EVALUATING SORGHUM AND MAIZE GERMPLASM FOR POST-ANTHESIS DROUGHT TOLERANCE

by

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Abstract

Drought is the single most limiting factor in crop production. This study was conducted to investigate if a cell viability assay could serve as an effective, efficient screen to determine post-anthesis drought tolerance in sorghum (*Sorghum bicolor* [L.] Moench) and maize (*Zea mays* [L]). The assay measured decline in chlorophyll fluorescence (Fv/Fm) over time from leaf punches collected from plants grown under optimum environmental conditions and placed in an incubator under high respiratory demand. A total of 300 lines of sorghum and 197 lines of maize were screened using this assay and potential post flowering drought tolerant staygreen lines and non-stay green lines were identified. Further testing of potential lines was done in both controlled and field environments, under drought conditions, to evaluate genotype performance for physiological, yield, and staygreen traits. Standard known staygreen and non-staygreen checks were also included in these studies for comparisons. Some relationships existed between results from the cell viability assay and performance measures under controlled environment and field conditions for both sorghum and corn. However, controlled experiments were limited due to space and time constraints, and field experiments were limited due to an absence of drought during the growing season. These studies showed that the staygreen trait was not clear in the known standards under controlled environment conditions. Few of the selected lines performed better under field condition. Further testing needs to be conducted to investigate the effectiveness of a cell viability assay as a feasible indicator of drought tolerance. Experiments under field conditions at different locations and with more replications would be necessary to evaluate relations between cell viability assay and expression of drought tolerance in field conditions.

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Dedication

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CHAPTER 1 - Literature Review

Introduction

Maize (*Zea mays* [L.]) and sorghum (*Sorghum bicolor* [L.] Moench) are staple grains in human and animal diets, ranking as the first and fifth leading cereals produced in the world, respectively. In the United States, maize and sorghum primarily serve as feed stock for the livestock industry (US Grain Council, 2008), as they are nutritionally similar (Hubbard et al., 1950; Waniska and Rooney, 1992). Additionally, maize and sorghum are used in ethanol and bio-fuels production, industrial manufacturing and as alternative food sources (Pimentel and Patzek, 2005; Zhan et al., 2003; Shukla and Cheryan, 2001; Schober et al., 2007). Maize is grown primarily in the Midwest, because productive soils and adequate rainfall make the crop an attractive and economically feasible choice in the region. Sorghum, however, is grown primarily in the Great Plains from the Texas Panhandle through Nebraska as the crop characteristics allow for maintenance of yield stability in environments where heat and drought stress can be problematic. In Kansas, maize is grown under irrigated conditions or in parts of the state where rainfall is adequate, whereas sorghum in generally grown in lower-yielding and drought-prone parts of the state.

With all crop production, the main causes of yield loss and instability are biotic and abiotic factors. Biotic factors that commonly affect maize and sorghum yields include, but are not limited to, disease outbreaks, insect infestations, weed competition and human interactions. Common abiotic factors that affect crop production include cold stress, heat stress and drought stress (Prasad et al., 2006; Campos et al., 2004). Drought stress is the single most limiting factor

in yield stability that can have a severe impact on agronomic and grain quality characteristics, as well as grain yield. Drought can occur at both pre-flowering and post-flowering stages of development, and has the most adverse effect on yield during and after anthesis (Tuinstra et al., 1997; Kebede et al., 2001). Drought stress usually coincides with periods of heat stress. Recent studies by Prasad et al. (2008) have shown that heat stress occurring at the pre-flowering, flowering, and post-flowering stages can affect sorghum plants. The authors concluded that the most sensitive stages to grain filling in sorghum were at flowering and ten days prior to flowering. It was noted that post-flowering heat stress caused yield losses up to 50% due to reduced seed filling duration.

Drought Effects on Plant Physiological Processes

Drought is the single most limiting factor to crop production world-wide. Lack of adequate soil moisture or water deficit, affect the plants ability to grow and complete a normal life cycle (Moussa et al., 2008). Changes in water balance and soil available water are crucial to crop yields by directly affecting plant physiological processes and responses (Miyashita, 2005; Kramer and Boyer, 1995; Hsiao, 1973). It has been reported that several physiological responses by the plant occur at water potential of less than -1.5 MPa. An increase in water deficit coincides with a dramatic decrease in water potential having differing effects depending on the crop. Atteya (2003) found that drought stress significantly altered internal water status in maize by lowering osmotic potential and relative water content, which inhibited photosynthetic rate. However, Giles et al. (1976) found that in comparison to maize, sorghum is less affected by water stress and offers that root factors and the ability of sorghum to maintain open stomata at lower water potential as explanations.

The main effect of drought is usually thought to be a decrease in photosynthesis and growth as a result of stomatal closure (Mwanamwenge et al., 1999). Stomata are highly sensitive to changes in soil water deficit, as conductance decreases quickly before much change in water potential (Atteya, 2003). Iljin (1957) reported that a water loss of 10% on a fresh weight basis will induce stomatal closure. Stomatal response to leaf water potential and environment are important for regulation of transpiration and photosynthesis (Ackerson et al., 1977), and essential for CO_2 acquisition (Medici et al., 2007).

Xu et al. (2008) offered that approximately two thirds of the decline in net photosynthetic rate is attributable to stomatal limitations under mild-to-moderate water stress, which limits CO_2 assimilation. When the plants photosynthetic machinery becomes susceptible to photoinhibition, CO_2 assimilation is reduced leading to a decline in photosynthetic efficiency, thereby reducing crop productivity and yield (Vitale et al., 2007). Xu et al. (2008) suggests that leaf dehydration is a factor in the rate at which net photosynthesis declines, particularly in older leaves. Additionally, drought stress causes decreases in photosynthesis prior to decreases in respiration, lowering the ratio between the two and allowing an increase in photorespiration (Prasad et al., 2008). Even though respiration is an important plant process, Ribas-Carbo et al. (2005) reports a limitation of studies examining respiration responses to drought and temperature stress. It was reported (Prasad et al., 2008), however, that respiration increases exponentially with increasing temperatures up to 40^{o}C and then decreases due to damage to the respiratory machinery. Additionally, drought stress can result in decreased leaf and root respiration in the short term (Byrla et al., 2001).

Ackerson et al. (1977) reported that stomata of maize and sorghum tend to be less responsive to drought if the plants have already been exposed to a mild water deficit, and that

stomates do not respond to changes in leaf water potential once reproductive plant growth has been initiated. This is contrasted by Tsuji et al. (2003) who found that drought stress reduced the stomatal conductance, transpiration rate, and net photosynthetic rate of sorghum cultivars 90 days after planting, and those of Miyashita et al. (2005) who noted that photosynthesis in maize decreases during reproductive growth as a result of diminishing leaf water potential.

It is important to note that drought-induced decreases in photosynthesis are not only attributed to stomatal limitations that decrease flow of CO_2 to mesophyll tissue, but non-stomatal limitations as well, which impair metabolic activities (Atteya, 2003; Graan and Boyer, 1990; Chaves et al., 2003; Farquhar et al., 1988). Under severe water deficit the amount of non-stomatal limitation to photosynthesis may increase (Thiagarajah et al., 1981). Major metabolic changes caused by non-stomatal limitation include impairment and reduced regeneration of ribulose bisphospahte (RuBP) and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Vitale et al., 2007; Bota et at., 2004). Adenosine tri-phosphate (ATP) synthesis and inorganic phosphorous accumulation are also adversely affected (Prasad et al., 2008), under limited photosynthesis.

While it is known that both stomatal and non-stomatal limitations cause reductions in photosynthesis during drought (Atteya, et al., 2003; Zhou et al., 2007), the limitations also have a negative impact on plant biochemistry and primary photochemistry associated with photosystem II (PSII) (Cechin, 1998). Chlorophyll fluorescence of the PSII is known as a significant indicator in the response of leaf photosynthesis to environmental stresses (Chechin, 1998; Baker, 1991). Chechin (1998) demonstrated that chlorophyll fluorescence indicated tolerance of PSII to water stress based on only a slight decrease in the efficiency of excitation capture by open PSII reaction centers, measured as F_v/F_m. Declining values of F_v/F_m are an indicator of stress. F_v/F_m

values for healthy plants are generally 0.8 (Burke, 2007). Xu et al. (2008) suggest that leaf age in maize plants may have an effect on the plasticity to rapid water deficit in terms of dehydration of photosynthesis and PSII activity limited by both stomatal and non-stomatal factors. In the same study, net photosynthetic rate, stomatal conductance and F_v/F_m were reduced to a greater degree in older leaves of maize plants. A close correlation was found between net photosynthetic rate and F_v/F_m and electron transport rate indicating that PSII function might partially explain a reduction in photosynthesis. It was also demonstrated in kidney beans (*Phaseolus vulgaris* [L.]) that after a water deficit of seven days, F_v/F_m values were slightly decreased, suggesting that photosynthetic activity was affected by the injury of electron transfer in PSII (Miyashita et al., 2005). In this experiment the F_v/F_m values were greatly decreased only under severe water stress, when leaf water potential was below -1.9 MPa because values maintained a constant between 0.8 and 0.81 for up to six days without watering, only greatly decreasing on the seventh day.

Maintenance of cellular membrane integrity is important for plants to withstand severe water stress, as a drop in soil moisture causes cell destruction, changes in growth hormone levels within the plant, and alters chloroplast and mitochondrial structure (Giles et al., 1976). Ion leakage from thylakoid membranes by a dehydration induced increase in free radicals causing lipid peroxidation (Moussa et al., 2008) adversely affecting photosynthesis (Xu et al., 2008). Differences in water stressed alterations of cell membrane ultrastructure have been reported between maize and sorghum. Giles et al. (1976) found that changes in chloroplast ultrastructure for sorghum and maize occurred at water potentials of -14 bars and -18 bars respectively and suggested that sorghum may be less susceptible than maize to drought induced cellular damage. They also noted that bundle sheaths in both species seemed to be more tolerant under drought

than mesophyll cells. Ristic and Cass (1991) noted however that thylakoids were damaged only under severe water stress in maize.

In conclusion, water deficit causes stomatal closure, reducing CO_2/O_2 ratio, thereby inhibiting photosynthesis (Moussa et al., 2008). In sorghum, drought mainly decreases photosynthesis, stomatal conductance and transpiration with decreasing leaf water potential. The same effects have been noted in maize, although leaf age seems to play a bigger role in the magnitude of decline for each physiological process (Tsuji et al., 2003; Xu et al., 2008).

Drought Effects on Growth and Development

Pre- or post-flowering drought can have dramatic effects on the agronomics of crop production. Symptoms of post-flowering drought can include but are not limited to premature leaf and stem death, accelerated leaf senescence, stalk brittleness or collapse and reduction in seed size (Xu et al., 2000). Stalk rot is one of the most problematic symptoms associated with drought, causing lodging which decreases harvest ability. Burgess et al. (2002), report that lesions caused by charcoal rot (*Macrophomina phaseolina*) in susceptible varieties of grain sorghum were 27% shorter in irrigated trials versus non-irrigated trials. Post-flowering drought can have similar effects on maize as well. Water deficits tend to shift the source-sink relation out of balance one way or the other. Excess source capacity causes purpling of the leaf, sheath and stalk tissues during grain filling. Excess sink capacity results in typical drought symptoms of premature tissue senescence and reduced yields (Lee and Tollenaar, 2007). Drought also can reduce leaf area development, leaf size and leaf dry matter accumulation, lowering resource capture and leading to lower canopy photosynthesis. Under mild water stress, shoot growth is restricted while root growth continues (Burke, 2007). The general effects include reduction in

leaf numbers and reduction in leaf expansion. Under severe stress, leaf elongation decreases and leaf growth can cease (Cakir, 2004; Prasad et al., 2008).

Post-Flowering Drought Effects on Yield

Drought stress can have a significant impact on final yield in cereal crops. A moisture deficit at the time of flowering causes the largest reduction in yield, as compared to drought events taking place at other stages of development. The amount of yield reduction depends not only on the timing of stress, but also on the severity of the stress (Wilson, 1968; Claasen and Shaw, 1970). Westgate and Grant (1989) and Saini (1997) suggest that when drought coincides with the onset of meiosis and early grain development, it has the most dramatic effect on yield.

During meiosis, water stress inhibits development of pollen grains and can cause male sterility. Disturbances in carbohydrate metabolism inhibit the internal pollen wall from developing normally as a result of lowered amounts of reserve starch supplies (Sheoran and Saini, 1996). The lack of starch also causes insufficient pollen tube growth on the female florets, inhibiting the pollen tubes from reaching the ovules, and disturbs the adherence of pollen grains to the surface of stigma papilla cells and normal pollen tube growth (Clement et al., 1994; Barnabas et al., 2008). In order for successful seed set, pollen must remain viable and stigmas must remain receptive, pollen tubes and ovules must function properly after fertilization, and embryo and endosperm development must proceed properly. Pollen viability and germination of the pollen grain is one of the most sensitive processes to moisture stress (Stone, 2001). Consequently, Cakir (2004) showed that in maize, a drought event at tasselling lead to a 20% decrease in kernel number on average in addition to reducing leaf area index.

Moisture stress also has an impact on female reproductive parts. In maize for example, silking is a relationship between ear growth and plant growth rate at the time of anthesis (Borras et al., 2007). In addition, an ear needs to reach a certain amount of accumulated biomass before reaching the silking stage. However, Schussler and Westgate (1991) report that stem development was greatly favored over ear biomass accumulation when plant growth is decreased due to water stress.

The time period between pollen shed and female stigma receptivity is known as the anthesis-silk interval (ASI) in maize. For maize, as well as most crops, anthesis for male flowers is defined as the beginning of pollen shed from the tassel or other male flower part; for females, it is defined by the appearance of pollen receptive stigmas. For populations of plants, these stages are defined when 50% of the total plants reach pollen shed or sigma receptivity (Borras et al., 2007). Water stress during floral induction and inflorescence development can lead to a delay in flowering or even complete inhibition, as has been reported in sorghum (Winkel et al., 1997; Wopereis et al., 1996). Moderate water stress can decrease time to flowering while severe water stress can increase the interval. Prasad et al. (2008) reports that panicle initiation can be delayed by 2-25 days and flowering can be delayed up to 59 days under drought stress conditions.

Yang et al. (2001) offer that high levels of absicic acid in early reproductive structures under moisture stress may impair floret and seed development. Damptey et al. (1976) and Blum (2000) say that absicic acid may also have a role in causing considerable delays in female organ development, while affecting male inflorescences to a lesser degree.

Ogretir (1994), states that maize is most sensitive to soil water deficits at tasseling and silking stages, but tends to be more tolerant at milk stage. Robins and Domingo (1953) indicated

that maize grown in pots can suffer yield reductions of up to 22 and 50% when subjected to 2-day and 7-day water stress at the tasseling stage, respectively. Westgate et al. (2004) conclude that a close synchrony between tasselling and silking is required for a high kernel set in maize and a negative relationship exists between final kernel number and a lengthier ASI.

Stresses during flowering and anthesis lead to failure of fertilization because of the impairment of pollen and ovule function. Drought can inhibit pollen development and cause sterility, shorten spike development thereby reducing potential seed number, and reduce grain filling duration thereby reducing seed weight (Prasad et al., 2008). It was found in maize (Claasen and Shaw, 1970) and peanut (Prasad et al., 1999) that the occurrence of stress just before anthesis caused significant increases in floral abortions and decreases in seed number. Water stress before pollination can result in aborted ovules even if ample water is available at pollination (Westgate and Boyer, 1986). Raper and Kramer (1987) imply that the increase in abortion rate could be a result of fewer photosynthates being allocated to floral organs during drought events at anthesis. Zinselmeier et al. (1999) and Setter et al. (2001) both show that embryo abortion was higher and kernel number decreased markedly under 5-day water stress around pollination time compared to an absence in stress at flowering. An insufficient nutrient supply can block the development of reproductive structures, causing ovule abortion. Barnabas et al. (2008) report that 15-45% of ovules develop abnormally when subjected to drought stress, while the value for irrigated plants was only 2.5%.

