixed effects were found to be significantly different for stomatal resistance at an α level of 0.05. Differences between day and treatment were observed. Experiment was not tested as a fixed effect for stomatal resistance due to lack of measurements from the second experiment. Once again, no significant differences were observed across all genotypes. Error levels increased greatly towards the end of the experiment.

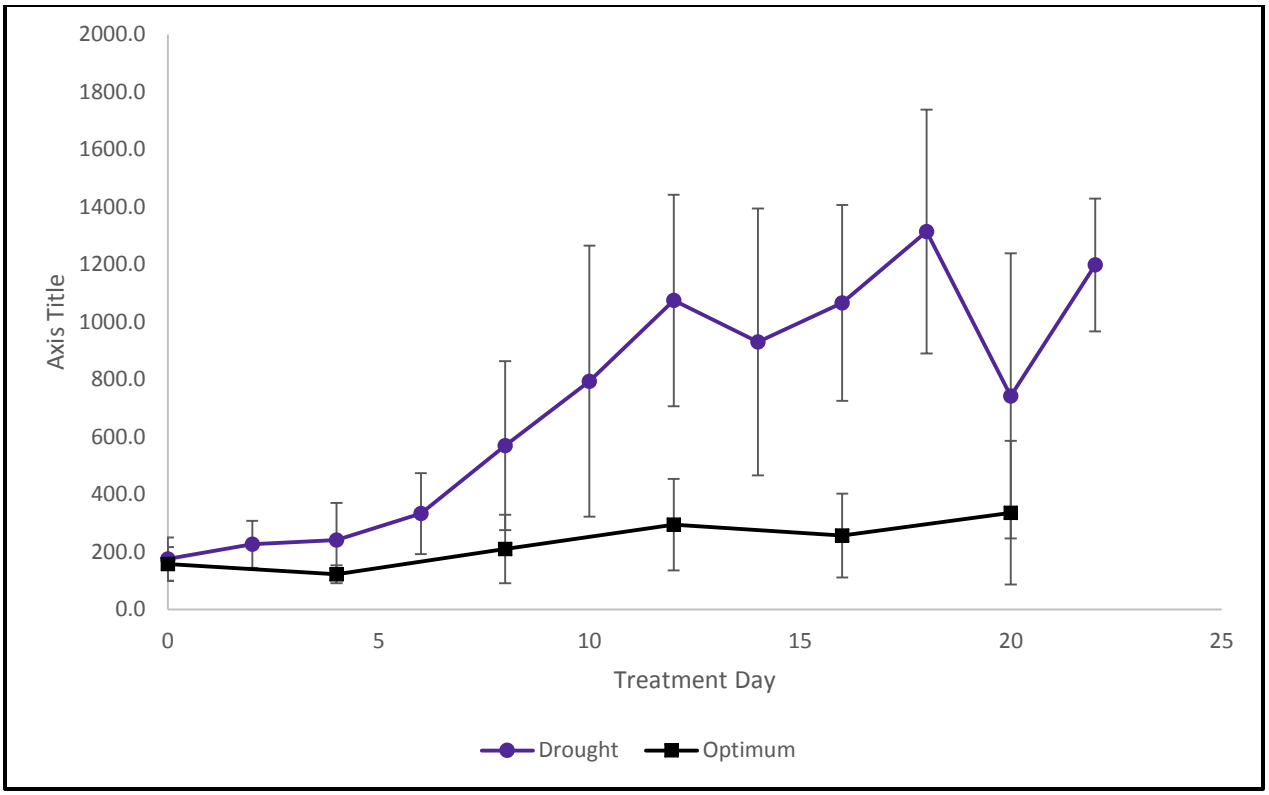

Figure 10: Analysis of stomatal resistance for Experiment One for the drought and optimum treatments

The drought treatment ended up with a grand mean of approximately 1,200 s m^{-1} while the optimum treatment fell at approximately 300 s m^{-1} (Fig. 10). The slope of the drought treatment was 49.7 s m^{-1} per treatment day, while the slope of the optimum was 9.9 s m^{-1} per treatment day. After an analysis within group two, it was observed that the rank order for stomatal resistance was similar to that of the chlorophyll index readings. It is difficult to define the final end rank due to plant variability that increased with increasing stress and resulted in a large standard error. Parent D ended the treatment with a stomatal resistance of approximately 1,000 s m^{-1} greater than the hybrid entry and other parent (Fig. 11).

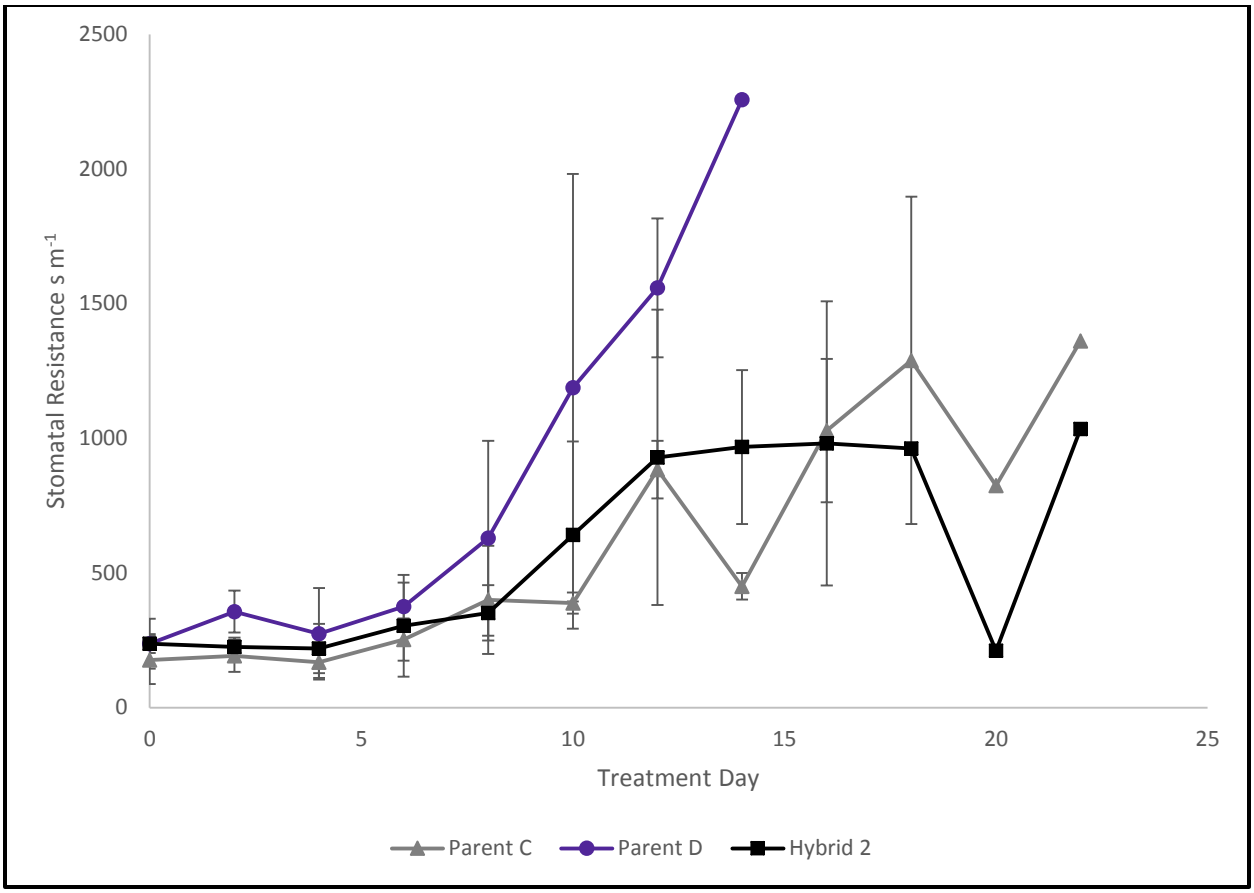

Figure 11: Stomatal resistance of the drought treatment in Experiment One for group two

Variable Fluorescence

Similar fixed effects were noted when analyzing variable fluorescence. Day and treatment were significant factors for both experiments, while genotype and experiment were non-significant. The standard error followed a trend similar to that of stomatal resistance. Following approximately treatment day 10, error began to increase rapidly, making it difficult to draw sound conclusions. It was observed that photosynthetic activity began to drop off between treatment day 12 and 16 for the drought treatment during both experiments. The Fv/Fm ratio for the optimum treatment ended at approximately 0.78 while, for the drought treatment, it fell below 0.6 (Fig. 12).

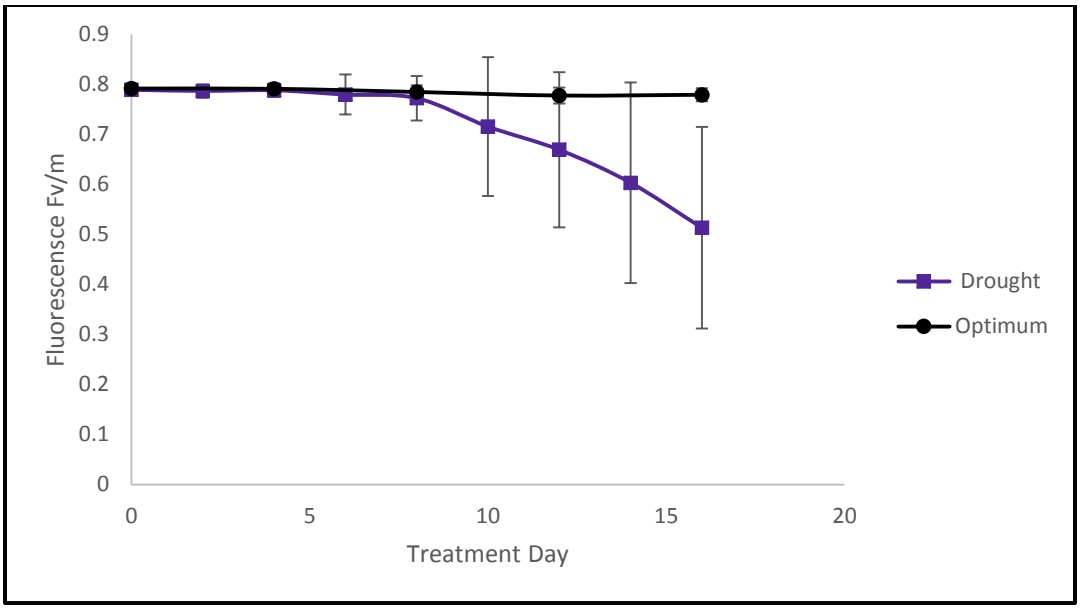

Figure 12: Fluorescence averaged over both experiments for the drought and optimum treatments

Within group analysis of group 1 for Experiment Two showed that Hybrid 1 maintained a ratio of greater than 0.7 throughout the treatment (Fig. 13). This was greater than the parent entries, but was not significant. Error became too large towards the end of the treatment due to plant variability.

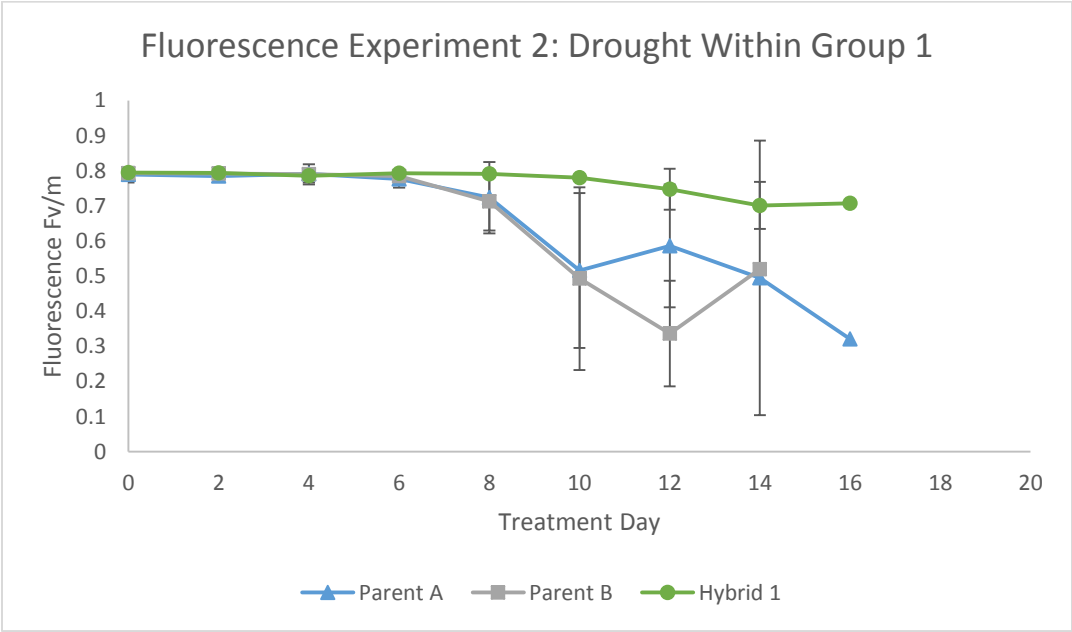

Figure 13: Fluorescence in Experiment Two of group one

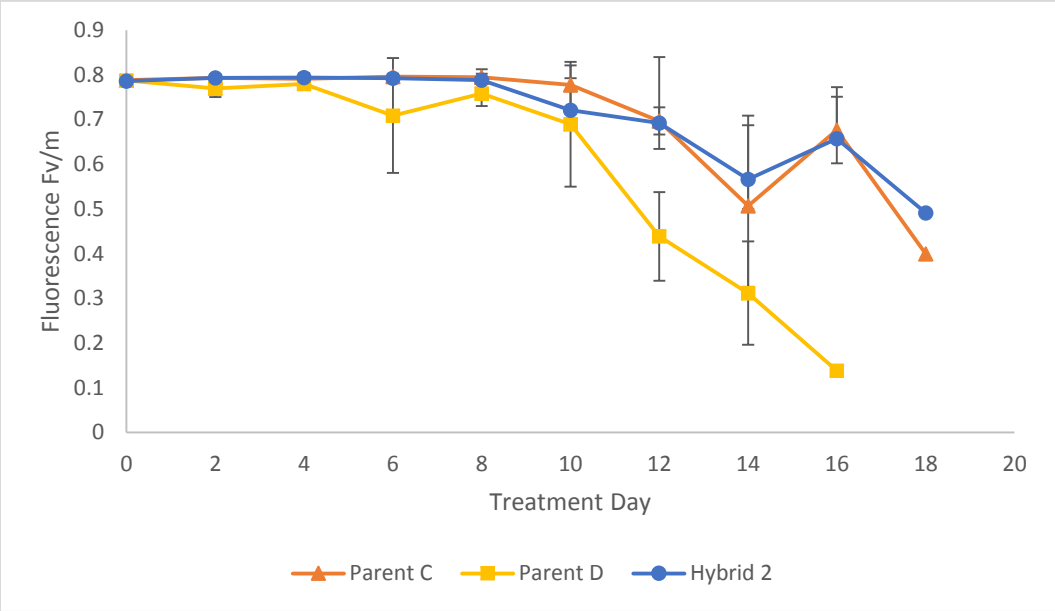

Figure 14: Fluorescence in Experiment Two of group two

Parent D once again had significantly lower photosynthetic activity than the hybrid and other parent entry. It ended with a ratio below 0.2 while the other two entries were virtually indistinguishable with ratios of approximately 0.5 (Fig. 14).