Drought decreases seed filling duration (Frederick et al., 1991; de Souza et al., 1997), defined as the time from fertilization to physiological maturity, leading to smaller seed size. Prasad et al. (2008) report that drought occurring after flowering has little effect on seed filling rate, but shortens seed filling duration, leading to smaller seed size and less yield. Seed size is

largely dependent on photosynthetic reserves that can be mobilized in the plant. Additional reductions in carbohydrates and nitrogen supplies, either from a decrease in photosynthetic activity or a reduction in leaf area, further decrease seed size and can shorten seed filling duration, all resulting in smaller seed size.

Grain filling is the final stage of growth in cereals where ovaries that were fertilized at pollination develop into caryopses. Its duration and rate determine the final grain weight, which is a key component of overall yield. Drought events during the grain filling stage can cause major reduction in yield by reducing starch accumulation as a result of limited assimilate partitioning to the developing grain (Blum, 1998) or by direct effects on processes of grain growth (Yang et al., 2004). In the early stages of grain fill, endosperm cells determine the maximum amounts of starch and protein that can be accumulated in each kernel (Egli, 1998) as influenced by the rate and duration of grain fill. Water stress during the grain filling period reduces photosynthesis, induces early senescence, and shortens the grain filling period, which is more affected by water stress than grain filling rate (Brooks et al., 1982; Westgate, 1994; Altenbach et al., 2003; Borras et al., 2003). Grain filling is closely related to senescence and utilization of stem reserves (Barnabas et al., 2008). Pre-anthesis stem reserves can contribute as much as 10-40% of final grain weight in wheat and rice. van Herwaarden et al. (1998) report that under drought stress, stem reserve mobilization can account for as much as 75-100% of grain yield in wheat.

Complex enzymatic processes account for starch accumulation in cereal grains (Morell et al., 2001). Sucrose synthase, adenosine 5'-diphosphate pyrophosphorylase (AGPase), soluble starch synthase, and starch branching enzymes A and B (SBE A, SBE B) are main enzymes affecting starch accumulation. Depending on severity of stress, the activity of these enzymes are

altered. Under severe dehydration, cessation of grain growth is attributed to reduced AGPase activity, but soluble starch synthase's decreased activity is responsible in limiting grain growth under moderate water stress (Ahmadi and Baker, 2001). The activity of these key enzymes result in increased sink activity, ultimately leading to an increase in the rate of grain fill under drought conditions (Barnabas et al., 2008).

Kernel number and size are a function of the rate and amount of biomass accumulation at the ear level around the flowering period (Andrade et al., 1999; Vega et al., 2001; Echarte et al., 2004), and is dependent on two main carbon sources: assimilates from photosynthetic activity and reserve carbohydrate stores (Plaut et al., 2004). Both are hampered by drought conditions as photosynthesis is reduced, resulting in reduction of assimilate supply.

One of the most important parts of grain development is grain yield, and is mainly due to the number of harvested kernels versus the variation in individual kernel weight (Earley, 1966; Borras, et al., 2004). Yazar et al. (1999) stated the kernel number per plant is a result of moisture availability and concluded that decrease in kernel number is the primary effect of water deficit on maize grain yield. In conclusion, drought mainly influences yield by limiting seed numbers. Drought also has an effect on pollen viability, pollen tube germination and also increases ovule abortion rates caused by reduction in assimilate supplies which are necessary for proper grain development.

Post-Flowering Drought Tolerance

The progress of leaf senescence is usually associated with a characteristic yellowing of leaf tissue indicating chlorophyll loss from the pigment-protein complexes of the photosynthetic

apparatus (Thomas and Howarth, 2000; Borrell et al., 2000). Tolerance of senescence in the advent of post-flowering drought stress is known as the stay-green trait, and is important in sorghum (Xu et al., 2000) and has been reported in maize (Tollenaar and Daynard, 1978; Crafts-Bander et al., 1984; Gentinetta et al., 1986; Rajcan and Tollenaar, 1999).

Thomas and Howarth (2000), report four distinct stay-green types. In type A stay-greens, senescence is initiated later than normal but proceeds at a normal rate. Type B stay-greens initiate senescence normally but it progresses rather slowly. Type C stay-greens retain green leaf area almost indefinitely, however photosynthetic activity declines over time under the cosmetics of the retained pigment. Type D stay-greens are acquired by artificial means such as boiling, drying or freezing in order to maintain green leaf color. The stay-green trait in sorghum is genetically and physiologically complex, both in expression and function, and can be classified as a mixture of both Type A and Type B (van Oosterom et al., 1996), whereas as modern maize hybrids can be classified in expression as Type C (Lee and Tollenaar, 2007). Stay-green has been associated with reduced lodging and resistance to stalk rots (Mughogho and Pande, 1983; Rosenow, 1984), higher levels of stem carbohydrates both during and after grain filling (McBee, 1984; Duncan, 1984;), and improved grain filling and grain yield under stress (Rosenow et al., 1983; Rosenow and Clark, 1981).

Stay-green genotypes appear to have higher leaf nitrogen amounts during flowering and are able to maintain green leaf area through grain filling (Borrell and Hammer, 2000). High leaf nitrogen content in maize at silking is highly correlated with yield, and photosynthetic ability is intimately related to nitrogen status at the leaf level (Xu et al., 2000). At the whole plant level, stay-green can be viewed as the result of balance between nitrogen demanded by the grain and nitrogen supplied during grain filling. Nitrogen comes from the soil as well as remobilization

from vegetative tissues (Borell et al., 2001). Rajcan and Tollenaar (1999) show that nitrogen derived from the soil during grain filling in maize was 60% for stay-green types and 40% for non stay-green types, meaning more nitrogen from tissue stores was available for maintenance of photosynthesis. This was shown to have an association with greater yields. Furthermore, Xu et al. (2000) showed that relative water content in stay-green lines of sorghum was 81%, much higher than non stay-green lines (38%), indicating that stay-green genotypes keep stalk transport functional under drought conditions.

As future crop water resources decline and world population increases, development of drought tolerant and water use efficient crops will be important. Plant resistance to drought happens at the molecular, cellular and physiological levels and can be subdivided into escape, avoidance, and tolerance (Barnabas et al., 2008). Drought escape relies on shorter life cycles and higher growth rates. Drought avoidance is the result of minimizing water loss through stomatal closure, or reduced leaf area or to maximizing water uptake by increased root growth. Drought tolerance may include osmotic adjustment and scavenging of reactive oxidative species (Barnabas et al., 2008; Tuinstra et al., 1997). Also, increased concentration of absicic acid as a result of drought, can maintain root growth while increasing hydraulic conductivity.

Improvements of Post-Flowering Drought Tolerance

Climates are changing across the globe, gradually leading towards higher temperatures, increases in evapotranspiration and increases in the incidence of drought in crop producing regions (Campos et al., 2004). The use of genetics and genomic technology in improving crop tolerance to drought stress will be important. In traditional plant breeding programs, post-

flowering drought stress is evaluated by growing the plants under irrigation in the vegetative stage to allow for sufficient growth. Just prior to anthesis, irrigation is terminated to allow a water deficit to develop during flowering and intensify during the grain filling period (Xu et al., 2000). Although environmental conditions should allow for good leaf growth in the vegetative stage and allow a stress period during flowering and grain filling, environmental effects are not always viable options and molecular markers might serve as a good alternative (Mahalakshmi and Bidinger, 2002).

Visual ratings of the stay-green trait are useful to breeders, they are quick and easy to perform and less expensive than quantitative measurements of stay-green. Ratings of visual leaf score for stay-green have been used to select for drought stress tolerance in maize (Bolanos and Edmeades, 1996) and with even better results in sorghum (Xu et al., 2000). Personal biases and differences in ratings among scientists can serve as limitations, however (Borrell et al., 2000).

Genetic analyses of stay-green have also been conducted. Tuinstra et al. (1997) indicated that stay-green was controlled by a single dominant factor with some epistatic interactions in sorghum. Additional results indicated that quantitative trait loci (QTL) for stay-green were located on linkage groups F and I and showed positive association with grain yield under post-flowering drought conditions. Kebede et al. (2001) concluded that two stay-green QTL's in sorghum corresponded to stay-green QTL regions in maize, and demonstrated congruency with these regions and other agronomic and physiological traits. Campos et al. (2004) report that individual drought associated QTL generally account for less than 10% of phenotypic variance for grain yield, ASI or barrenness under stress. Conventional selection based on wide testing has been successful at improving yield, but gains under terminal drought are minimal and novel sources of genetic variability will be needed in future improvement. Nguyen (1999) states that

genetic studies of drought tolerance in sorghum and maize show that multiple genes control tolerance associated with stay-green in sorghum and ASI in maize.

Screening for Drought Tolerance

Several physiological plant processes and the alterations caused by drought stress can be investigated and exploited to determine species and genotypes that show usefulness for drought tolerance. Grzeskiak et al. (2003) evaluated triticale response to drought via several physiological tests. Leaf gas exchange (photosynthesis), leaf water potential, chlorophyll content, chlorophyll fluorescence, and leaf temperature were all investigated as potential screening methods to determine drought tolerance. Results indicated that water potential and chlorophyll fluorescence were good indicators of drought stress during the vegetative stages. No correlations were found between the same tests and drought stress during the reproductive stage. Measuring chlorophyll fluorescence is a useful and non-invasive technique in ecological and physiological studies in terms of assessing plants response to environmental stress (Sayed, 2003). This method aims to estimate maximal quantum yield of PSII by measuring the ratio of maximum chlorophyll yield and chlorophyll yield in the exited state (F_v/F_m). F_v/F_m has been used in screening both maize (Jovanovic et al., 1991) and sorghum (Masojidek et al., 1991) and was correlated with decreased CO_2 assimilation and electron transport (Sayed, 2003). F_v/F_m however only exhibits changes in its values over time under strong environmental stress.

Other methods of screening plants for responses to stress include in vivo electrolyte leakage methods. It is readily available and inexpensive, however it is minimally destructive and results can be markedly influenced by various experimental procedures (Bajji et al., 2002). In

the last decade, use of handheld chlorophyll meters (SPAD) has increased dramatically. Chlorophyll content meters are relatively inexpensive and can acquire readings in rapid succession without damaging the plant. Readings are logged by exploiting the optical properties of leaves and are based on the reflectance and/or absorbance of radiation by chlorophyll. It has been shown, that linear functions do not always best fit the data that chlorophyll content meters provide (Uddling et al., 2007). Recent studies in wheat and maize have shown that chlorophyll content readings under heat stress are closely correlated with chlorophyll a fluorescence (Ristic et al., 2008).

A recent study (Burke, 2007) compared the decline in chlorophyll fluorescence over time between irrigated and non-irrigated treatments of cotton. It was found that leaf tissue harvested at sunrise, incubated at elevated temperatures in the dark, from well watered plants exhibited a greater decline in chlorophyll fluorescence yield over time when compared to the non-irrigated treatment. The initial premise for the assay was that large nighttime mobilization of photosynthates from source leaves to sinks of non-stressed plants might result in less photosynthate remaining in the source leaves compared to the stressed plants. The results suggest that a delayed decline in chlorophyll fluorescence yield can be used as an indicator of water-deficit stress responses.

Research was also conducted by Mr. Raymond Mutava (2008, personal communication) that expressed similar results between irrigated lines of staygreen and non-staygreen sorghum. The staygreen lines exhibited a greater decline in chlorophyll fluorescence yield over time than did non-staygreen lines. These results are similar to what Burke (2007) found in irrigated versus non-irrigated treatments of cotton.

Research Objectives

The objective of this research study is to investigate a new cell viability assay developed for cotton (Burke, 2007) as a viable, high-throughput, time efficient and inexpensive screen for large populations of maize and sorghum under post-flowering drought conditions. The assay investigates source leaf responses to water-deficit stresses. The hypothesis is that the values obtained from the cell viability assay can serve as an early indicator of plant performance (stay-green expression and yield) under post-flowering drought stress.

CHAPTER 2 - Cell Viability Assay Screen in Sorghum and Maize

Abstract

Drought is the single most limiting factor to crop yields and is most detrimental during and after anthesis. This greenhouse study was conducted to determine if measuring the decline in chlorophyll fluorescence yield (Fv/Fm) under elevated respiratory demand could identify staygreen genotypes versus non-staygreen genotypes. Sorghum (*Sorghum bicolor* [L.] Moench) and maize (*Zea mays* [L.]) were evaluated consisting of 300 lines from the sorghum diversity panel and 197 lines from the Monsanto Corporation. Leaf discs were collected at the V5 growth stage and Fv/Fm values were recorded with a chlorophyll fluorometer initially and then placed in an incubator at 40^{o}C. Fv/Fm measurements were taken at 2, 4, and 6 hours while being incubated. The results showed differences in the rate of decline in Fv/Fm in different lines of sorghum and maize. Based on the rate of decline, genotypes were classified as potential staygreen and non-staygreen lines. The lines which declined faster were classified as staygreen lines and those that declined slower were classified as non-staygreen lines. In 2007 twenty-six lines (thirteen in group 1; thirteen in group 2) of sorghum and twenty lines of maize (ten in group 1; ten in group 2) were identified as potential staygreen (group 2) or non-staygreen genotypes (group 1), based on results from a previous assay of known staygreen and non-staygreen material. The results suggest that the rate of decline in chlorophyll fluorescence measured over time can help identify staygreen and non-staygreen genotypes of sorghum and maize. However,

more investigation needs to be conducted in regards to incubation temperature to assess the effectiveness of the cell viability assay in sorghum and maize.

Introduction

Sorghum (*Sorghum bicolor* [L.] Moench) and maize (*Zea mays* [L.]) are two main crops grown in the Midwest and the Southern Plains. They serve as staples in human and animal consumption in a variety of ways (Schober et al., 2007; Pimental and Patzek, 2005; Zhan et al., 2003; Shukla and Cheryan, 2001;). Researchers are constantly trying to improve the yield of these crops, as well as develop varieties that are adapted to Kansas and other regions.

The main causes of yield loss and instability are biotic and abiotic factors. Biotic factors that commonly affect maize and sorghum yields include, but are not limited to, disease, insect infestations, weed competition, and human interactions. Common abiotic factors that affect crop productivity include cold stress, heat stress, and drought stress (Prasad et al., 2006; Campos et al., 2004). Drought stress is the single most limiting factor in yield instability and can severely reduce grain yield and grain quality. Drought is characterized at both pre-flowering and post-flowering stages of growth and development and has the most dramatic effect on yield during and after anthesis (Kebede et al., 2001; Tuinstra et al., 1997). Tolerance to post-flowering drought can help increase grain fill duration and results in greater yields.

Finding an efficient screening tool to effectively and efficiently screen for staygreen lines would greatly benefit researchers in advancing varieties adapted to post-anthesis drought stress. Measuring chlorophyll fluorescence can be a useful and non-invasive technique in ecological and physical studies in assessing plant response to environmental stress (Sayed, 2003). Methods have been used in the past to estimate maximum quantum yield of PSII by measuring the ratio of

maximum chlorophyll yield and chlorophyll yield in the exited state (Fv/Fm). Fv/Fm has been used in screening both maize and sorghum genotypes (Jovanovic et al., 1991; Masojidek et al., 1991).

An assay conducted on lines of cotton revealed that chlorophyll fluorescence declines over time to a greater degree in irrigated lines compared to lines that were not irrigated. The initial premise for the assay was that large nighttime mobilization of photosynthates from source leaves to sinks of non-stressed plants might result in less photosynthate remaining in the source leaves compared to the stressed plants. The results suggest that a delayed decline in chlorophyll fluorescence can be used as an indicator of water-deficit stress responses (Burke, 2007). Results involving the same assay run on known staygreen and non-staygreen genotypes of sorghum show the same trend over time for chlorophyll fluorescence. The staygreen lines showed a greater decline in chlorophyll fluorescence yield over time compared to non-staygreen lines.

The objective of this research was to test a novel stress cell viability assay (Burke, 2007) as a viable, high throughput, time efficient, and inexpensive way to screen, identify, and rank lines of maize and sorghum based on a change in chlorophyll fluorescence over time. If successful, the cell viability assay could potentially serve as a good indicator of staygreen expression, physiological performance, and yield performance under post-anthesis drought stress.

Materials and Methods

I. Sorghum

In 2007, 300 lines from the sorghum diversity panel (SB Panel) were grown in a controlled greenhouse environment in Manhattan, KS and tested for differences in chlorophyll fluorescence over time. Four replications of individual plants were grown in fifteen centimeter pots filled with Metro-Mix 360 growing medium (Hummert International, Topeka, KS). Five seeds pot^{-1} were planted on October 16th, 2007, and thinned to one plant pot^{-1} when stands became established. Hand rouging of weed species was done as needed. Slow release Osmocote brand fertilizer 15-9-12 (3.1 grams) was applied at planting. Pots were watered daily to provide a non-stressed environment. Greenhouse controls for daytime and nighttime temperature were set at 26.7 ^{o}C and 21.1^{o}C respectively, with a twelve hour photoperiod.

Leaf punches (1.3 cm in diameter) were collected from each individual plant after plants had obtained the fifth main stem leaf in the vegetative stage (V5). Leaf punches were taken halfway between the leaf collar and leaf tip, and halfway between the leaf margin and midrib. Leaf punches were placed in test tubes filled with distilled water at collection, and then transferred onto a piece of germination paper moistened with distilled water and placed on a glass plate. CO_2 permeable plastic wrap (GLAD Brand) was used to cover the leaf punches once they were in place on the germination paper. A handheld pulse modulated chlorophyll fluorometer (OptiSciences Inc., Hudson, NH) was used to take initial fluorescence readings (Fo, Fv/Fm) of each leaf sample. Samples were then placed in an incubator at 40^{o}C (Eco-Therm, Gahanna, OH). Thereafter fluorescence readings were taken on each leaf sample at two hours,

four hours, and six hours after the initial time reading. Samples were removed from the incubator for each fluorescence reading and replaced immediately after each fluorescence reading.

The experiment was designed in a randomized complete block with four replications. Lines from the diversity panel were treated as the main effects and replications served as block effects. Statistical analysis was conducted using the general linear model procedure (PROC GLM) of the Statistical Analysis Software (SAS v9.1) to test for differences in chlorophyll fluorescence over six hours between the 300 lines of sorghum.

II. Maize

In 2007, 197 experimental maize lines from the Monsanto Corporation were grown in a controlled greenhouse environment in Manhattan, KS and tested for differences in chlorophyll fluorescence over time. Five replications of individual plants were grown in fifteen centimeter pots filled with Metro-Mix 360 growing medium (Hummert International, Topeka, KS). Two seeds pot^{-1} were planted on May 30th, 2007 originally, and thinned to one plant pot^{-1} as stands became established. Hand rouging of weed species occurred as needed. Slow release Osmocote brand fertilizer 15-9-12 (3.1 grams) was applied at planting. Pots were watered daily to provide a non-stressed environment. Greenhouse controls for daytime and nighttime temperature were set at 26.7 ^{o}C and 21.1^{o}C respectively, with a twelve hour photoperiod.

Leaf punches (1.3 cm in diameter) were collected from each individual plant after plants had obtained the fifth main stem leaf in the vegetative stage (V5). Leaf punches were taken halfway between the leaf collar and leaf tip, and halfway between the leaf margin and midrib.

Leaf punches were placed in test tubes filled with distilled water at collection, and then transferred onto a piece of germination paper moistened with distilled water and placed on a glass plate. CO_2 permeable plastic wrap (GLAD Brand) was used to cover the leaf punches once they were in place on the germination paper. A handheld pulse modulated chlorophyll fluorometer (OptiSciences Inc., Hudson, NH) was used to take initial fluorescence readings (Fo, Fv/Fm) of each leaf sample. Samples were then placed in an incubator (Eco-Therm, Gahanna, OH) at 40^{o}C. Thereafter, fluorescence readings were taken on each leaf sample at two hours, four hours, and six hours after the initial reading. Samples were removed from the incubator for fluorescence readings and replaced immediately after each fluorescence reading was taken.

The experiment was designed in a randomized complete block with five replications. Experimental maize lines were treated as the main effects and replications served as block effects. Statistical analysis was conducted using the general linear model procedure (PROC GLM) of the Statistical Analysis Software (SAS v9.1) to test for differences in chlorophyll fluorescence over six hours among the 197 experimental maize lines.

Results

I. Sorghum

Cell viability assay results on 300 lines of sorghum from the sorghum diversity panel are given in Appendix A. Differences between lines were tested at the initial time reading (0 h), 2 h, 4 h, and 6 h time readings after incubation. Differences in lines also were tested for the decline in chlorophyll fluorescence at the time interval of Δ 2 hour (change from 0-2h), Δ 4 hour (change

from 0-4h), and Δ 6 hour (change from 0-6h). Mean values of Fv/Fm for all lines and all time readings and P-values associated with the F-test for differences between lines are shown in Table 2.1. Differences in Fv/Fm readings between lines at 2 hours, 4 hours, Δ 2 hours, and Δ 4 hours were significant at the $P < 0.1$ level. Also, a decreasing trend is noticeable in chlorophyll fluorescence values (Fv/Fm) over time.

Table 2.2 displays the 26 lines of sorghum selected from the sorghum diversity panel and the rank assigned to each line. Fv/Fm values for each time interval are represented in the table (ranks correspond to Δ 6 hour values). Those lines ranked 1 through 13 are denoted group 1 and tested as potential non-staygreen lines. Those lines ranked 14-26 are denoted as group 2 and tested as potential staygreen lines. Four additional lines are also represented as known non-staygreen (group 3) and staygreen (group 4) genotypes, assigned rank 00 and 0 respectively.

Least significant difference ($\alpha = 0.1$) between groups for the change in Fv/Fm over time are shown in Table 2.3. The change in Fv/Fm over two hours was not significant between groups. At Δ 4 hour, Fv/Fm values for the non-staygreen check group 3 and the non-staygreen group 1, were significantly different. At Δ 6 hours, there was a significant difference between the staygreen check group 4 and the non-staygreen group 1. Significant differences were also noted for Fv/Fm values between the non-staygreen check group 3 and the non-staygreen group 1. There was no significant difference in Fv//Fm values between groups 3 and 4.

Figure 2.1 displays the decline in chlorophyll fluorescence measured as Fv/Fm over time. At the initial and two hour time readings, there was no group effect on the Fv/Fm values. However, there was a group effect at four and six hours after incubation. At the four hour reading, differences in Fv/Fm between groups were significant at the $P = 0.05$ level. At the six hour time reading, differences in Fv/Fm between groups were significant at the $P = 0.01$ level.

The non-staygreen group 1 had significantly higher Fv/Fm values compared to staygreen group 2 at 4 and 6 hours after incubation. However there were no significant differences between the known non-staygreen checks (group 3) and the known staygreen checks (group 4).

II. Maize

Cell viability assay results for all 197 experimental lines of maize are represented in Appendix B. Analysis of variance results (Table 2.4) show significance differences existed between lines for mean Fv/Fm values at the initial, 4 hour, 6 hour, Δ 4 hour, and Δ 6 hour values.

Table 2.5 displays the 20 lines of maize selected from the set of experimental maize lines and a rank assigned to each line. Ranks correspond to Δ 6 hour Fv/Fm values. Fv/Fm values for each time interval are also represented on the table. Those lines ranked 1 through 10 are denoted group 1 and tested as potential non-staygreen lines. Those lines ranked 11-20 are denoted as group 2 and tested as potential staygreen lines.

Significant difference ($\alpha = 0.1$) for change in Fv/Fm over time were observed (Table 2.6) between groups at Δ 4 hour and Δ 6 hours. Lines in staygreen group 2 had significantly higher declines in Fv/Fm compared to lines in non-staygreen group 1 at 4 and 6 hours after incubation.

Figure 2.2 displays the decline in chlorophyll fluorescence measured as Fv/Fm over the four time measurements. Significance for group effect was noted at the initial time reading ($P = 0.01$) and for the four and six hour time readings ($P = 0.001$). These data show that non-staygreen group 1 had significantly higher Fv/Fm values compared to staygreen group 2.

Discussion

Chlorophyll fluorescence has been identified as a good indicator of drought tolerance (Grzeskiak et al., 2003), and has been used in screening both sorghum (Masojidek et al., 1991) and maize (Javanovic et al., 1991). Burke (2007) suggests that a decline in chlorophyll fluorescence yield exposed to elevated respiratory demand over time can be used as a sensitive indicator of water stress responses. A novel stress cell viability assay was run on lines of sorghum and maize to see how Fv/Fm values changed over time. Results from earlier experiments conducted by Mutava (2008) show that known staygreen varieties exhibit a greater decline in Fv/Fm over time than varieties known to be non-staygreen types. It was hypothesized based on these results that lines of sorghum and maize, when placed under elevated respiratory demand, showing a greater decline in Fv/Fm over time were potential staygreen lines. Likewise, those lines showing a lesser decline in Fv/Fm over time were potential non-staygreen lines.

The assay results for all 300 lines of sorghum and 197 lines of maize exposed to elevated respiratory demand over time, showed a decreasing trend over six hours for values of chlorophyll fluorescence (Fv/Fm). Sayed (2003) noticed that Fv/Fm also exhibits changes over time under strong environmental stress, which is similar to the results for the sorghum and maize lines.

Change in Fv/Fm values over six hours (Δ 6 hour) were examined and subsets of 26 lines of sorghum and 20 lines of maize were selected to evaluate the effectiveness of the assay. Those exhibiting low Δ 6 hour values were denoted as non-staygreen group 1, while those exhibiting high Δ 6 hour values were denoted as staygreen group 2. For sorghum, an additional four lines were added, two known staygreen types (SC35 and MN 7645) and two known non-staygreen types (SC599 and TX7078), to serve as checks. These were denoted as NSG check group 3 and SG check group 4.

It was observed in Figures 2.1 and 2.2 that a general trend of declining Fv/Fm under elevated respiratory demand was taking place, which coincides with both Burke (2007) and Sayed (2003). Significant differences between groups for both maize and sorghum were also noted, coinciding with Mutava (2008) that Fv/Fm is declining over time. In this case, the hypothesized non-staygreen and staygreen groups both correspond to what Mutava (2008) found. It is also known that staygreen offers tolerance to post-anthesis drought (Xu et al., 2000). Therefore this assay could potentially serve as an indicator of staygreen expression under post-anthesis drought conditions. However further studies are necessary to see if relationships exist between the cell viability assay values and plant physiology, staygreen expression and yield under post-anthesis drought.

Conclusion

This experiment showed that differences in Fv/Fm measured under elevated respiratory demand over time exist across the sorghum diversity panel and across experimental maize lines from the Monsanto Corporation. Also, groups of lines thought to be non-staygreen and staygreen show similar trends in the decline of Fv/Fm values when compared to data on known staygreen and non-staygreen lines. It is suggested in the literature that decline in chlorophyll fluorescence over time could be used as an indicator of drought tolerance. The results of this experiment and other research at Kansas State University suggest that sampling a large number of lines and evaluating chlorophyll fluorescence under elevated respiratory demand can be useful as a preliminary screen in determining staygreen and non-staygreen genotypes.

Table 2.1. Mean Fv/Fm for 300 lines of sorghum screened from the diversity panel at different time intervals after incubation.

Time	Lines Fv/Fm	P-Value
Initial	0.762	0.704
2 Hours	0.436	0.088
4 Hours	0.232	0.097
6 Hours	0.128	0.159
Δ 2hours	0.326	0.094
Δ 4hours	0.530	0.091
Δ 6hours	0.634	0.153

Table 2.2. Time series data of selected sorghum lines from the sorghum diversity panel and their corresponding rank based on the cell viability (CVA) at assay Δ 6 hour values for Fv/Fm from initial screening experiment.

CVA Rank	Pedigree	Initial	2 H	4 H	6 H	Δ 2hour	Δ 4hour	Δ 6hour
1	SC124	0.760	0.437	0.407	0.370	0.323	0.353	0.390
2	El Mota	0.757	0.589	0.427	0.337	0.168	0.330	0.420
3	SC1214	0.753	0.282	0.272	0.324	0.471	0.481	0.429
4	SC305	0.750	0.513	0.320	0.283	0.237	0.430	0.467
5	SC213	0.758	0.527	0.424	0.289	0.231	0.334	0.469
6	SC58	0.747	0.450	0.300	0.262	0.297	0.447	0.485
7	SC110	0.764	0.456	0.314	0.263	0.308	0.450	0.502
8	B.OK11	0.761	0.500	0.326	0.250	0.262	0.436	0.512
9	SC132	0.759	0.505	0.388	0.238	0.255	0.371	0.521
10	SC1047	0.759	0.478	0.285	0.232	0.282	0.475	0.527
11	SC265	0.764	0.395	0.278	0.235	0.369	0.486	0.529
12	SC1019	0.769	0.473	0.288	0.211	0.296	0.481	0.557
13	B.Tx2752	0.742	0.339	0.283	0.160	0.403	0.459	0.583
14	SC480	0.760	0.350	0.109	0.030	0.410	0.651	0.730
15	SC337	0.760	0.421	0.138	0.025	0.339	0.623	0.735
16	R.TX2536	0.762	0.471	0.215	0.026	0.291	0.547	0.736
17	SC142	0.769	0.475	0.162	0.034	0.294	0.607	0.736
18	SC855	0.754	0.294	0.069	0.018	0.460	0.685	0.736
19	B.Tx615	0.763	0.452	0.083	0.026	0.311	0.680	0.737
20	SC452	0.763	0.464	0.136	0.021	0.299	0.627	0.742
21	SC1471	0.757	0.281	0.099	0.014	0.476	0.658	0.743
22	SC1201	0.769	0.447	0.116	0.020	0.322	0.653	0.749
23	SC1103	0.768	0.384	0.111	0.017	0.384	0.657	0.751
24	SC1319	0.778	0.614	0.135	0.026	0.164	0.643	0.752
25	SC929	0.775	0.360	0.076	0.018	0.415	0.700	0.757
26	SC329	0.769	0.237	0.089	0.010	0.533	0.681	0.759
NSG Check (00)	SC599	0.767	0.374	0.146	0.109	0.394	0.621	0.658
NSG Check (00)	Tx7078	0.761	0.497	0.344	0.161	0.264	0.417	0.600
SG Check (0)	SC35	0.759	0.453	0.091	0.010	0.306	0.668	0.749
SG Check (0)	00MN7645	0.772	0.352	0.070	0.010	0.420	0.702	0.762

Table 2.3. Mean change (Δ) in chlorophyll fluorescence (Fv/Fm) for groups of sorghum at given time intervals.