Discussion

The results from this study show that the hybrid offspring did not outperform the parents when exposed to severe post-anthesis drought stress. Hybrids failed to produce significantly greater biomass or root structure than the inbred parent lines. This does not agree with previous literature about hybrid vigor, but it could possibly be explained by variability within the system. The results of the drought treatment are in agreement with results from the field study of Oury et al. (1993) in which a severe drought affected the grain fill stage of wheat hybrids. Water deficit led to premature senescence and loss of valuable time required for grain fill. In another study, Oury et al. (1995) observed that heterosis for biomass and grain yield was associated with greater

assimilation post anthesis due to a greater capacity to fill grain. Hybrid yield stability stemming from heterosis is likely due to an increased biomass from early growth. This is a part of the important source-sink relationship. When exposed to a severe water deficit, the source or biomass was lost due to senescence, with a premature ending to the growth cycle. Without a full grain filling period, the hybrids were unable to out yield the parents under the drought treatment in the current experiment. The Profile substrate properties resulted in a more rapid and severe stress than would likely be seen in the field. This may have masked the grain filling effect for the hybrids. That being said, it also wasn't apparent in the control treatment. Further investigation is need in order to reach a solid conclusion.

In the current study, a depression in harvest weight and thousand kernel weight was observed due to drought. Total seed number stayed the same, which followed results from previous literature. The opposite would be expected for the optimum treatment. Hybrids would be expected to outperform their parents in an optimal environment. A significant advantage for the hybrids in the study was not observed here either. Some of this could be explained by limited replication or experimental error associated with growing a limited number of plants under artificial conditions. Something else to keep in mind is that only two experimental hybrids were tested. The results from this experiment do not necessarily extend to all hybrid wheat. Thousands of experimental hybrids exist across the globe, and it will require extensive testing of multiple hybrids to get a better understanding of the genetic and physiological basis of heterosis under drought stress.

Another goal of this study was to gain an understanding of different types of drought tolerance mechanisms. A point to be made is that two of the parents (Parent D and Parent B) came from the Kansas State University breeding program at Hays, while the other two (Parent C

and Parent A) came from the K-State breeding program of Manhattan, Kansas. The Hays program has a greater focus on drought tolerance while the Manhattan program has a greater focus on disease resistance and heat tolerance. Significant physiological differences were seen across all genotypes for the moisture treatment effect. Parent D had a shorter grain fill period and lower biomass. Its premature senescence was observed through a significantly lower chlorophyll content, lower fluorescence ratio, and higher stomatal resistance as shown in Figures 7, 9, and 12. Even though it appeared to be drought susceptible, it ranked highly for yield and yield components. The mechanism that allowed it to maintain yield is not exactly known, but it can be conjectured that this line was translocating assimilates from other parts of the plant. The exact opposite response was observed for Parent C. It held onto green leaf area up until physiological maturity, yet it did not yield well in the drought treatment. The opposite responses seen between Parent C and Parent D can be associated with recovery. A plant like Parent D will be able to survive in areas with terminal drought stress or areas where there is little rainfall expected during the grainfill stage as it is able to translocate assimilates after losing photosynthetic capacity. Lines with a response similar to Parent C would benefit more in an environment where there is a chance for intermittent rainfall allowing them to recover. Another parent, Parent B, was one of the highest yielders overall for both moisture treatments. It produced large amounts of tillers and biomass along with maintaining its green leaf area. Its ability to do this allowed for better performance than other genotypes by maintaining a proper source sink relationship. Another physiological trait considered to be an important indicator of drought tolerance is water soluble carbohydrates of the stem. These reserves can serve as an important source for adequate grain filling during water deficit (Blum, 1998). This trait was not measured in this experiment, but it could be the explanation for why Parent D still yielded well under drought stress. Stem reserves

may have been able to maintain adequate grain fill during the stress period while other sources declined due to senescence. This same trait may have played a role in the performance of Parent B. Going back to the point that both of these parent lines were selected for in Western Kansas, it is possible that water soluble carbohydrates in the stem played a role in their ability to yield in this environment.

Most of the traits measured did not differ significantly between hybrids and parents. The hybrids were often intermediate to the the two parents, which would be expected if the traits are controlled by additive genetic variance. One important observation is that where differences were observed, there were instances where the hybrid was more like one parent. Examples include Experiment Two: Hybrid 1 and Experiment One: Hybrid 2 for variable fluorescence as well as Experiment One: Hybrid 2 for stomatal resistance and cholophyll index. This suggests that these traits may be controlled by dominance. Additional research would be required to verify these effects, but these results would have implication for breeding hybrid wheat for drought-prone environments.

A lot of variability was observed throughout the drought treatment. Error for physiological measurements became very large towards the end of the treatment. Both experiments had to be analyzed separately due to greenhouse effects. During the first experiment, light pollution from a neighboring greenhouse had a small, but noticeable effect on plant health for the optimum treatment. The second experiment was conducted relatively late in the spring plants were exposed to higher air temperatures than in Experiment One. It is likely that the differences between the results of the two experiments can be at least partially explained by the effects of heat stress on the second experiment. Several measurements for the second experiment decreased due to increased temperatures. These complications to the experiment

could also explain some of the lack of significance between hybrids and parents. Plant variability within tubes was rather high with some plants being completely senesced while others still had measurable leaves. This could be explained by the idea of a dominant plant factor. Certain plants outcompeted others for resources within each tube leading to variable plant health. This constraint could be alleviated through greater replication in the system. Overall, this comprehensive method is good for identifying mechanisms of drought stress, but there are still constraints. Greenhouse experiments will always have the chance of light or heat contamination, and the overall cost effectiveness of the system remains a challenge for increasing replication.

Conclusion

In summary, the hypothesis stating that the hybrid genotypes would outperform the inbred parent lines under post-anthesis drought stress is rejected. It is important to understand that, though these were two high yielding hybrid lines, thousands of others have been created. Future expansion of measurements to reduce experimental variability and replication must be conducted in order to reach a more solid conclusion. We were able to identify some possible drought resistant traits and Parent C was added back into the Manhattan Kansas State University breeding program as a possible source for drought tolerance. This comprehensive screening method will continue to be useful in isolating drought stress in order to observe differences between elite varieties, wild relatives, hybrids, and new experimental lines.

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Appendix A - Supplemental Information

Table 4: List of all genotypes and designation as a hybrid or parent.

Genotype	Entry Type	Entry Group
Hybrid 1	Hybrid	1
Parent A	Parent	1
Parent B	Parent	1
Hybrid 2	Hybrid	2
Parent C	Parent	2
Parent D	Parent	2

Table 5: Final analysis of nutrient concentration applied through irrigation (Green, 2016).

Nutrient	Abbreviation	Concentration (ppm)
Nitrate	N	150
Phosphorous	P	48
Potassium	K	216
Calcium	Ca	116
Magnesium	Mg	31
Sulfate	SO_4	125
Iron	Fe	3
Manganese	Mn	0.5
Zinc	Sn	0.15
Copper	Cu	0.15
Boron	B	0.5
Molybdenum	Mo	0.1

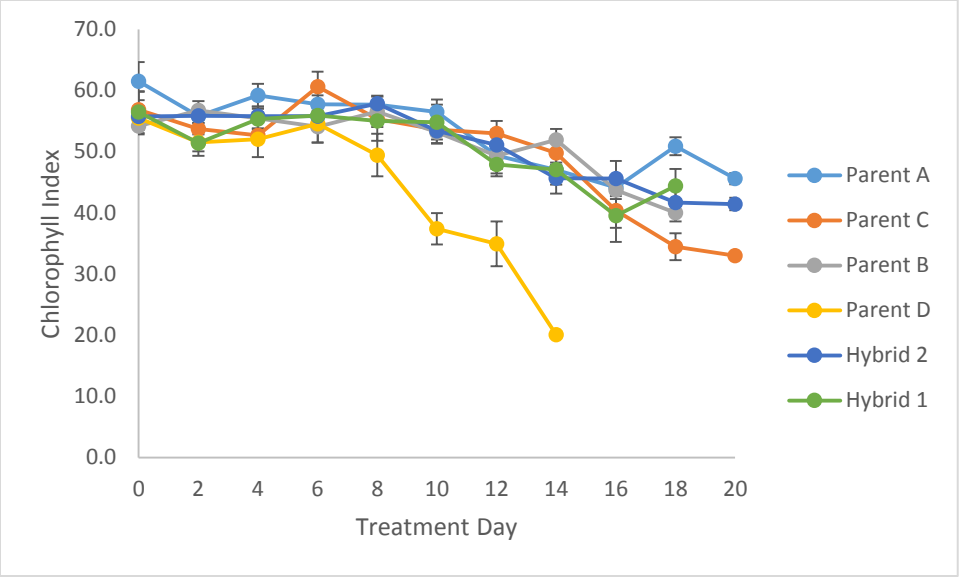

Figure 15: Chlorophyll index measurements from Experiment One for drought across all genotypes

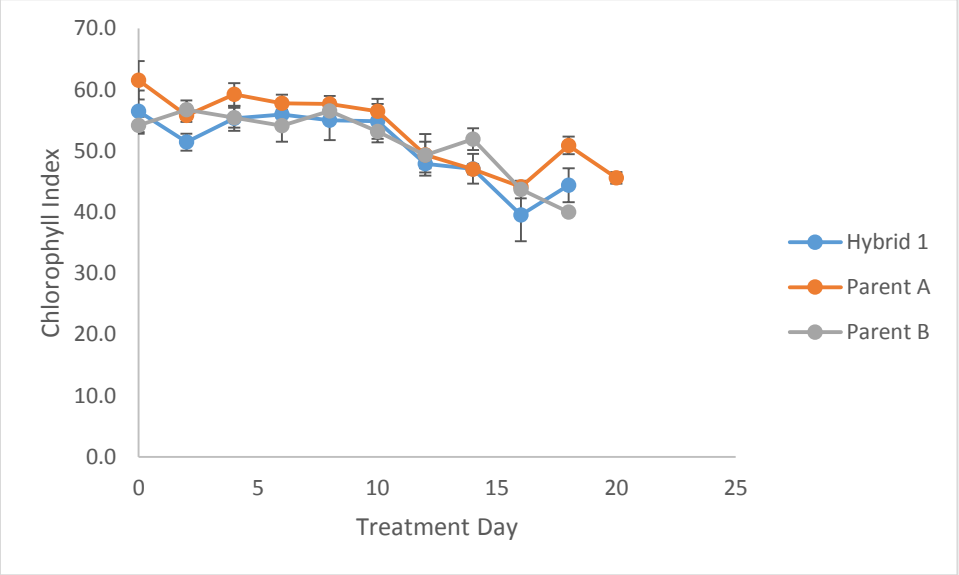

Figure 16: Chlorophyll index measurements for Experiment One for drought within group one

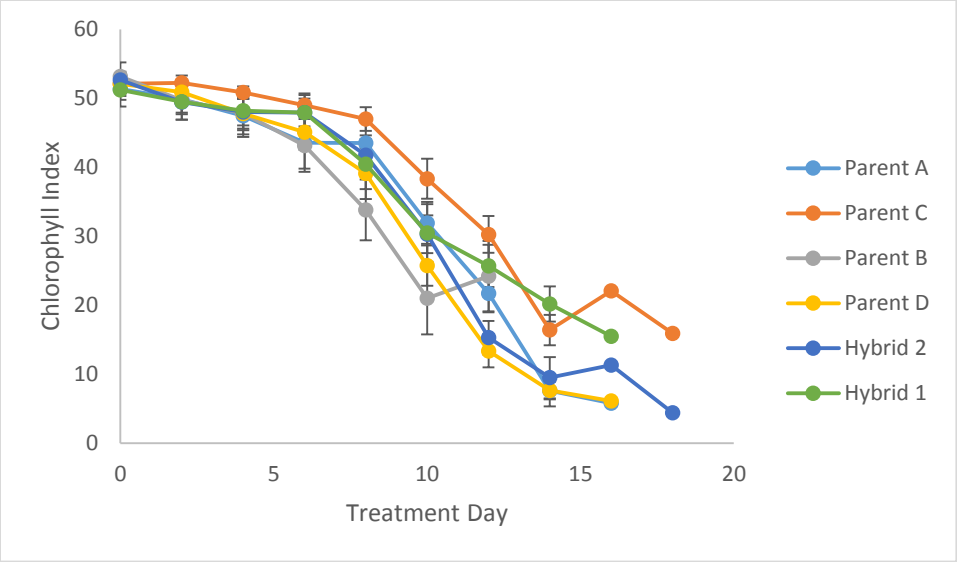

Figure 17: Chlorophyll index measurements for drought across all genotypes within experiment two

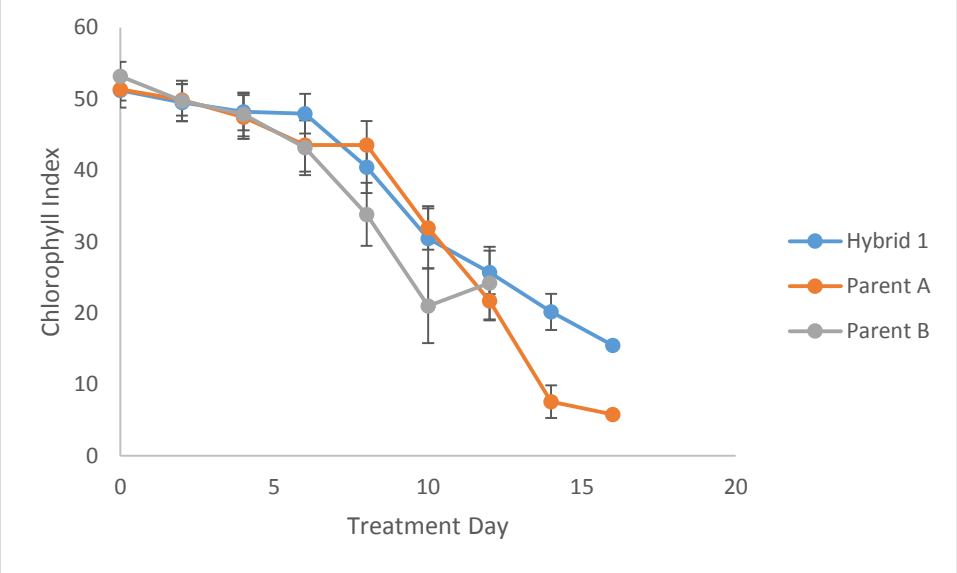

Figure 18: Chlorophyll index measurements for drought within group one in the second experiment