	Group	Δ 2hours	Δ 4hours	Δ 6hours
1	NSG	0.311	0.493[b]	.595[b]
2	SG	0.328	0.546[ab]	.654[ab]
3	NSG Check	0.372	0.610[a]	.703[a]
4	SG Check	0.366	0.590[a]	.691[a]
	LSD ($\alpha < 0.05$)	NS	0.103	0.088

Table shows change (Δ) in chlorophyll fluorescence (Fv/Fm) values at 2, 4, and 6 hours respectively. Least significant differences (LSD) across groups were calculated at the $\alpha = 0.05$ level. NSG and SG represent non-staygreen and staygreen testing groups respectively.

Table 2.4. Mean Fv/Fm for 197 lines of maize screened at different time intervals after incubation.

Time	Lines Fv/Fm	P-Value
Initial	0.753	0.091
2 Hours	0.610	0.223
4 Hours	0.468	< .0001
6 Hours	0.397	< .0001
Δ 2hours	0.143	0.498
Δ 4hours	0.284	< .0001
Δ 6hours	0.355	< .0001

This table shows the analysis of variance (ANOVA) tested at the α level of $P < 0.05$ for the 197 experimental maize lines from Monsanto Corporation screened with the novel stress cell viability assay. Fv/Fm values represent the mean value of chlorophyll fluorescence across lines at the given time interval.

Table 2.5. Time series data for selected corn lines from the Monsanto Corporation and their corresponding rank based on the cell viability assay Δ 6 hour values of Fv/Fm.

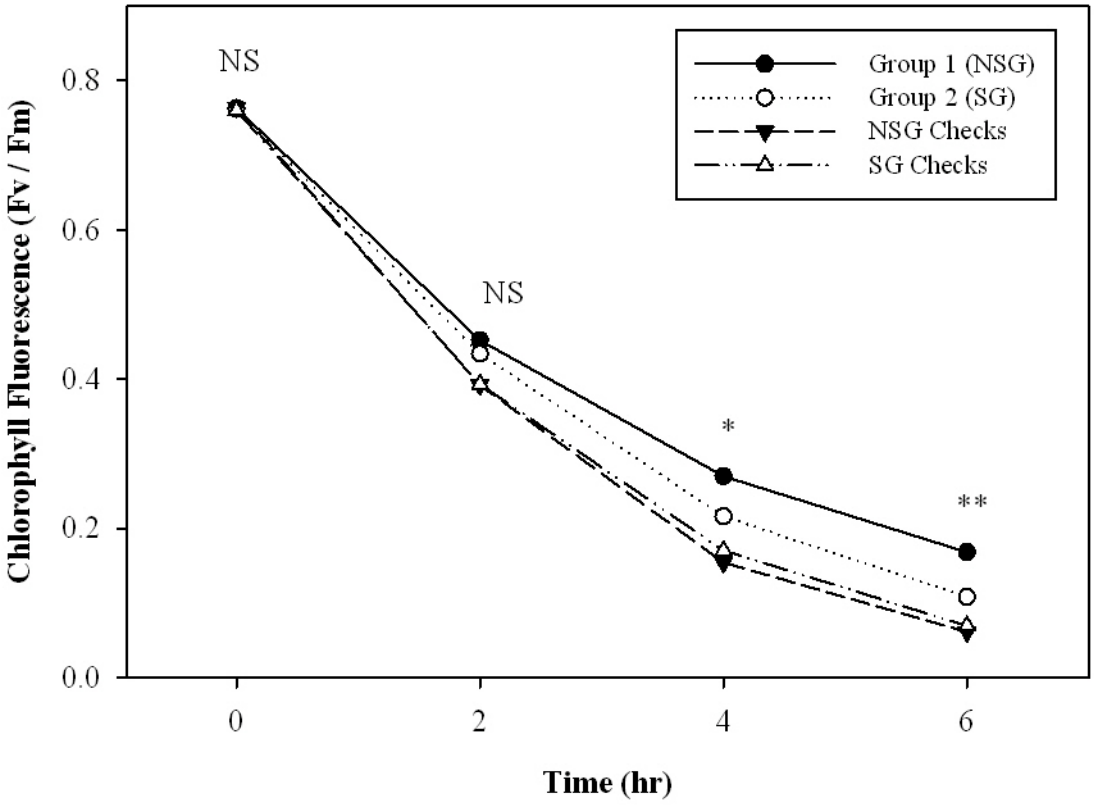
CVA Rank	Pedigree	Initital	2 Hours	4 Hours	6 Hours	Δ 2hour	Δ 4hour	Δ 6hour
1	EXP006	0.742	0.629	0.571	0.552	0.114	0.171	0.190
2	EXP156	0.747	0.613	0.586	0.540	0.134	0.160	0.207
3	EXP157 (B73xMO17)	0.746	0.637	0.533	0.535	0.109	0.213	0.211
4	EXP126	0.747	0.569	0.558	0.517	0.178	0.189	0.229
5	EXP180	0.745	0.588	0.536	0.514	0.157	0.208	0.230
6	EXP132	0.748	0.548	0.554	0.511	0.200	0.194	0.237
7	EXP102	0.763	0.643	0.582	0.522	0.120	0.181	0.240
8	EXP160	0.749	0.619	0.584	0.503	0.130	0.165	0.246
9	EXP169	0.748	0.618	0.571	0.498	0.130	0.177	0.249
10	EXP049	0.748	0.593	0.568	0.484	0.155	0.180	0.263
11	EXP041	0.749	0.597	0.375	0.314	0.152	0.374	0.435
12	EXP121	0.758	0.615	0.367	0.314	0.142	0.390	0.444
13	EXP134	0.752	0.616	0.403	0.290	0.137	0.349	0.462
14	EXP015	0.757	0.619	0.388	0.293	0.138	0.369	0.465
15	EXP031	0.748	0.589	0.357	0.283	0.159	0.391	0.465
16	EXP125	0.755	0.620	0.380	0.285	0.135	0.375	0.470
17	EXP092	0.758	0.621	0.376	0.280	0.137	0.381	0.477
18	EXP009	0.756	0.615	0.377	0.269	0.141	0.379	0.487
19	EXP148	0.767	0.635	0.398	0.274	0.132	0.369	0.493
20	EXP130	0.768	0.619	0.347	0.208	0.149	0.421	0.560

Table 2.6. Means values for change (Δ) of Fv/Fm between groups of maize over time.

	Group	Δ 2hours	Δ 4hours	Δ 6hours
1	NSG	0.143	0.185[b]	0.300[b]
2	SG	0.142	0.380[a]	0.476[a]
	LSD (α = 0 .05)	NS	0.039	0.045

Table shows change (Δ) in chlorophyll fluorescence (Fv/Fm) values at 2, 4, and 6 hours respectively for group 1 and group 2. Least significant differences (LSD) across groups were calculated at the $\alpha = 0.05$ level. NSG and SG represent non-staygreen and staygreen testing groups respectively.

Figure 2.1 Chlorophyll fluorescence (Fv/Fm) decline over time in non-staygreen and staygreen groups of sorghum.

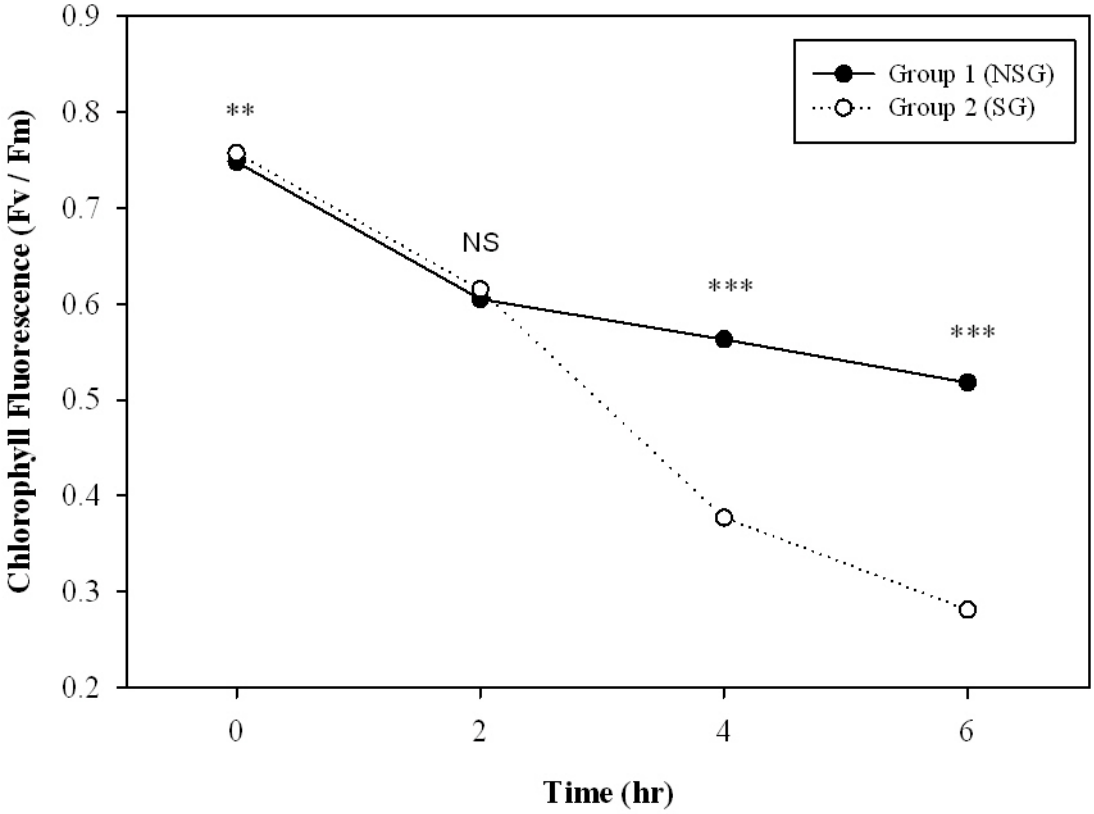


*, **, *** significant at P < 0.05, 0.01, and 0.001 respectively

NS – not significant

Graph displays chlorophyll fluorescence decline in sorghum between groups at each time interval.

Figure 2.2. Chlorophyll fluorescence (Fv/Fm) decline over time in non-staygreen and staygreen groups of corn.



*, **, *** significant at P < 0.05, 0.01, and 0.001 respectively

NS – not significant

Graph displays chlorophyll fluorescence decline in maize between groups at each time interval.

CHAPTER 3 - Greenhouse Evaluation of Cell Viability Assay

Abstract

Decline in chlorophyll fluorescence under elevated respiratory demand can be used as a sensitive indicator of plant responses to water-deficit. This greenhouse study was conducted to investigate if relationships existed between data from a cell viability assay and plant performance based on both physiological and yield traits, when plants were exposed to post-anthesis drought stress. Sorghum (30 lines) and maize (20 lines) were grown in a controlled greenhouse environment and tested across two groups based upon cell viability assay results (non-staygreen group 1, staygreen group 2). Water stress was induced at the beginning of anthesis for a seven day duration. Data on leaf chlorophyll content (SPAD meter), leaf temperature and chlorophyll fluorescence were taken at 7 d intervals starting at the completion of anthesis for six weeks. Yield components and staygreen scores were collected at harvest. Staygreen was scored on a 1 to 5 scale (1 = 100% green leaf retention, 5 = complete senescence). In sorghum, a time effect was observed for chlorophyll content and leaf temperature and chlorophyll content values were negatively correlated over time. There was also a difference between staygreen and non-staygreen lines in seed number. In maize, chlorophyll content and leaf temperature were both negatively correlated with time. Both chlorophyll content and chlorophyll fluorescence were correlated with an associated ranking from the cell viability assay data. Further investigation is needed on a larger scale to determine the extent of the relationship between cell viability assay

rankings and plant performance. A field study would be more indicative of the relationship between the assay rankings and physiological and yield traits under post-anthesis drought stress.

Introduction

Maize (*Zea mays* [L.]) and sorghum (*Sorghum bicolor* [L.] Moench) are the first and third largest crops grown in the United States respectively (US Grains Council, 2008). They offer growers in the Midwest and Southern Plains states an attractive choice for crop production due to their ability to grow in a wide array of environmental conditions. Maize and sorghum are also major constituents in food and feedstuffs, and are nutritionally similar (Waniska and Rooney, 1992; Hubbard et al., 1950).

It is a constant challenge for plant breeders and physiologists to find quick and reliable ways of screening for stress tolerance. Drought is most detrimental to overall yield during and after anthesis (Tuinstra et al., 1997; Kebede et al., 2007). The duration of drought stress plays an important role in yield reduction (Wilson, 1968; Classen and Shaw, 1970). Water stress affects the plants ability to grow and complete a normal life cycle (Moussa et al., 2008) by directly affecting plant physiological processes and yield responses (Miyashita, 2005; Kramer and Boyer, 1995; Hsiao, 1973).

Finding ways to effectively and efficiently screen experimental lines in a high throughput fashion could significantly shorten the amount of time needed to evaluate germplasm and develop new varieties. High yielding, drought tolerant lines of maize and sorghum could be brought into production faster. Chechin (1998) demonstrated that chlorophyll fluorescence could be used as a potential screen for drought stress tolerance. Several other physiological screens,

(eg. Cell membrane thermo-stability, relative leaf chlorophyll content, stomatal conductance, leaf canopy temperature depression) have also been reported in literature as indicators of drought stress tolerance. However, it is equally important to know how values from a high throughput screen might relate to other plant processes.

The objective of this research was to investigate if relationships could be found between a cell viability assay measuring chlorophyll fluorescence and various measures of plant performance. Rankings were assigned to lines of sorghum and maize based on chlorophyll fluorescence (Chapter 2). These lines were tested in a controlled greenhouse environment to determine if there were any relationships between cell viability assay rankings and physiological, yield, and staygreen ratings in staygreen and non-staygreen lines.

Materials and Methods

I. Sorghum

In 2008, 30 lines from the sorghum diversity panel (diverse group of sorghum germplasm researched extensively at K-State) were grown in a controlled greenhouse environment in Manhattan, KS and tested for differences in leaf chlorophyll content, infrared leaf temperature, chlorophyll fluorescence, staygreen expression and yield components. The 30 lines were a subsample of 300 lines from the sorghum diversity panel. A cell viability assay (Chapter 2) was used to select these lines based on change in chlorophyll fluorescence over six hours (Δ 6 hour). The 30 lines were tested as four separate groups (Non-Staygreen, Group 1, 13 lines; Staygreen, Group 2, 13 lines; Non-Staygreen Checks, Group 3, 2 lines; Staygreen Checks, Group 4, 2 lines). Lines within group 1 and group 2 were selected based on the experiment hypothesis that non-staygreen lines have a smaller Δ 6 hour Fv/Fm value, while staygreen lines have a higher Δ 6 hour Fv/Fm value, and were assigned a rank (1-13; 14-26). Checks were selected as known non-

staygreen or staygreen genotypes (suggested by Dr. Mitch Tuinstra, Purdue Univ.). Two replications were grown in 30.5 centimeter pots filled with equal parts of Metro-Mix 360 (Hummert Intl, Topeka, KS) growing medium, sand, and soil. Five seeds pot^{-1} were planted on April 18th, 2008 originally and thinned to one plant pot^{-1} when stands became established. Hand rouging of weed species occurred as needed. Slow release Osmocote 14-14-14 fertilizer (19.7 g) was applied at planting and incorporated into the growing medium. Greenhouse controls were set at daytime and nighttime temperatures of 29.4°C and 23.9°C respectively, with a twelve hour photoperiod. Pots were kept under full irrigation until the onset of anthesis, when watering was withheld for a seven-day period. After seven days, watering was resumed as needed until the grain filling period was complete.

Physiological measurements were recorded at the completion of anthesis and were recorded at seven day intervals for a six week period. Measurements were recorded on the flag leaf. Leaf chlorophyll content was measured with a SPAD 502 meter (Minolta Corp., Tokyo, Japan). Infrared leaf temperature was measured with an infrared thermal imaging camera (Flir Corp., Wilsonville, OR). Chlorophyll fluorescence was measured with a handheld pulse modulated chlorophyll fluorometer (OptiSciences Inc., Hudson, NH).

Pots were harvested on August 7th and August 19th, depending on maturity, and staygreen was scored on a 1-5 scale (1 = 100% green leaf retention, 5 = complete senescence) as described by Xu et al. (2000). Panicles were clipped off and placed in a grain dryer for seven days at 37.2°C, then removed and threshed with a portable threshing device (Almaco Ind., Nevada, IA). Seed weight and seed number pot^{-1} were also recorded. Seed was counted using a model 850-3 seed counter (International Marketing and Design Co., San Antonio, TX).

The experimental design was a randomized complete block with two replications. Groups of sorghum lines were treated as the main effects and replications served as the block effect. Statistical analyses were conducted using PROC CORR (Spearman and Pearson tests), PROC MIXED, and PROC GLM within the Statistical Analysis Software (SAS v9.1) program. PROC CORR using the Spearman test was used to determine if correlations existed between the ranking assigned to each line from the cell viability assay (Chapter 2) and physiological measurements, yield components, and staygreen score. PROC CORR using the Pearson test was used to determine if a correlation existed between grouping and time and the various physiological measurements, yield components and staygreen scores. PROC GLM was used to test for differences between groups for yield characteristics and staygreen expression. PROC MIXED was used to test for differences between groups and the time of measurement, as well as a group*time interaction in regards to the physiological measurements recorded.

II. Maize

In 2008, 20 experimental lines of maize from the Monsanto Corporation were grown in a controlled greenhouse environment in Manhattan, KS and tested for differences in leaf chlorophyll content, infrared leaf temperature, chlorophyll fluorescence, staygreen expression, and yield components. The 20 lines were a subsample of 197 experimental lines supplied by the Monsanto Corporation. A cell viability assay (Chapter 2) was used to categorize these lines based on the change in chlorophyll fluorescence over six hours (Δ 6 hour). The 20 lines were tested as two separate groups (Non-staygreen, Group 1, 10 lines; Staygreen, Group 2, 10 lines). Lines within group 1 and group 2 were selected based on the experiment hypothesis that non-staygreen lines have a smaller Δ 6 hour value, while staygreen lines have a higher Δ 6 hour

value, and were assigned a rank (1-10; 11-20). Two replications were grown in 30.5 centimeter pots filled with equal parts of Metro-Mix 360 growing medium, sand, and soil. Three seeds pot^{-1} were planted on April 28th, 2008 and thinned to one plant pot^{-1} as stands became established. Hand rouging of weed species occurred as needed. Slow release Osmocote 14-14-14 fertilizer (19.7 g) was incorporated into the growing medium at planting. Greenhouse controls were set at daytime and nighttime temperatures of 29.4°C and 23.9°C respectively, with a twelve hour photoperiod. Pots were fully watered until the onset of anthesis, at which time watering was withheld for a seven day period. After seven days, watering was resumed as needed until the grain filling period was complete.

Physiological measurements were recorded at the completion of anthesis and were recorded at seven day intervals over a six week period. Measurements were recorded on the ear leaf. Leaf chlorophyll content was measured with a SPAD 502 meter (Minolta, Tokyo, Japan). Infrared leaf temperature was measured with an infrared thermal imaging camera (FLIR Corp., Wilsonville, OR). Chlorophyll fluorescence was measured with a handheld pulse modulated chlorophyll fluorometer (OptiSciences Inc., Hudson, NH).

Pots were harvested on August 7th and staygreen was scored on a 1-5 scale (1 = 100% green leaf retention, 5 = complete senescence) as described by Xu et al. (2000). Ears were removed and placed in a grain dryer for seven days at 37.2°C then removed and threshed with a portable ear sheller (Almaco Ind., Nevada, IA). Ear length measured in centimeters, number of kernel rows ear^{-1}, number of kernels row^{-1}, seed weight and seed number pot^{-1} were collected as yield components. Seed was counted using a model 850-3 seed counter (International and Design Corporation, San Antonio, TX).

The experimental design was a randomized complete block with two replications. Two groups of maize lines were treated as the main effects and replications served as the block effect. Statistical analyses were conducted using PROC CORR (Spearman and Pearson tests), PROC MIXED and PROC GLM within the Statistical Analysis Software (SAS v9.1) program. PROC CORR using the Spearman test was used to determine if correlations existed between the rankings assigned to each line from the cell viability assay (Chapter 2) and the physiological measurements, yield components, and staygreen scores. PROC CORR using the Pearson option was used to determine if a correlation existed between grouping and time and the various physiological measurements, yield components and staygreen scores. PROC GLM was used to test for differences between groups for yield components and staygreen expression. PROC MIXED was used to test for differences between groups and the time of measurement, as well as a group*time interaction in regards to the physiological measurements recorded.

Results

I. Sorghum

This experiment was conducted in a controlled greenhouse environment, and tested the four groups identified by the cell viability assay for differences between groups and variability within groups for staygreen expression and various physiological and yield components. Lines are shown in Table 2.2

Table 3.1 shows analysis of variance for physiological traits for effects of group and time, and a group*time interaction. No significant group effect existed for any physiological measurements. A significant time effect was observed for chlorophyll content readings and leaf temperature. A significant group*time interaction for leaf temperature was also observed. Leaf

chlorophyll content and leaf temperature decreased in value over time, and leaf temperature decreased to varying degrees over time based on group effect.

Correlation analysis revealed few relationships. Spearman correlations between the ranks assigned to each individual line tested (Table 2.2) and all physiological, yield, and staygreen data were found to be non-significant. The Spearman coefficients and the associated P-values are listed in Table 3.2. Additionally, Pearson correlations were ran to test for relationships between physiological, yield, and staygreen data and the group and time effects (Table 3.3). A significant negative relationship was observed between chlorophyll content values and time. All other correlations in relation to group and time were non-significant.

Figure 3.1 shows each of the four groups and their chlorophyll content values over time. No group effect or group*time interaction was observed. A time effect was observed for the decline in chlorophyll content values over time. Lines in the non-staygreen check group 3 had lower chlorophyll content than the staygreen check group 4. However, there were no significant differences between the lines in group 1 and group 2 which were selected based on the cell viability assay. Figure 3.2 shows each of the four groups and their leaf temperature values over a six week period after anthesis. There was no overall group effect, but a time effect and group*time interaction were present. There were significant differences in leaf temperature between staygreen checks and lines indentified as staygreen from cell viability assay, while there was no significant difference between staygreen group 2 and the non-staygreen checks. Figure 3.3 and Figure 3.4 show a response between Fo (minimum chlorophyll fluorescence) values and Fv/Fm values over three time measurements after anthesis. No group or time effects were observed, and no group*time interaction was observed for either measurement.

Measurements for yield components and staygreen scores were recorded at harvest (Table 3.7). Group differences in mean values for seed weight and staygreen scores were non-significant. However, group differences in mean values for but seed number were found to be significant. The non-staygreen check group 3 was significantly different from the other three groups ($\alpha = 0.1$).

II. Maize

This experiment tested the two groups identified by the cell viability assay for differences between groups and variability within groups for staygreen expression and various physiological and yield components. Lines tested are shown in Table 2.5.

Table 3.4 shows analysis of variance for physiological traits for effects of group, time, and group*time interaction. No group*time interactions were observed. No significant group effect existed for any physiological traits. A significant time effect was observed for chlorophyll content and leaf temperature, as both traits showed a decline over time.

Correlation analysis showed few relationships. The Spearman test showed positive correlations between chlorophyll readings and Fv/Fm readings in relation to cell viability assay ranks (Table 3.5). No other significant relationships between physiological, yield, and staygreen data and cell viability assay rankings were observed. Pearson correlations between physiological, yield, and staygreen data and the group and time of measurement effects are represented in Table 3.6. Positive relationships were observed between group and chlorophyll content readings as well as group and Fv/Fm readings. Also, negative relationships were observed between time and chlorophyll content values, time and leaf temperature, and time and Fo. No other significant relationships between physiological data and group and time were

observed, and no relationships between yield and staygreen data were observed with the group effect.

Non-staygreen group 1 and staygreen group 2 were tested for group and time effects, and group*time interactions in relation to physiological traits. Chlorophyll content response to time showed no overall group effect or group*time interaction, but a time effect was observed. Additionally, a significant difference between groups was observed at the sixth time measurement for chlorophyll content readings. Lines in the non-staygreen group 1 had significantly lower relative leaf chlorophyll content six weeks after anthesis when compared to lines from staygreen group 2 (Figure 3.5). Leaf temperature response to time showed no group effect and no group*time interaction, but there was a time effect observed, similar to chlorophyll content responses over time. Leaf temperatures declined with time in both groups (Figure 3.6). Fo and Fv/Fm showed no group or time effect and no group*time interaction. These responses are shown in Figures 3.7 and 3.8 respectively.

Data on yield components showed no differences between the non-staygreen group 1 and the staygreen group 2 lines for ear length, kernel rows ear^{-1}, number of kernels row^{-1}, seed number pot^{-1}, seed weight, and staygreen score (Table 3.8).

Discussion

In a previous study (Chapter 2) a cell viability assay was conducted that identified 26 lines of sorghum and 20 lines of maize as potential staygreen or non-staygreen genotypes. The sorghum lines were divided into groups of thirteen and ranked according their Δ 6 hour value of Fv/Fm. Similarly, the maize lines were further divided into groups of ten and ranked based on their Δ 6 hour values of Fv/Fm. Lines were tested on a group basis (non-staygreen, group 1; staygreen, group 2). The purpose of this experiment was to investigate if a cell viability assay, conducted in the vegetative stage (V5), could serve as an early indicator of staygreen expression, physiological performance, and yield performance when plants are under post-anthesis drought stress. Group differences in measures of plant performance could indicate how well the assay performed at predicting plant performance and staygreen expression under post-anthesis drought stress.

I. Sorghum

A time effect was found to be significant for chlorophyll content. A significant correlation showed a negative relationship between chlorophyll content values and time. Findings by Xu et al. (2008) suggest that leaf age, particularly older leaves, play a role in the decline for physiological processes under drought stress. Spearman correlations revealed no relationships between cell viability assay rankings and physiological, yield, and staygreen traits measured, suggesting that the assay does not indicate how well a line will perform from physiological, yield, and staygreen aspects.

It is known that drought during anthesis leads to major reduction in yield (Wilson, 1968; Claasen and Shaw, 1970; Cakir, 2004). There was a significant difference between groups in seed number. The non-staygreen check group 3 had a higher seed count than the other three groups. Since these lines are known to be senescent type, one could predict that they would have been lower in seed number than the known staygreen type group 4 or staygreen group 2. Kernel numbers come from assimilates from photosynthetic activity and reserve carbohydrate stores (Plaut et al., 2004), and both are hampered under drought conditions. Although McBee (1984) and Duncan (1984) suggest that staygreen genotypes are associated with higher levels of stem carbohydrates under stress levels. The discrepancy most likely lies in the fact that there were only two replications for each group, in addition to having only two lines each in group 3 and 4, reducing precision of the data. The LSD value in this case was extremely high at 379.23. Future studies with more replications are necessary to determine if correlations exist between rankings based on the cell viability assay and performance in terms of physiological traits and yield and yield components.

II. Maize

Results for maize were slightly different than for sorghum. Positive correlations between assay rank and group and time were present for chlorophyll content. Additionally, positive correlations were found between assay rank and Fv/Fm, and between group and Fv/Fm. Leaf temperature decreased with time but there was no group interaction.

There were also no significant group effects for any yield component that was measured. This is contradictory to what has appeared in the literature. Rosenow et al., (1983) reported that staygreen genotypes have been associated with improved grain filling and grain yield under

stress. This could be due to higher levels of stem carbohydrates during grain filling under stress conditions (McBee, 1984; Duncan, 1984). The contradiction between the results of the experiment and the literature could be due to a low number of replications, small pot size and stress conditions.

Conclusion

Although results were not strongly correlated with assay rankings and group effects were not as expected, future research should be conducted before any decisions are made about the effectiveness of this cell viability assay. In order for physiological data to be more accurate, the experiment should be repeated with more replications before any inferences can be made between the assay and plant performance under post-anthesis drought stress. The same could be said for yield data. More replications would be helpful, however yield trials should be conducted in a field environment where one can either control or predict post-anthesis drought stress. A rain out shelter or a part of the state where drought is almost always problematic would be ideal for testing yield response and staygreen expression in regards to the information derived from the cell viability assay.

Table 3.1. Analysis of variance (ANOVA) for fixed effects and group*time interaction on physiological measurements for 30 sorghum lines.

Effect	Chlorophyll Content	Leaf Temperature	Fo	Fv / Fm
Group	0.144	0.209	0.338	0.286
Time	< 0.001	0.001	0.976	0.322
Group*Time	0.774	< 0.001	0.345	0.704

Table 3.2. Spearman correlation coefficients for various traits measured in sorghum and cell viability assay rankings.

Trait	Spearman Coefficient	P-Value
Chlorophyll Content	0.072	0.187
Leaf Temperature	-0.053	0.4076
Fo	-0.097	0.391
Fv / Fm	0.031	0.735
Seed Weight	0.07	0.599
Seed Number	-0.136	0.309
Staygreen	-0.045	0.741

This table shows the yield component traits and physiological parameters and the correlations to the assigned rankings determined by the cell viability assay.

Table 3.3. Pearson correlation coefficients for traits measured in sorghum and the group and time main effects.

	Pearson Coefficients			
Trait	Group	P-Value	Time	P-Value
Chlorophyll Content	-0.087	0.109	-0.349	< .0001
Leaf Temperature	-0.029	0.642	-0.095	0.131
Fo	0.012	0.919	-0.078	0.489
Fv / Fm	-0.134	0.145	0.044	0.631
Seed Weight	-0.117	0.383	NA	NA
Seed Number	-0.087	0.518	NA	NA
Staygreen	0.038	0.777	NA	NA

This table shows the correlation between the group (NSG, SG, NSG Check, SG Check) and yield components and the group and time of the measurement for the different physiological parameters.

Table 3.4. ANOVA results for fixed effects of group, time and group*time interaction with physiological traits for 20 maize lines.

Effect	Chlorophyl Content	Leaf Temperature	Fo	Fv / Fm
Group	0.192	0.556	0.547	0.286
Time	<0.001	< 0.001	0.102	0.835
Group*Time	0.713	0.946	0.948	0.579

Table 3.5. Spearman correlation coefficients for cell viability assay rankings and maize plant performance traits.

Trait	Spearman Coefficient	P-Value
Chlorophyll Content	0.222	0.0006
Leaf Temperature	0.042	0.526
Fo	0.167	0.267
Fv / Fm	0.262	0.037
Ear Length	0.098	0.568
Rows / Ear	0.052	0.765
Seed / Row	0.146	0.397
Seed Number	-0.07	0.685
Seed Weight	0.025	0.885
Staygreen	-0.071	0.664

This table shows the yield component traits and physiological traits and the correlations to the assigned rankings determined by the cell viability assay.

Table 3.6. Pearson correlation coefficients for group and time effects and maize plant performance traits.

	Pearson Coefficients			
Trait	Group	P-Value	Time	P-Value
Chlorophyll Content	0.216	0.001	-0.297	< 0.001
Leaf Temperature	0.043	0.515	-0.532	< 0.001
Fo	0.123	0.417	-0.312	0.035
Fv / Fm	0.279	0.025	0.023	0.856
Ear Length	0.126	0.463	NA	NA
Rows / Ear	0.047	0.784	NA	NA
Seeds / Row	0.076	0.660	NA	NA
Seed Number	0.150	0.382	NA	NA
Seed Weight	0.214	0.210	NA	NA
Staygreen	-0.128	0.430	NA	NA

This table shows the correlation between the group factor and yield components and the group and time factors for the different physiological parameters.