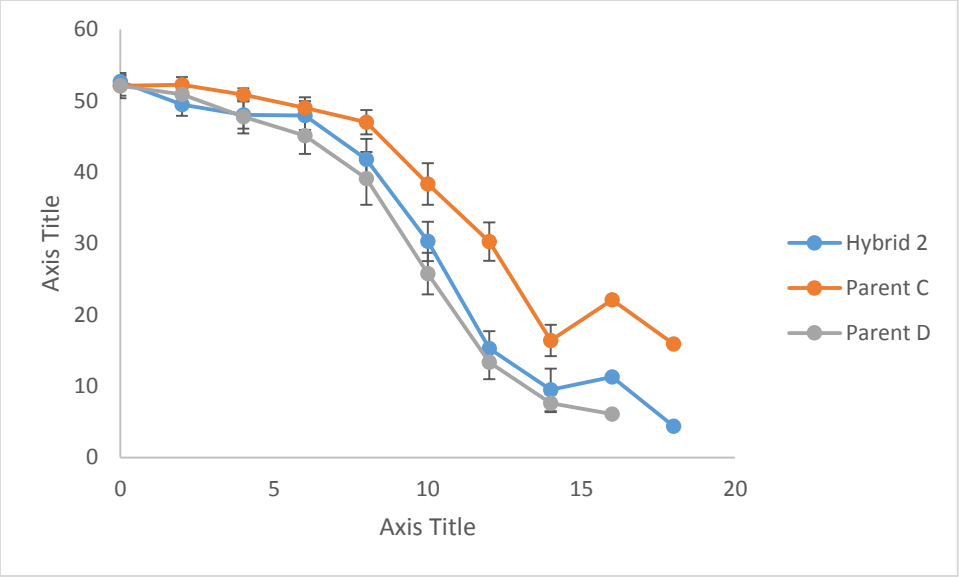

Figure 19: Chlorophyll index measurements for drought within group two in the second experiment

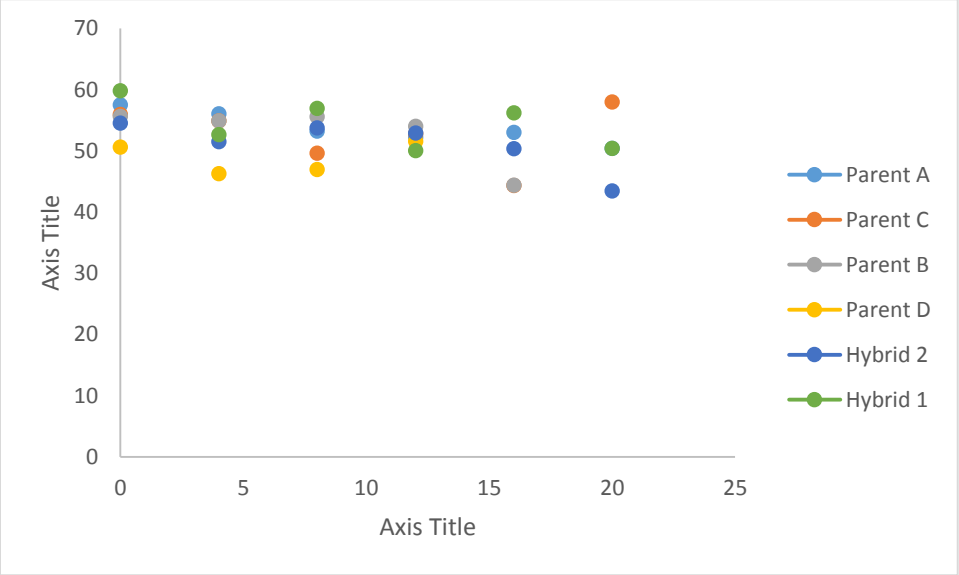

Figure 20: Chlorophyll index measurements for the optimum treatment across all genotypes in experiment one

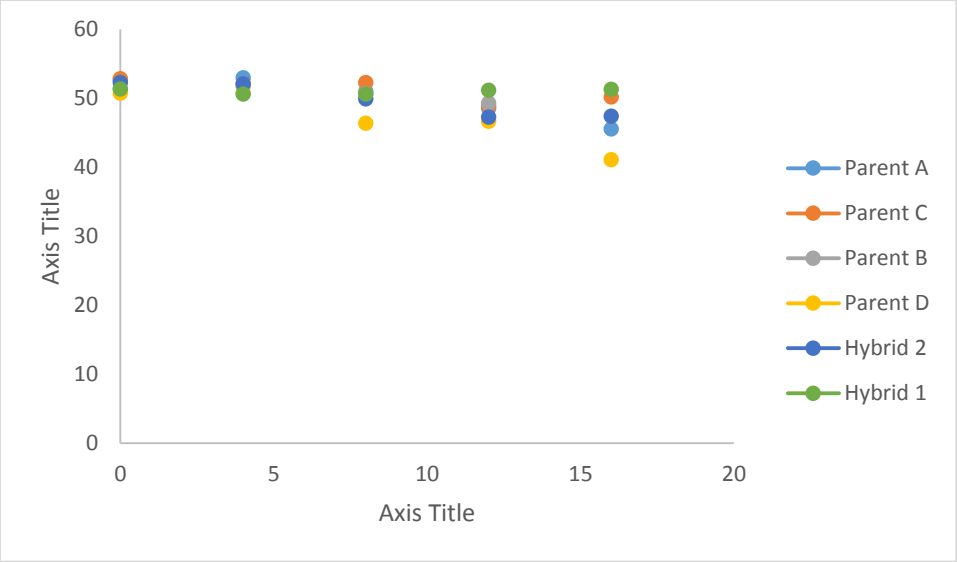

Figure 21: Chlorophyll index measurements for the optimum treatment across all genotypes for experiment 2

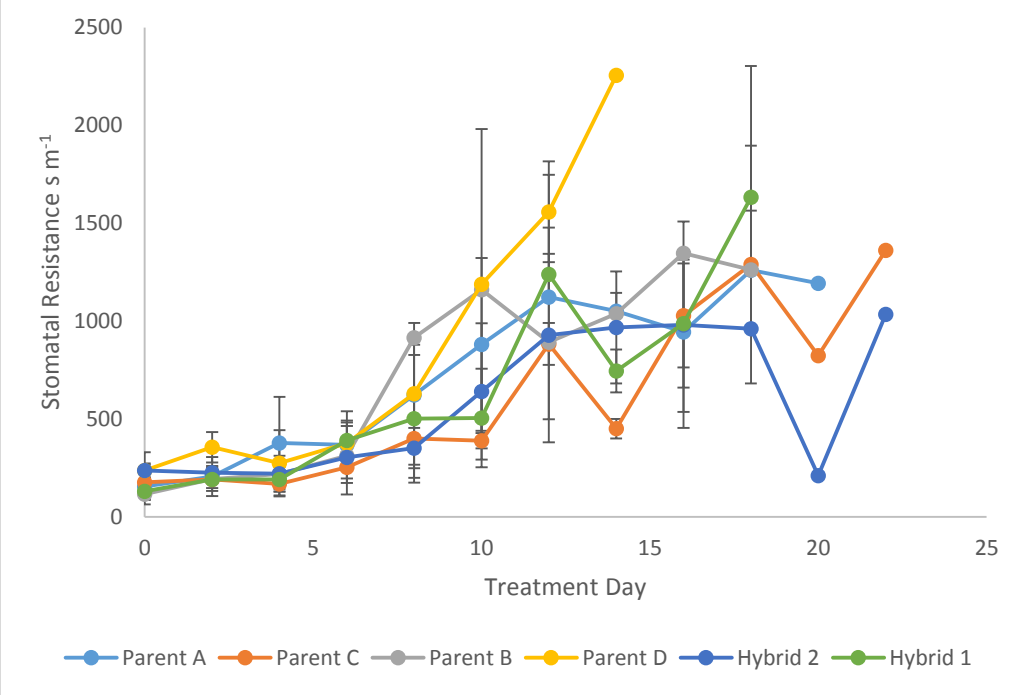

Figure 22: Stomatal resistance measurements for drought across all genotypes

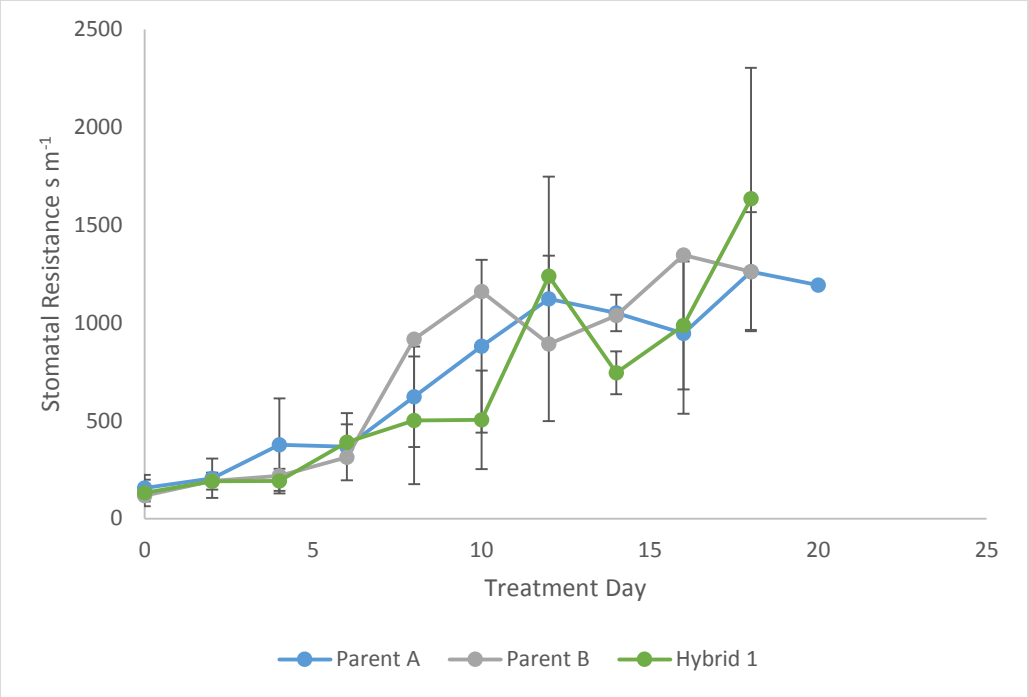

Figure 23: Stomatal resistance for drought within group one

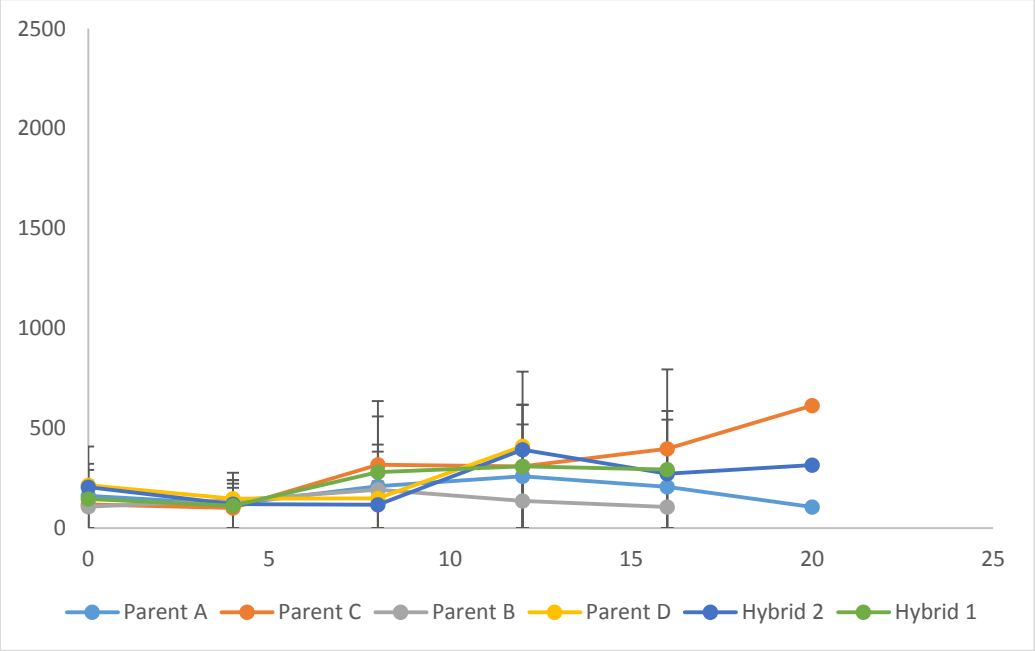

Figure 24: Stomatal Resistance optimum treatment across all genotypes

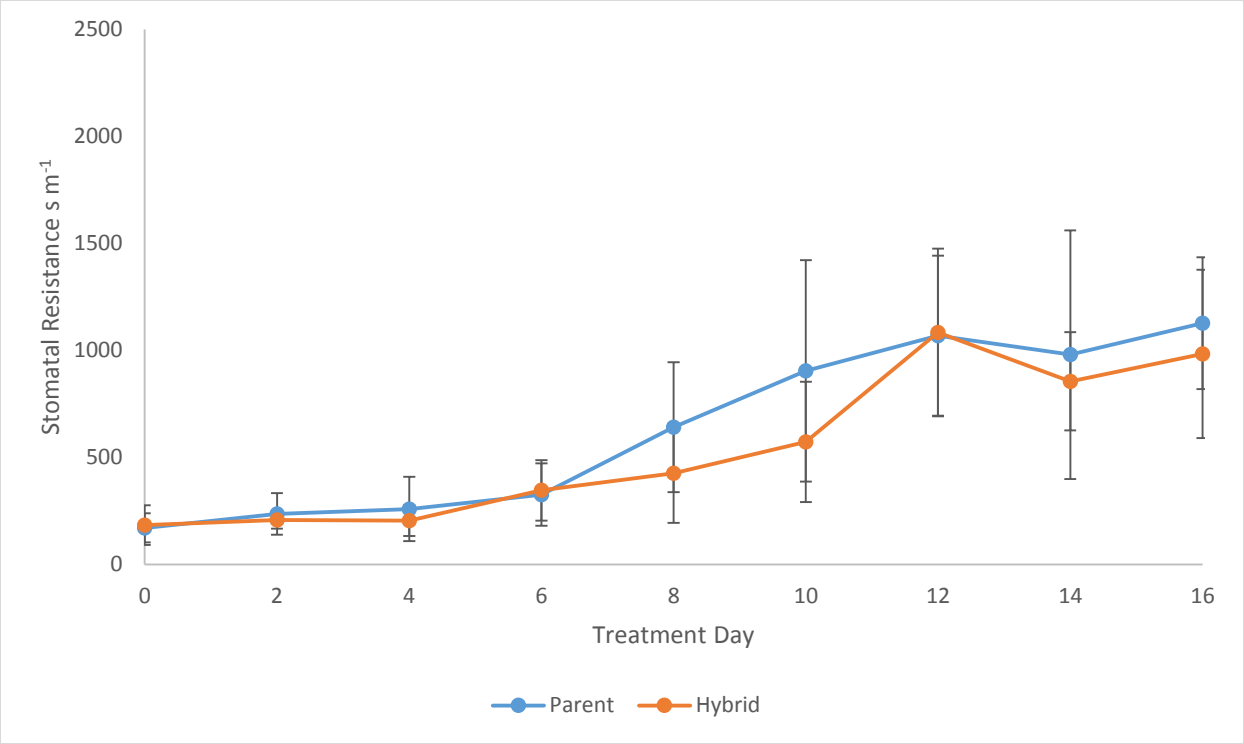

Figure 25: Stomatal resistance parents vs. hybrids for the drought treatment.

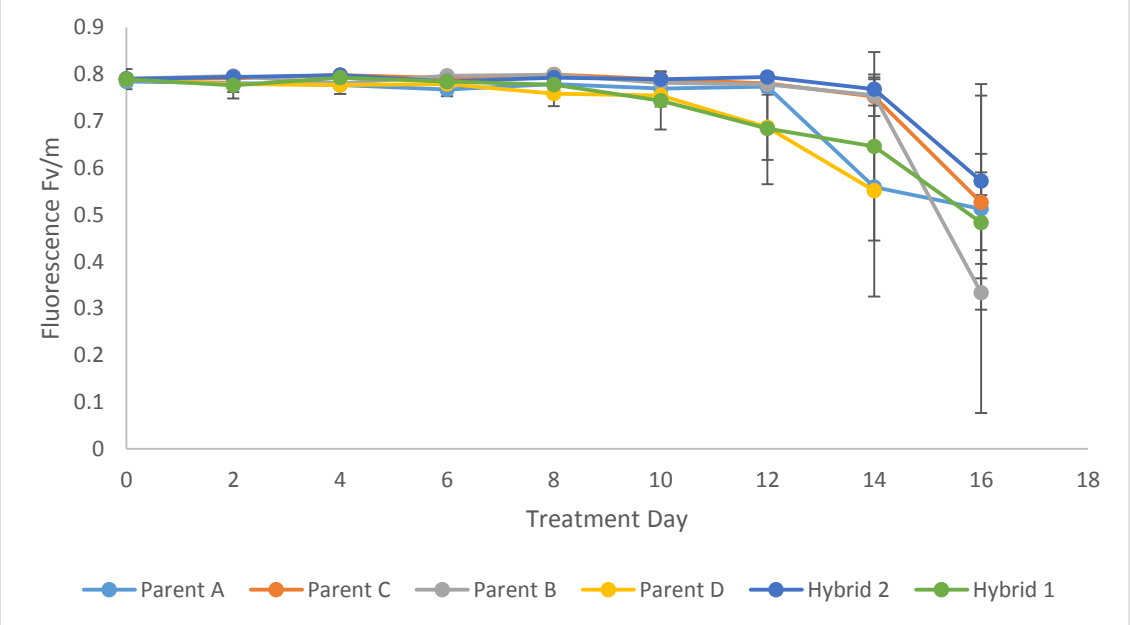

Figure 26: Fluorescence for drought in experiment one across all genotypes.

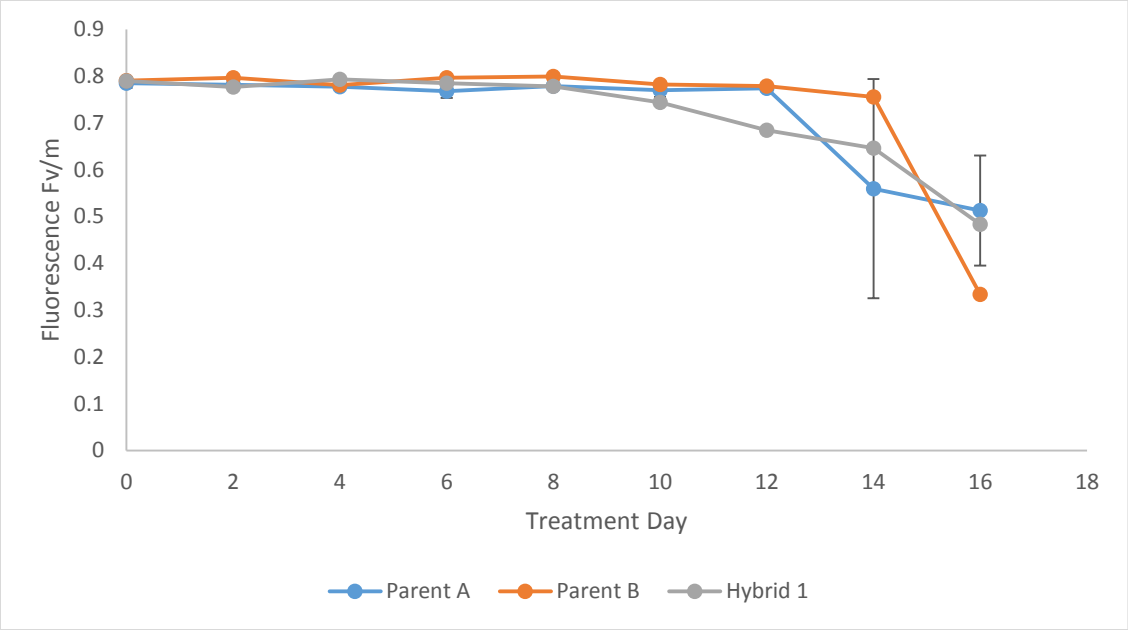

Figure 27: Fluorescence for drought in experiment one within group one.

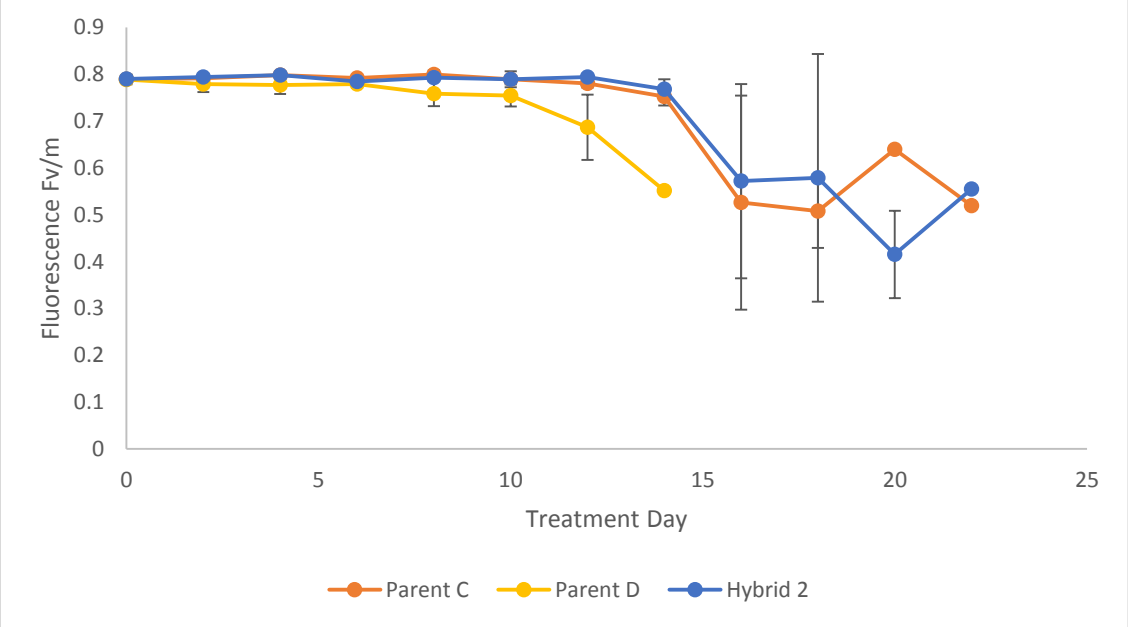

Figure 28: Fluorescence for drought in experiment one within group two.

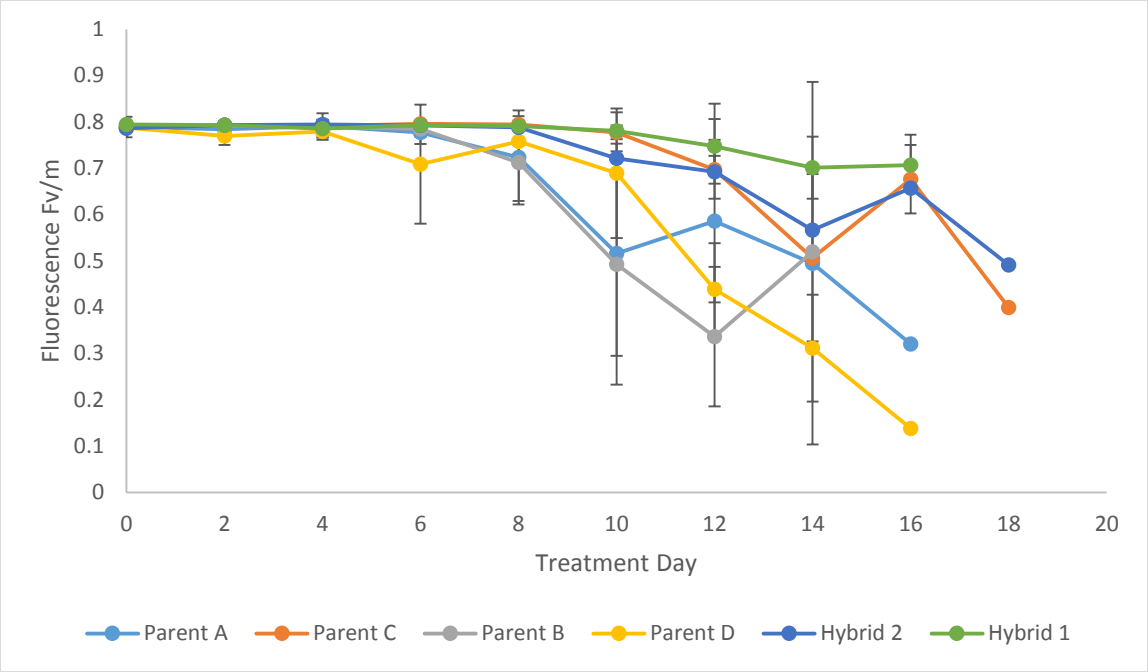

Figure 29: Fluorescence for drought in experiment two across all genotypes.

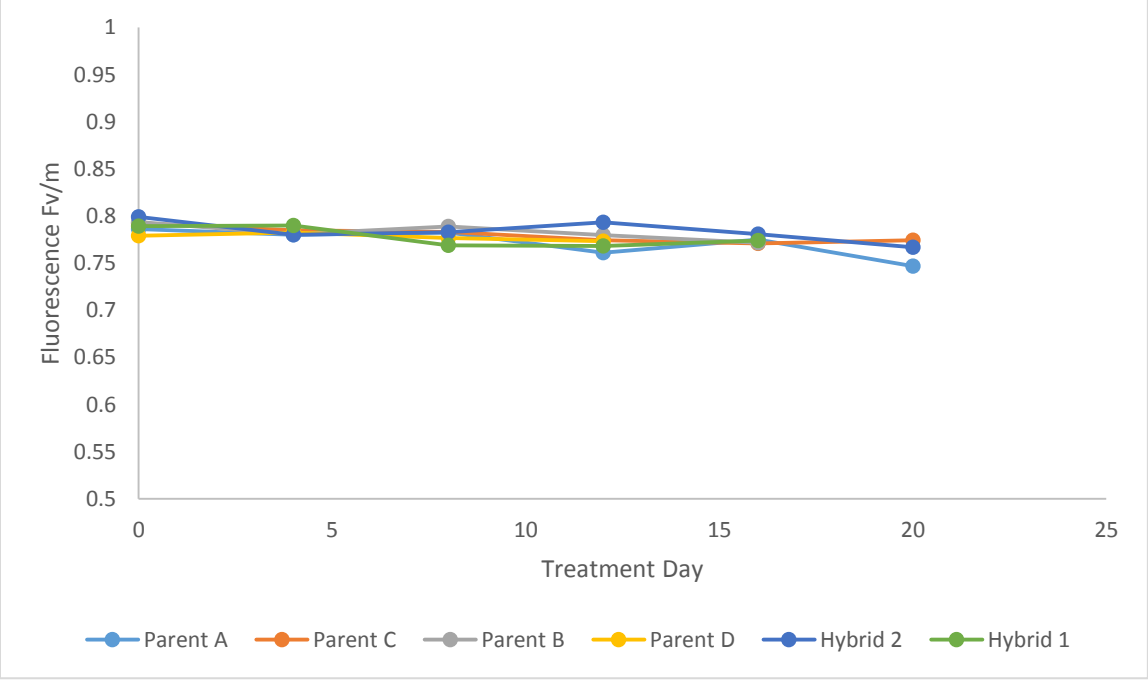

Figure 30: Fluorescence for optimum treatment in experiment one across all genotypes in.