Table 3.7. Comparison between sorghum groups for yield components and staygreen scores.

Group	Seed Number Plant^{-1}	Seed Weight Plant^{-1}	Staygreen Score
NSG	672.0 b	13.0 a	3.2 a
SG	491.1 b	11.5 a	3.1 a
NSG Checks	1083.3 a	14.5 a	3.7 a
SG Checks	379.8 b	9.0 a	3.2 a

*LSD computed at $\alpha = 0.1$

Table 3.8. Comparison between maize groups for yield components and staygreen scores.

	Group	Ear Length	Rows Ear^{-1}	Kernels Row^{-1}	Seed Number $Plant^{-1}$	Seed Weight $Plant^{-1}$	Staygreen Score
1	NSG	11.7 a	13.3 a	35.7 a	172.3 a	30.6 a	3.9 a
2	SG	12.5 a	13.5 a	36.8 a	216.1 a	45.1 a	3.5 a

*LSD computed at $\alpha = 0.1$

Figure 3.1 Response among sorghum groups to leaf chlorophyll content over time.

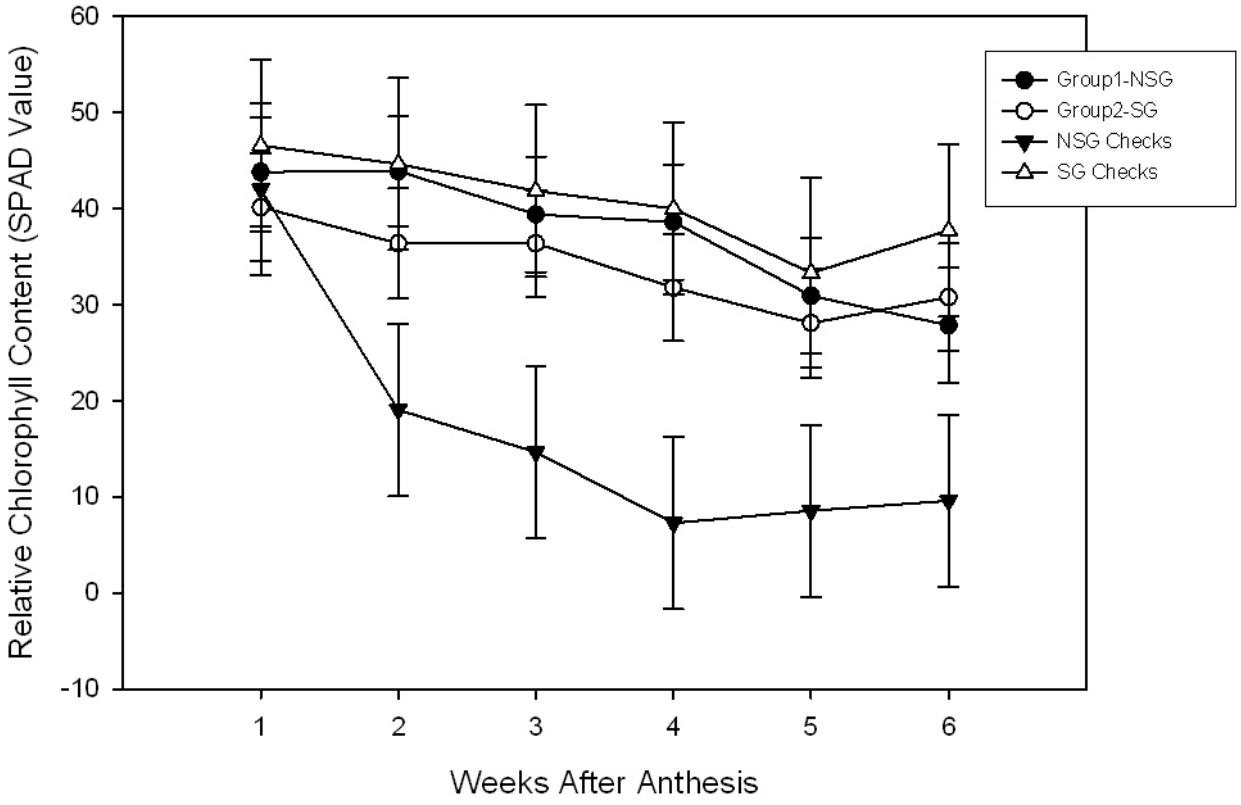


Figure 3.2. Leaf temperature among sorghum groups over time for sorghum.

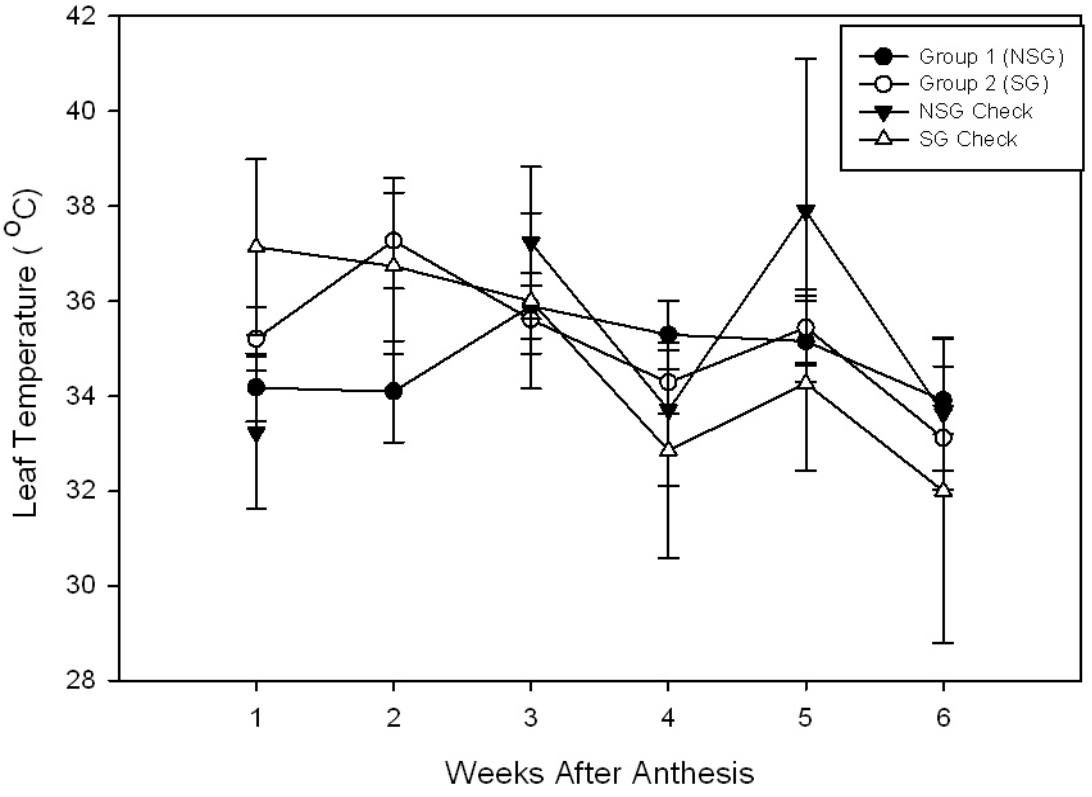


Figure 3.3. Minimum fluorescence values (Fo) values among sorghum groups over the first three weeks after anthesis for sorghum lines.

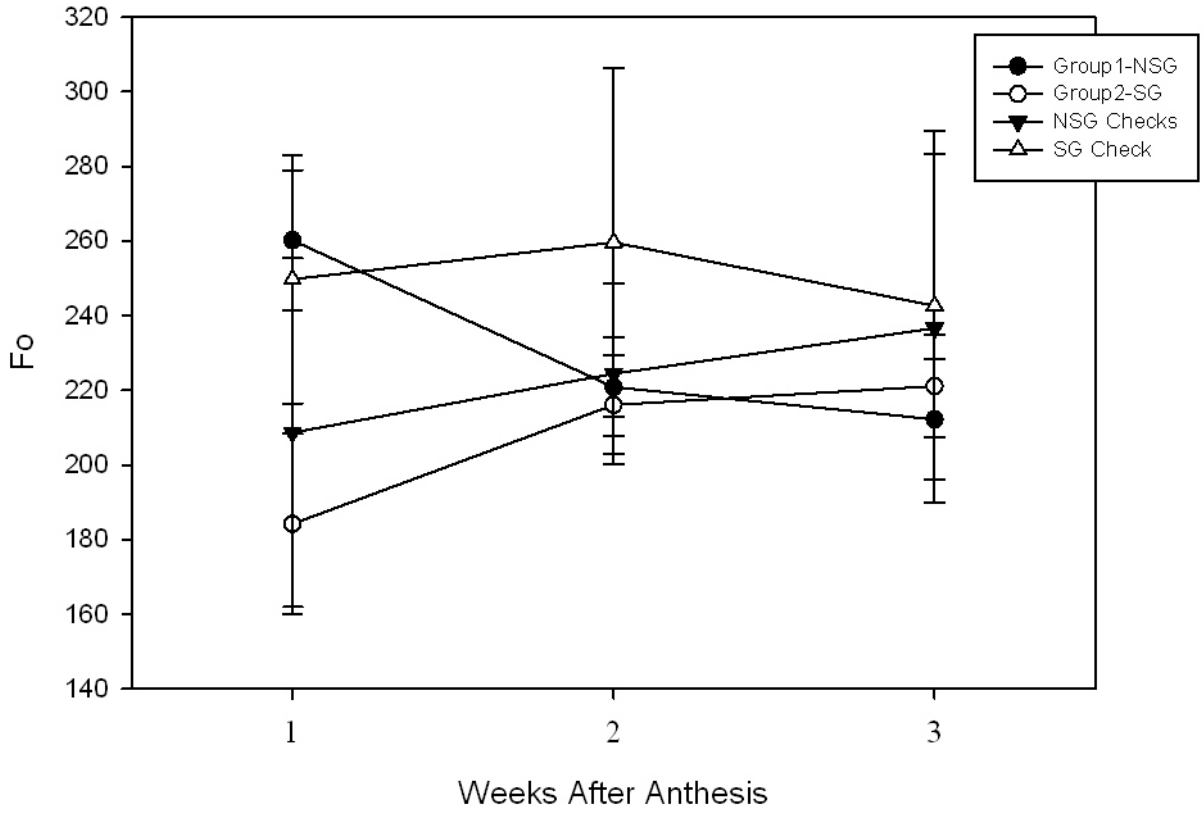


Figure 3.4. Time response of Fv/Fm among groups for sorghum.

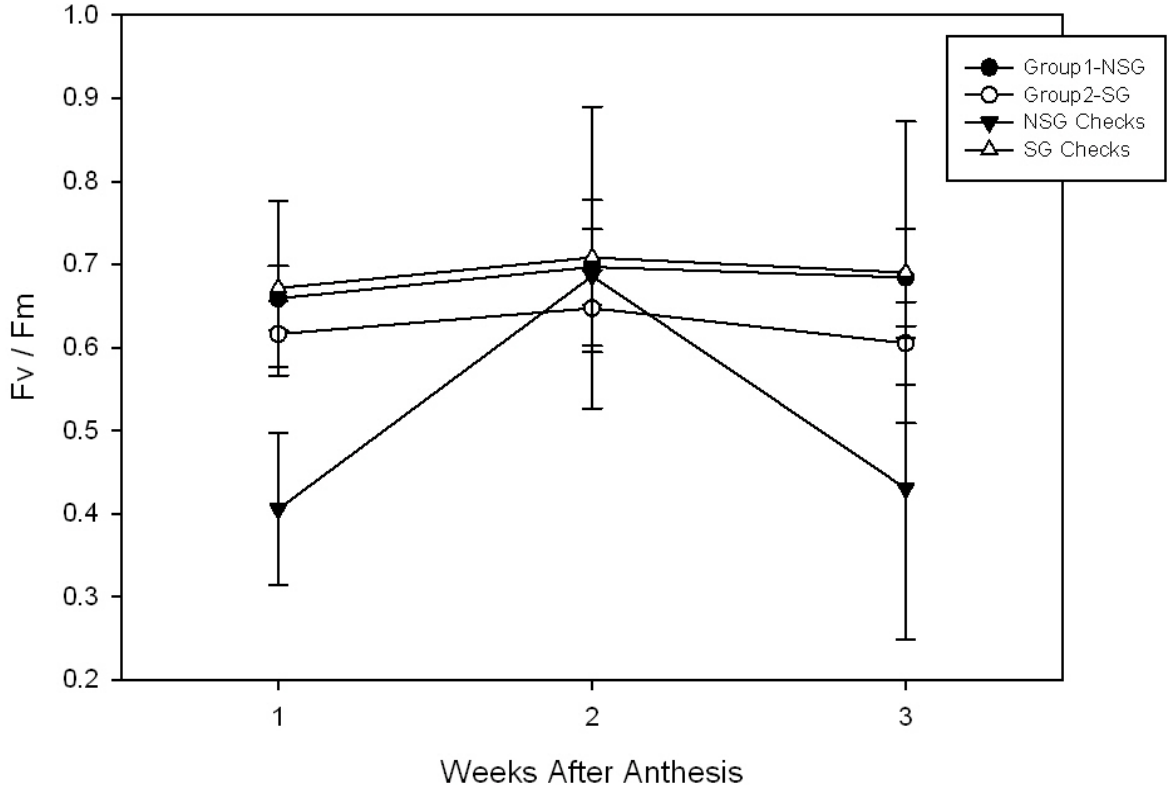
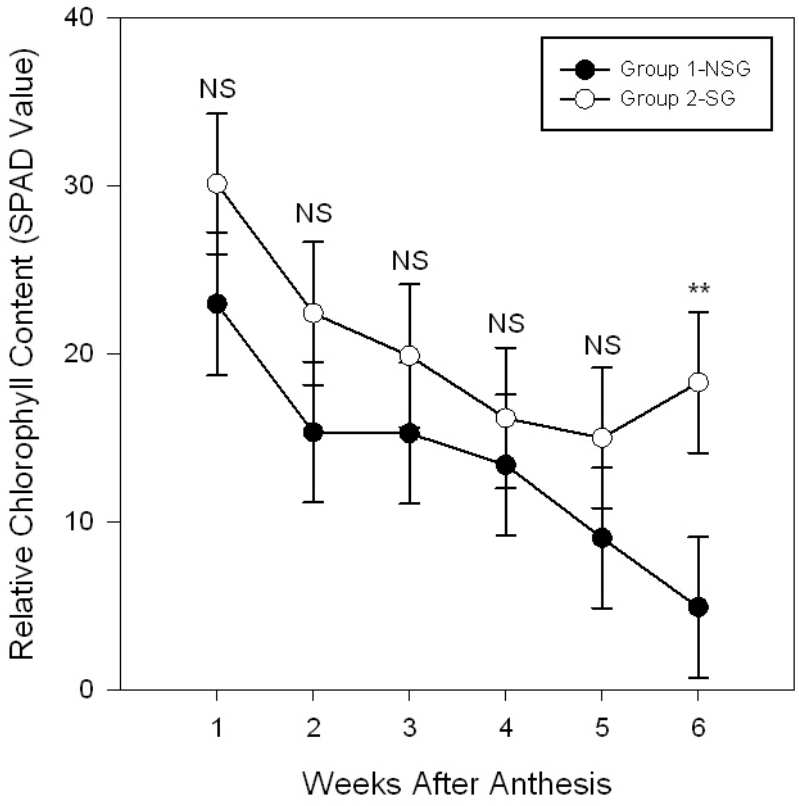


Figure 3.5. Relative leaf chlorophyll content among groups of maize over six weeks following anthesis.