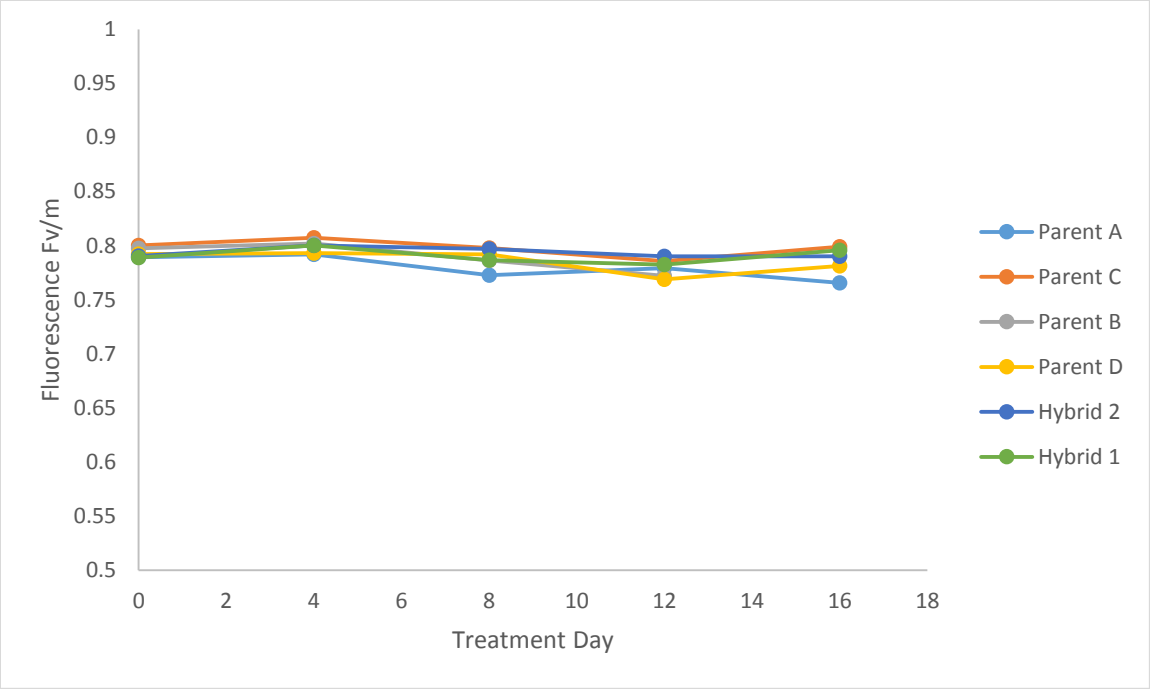

Figure 31: Fluorescence for the optimum treatment in experiment two across all genotypes.

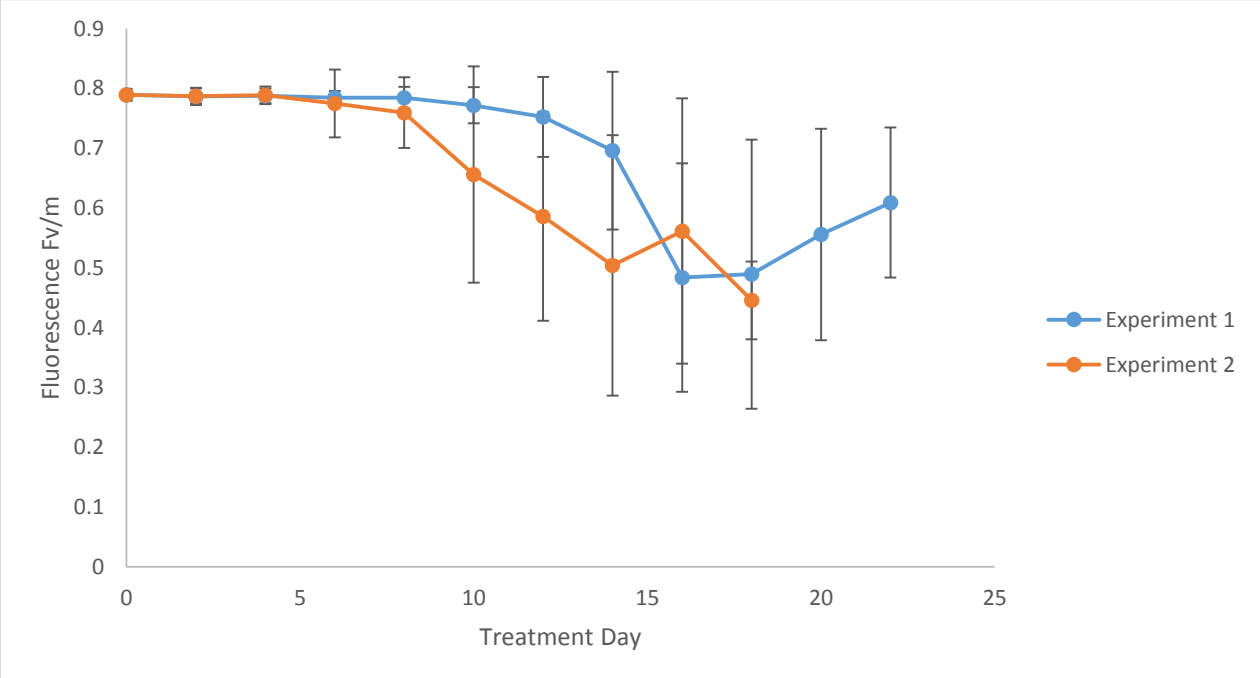

Figure 32: Fluorescence for drought between experiment one and experiment 2.

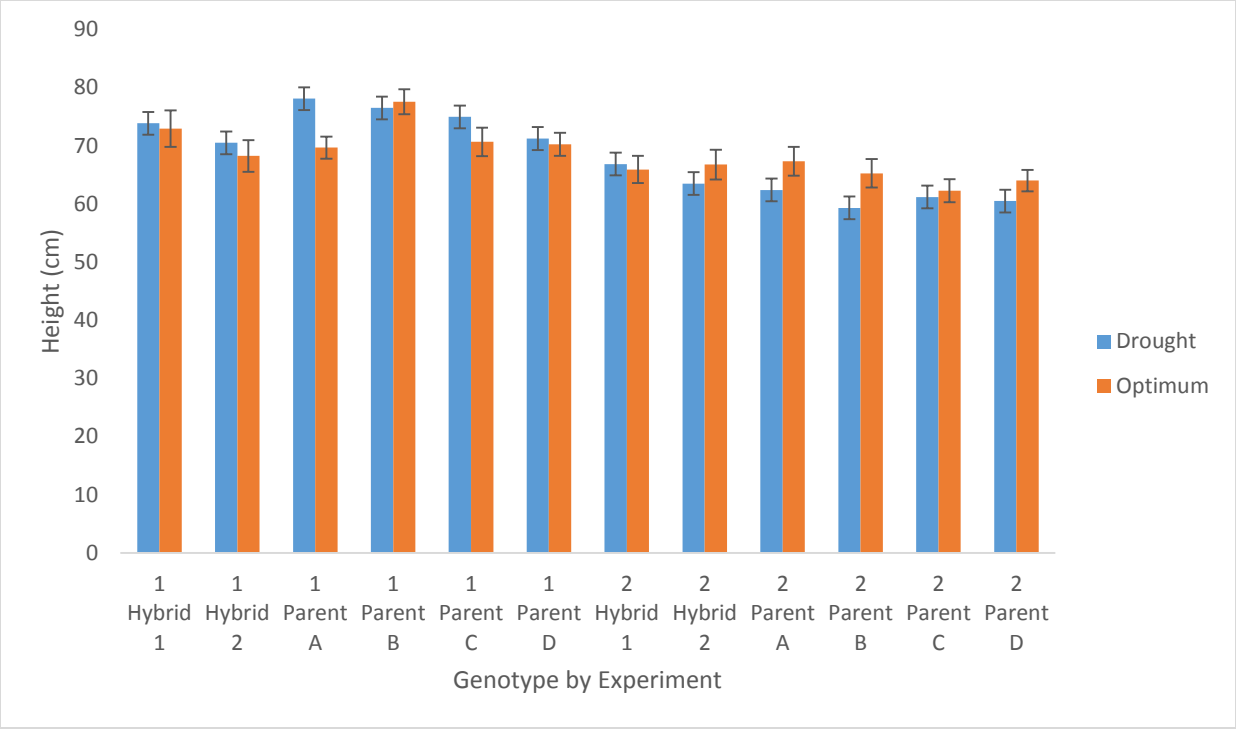

Figure 33: Plant height across all genotypes for both treatments and experiments

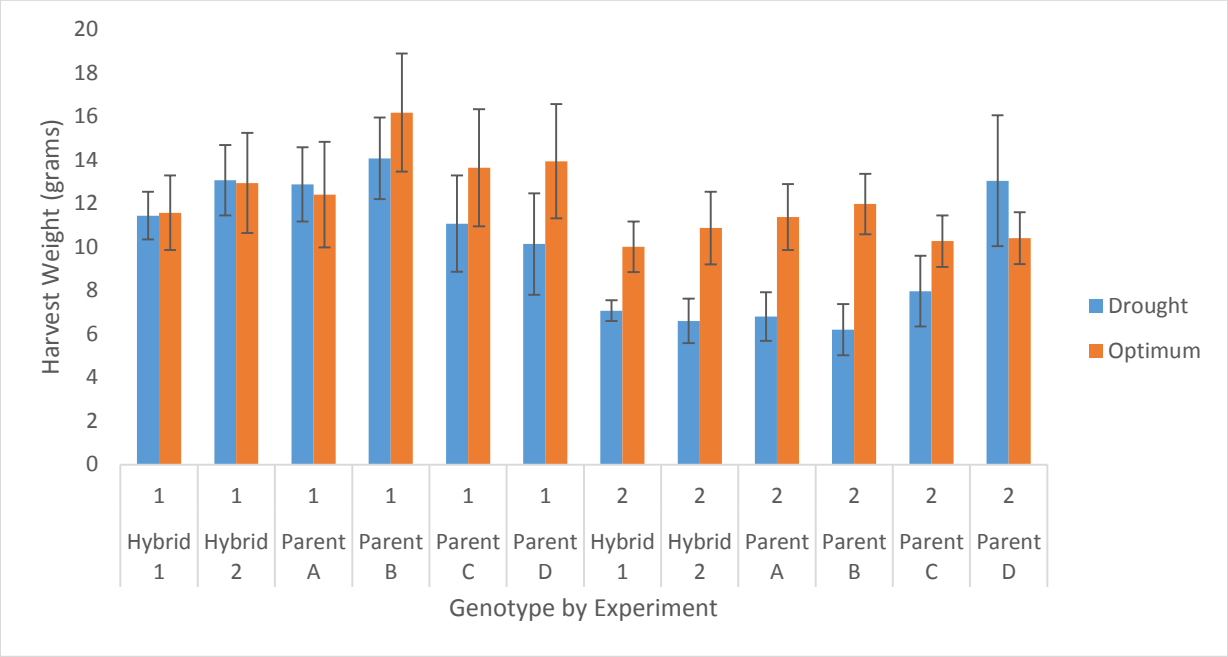

Figure 34: Harvest weight or mass of all the spikes across all genotypes for both treatments and experiments

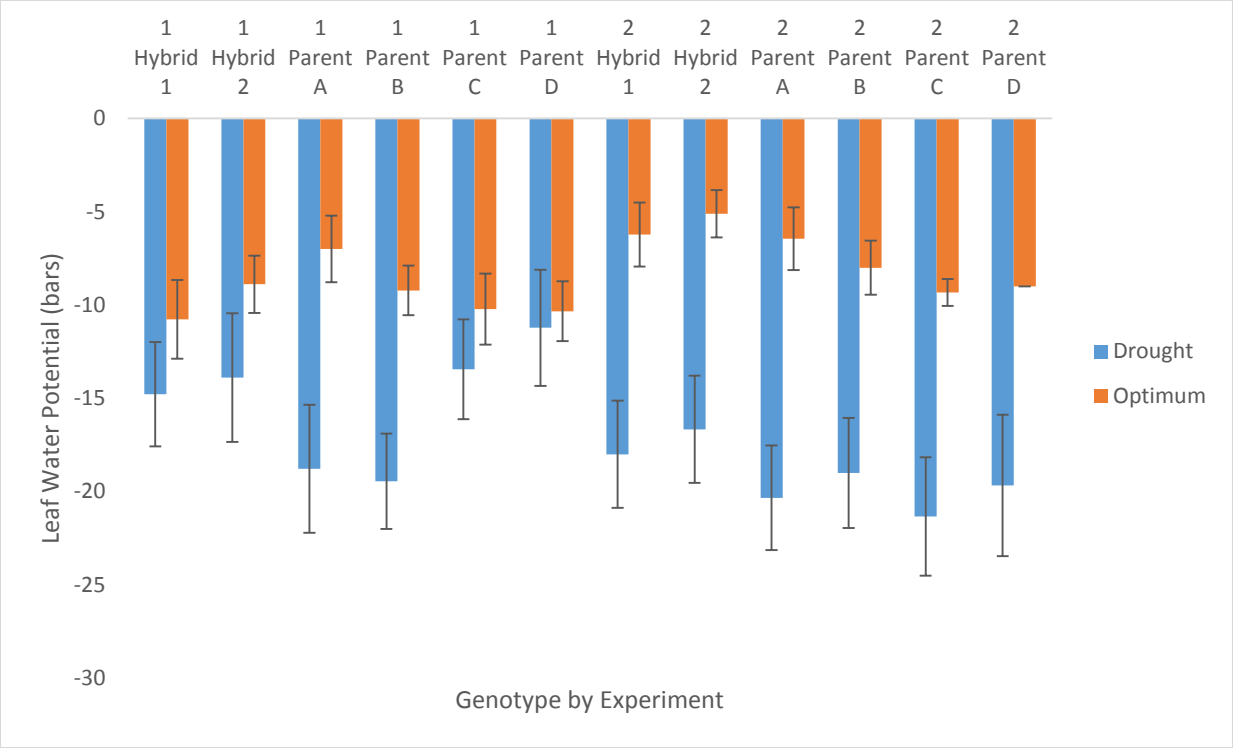

Figure 35: F-1 leaf water potential across all genotypes for both treatments and experiments

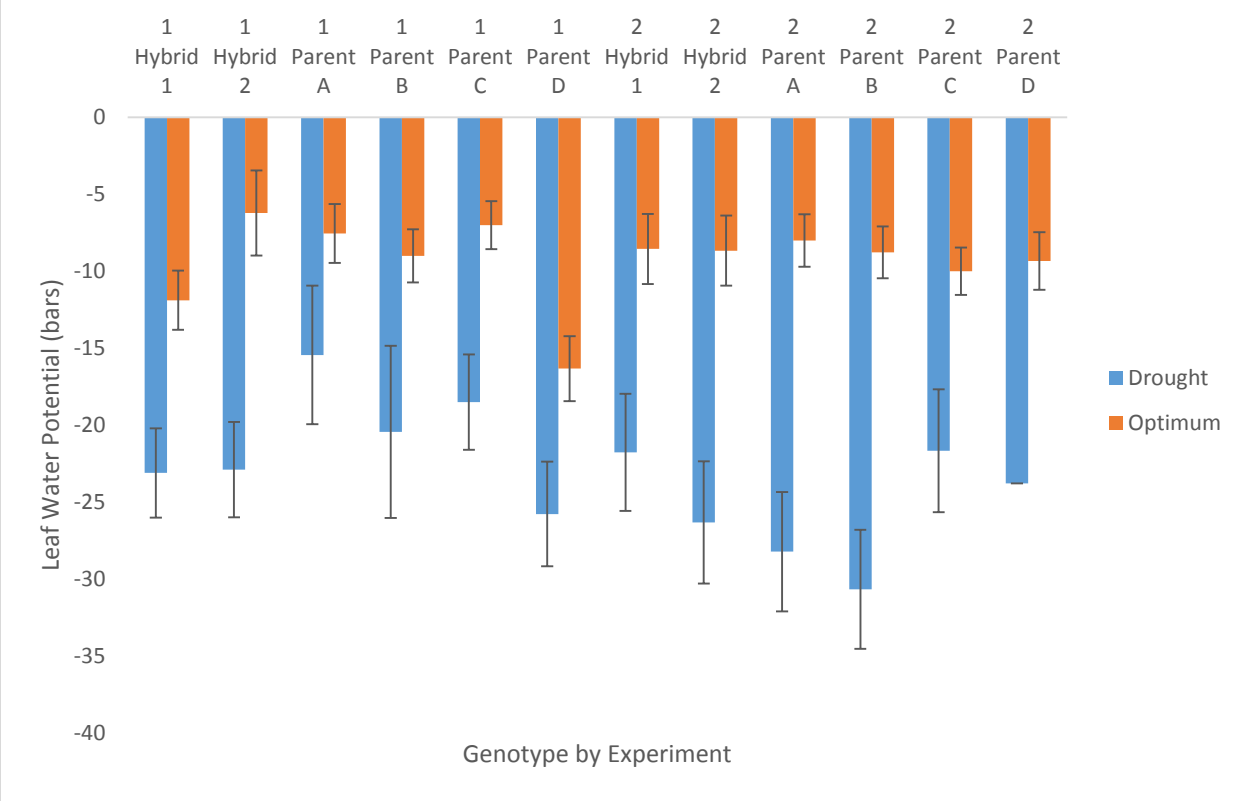

Figure 36: Flag leaf water potential across all genotypes for both treatments and experiments

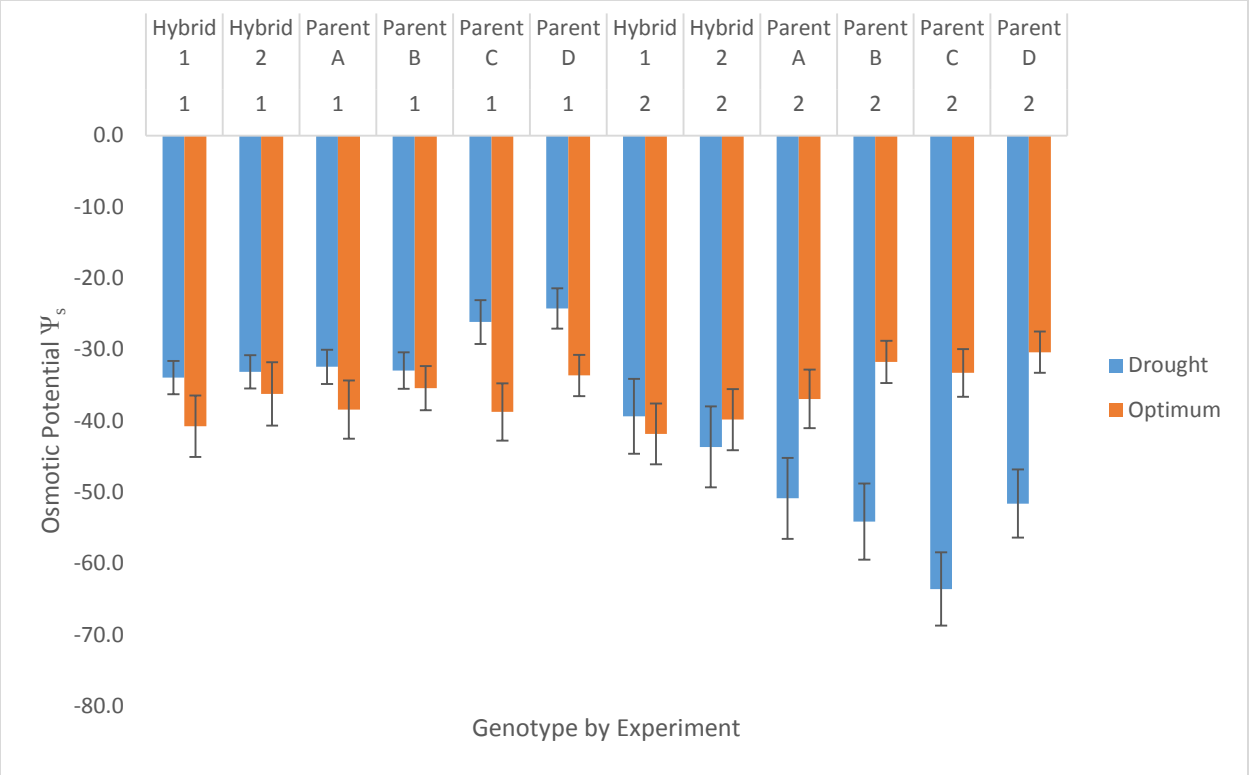

Figure 37: Osmotic potential values for all genotypes across both treatments and experiments.

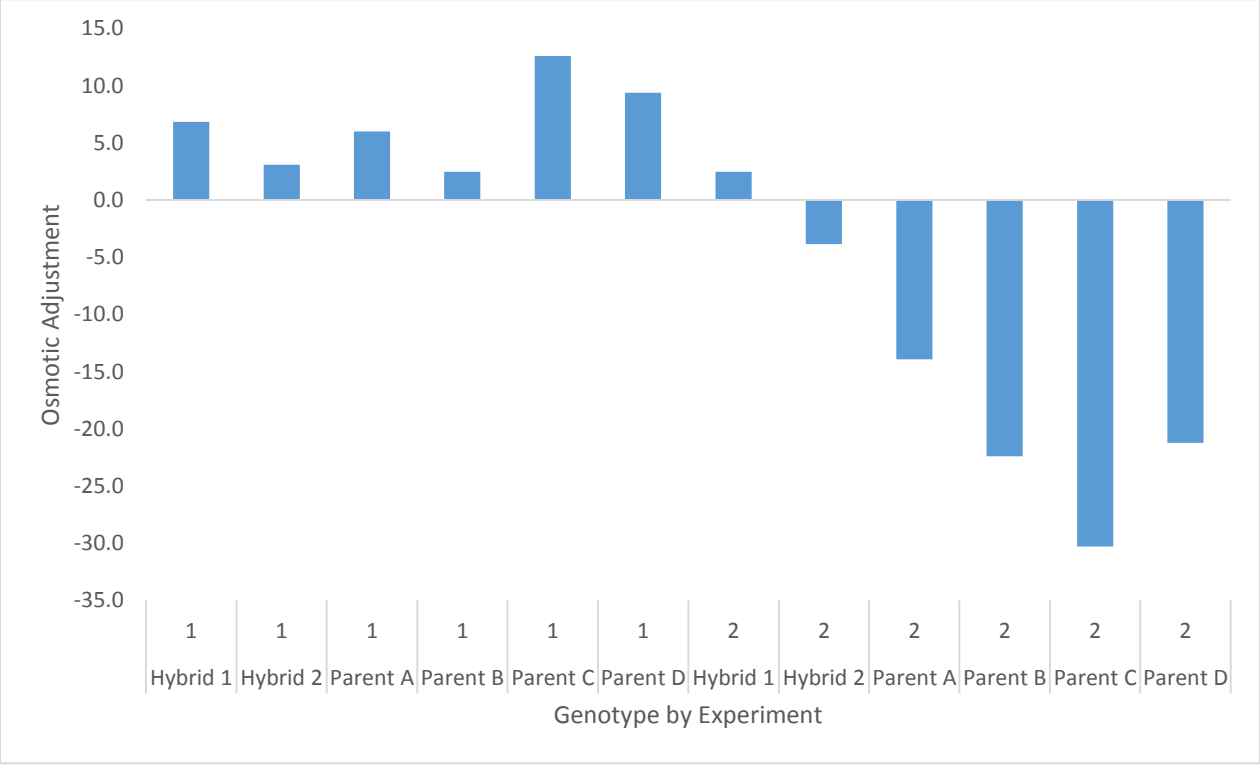

Figure 38: Osmotic Adjustment values among genotypes for both experiments

Appendix B - Programming Code for Sensor Data and Automatic Irrigation System

*Programming code was the same used in (Green, 2016)

```
'Declare Variables and Units
Public VWC_1
Public VWC_5
Public VWC_9
Public VWC_13
Public VWC_17
Public VWC_21
Public VWC_25
Public VWC_29
Public VWC_33
Public VWC_37
Public VWC_41
Public VWC_45
Public VWC_49
Public VWC_53
Public VWC_57
Public VWC_61
Public VWC_65
Public VWC_69
Public VWC_73
Public VWC_77
Public VWC_81
Public VWC_85
```