** = significant at the P < 0.01 level

NS – non-significant

Figure 3.6. Leaf temperature response to time among two different groups of maize.

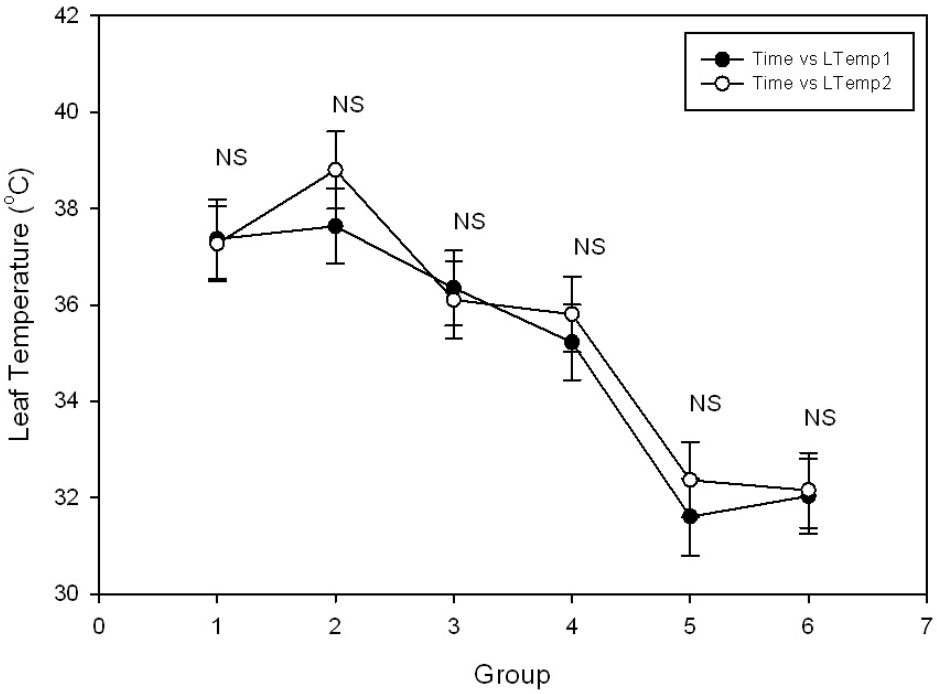


Figure 3.7. Minimum chlorophyll fluorescence (Fo) response over the first three weeks after anthesis among groups of maize.

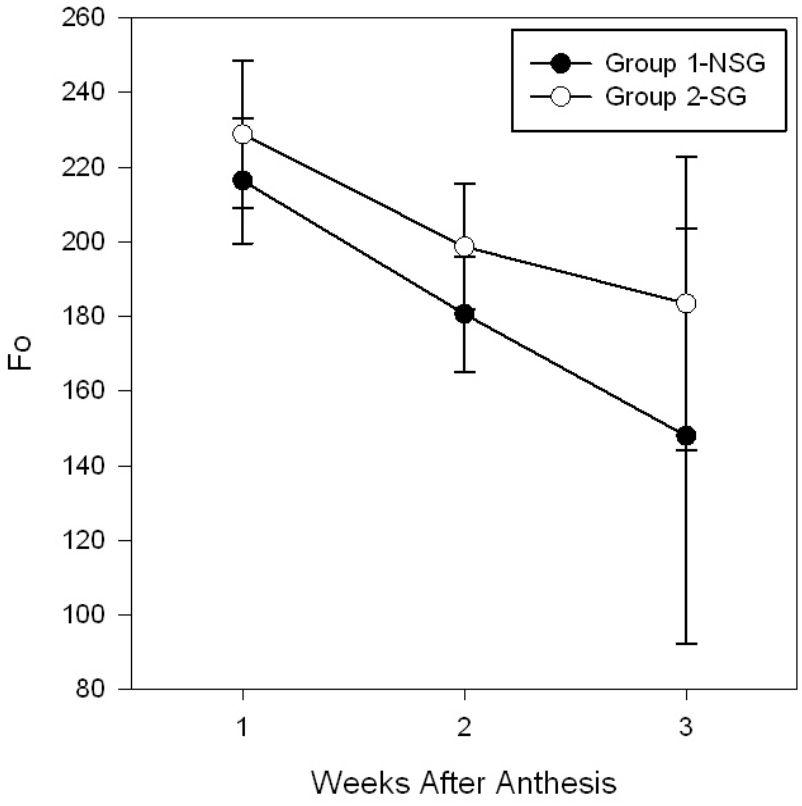
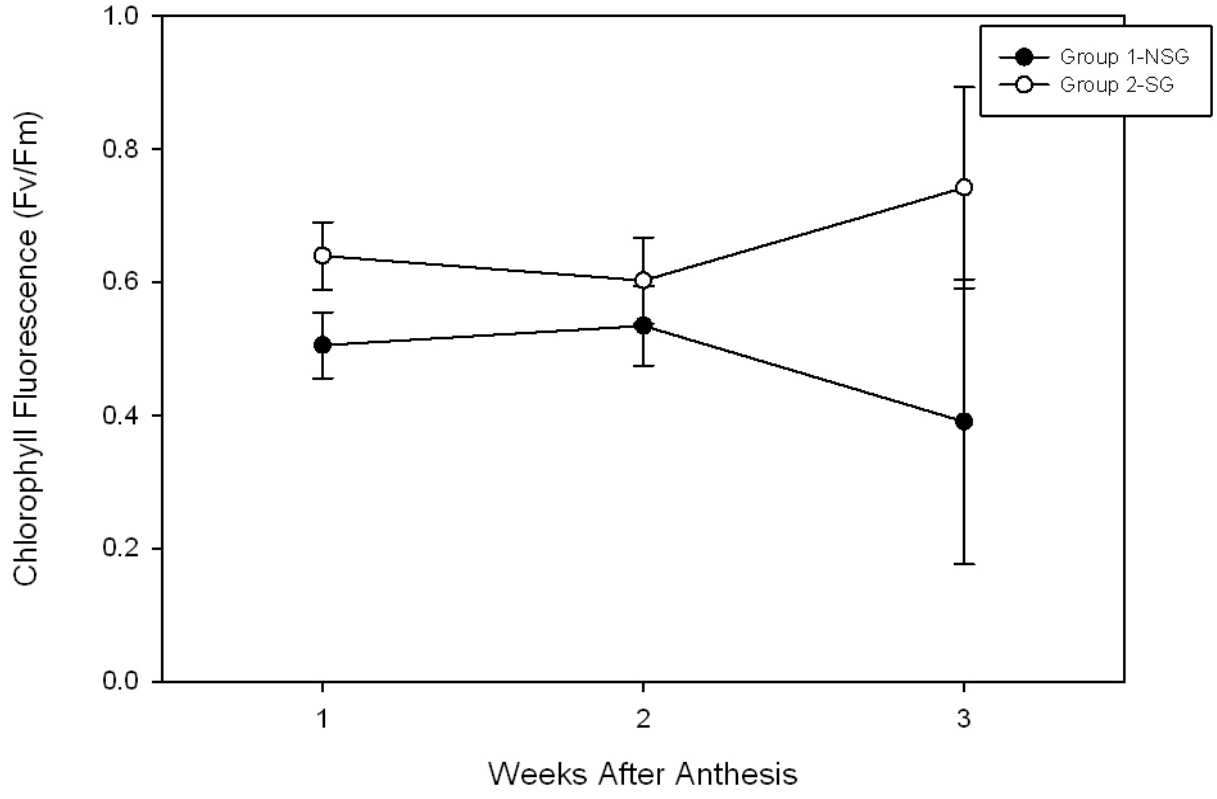


Figure 3.8. Fv/Fm response over the first three weeks after anthesis among groups of maize.



CHAPTER 4 - Field Evaluations of Cell Viability Assay

Abstract

Decline in chlorophyll fluorescence yield under elevated respiratory demand can be used as a sensitive indicator of plant responses to water stress. This field study was conducted to investigate if relationships existed between data from a cell viability assay conducted in controlled environments and plant performance, for both physiological and yield traits, when plants were exposed to post-anthesis drought stress under field conditions. Sorghum (30 lines) and maize (20 lines) were grown in a field environment and tested across two groups based upon cell viability assay results (non-staygreen group 1, staygreen group 2). Analysis of variance results for both sorghum and maize showed time effects for leaf chlorophyll content (SPAD), leaf temperature, and Fv/Fm values. A group effect was observed for leaf chlorophyll content in sorghum. Yield differences between groups were not present in sorghum. In maize, a planting date effect was noted for both physiological and yield traits. Planting date, however, was not considered as a treatment but only present to induce a drought response. Drought stress was not present during the evaluation period for either planting date. Further investigation is needed to determine what relationships exist between the data from a cell viability assay and plant performance when subjected to a post-anthesis drought stress.

Introduction

Maize (*Zea mays* [L.]) and sorghum (*Sorghum bicolor* [L.] Moench) are the first and third largest crops grown in the United States respectively (US Grains Council, 2008). They offer growers in the Midwest and Southern Plains states an attractive choice for crop production due to their ability to grow in a wide array of environmental conditions. Maize and sorghum are also major constituents in food and feedstuffs, and are nutritionally similar (Waniska and Rooney, 1992; Hubbard et al., 1950).

It is a constant challenge for plant breeders and physiologists to find effective, reliable, and quick ways of screening for stresses. Drought is most detrimental to total yield during and after anthesis (Tuinstra et al., 1997; Kebede et al., 2001). The duration of drought stress plays an important role in yield reduction (Wilson, 1968; Classen and Shaw, 1970). Water stress affects the plants ability to grow and complete a normal life cycle (Moussa et al., 2008) by directly affecting plant physiological processes and yield components (Miyashita, 2005; Kramer and Boyer, 1995; Hsiao, 1973).

Finding ways to effectively and efficiently screen experimental lines for drought tolerance in a high throughput fashion could significantly shorten the amount of time needed to evaluate germplasm and develop new varieties. High yielding, drought tolerant lines of maize and sorghum could therefore be brought into production faster. Chechin (1998) demonstrated that chlorophyll fluorescence could be used as a potential screen for drought stress tolerance. Several other physiological screens (eg. Cell membrane thermostability, canopy temperature depression, and harvest index) also have been reported in literature as good indicators of drought

stress tolerance. However, it is equally important to know how values from a high throughput screen might relate to other plant processes and their performance under field conditions.

The objective of this research was to investigate if relationships could be found between a cell viability assay that measured chlorophyll fluorescence under controlled environments and various measures of plant performance under field conditions. Rankings were assigned to lines of sorghum and maize based on data from a previous experiment (Chapter 2). In this experiment, these lines were tested in a field environment to determine if relationships could be observed between cell viability assay rankings and physiological traits, yield traits, and staygreen scores across different groups of genotypes.

Materials and Methods

I. Sorghum

In 2008, 30 lines from the sorghum diversity panel (diverse group of sorghum germplasm researched extensively at Kansas State University) were grown at the Ashland Bottoms south of Manhattan, KS and tested for differences in leaf chlorophyll content, infrared leaf temperature, chlorophyll fluorescence, staygreen expression and yield components. The 30 lines were a subsample out of 300 lines in the sorghum diversity panel. Results from a cell viability assay (Chapter 2) were used to select these lines based on the change in chlorophyll fluorescence over six hours (Δ 6h). The 30 lines were tested as four separate groups (Non-Staygreen, Group 1, 13 lines; Staygreen, Group 2, 13 lines; Non-Staygreen Checks, Group 3, 2 lines; Staygreen Checks, Group 4, 2 lines). Lines within group 1 and group 2 were selected based on the experiment hypothesis that non-staygreen lines have a smaller Δ 6h Fv/Fm value, while staygreen lines have

a higher Δ 6h Fv/Fm value, and were assigned a rank (1-13 and 14-26 respectively). Checks were selected as known senescent or staygreen genotypes. Four replications were grown in 0.76 meter x 6.10 meter plots on a Eudora silt loam soil. Plots were planted on two different dates: May 21st and June 17th. The second planting of sorghum was abandoned due to a planting error and extreme weed pressure. Plots were seeded at approximately 100 seeds plot^{-1} into a conventionally tilled seedbed with soybean as the previous crop. Nitrogen (45.4 kilograms ha^{-1}) and s-metolachlor + atrazine (3.51 L ha^{-1}) were applied prior to planting. Hand rouging of weeds was conducted as needed, and a mechanical cultivation to eliminate weeds between plot rows was conducted just prior to canopy closure.

Physiological measurements were taken at the beginning of grain filling, and were measured on the flag leaf. Leaf chlorophyll content was measured with a SPAD 502 meter (Minolta Corp., Tokyo, Japan) and was measured over a five week period. Infrared leaf temperature was measured with an infrared thermal imaging camera (FLIR Corp., Wilsonville, OR), and chlorophyll fluorescence was measured with a handheld pulse modulated chlorophyll fluorometer (OptiSciences Inc., Hudson, NH). Both leaf temperature and chlorophyll fluorescence were measured at early grain fill, 50% grain fill, and late grain fill.

Plots were harvested on September 30th and staygreen was scored on a 1-5 scale (1 = 100% green, 5 = complete senescence) as described by Xu et al. (2000). Panicles from 2 m of each plot were harvested and placed in a grain dryer at 37.5^{o}C for seven days, then removed and threshed with a portable threshing device (Almaco Ind., Nevada, IA). Seed weight was measured from 2 m of row and seed number was counted and averaged on two heads plot^{-1} . Seed was counted using a model 850-3 seed counter (International Marketing and Design Corp., San Antonio, TX).

The experimental design was a randomized complete block with four replications. Groups were treated as main effects and replications served as the block effect. Statistical analyses were conducted using PROC CORR (Spearman and Pearson methods), PROC MIXED, and PROC GLM within the Statistical Analysis Software (SAS v9.1) program. PROC CORR with the Spearman option was used to determine correlations between the ranking assigned to each line from the cell viability assay (Chapter 2) and physiological traits, yield components, and staygreen scores. PROC CORR using the Pearson option was used to determine if a correlation existed between group and the various physiological traits, yield components, and staygreen scores. PROC GLM was used to test for differences among groups for yield characteristics and staygreen expression. PROC MIXED was used to test for differences between groups and time of measurement, as well as group*time and group*line interactions for the physiological measurements recorded.

II. Maize

In 2007, 197 experimental maize lines from Monsanto Corporation were evaluated at the Ashland Bottoms south of Manhattan, KS and tested for differences in leaf chlorophyll content, infrared leaf temperature, staygreen expression and yield components. Results from a cell viability assay (Ch 2) were used to select 20 lines based on the change in chlorophyll fluorescence over six hours (Δ 6h). The 20 lines were tested as two separate groups (Non-Staygreen, Group 1, 10 lines; Staygreen, Group 2, 10 lines). Lines within group 1 and group 2 were selected based on the experiment hypothesis that non-staygreen lines have a smaller Δ 6h Fv/Fm value, while staygreen lines have a higher Δ 6h Fv/Fm value, and were assigned a rank (1-10 and 10-20 respectively). Two replications were grown in 0.76 meter x 6.10 meter plots on

a Eudora silt loam soil. The sub set of 20 lines was evaluated again in 2008 with four replications. Plots were planted on May 18th, 2007 and on two different planting dates of May 21st and June 17th respectively in 2008. Plots were seeded at approximately 100 seeds per plot into no-till soybean stubble in 2007, and a conventionally tilled seedbed with soybean as the previous crop in 2008. 36.3 kg ha^{-1} nitrogen, 0.68 kg ha^{-1} of atrazine and 0.45 kg ha^{-1} of glyphosate were applied before planting in 2007. In 2008, 45.4 kg ha^{-1} nitrogen and 3.51 L ha^{-1} of s-metolachlor + atrazine were applied just prior to planting. Hand rouging of weeds was conducted as needed, and a mechanical cultivation to eliminate weeds between plot rows was conducted just prior to canopy closure.