Public VWC_89
Public VWC_93
Public VWC_97
Public VWC_101
Public VWC_105
Public VWC_109
Public VWC_113
Public VWC_117
Public VWC_121
Public VWC_125
Public VWC_129
Public VWC_133
Public VWC_137
Public VWC_141
Public MPS1 162

```
Public MPS3
Public MPS5
Public MPS7
Public MPS9
Public MPS11
Public MPS13
Public MPS15
Public MPS17
Public MPS19
Public MPS21
Public MPS23
Public MPS25
Public MPS27
Public MPS29
Public MPS31
Public MPS33
Public MPS35
Public BattV
Public PTemp_C
Public LCount
Public LCount2
Public FullBR_3(16
Public FullBR(6)
Public Mult(6)={1,1,1,1,1,1}
Public Offs(6)={0,0,0,0,0,0}
Public Mult_3(16)={1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1}
Public Offs_3(16)={0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0}
Public ResultCode
Public ValveCtrl(48)
Units BattV=Volts
Units PTemp_C=Deg C
Units FullBR=mV/V
Units FullBR_3=mV/V
Public T_kPa_33
Public T_kPa_34
Public T_kPa_35
Public T_kPa_36
Public T_kPa_37
Public T_kPa_38
Public T_kPa_39
Public T_kPa_40
Public T_kPa_41
Public T_kPa_42
Public T_kPa_43
Public T_kPa_44
Public T_kPa_45 163
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```
Public T_kPa_46
Public T_kPa_47
Public T_kPa_48
Public T_kPa_49
Public T_kPa_50
Public T_kPa_51
Public T_kPa_52
Public T_kPa_53
Public T_kPa_54
'Define Data Tables
DataTable(Tens2,True,-1)
DataInterval(0,360,min, 10)
Sample(1,T_kPa_33,FP2)
Sample(1,T_kPa_34,FP2)
Sample(1,T_kPa_35,FP2)
Sample(1,T_kPa_36,FP2)
Sample(1,T_kPa_37,FP2)
Sample(1,T_kPa_38,FP2)
Sample(1,T_kPa_39,FP2)
Sample(1,T_kPa_40,FP2)
Sample(1,T_kPa_41,FP2)
Sample(1,T_kPa_42,FP2)
Sample(1,T_kPa_43,FP2)
Sample(1,T_kPa_44,FP2)
Sample(1,T_kPa_45,FP2)
Sample(1,T_kPa_46,FP2)
Sample(1,T_kPa_47,FP2)
Sample(1,T_kPa_48,FP2)
Sample(1,T_kPa_49,FP2)
Sample(1,T_kPa_50,FP2)
Sample(1,T_kPa_51,FP2)
Sample(1,T_kPa_52,FP2)
Sample(1,T_kPa_53,FP2)
Sample(1,T_kPa_54,FP2)
Sample(1,FullBR_3(1),FP2)
Sample(1,FullBR_3(2),FP2)
Sample(1,FullBR_3(3),FP2)
Sample(1,FullBR_3(4),FP2)
Sample(1,FullBR_3(5),FP2)
Sample(1,FullBR_3(6),FP2)
Sample(1,FullBR_3(7),FP2)
Sample(1,FullBR_3(8),FP2)
Sample(1,FullBR_3(9),FP2) 164
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Sample(1,FullBR_3(10),FP2)
Sample(1,FullBR_3(11),FP2)
Sample(1,FullBR_3(12),FP2)
Sample(1,FullBR_3(13),FP2)
Sample(1,FullBR_3(14),FP2)
Sample(1,FullBR_3(15),FP2)
Sample(1,FullBR_3(16),FP2)
Sample(1,FullBR(1),FP2)
Sample(1,FullBR(2),FP2)
Sample(1,FullBR(3),FP2)
Sample(1,FullBR(4),FP2)
Sample(1,FullBR(5),FP2)
Sample(1,FullBR(6),FP2)
EndTable
DataTable(Table2,True,-1)
DataInterval(0,1440,Min,10)
Minimum(1,BattV,FP2,False,False)
EndTable
DataTable (WateringRecord, True, -1)
DataInterval (0,60, Min, 10) 'change back to 480 for an 8 hour scan interval which will record the watering status at 8am
Sample (48, ValveCtrl(), FP2) 'change first number for number of repetitions
EndTable
'Main Program
BeginProg
'Main Scan
Scan(1,min,1,0)'change scan to five minutes for a program that waters for five minutes to ensure proper start and stop
'Default Datalogger Battery Voltage measurement 'BattV'
Battery(BattV)
'Default Wiring Panel Temperature measurement 'PTemp_C'
PanelTemp(PTemp_C,_60Hz)
'Turn AM16/32 Multiplexer On
PortSet(8,1)
Delay(0,150,mSec)
LCount=1
SubScan(0,uSec,6)
'Switch to next AM16/32 Multiplexer channel
PulsePort(4,10000)
'Generic Full Bridge measurements 'FullBR()' on the AM16/32 Multiplexer 165
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```
BrFull(FullBR(LCount),1,mv25,2,1,1,2500,True,True,0,_60Hz,Mult(LCount),Offs(LCount))
LCount=LCount+1
NextSubScan
'Turn AM16/32 Multiplexer Off
PortSet(8,0)
Delay(0,150,mSec)
PortSet(7,1)
Delay(0,150,mSec)
LCount2=1
SubScan(0,uSec,16)
'Switch to next AM16/32 Multiplexer channel
PulsePort(4,10000)
'Generic Full Bridge measurements 'FullBR_3()' on the AM16/32 Multiplexer
BrFull(FullBR_3(LCount2),1,mv25,1,1,1,2500,True,True,0,_60Hz,Mult_3(LCount2),Offs_3(LCount
2))
LCount2=LCount2+1
NextSubScan
'Turn AM16/32 Multiplexer Off
PortSet(7,0)
Delay(0,150,mSec)
'The GetVariables commands will collect EC-5 and MPS data from the specified sensors, to be used
in making the automatic irrigation decisions
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_1",VWC_1,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_5",VWC_5,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_9",VWC_9,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_13",VWC_13,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_17",VWC_17,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_21",VWC_21,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_25",VWC_25,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_29",VWC_29,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_33",VWC_33,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_37",VWC_37,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_41",VWC_41,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_45",VWC_45,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_49",VWC_49,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_53",VWC_53,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_57",VWC_57,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_61",VWC_61,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_65",VWC_65,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_69",VWC_69,1) 166
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GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_73",VWC_73,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_77",VWC_77,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_81",VWC_81,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_85",VWC_85,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_89",VWC_89,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_93",VWC_93,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_97",VWC_97,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_101",VWC_101,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_105",VWC_105,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_109",VWC_109,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_113",VWC_113,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_117",VWC_117,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_121",VWC_121,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_125",VWC_125,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_129",VWC_129,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_133",VWC_133,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_137",VWC_137,1)
GetVariables (ResultCode,Com3,0,1,0000,5,"VWC","VWC_141",VWC_141,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M1_kPa", MPS1,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M4_kPa", MPS3,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M7_kPa", MPS5,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M10_kPa", MPS7,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M13_kPa", MPS9,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M16_kPa", MPS11,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M19_kPa", MPS13,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M22_kPa", MPS15,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M25_kPa", MPS17,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M28_kPa", MPS19,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M31_kPa", MPS21,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M34_kPa", MPS23,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M37_kPa", MPS25,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M40_kPa", MPS27,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M43_kPa", MPS29,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M46_kPa", MPS31,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M49_kPa", MPS33,1)
GetVariables (ResultCode,Com3,0,1,0000,5, "MPS","M52_kPa", MPS35,1)
If IfTime (479, 1440, Min) Then ValveCtrl(48)=1 'turn on fertilizer tank pump 1 minutes before
watering scheduled
'If IfTime(480, 1440,Min)AND VWC_1<0.38 AND VWC_1>0.1 Then ValveCtrl(1)=1
'If IfTime(480,1440,Min) AND VWC_9<0.38 AND VWC_9>0.1 Then ValveCtrl(2)=1
'If IfTime(480,1440,Min) AND VWC_17<0.38 AND VWC_17>0.1 Then ValveCtrl(3)=1
'If IfTime(480,1440,Min) AND VWC_25<0.38 AND VWC_25>0.1 Then ValveCtrl(4)=1
'If IfTime(480,1440,Min) AND VWC_33<0.38 AND VWC_33>0.1 Then ValveCtrl(5)=1
'If IfTime(480,1440,Min) AND VWC_41<0.38 AND VWC_41>0.1 Then ValveCtrl(6)=1 167
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'If IfTime(480,1440,Min) AND VWC_49<0.38 AND VWC_49>0.1 Then ValveCtrl(7)=1
'If IfTime(480,1440,Min) AND VWC_57<0.38 AND VWC_57>0.1 Then ValveCtrl(8)=1
'If IfTime(480,1440,Min) AND VWC_65<0.38 AND VWC_65>0.1 Then ValveCtrl(9)=1
'If IfTime(480,1440,Min) AND VWC_73<0.38 AND VWC_73>0.1 Then ValveCtrl(10)=1
'If IfTime(480,1440,Min) AND VWC_81<0.38 AND VWC_81>0.1 Then ValveCtrl(11)=1
'If IfTime(480,1440,Min) AND VWC_89<0.38 AND VWC_89>0.1 Then ValveCtrl(12)=1
'If IfTime(480,1440,Min) AND VWC_97<0.38 AND VWC_97>0.1 Then ValveCtrl(13)=1
'If IfTime(480,1440,Min) AND VWC_105<0.38 AND VWC_105>0.1 Then ValveCtrl(14)=1
'If IfTime(480,1440,Min) AND VWC_113<0.38 AND VWC_113>0.1 Then ValveCtrl(15)=1
'If IfTime(480,1440,Min) AND VWC_121<0.38 AND VWC_121>0.1 Then ValveCtrl(16)=1
'If IfTime(480,1440,Min) AND VWC_129<0.38 AND VWC_129>0.1 Then ValveCtrl(17)=1
'If IfTime(480,1440,Min) AND VWC_137<0.38 AND VWC_137>0.1 Then ValveCtrl(18)=1
If IfTime (480, 1440, Min) AND MPS1<-500 AND MPS1<-10 Then ValveCtrl(1)=1
If IfTime (480, 1440, Min) AND MPS3<-500 AND MPS3<-10 Then ValveCtrl(2)=1
If IfTime (480, 1440, Min) AND MPS5 <-500 AND MPS5<-10 Then ValveCtrl(3)=1
If IfTime (480, 1440, Min) AND MPS7<-500 AND MPS7<-10 Then ValveCtrl(4)=1
If IfTime (480, 1440, Min) AND MPS9<-500 AND MPS9<-10 Then ValveCtrl(5)=1
If IfTime (480, 1440, Min) AND MPS11<-500 AND MPS11<-10 Then ValveCtrl(6)=1
If IfTime (480, 1440, Min) AND MPS13<-500 AND MPS13<-10 Then ValveCtrl(7)=1
If IfTime (480, 1440, Min) AND MPS15 <-500 AND MPS15<-10 Then ValveCtrl(8)=1
If IfTime (480, 1440, Min) AND MPS17 <-500 AND MPS17<-10 Then ValveCtrl(9)=1
If IfTime (480, 1440, Min) AND MPS19 <-500 AND MPS19<-10 Then ValveCtrl(10)=1
If IfTime (480, 1440, Min) AND MPS21 <-500 AND MPS21<-10 Then ValveCtrl(11)=1
If IfTime (480, 1440, Min) AND MPS23 <-500 AND MPS23<-10 Then ValveCtrl(12)=1
If IfTime (480, 1440, Min) AND MPS25 <-500 AND MPS25<-10 Then ValveCtrl(13)=1
If IfTime (480, 1440, Min) AND MPS27 <-500 AND MPS27<-10 Then ValveCtrl(14)=1
If IfTime (480, 1440, Min) AND MPS29 <-500 AND MPS29<-10 Then ValveCtrl(15)=1
If IfTime (480, 1440, Min) AND MPS31 <-500 AND MPS31<-10 Then ValveCtrl(16)=1
If IfTime (480, 1440, Min) AND MPS33 <-500 AND MPS33<-10 Then ValveCtrl(17)=1
If IfTime (480, 1440, Min) AND MPS35 <-500 AND MPS35<-10 Then ValveCtrl(18)=1
'If IfTime (483, 1440, Min) AND MPS1<-750 AND MPS1<-10 Then ValveCtrl(1)=1
'If IfTime (483, 1440, Min) AND MPS3<-750 AND MPS3<-10 Then ValveCtrl(2)=1
'If IfTime (483, 1440, Min) AND MPS5 <-750 AND MPS5<-10 Then ValveCtrl(3)=1
'If IfTime (483, 1440, Min) AND MPS7<-750 AND MPS7<-10 Then ValveCtrl(4)=1
'If IfTime (483, 1440, Min) AND MPS9<-750 AND MPS9<-10 Then ValveCtrl(5)=1
'If IfTime (483, 1440, Min) AND MPS11<-750 AND MPS11<-10 Then ValveCtrl(6)=1
'If IfTime (483, 1440, Min) AND MPS13<-750 AND MPS13<-10 Then ValveCtrl(7)=1
'If IfTime (483, 1440, Min) AND MPS15 <-750 AND MPS15<-10 Then ValveCtrl(8)=1
'If IfTime (483, 1440, Min) AND MPS17 <-750 AND MPS17<-10 Then ValveCtrl(9)=1
'If IfTime (483, 1440, Min) AND MPS19 <-750 AND MPS19<-10 Then ValveCtrl(10)=1
'If IfTime (483, 1440, Min) AND MPS21 <-750 AND MPS21<-10 Then ValveCtrl(11)=1
'If IfTime (483, 1440, Min) AND MPS23 <-750 AND MPS23<-10 Then ValveCtrl(12)=1
'If IfTime (483, 1440, Min) AND MPS25 <-750 AND MPS25<-10 Then ValveCtrl(13)=1
'If IfTime (483, 1440, Min) AND MPS27 <-750 AND MPS27<-10 Then ValveCtrl(14)=1 168

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'If IfTime (483, 1440, Min) AND MPS29 <-750 AND MPS29<-10 Then ValveCtrl(15)=1
'If IfTime (483, 1440, Min) AND MPS31 <-750 AND MPS31<-10 Then ValveCtrl(16)=1
'If IfTime (483, 1440, Min) AND MPS33 <-750 AND MPS33<-10 Then ValveCtrl(17)=1
'If IfTime (483, 1440, Min) AND MPS35 <-750 AND MPS35<-10 Then ValveCtrl(18)=1
If IfTime (1259, 1440, Min) Then ValveCtrl(48)=1
If IfTime (1260, 1440, Min) AND MPS1<-500 AND MPS1<-10 Then ValveCtrl(1)=1
If IfTime (1260, 1440, Min) AND MPS3<-500 AND MPS3<-10 Then ValveCtrl(2)=1
If IfTime (1260, 1440, Min) AND MPS5 <-500 AND MPS5<-10 Then ValveCtrl(3)=1
If IfTime (1260, 1440, Min) AND MPS7<-500 AND MPS7<-10 Then ValveCtrl(4)=1
If IfTime (1260, 1440, Min) AND MPS9<-500 AND MPS9<-10 Then ValveCtrl(5)=1
If IfTime (1260, 1440, Min) AND MPS11<-500 AND MPS11<-10 Then ValveCtrl(6)=1
If IfTime (1260, 1440, Min) AND MPS13<-500 AND MPS13<-10 Then ValveCtrl(7)=1
If IfTime (1260, 1440, Min) AND MPS15 <-500 AND MPS15<-10 Then ValveCtrl(8)=1
If IfTime (1260, 1440, Min) AND MPS17 <-500 AND MPS17<-10 Then ValveCtrl(9)=1
If IfTime (1260, 1440, Min) AND MPS19<-500 AND MPS19<-10 Then ValveCtrl(10)=1
If IfTime (1260, 1440, Min) AND MPS21 <-500 AND MPS21<-10 Then ValveCtrl(11)=1
If IfTime (1260, 1440, Min) AND MPS23 <-500 AND MPS23<-10 Then ValveCtrl(12)=1
If IfTime (1260, 1440, Min) AND MPS25 <-500 AND MPS25<-10 Then ValveCtrl(13)=1
If IfTime (1260, 1440, Min) AND MPS27 <-500 AND MPS27<-10 Then ValveCtrl(14)=1
If IfTime (1260, 1440, Min) AND MPS29 <-500 AND MPS29<-10 Then ValveCtrl(15)=1
If IfTime (1260, 1440, Min) AND MPS31 <-500 AND MPS31<-10 Then ValveCtrl(16)=1
If IfTime (1260, 1440, Min) AND MPS33 <-500 AND MPS33<-10 Then ValveCtrl(17)=1
If IfTime (1260, 1440, Min) AND MPS35 <-500 AND MPS35<-10 Then ValveCtrl(18)=1
If IfTime(1260,1440,Min) AND VWC_5<0.38 AND VWC_5>0.1 Then ValveCtrl(19)=1
If IfTime(1260,1440,Min) AND VWC_13<0.38 AND VWC_13>0.1 Then ValveCtrl(20)=1
If IfTime(1260,1440,Min) AND VWC_21<0.38 AND VWC_21>0.1 Then ValveCtrl(21)=1
If IfTime(1260,1440,Min) AND VWC_29<0.38 AND VWC_29>0.1 Then ValveCtrl(22)=1
If IfTime(1260,1440,Min) AND VWC_37<0.38 AND VWC_37>0.1 Then ValveCtrl(23)=1
If IfTime(1260,1440,Min) AND VWC_45<0.38 AND VWC_45>0.1 Then ValveCtrl(24)=1
If IfTime(1260,1440,Min) AND VWC_53<0.38 AND VWC_53>0.1 Then ValveCtrl(25)=1
If IfTime(1260,1440,Min) AND VWC_61<0.38 AND VWC_61>0.1 Then ValveCtrl(26)=1
If IfTime(1260,1440,Min) AND VWC_69<0.38 AND VWC_69>0.1 Then ValveCtrl(27)=1
If IfTime(1260,1440,Min) AND VWC_77<0.38 AND VWC_77>0.1 Then ValveCtrl(28)=1
If IfTime(1260,1440,Min) AND VWC_85<0.38 AND VWC_85>0.1 Then ValveCtrl(29)=1
If IfTime(1260,1440,Min) AND VWC_93<0.38 AND VWC_93>0.1 Then ValveCtrl(30)=1
If IfTime(1260,1440,Min) AND VWC_101<0.38 AND VWC_101>0.1 Then ValveCtrl(31)=1
If IfTime(1260,1440,Min) AND VWC_109<0.38 AND VWC_109>0.1 Then ValveCtrl(32)=1
If IfTime(1260,1440,Min) AND VWC_117<0.38 AND VWC_117>0.1 Then ValveCtrl(33)=1
If IfTime(1260,1440,Min) AND VWC_125<0.38 AND VWC_125>0.1 Then ValveCtrl(34)=1
If IfTime(1260,1440,Min) AND VWC_133<0.38 AND VWC_133>0.1 Then ValveCtrl(35)=1
If IfTime(1260,1440,Min) AND VWC_141<0.38 AND VWC_141>0.1 Then ValveCtrl(36)=1
If IfTime (899, 1440, Min) Then ValveCtrl(48)=1
If IfTime (900, 1440, Min) AND MPS1<-500 AND MPS1<-10 Then ValveCtrl(1)=1 169
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If IfTime (900, 1440, Min) AND MPS3<-500 AND MPS3<-10 Then ValveCtrl(2)=1
If IfTime (900, 1440, Min) AND MPS5 <-500 AND MPS5<-10 Then ValveCtrl(3)=1
If IfTime (900, 1440, Min) AND MPS7<-500 AND MPS7<-10 Then ValveCtrl(4)=1
If IfTime (900, 1440, Min) AND MPS9<-500 AND MPS9<-10 Then ValveCtrl(5)=1
If IfTime (900, 1440, Min) AND MPS11<-500 AND MPS11<-10 Then ValveCtrl(6)=1
If IfTime (900, 1440, Min) AND MPS13<-500 AND MPS13<-10 Then ValveCtrl(7)=1
If IfTime (900, 1440, Min) AND MPS15 <-500 AND MPS15<-10 Then ValveCtrl(8)=1
If IfTime (900, 1440, Min) AND MPS17 <-500 AND MPS17<-10 Then ValveCtrl(9)=1
If IfTime (900, 1440, Min) AND MPS19<-500 AND MPS19<-10 Then ValveCtrl(10)=1
If IfTime (900, 1440, Min) AND MPS21 <-500 AND MPS21<-10 Then ValveCtrl(11)=1
If IfTime (900, 1440, Min) AND MPS23 <-500 AND MPS23<-10 Then ValveCtrl(12)=1
If IfTime (900, 1440, Min) AND MPS25 <-500 AND MPS25<-10 Then ValveCtrl(13)=1
If IfTime (900, 1440, Min) AND MPS27 <-500 AND MPS27<-10 Then ValveCtrl(14)=1
If IfTime (900, 1440, Min) AND MPS29 <-500 AND MPS29<-10 Then ValveCtrl(15)=1
If IfTime (900, 1440, Min) AND MPS31 <-500 AND MPS31<-10 Then ValveCtrl(16)=1
If IfTime (900, 1440, Min) AND MPS33 <-500 AND MPS33<-10 Then ValveCtrl(17)=1
If IfTime (900, 1440, Min) AND MPS35 <-500 AND MPS35<-10 Then ValveCtrl(18)=1
If IfTime (482,1440,Min) Then ValveCtrl(1)=0
If IfTime (482,1440,Min) Then ValveCtrl(2)=0
If IfTime (482,1440,Min) Then ValveCtrl(3)=0
If IfTime (482,1440,Min) Then ValveCtrl(4)=0
If IfTime (482,1440,Min) Then ValveCtrl(5)=0
If IfTime (482,1440,Min) Then ValveCtrl(6)=0
If IfTime (482,1440,Min) Then ValveCtrl(7)=0
If IfTime (482,1440,Min) Then ValveCtrl(8)=0
If IfTime (482,1440,Min) Then ValveCtrl(9)=0
If IfTime (482,1440,Min) Then ValveCtrl(10)=0
If IfTime (482,1440,Min) Then ValveCtrl(11)=0
If IfTime (482,1440,Min) Then ValveCtrl(12)=0
If IfTime (482,1440,Min) Then ValveCtrl(13)=0
If IfTime (482,1440,Min) Then ValveCtrl(14)=0
If IfTime (482,1440,Min) Then ValveCtrl(15)=0
If IfTime (482,1440,Min) Then ValveCtrl(16)=0
If IfTime (482,1440,Min) Then ValveCtrl(17)=0
If IfTime (482,1440,Min) Then ValveCtrl(18)=0
If IfTime (484,1440,Min) Then ValveCtrl(1)=0
If IfTime (484,1440,Min) Then ValveCtrl(2)=0
If IfTime (484,1440,Min) Then ValveCtrl(3)=0
If IfTime (484,1440,Min) Then ValveCtrl(4)=0
If IfTime (484,1440,Min) Then ValveCtrl(5)=0
If IfTime (484,1440,Min) Then ValveCtrl(6)=0
If IfTime (484,1440,Min) Then ValveCtrl(7)=0
If IfTime (484,1440,Min) Then ValveCtrl(8)=0
If IfTime (484,1440,Min) Then ValveCtrl(9)=0 170
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If IfTime (484,1440,Min) Then ValveCtrl(10)=0
If IfTime (484,1440,Min) Then ValveCtrl(11)=0
If IfTime (484,1440,Min) Then ValveCtrl(12)=0
If IfTime (484,1440,Min) Then ValveCtrl(13)=0
If IfTime (484,1440,Min) Then ValveCtrl(14)=0
If IfTime (484,1440,Min) Then ValveCtrl(15)=0
If IfTime (484,1440,Min) Then ValveCtrl(16)=0
If IfTime (484,1440,Min) Then ValveCtrl(17)=0
If IfTime (484,1440,Min) Then ValveCtrl(18)=0
If IfTime (1265,1440, Min) Then ValveCtrl(19)=0
If IfTime (1265,1440, Min) Then ValveCtrl(20)=0
If IfTime (1265,1440, Min) Then ValveCtrl(21)=0
If IfTime (1265,1440, Min) Then ValveCtrl(22)=0
If IfTime (1265,1440, Min) Then ValveCtrl(23)=0
If IfTime (1265,1440, Min) Then ValveCtrl(24)=0
If IfTime (1265,1440, Min) Then ValveCtrl(25)=0
If IfTime (1265,1440, Min) Then ValveCtrl(26)=0
If IfTime (1265,1440, Min) Then ValveCtrl(27)=0
If IfTime (1265,1440, Min) Then ValveCtrl(28)=0
If IfTime (1265,1440, Min) Then ValveCtrl(29)=0
If IfTime (1265,1440, Min) Then ValveCtrl(30)=0
If IfTime (1265,1440, Min) Then ValveCtrl(31)=0
If IfTime (1265,1440, Min) Then ValveCtrl(32)=0
If IfTime (1265,1440, Min) Then ValveCtrl(33)=0
If IfTime (1265,1440, Min) Then ValveCtrl(34)=0
If IfTime (1265,1440, Min) Then ValveCtrl(35)=0
If IfTime (1265,1440, Min) Then ValveCtrl(36)=0
If IfTime (1265,1440, Min) Then ValveCtrl(48)=0
If IfTime (1262,1440, Min) Then ValveCtrl(1)=0
If IfTime (1262,1440,Min) Then ValveCtrl(2)=0
If IfTime (1262,1440,Min) Then ValveCtrl(3)=0
If IfTime (1262,1440,Min) Then ValveCtrl(4)=0
If IfTime (1262,1440,Min) Then ValveCtrl(5)=0
If IfTime (1262,1440,Min) Then ValveCtrl(6)=0
If IfTime (1262,1440,Min) Then ValveCtrl(7)=0
If IfTime (1262,1440,Min) Then ValveCtrl(8)=0
If IfTime (1262,1440,Min) Then ValveCtrl(9)=0
If IfTime (1262,1440,Min) Then ValveCtrl(10)=0
If IfTime (1262,1440,Min) Then ValveCtrl(11)=0
If IfTime (1262,1440,Min) Then ValveCtrl(12)=0
If IfTime (1262,1440,Min) Then ValveCtrl(13)=0
If IfTime (1262,1440,Min) Then ValveCtrl(14)=0
If IfTime (1262,1440,Min) Then ValveCtrl(15)=0
If IfTime (1262,1440,Min) Then ValveCtrl(16)=0 171
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If IfTime (1262,1440,Min) Then ValveCtrl(17)=0
If IfTime (1262,1440,Min) Then ValveCtrl(18)=0
If IfTime (902,1440, Min) Then ValveCtrl(48)=0
If IfTime (902,1440, Min) Then ValveCtrl(1)=0
If IfTime (902,1440,Min) Then ValveCtrl(2)=0
If IfTime (902,1440,Min) Then ValveCtrl(3)=0
If IfTime (902,1440,Min) Then ValveCtrl(4)=0
If IfTime (902,1440,Min) Then ValveCtrl(5)=0
If IfTime (902,1440,Min) Then ValveCtrl(6)=0
If IfTime (902,1440,Min) Then ValveCtrl(7)=0
If IfTime (902,1440,Min) Then ValveCtrl(8)=0
If IfTime (902,1440,Min) Then ValveCtrl(9)=0
If IfTime (902,1440,Min) Then ValveCtrl(10)=0
If IfTime (902,1440,Min) Then ValveCtrl(11)=0
If IfTime (902,1440,Min) Then ValveCtrl(12)=0
If IfTime (902,1440,Min) Then ValveCtrl(13)=0
If IfTime (902,1440,Min) Then ValveCtrl(14)=0
If IfTime (902,1440,Min) Then ValveCtrl(15)=0
If IfTime (902,1440,Min) Then ValveCtrl(16)=0
If IfTime (902,1440,Min) Then ValveCtrl(17)=0
If IfTime (902,1440,Min) Then ValveCtrl(18)=0
'Watered Tubes- These lines should never be changed'
If IfTime(480,1440,Min) AND VWC_5<0.38 AND VWC_5>0.1 Then ValveCtrl(19)=1
If IfTime(480,1440,Min) AND VWC_13<0.38 AND VWC_13>0.1 Then ValveCtrl(20)=1
If IfTime(480,1440,Min) AND VWC_21<0.38 AND VWC_21>0.1 Then ValveCtrl(21)=1
If IfTime(480,1440,Min) AND VWC_29<0.38 AND VWC_29>0.1 Then ValveCtrl(22)=1
If IfTime(480,1440,Min) AND VWC_37<0.38 AND VWC_37>0.1 Then ValveCtrl(23)=1
If IfTime(480,1440,Min) AND VWC_45<0.38 AND VWC_45>0.1 Then ValveCtrl(24)=1
If IfTime(480,1440,Min) AND VWC_53<0.38 AND VWC_53>0.1 Then ValveCtrl(25)=1
If IfTime(480,1440,Min) AND VWC_61<0.38 AND VWC_61>0.1 Then ValveCtrl(26)=1
If IfTime(480,1440,Min) AND VWC_69<0.38 AND VWC_69>0.1 Then ValveCtrl(27)=1
If IfTime(480,1440,Min) AND VWC_77<0.38 AND VWC_77>0.1 Then ValveCtrl(28)=1
If IfTime(480,1440,Min) AND VWC_85<0.38 AND VWC_85>0.1 Then ValveCtrl(29)=1
If IfTime(480,1440,Min) AND VWC_93<0.38 AND VWC_93>0.1 Then ValveCtrl(30)=1
If IfTime(480,1440,Min) AND VWC_101<0.38 AND VWC_101>0.1 Then ValveCtrl(31)=1
If IfTime(480,1440,Min) AND VWC_109<0.38 AND VWC_109>0.1 Then ValveCtrl(32)=1
If IfTime(480,1440,Min) AND VWC_117<0.38 AND VWC_117>0.1 Then ValveCtrl(33)=1
If IfTime(480,1440,Min) AND VWC_125<0.38 AND VWC_125>0.1 Then ValveCtrl(34)=1
If IfTime(480,1440,Min) AND VWC_133<0.38 AND VWC_133>0.1 Then ValveCtrl(35)=1
If IfTime(480,1440,Min) AND VWC_141<0.38 AND VWC_141>0.1 Then ValveCtrl(36)=1
If IfTime (485,1440, Min) Then ValveCtrl(1)=0
If IfTime (485,1440,Min) Then ValveCtrl(2)=0 172
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If IfTime (485,1440,Min) Then ValveCtrl(3)=0
If IfTime (485,1440,Min) Then ValveCtrl(4)=0
If IfTime (485,1440,Min) Then ValveCtrl(5)=0
If IfTime (485,1440,Min) Then ValveCtrl(6)=0
If IfTime (485,1440,Min) Then ValveCtrl(7)=0
If IfTime (485,1440,Min) Then ValveCtrl(8)=0
If IfTime (485,1440,Min) Then ValveCtrl(9)=0
If IfTime (485,1440,Min) Then ValveCtrl(10)=0
If IfTime (485,1440,Min) Then ValveCtrl(11)=0
If IfTime (485,1440,Min) Then ValveCtrl(12)=0
If IfTime (485,1440,Min) Then ValveCtrl(13)=0
If IfTime (485,1440,Min) Then ValveCtrl(14)=0
If IfTime (485,1440,Min) Then ValveCtrl(15)=0
If IfTime (485,1440,Min) Then ValveCtrl(16)=0
If IfTime (485,1440,Min) Then ValveCtrl(17)=0
If IfTime (485,1440,Min) Then ValveCtrl(18)=0
If IfTime (488,1440,Min) Then ValveCtrl(19)=0
If IfTime (488,1440,Min) Then ValveCtrl(20)=0
If IfTime (488,1440,Min) Then ValveCtrl(21)=0
If IfTime (488,1440,Min) Then ValveCtrl(22)=0
If IfTime (488,1440,Min) Then ValveCtrl(23)=0
If IfTime (488,1440,Min) Then ValveCtrl(24)=0
If IfTime (488,1440,Min) Then ValveCtrl(25)=0
If IfTime (488,1440,Min) Then ValveCtrl(26)=0
If IfTime (488,1440,Min) Then ValveCtrl(27)=0
If IfTime (488,1440,Min) Then ValveCtrl(28)=0
If IfTime (488,1440,Min) Then ValveCtrl(29)=0
If IfTime (488,1440,Min) Then ValveCtrl(30)=0
If IfTime (488,1440,Min) Then ValveCtrl(31)=0
If IfTime (488,1440,Min) Then ValveCtrl(32)=0
If IfTime (488,1440,Min) Then ValveCtrl(33)=0
If IfTime (488,1440,Min) Then ValveCtrl(34)=0
If IfTime (488,1440,Min) Then ValveCtrl(35)=0
If IfTime (488,1440,Min) Then ValveCtrl(36)=0
If IfTime (488,1440,Min) Then ValveCtrl(48)=0 'turn fertilizer tank pump off
SDMCD16AC (ValveCtrl(), 3,0)
T_kPa_33=(FullBR_3(1)*79.35+56.02)/10
T_kPa_34=(FullBR_3(2)*79.35+56.02)/10
T_kPa_35=(FullBR_3(3)*79.35+56.02)/10
T_kPa_36=(FullBR_3(4)*79.35+56.02)/10
T_kPa_37=(FullBR_3(5)*79.35+56.02)/10
T_kPa_38=(FullBR_3(6)*79.35+56.02)/10
T_kPa_39=(FullBR_3(7)*79.35+56.02)/10
T_kPa_40=(FullBR_3(8)*79.35+56.02)/10 173
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T_kPa_41=(FullBR_3(9)*79.35+56.02)/10
T_kPa_42=(FullBR_3(10)*79.35+56.02)/10
T_kPa_43=(FullBR_3(11)*79.35+56.02)/10
T_kPa_44=(FullBR_3(12)*79.35+56.02)/10
T_kPa_45=(FullBR_3(13)*79.35+56.02)/10
T_kPa_46=(FullBR_3(14)*79.35+56.02)/10
T_kPa_47=(FullBr_3(15)*79.35+56.02)/10
T_kPa_48=(FullBR_3(16)*79.35+56.02)/10
T_kPa_49=(FullBR(1)*79.35+56.02)/10
T_kPa_50=(FullBR(2)*79.35+56.02)/10
T_kPa_51=(FullBR(3)*79.35+56.02)/10
T_kPa_52=(FullBR(4)*79.35+56.02)/10
T_kPa_53=(FullBR(5)*79.35+56.02)/10
T_kPa_54=(FullBR(6)*79.35+56.02)/10
'Call Data Tables and Store Data
CallTable Tens2
CallTable Table2
CallTable WateringRecord
NextScan
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