Physiological measurements were taken at the beginning of grain filling, and were measured on the ear leaf. Leaf chlorophyll content was measured with a SPAD 502 meter (Minolta Corp., Tokyo, Japan) and was measured over a five week period. Infrared leaf temperature was measured with an infrared thermal imaging camera (FLIR Corp., Wilsonville, OR), and chlorophyll fluorescence was measured with a handheld pulse modulated chlorophyll fluorometer (OptiSciences Inc., Hudson, NH). Chlorophyll fluorescence was measured in 2008 only. Both leaf temperature and chlorophyll fluorescence were measured at early grain fill, 50% grain fill, and late grain fill.

Plots in 2007 were harvested on September 6th. In 2008, the first planting was harvested on September 30th and the second planting was harvested on October 16th. In both years staygreen was scored at harvest on a 1-5 scale (1 = 100% green, 5 = complete senescence) as described by Xu et al. (2000). Ears from 2 m of each plot were harvested and placed in a grain dryer at 37.5°C for seven days, then removed and threshed with a portable ear sheller (Almaco Ind., Nevada, IA). Ear length measured in centimeters, number of kernel rows ear^{-1}, and number

of kernels row^{-1} were measured as yield components. Additionally, seed weight was measured from 2 m of row for each plot, and seed number was counted and averaged on two ears plot^{-1}. Seed was counted using a model 850-3 seed counter (International Design and Marketing Corp., San Antonio, TX).

The experimental design was a randomized complete block with four replications. Groups were treated as the main effects and replications served as the block effect. Statistical analyses were conducted using PROC CORR (Spearman and Pearson methods), PROC MIXED, and PROC GLM within the Statistical Analysis Software (SAS v9.1) program. PROC CORR with the Spearman option was used to determine correlations between the ranking assigned to each line from the cell viability assay (Chapter 2) and the various physiological traits, yield components, and staygreen score. PROC CORR using the Pearson option was used to determine if a correlation existed between grouping and the various physiological traits, yield components and staygreen score. PROC GLM was used to test for differences between groups for yield characteristics and staygreen expression. PROC MIXED was used to test for differences between groups and the time of measurement, as well as group*time and group*line interactions for the physiological and yield traits.

Results

I. Sorghum

This experiment was conducted in a field environment, and tested the four groups identified by a cell viability assay (Table 2.2) for differences between groups and variability within groups for staygreen expression and various physiological and yield components.

An analysis of variance for physiological traits was conducted (Table 4.1). A group and time effect were observed for relative chlorophyll content, leaf temperature, and Fv/Fm. There was also a significant group*time interaction for Fv/Fm values.

Correlation analyses showed some relationships. Spearman correlation analyses showed positive relationships between the cell viability assay and relative chlorophyll content and staygreen scores (Table 4.2). No other correlations with rankings in the cell viability assay were observed. Pearson correlations were run to investigate if relationships between physiological traits, yield traits, and staygreen scoring and group and time effects existed (Table 4.3). A positive relationship between relative chlorophyll content and group existed. A negative relationship existed for Fv/Fm and seed number plant^{-1}.

Figure 4.1 shows each of the four groups and the response of relative chlorophyll content over time. A group effect ($P = 0.04$) and time effect ($P < 0.001$) were both observed for the decline in relative chlorophyll over time. No group*time interaction was observed. A time effect but no group effect, was observed on leaf temperature readings ($P < 0.001$) as shown in Figure 4.2. No group, time, or group*time interaction was observed for Fo values (Figure 4.3). There was, however, a noticeable time effect ($P = 0.025$) and group*time interaction ($P = 0.002$) for Fv/Fm values as observed in Figure 4.4.

Tests for least significant differences ($\alpha = 0.1$) were run between groups for mean values of the yield traits that were measured (Table 4.9). Group differences in mean values where significant for seed number and staygreen scores. Group 2 and group 4 showed differences in seed number and differences in staygreen scores were observed between group 2 and group 4. Group differences in mean values for seed weight were non-significant.

II. Maize

This experiment was conducted in a field environment, and tested the two groups identified by the cell viability assay (Table 2.5) for differences between groups and variability within groups for staygreen expression and various physiological traits and yield components.

Analysis of variance was conducted for both physiological traits and yield components (Table 4.4). Physiological data from 2007 showed no differences between groups for any physiological, yield, and staygreen trait measured. Therefore it will not be included in this section. Only physiological data from 2008 will be included. A planting date effect was observed for chlorophyll content ($P < 0.001$), leaf temperature ($P < 0.001$), Fo ($P = 0.05$) and Fv/Fm ($P = 0.031$). Values were noticeably lower for all physiological traits in the first planting, as compared to the second planting. Plantings were not considered treatments, however two different plantings were included to induce drought stress. A time effect was observed for relative chlorophyll content ($P < 0.001$), leaf temperature ($P < 0.001$), Fo ($P = 0.026$) and Fv/Fm ($P < 0.001$). All values showed a decline over time. No group effect, group*time interaction, or group*planting interaction were observed for any of the physiological traits. All differences were found to be non-significant for these main effects and interactions ($P > 0.05$).

No group effect was observed for yield components in 2007 (Table 4.5). In 2008, a significant difference between planting dates was observed for kernel rows ear^{-1} at the $P < 0.01$ level. Significant differences were also observed for kernels ear row^{-1}, seed weight plot^{-1}, and

staygreen score at the P <0.001 level. All other yield components in 2008 were insignificant at the $\alpha = 0.05$ level.

A test for least significant differences (LSD) between groups for yield data in 2007 showed no significant differences for ear length, kernel rows ear^{-1}, kernel number row^{-1}, seed weight, seed number, or staygreen scoring. All LSD values were tested at $\alpha = 0.1$ level. Similarly, in 2008 the results between non-staygreen group 1 and staygreen group 2 were the same as in 2007. There were no significant differences observed in 2008 between non-staygreen group 1 and staygreen group 2 for any of yield components or staygreen score. However, least significant differences were observed in 2008 between planting dates. Differences were observed between kernel rows ear^{-1}, kernels ear row^{-1}, seed weight plot^{-1}, and staygreen scores. All LSD values were computed at the $\alpha = 0.1$ level. These results are found in Table 4.6.

The Spearman test showed no correlations between cell viability assay rankings and physiological, yield and staygreen data. All P-values for physiological and yield traits as well as the staygreen scores were highly insignificant (Table 4.7). The Pearson test, however, showed relationships between time and chlorophyll content, leaf temperature, Fo, and Fv/Fm. The correlations for the physiological traits all showed negative relationships with time. Relationships were also observed between planting date and chlorophyll content, leaf temperature, Fo, kernel rows ear^{-1}, kernels ear row^{-1}, seed weight, and staygreen scores (Table 4.8).

Figure 4.5 shows the response of relative chlorophyll content over time among groups of maize compared between planting dates. No group effect was observed for either planting. Significant differences ($P < 0.001$) were observed between plantings during the first four weeks after anthesis. A significant difference was also observed at five weeks after anthesis between

plantings ($P < 0.05$). Additionally, leaf chlorophyll content declined over the growing season in both plantings.

Differences in leaf temperature were observed between plantings during the three periods of grain filling at which measurements were taken. In the first planting, leaf temperatures for both group 1 and group 2 declined about $4^{o}C$ between early and mid grain filling. Contrastingly, leaf temperatures for both group 1 and group 2 increased about 4^{oC} between mid grain filling and late grain filling. However, leaf temperature in the second planting of maize for group 1 and group 2 steadily declined over grain filling. No significant differences between groups were present within planting dates however (Figure 4.6).

Minimum chlorophyll fluorescence (Fo) was insignificant between groups and planting dates when measured during early and mid grain filling. However, a significant difference in Fo values between planting dates was observed when measured at the late grain filling stage and between groups for the second planting ($P < 0.001$, and $P = 0.154$). There was also a significant time effect ($P = 0.02$). Fo values for both groups in planting one show a decreasing trend over time with a substantial decline between mid and late grain filling, from about 250 to 180. While Fo values in the second planting increased over time (Figure 4.7). Fv/Fm values were insignificant among groups, but there was an effect of planting date observed when measured during mid grain fill ($P < 0.01$) (Figure 4.8). A significant time effect also existed for both groups in both planting dates. Fv/Fm measurements in early grain filling averaged around 0.75 and continually decreased over the duration of grain filling to between 0.4 and 0.5 in late grain fill.

Tests for least significant differences ($\alpha = 0.1$) in 2008 show group*planting interactions for kernels ear row^{-1}, kernel rows ear^{-1}, seed weight, and staygreen scores (Table 4.10). No

group*planting interaction was observed for ear length, or seed number ear^{-1}. Kernel rows ear^{-1} measured in the second planting were significantly greater for the non-staygreen group 2. Significant differences for kernels ear row^{-1}, seed weight, and staygreen scores were observed between planting dates, but differences in the mean values between groups for each yield component were non-significant within each planting date.

Discussion

This study produced noticeable results in regards to a decrease in activity for leaf chlorophyll content, and chlorophyll fluorescence over time for both sorghum and maize. The Spearman correlation test showed relationships to rankings for both relative chlorophyll content and staygreen scoring. This data coincides with previous research done at Kansas State University. It is also known that staygreen genotypes and chlorophyll content are directly related (Xu et al., 2000; Howard and Howarth, 2000). The decline in chlorophyll fluorescence over time (both Fo and Fv/Fm) was also discussed by Xu et al. (2008). The author suggests that Fv/Fm maintain relatively stable levels of Fv/Fm but levels are significantly decreased as leaves age. The trends in the decline of relative leaf chlorophyll content and the progress of Fv/Fm decline follow fairly closely to one another as noted by Thomas and Howarth (2000). The response of both sorghum and maize to leaf temperature is also interesting. Both sorghum and maize exhibited the lowest leaf temperatures at 50% grain fill. However, the weather data shows that leaf temperature was recorded on the day with the highest values for daily maximum and minimum temperatures, which is the opposite of what the trend shows over time. This is compared to the days that the other readings were taken on. It is noticeable that in the second

planting of maize, the temperature measurements from late grain fill were lowest, mainly due to being in a generally cooler part of the season.

While yield data from 2007 shows no significant differences among groups for yield components, the differences in 2008 were more noticeable mainly between the first and second planting. Seed weight was significantly higher in the second planting. Weather data shows that (Figure 4.9) a temperature difference of about 5-10^{oC} over anthesis for both planting dates. Also, the lines in the first planting where flowering during the hottest three days in growing season.

In maize, there were differences in staygreen across groups and across planting dates. Those planted later had a higher staygreen score meaning more leaf senescence. Prasad et al. (2008) suggest that higher temperatures during grain filling can have an effect of the rate of leaf senescence. The results from this experiment however were contradictory to that. Weather data across the growing season for both plantings is included in Figure 4.9. The data shows that temperatures were actually lower during the grain filling period for the second planting. There were varying results for staygreen scores between plantings and between groups (non-staygreen, staygreen and checks). It is suggested that personal biases and opinions can play a role in staygreen scoring from one person to another, and also one location to another (Xu et al. 2000; Howard et al. 2000). This could have also been due to the fact that the testing location never experienced a drought stress during the evaluation period. The weather data in Figure 4.9 shows that rainfall was well distributed across the growing season.

For both sorghum and maize, staygreen scoring was inconclusive. In sorghum the staygreen check group 4 had the lowest staygreen score, while the hypothesized staygreen group 2 had the highest. The non-staygreen check group 3 and non-staygreen group 1 fell in between. In maize, differences in staygreen scoring where not noted between groups, but rather between

plantings. However, in the absence of post-anthesis drought, these results might be misleading, the staygreen scores could have been a result of maturity and not stress in the environment. The literature reports that staygreen is only expressed under period of drought stress (Xu et al., 2000; Thomas and Howarth, 2000).

Although it is not reported in the results, group*line interaction for both sorghum and maize were also tested to look at variability within the groups for the various physiological, yield, and staygreen traits. The only significance was in seed weight for sorghum, ear length and rows ear^{-1} for corn in 2008. Since no drought stress was present, this was probably caused by a difference in genetics between the lines and not caused by grouping or environmental stress.

Results may be somewhat inconclusive in this study. Since no drought was experienced during the evaluation periods, it was difficult to ascertain the performance of the hypothesized groups in relation their associated rankings from the cell viability assay.

Conclusion

Material was grown and evaluated during a period of time when drought stress was not present. In order to determine the effectiveness of the cell viability assay in a field environment, future research needs to be conducted either in a season when drought is present or in an environment that is conducive to drought stress. Impacts on physiology were noted over time, and differences in yield components were observed. However, at this present time, the results are inconclusive in regards to the hypothesis. The cell viability assay did indeed reveal differences in lines of maize and sorghum in relation to the decline in chlorophyll fluorescence over time, but in the absence of drought stress, conclusions cannot be made as to whether or not the assay is a successful determinant of staygreen expression and plant performance.

Table 4.1. Analysis of variance (ANOVA) table for fixed effects and group*time interaction on physiological measurements for 30 sorghum lines.

Effect	Chlorophyll Content	Leaf Temperature	Fo	Fv/Fm
Group	0.04	0.179	0.580	0.118
Time	0.001	< 0.001	0.652	0.025
Group*Time	0.986	0.467	0.250	0.002

Table 4.2. Spearman correlation results for various traits measured in sorghum and the corresponding relationship with cell viability assay ranking.

Trait	Spearman Coefficient	P-Value
Chlorophyll Content	0.093	0.032
Leaf Temperature	0.001	0.983
Fo	0.006	0.914
Fv/Fm	0.069	0.227
Seed Weight	-0.086	0.380
Seed Number	-0.016	0.869
Staygreen	0.366	< 0.001

Table 4.3. Pearson correlation results and the corresponding relationships for traits measured in sorghum and group and time main effects.

	Pearson Coefficients			
Trait	Group	P-Value	Time	P-Value
Chlorophyll Content	0.103	0.018	-0.253	< .0001
Leaf Temperature	0.071	0.212	-0.087	0.121
Fo	-0.08	0.16	-0.057	0.318
Fv/Fm	-0.111	0.051	-0.301	< .0001
Seed Weight	-0.114	0.244	NA	NA
Seed Number	-0.224	0.021	NA	NA
Staygreen	0.004	0.967	NA	NA

This table shows the correlation between the group (NSG, SG, NSG Check, SG Check) and yield components and the group and time of the measurement for the different physiological parameters.

Table 4.4. ANOVA results for 20 maize lines testing fixed effects and interaction (group, planting, time, group*time, group*planting).

Effect	Chlorophyll Content	Leaf Temperature	Fo	Fv/Fm
Group	0.982	0.495	0.271	0.466
Planting	< 0.001	< 0.001	0.048	0.031
Time	< 0.001	< 0.001	0.026	< 0.001
Group*Time	0.917	0.718	0.887	0.850
Group*Planting	0.502	0.175	0.464	0.154

*planting was not considered as a treatment, but was used to induce stress.

Table 4.5. ANOVA results for maize yield traits across two separate years. In 2008 the planting date was tested as a fixed effect.

		Yield Traits					
Year		Ear Length	Rows Ear^{-1}	Seeds Row^{-1}	Seed Weight Plot^{-1} (g)	Seed Number Plant^{-1}	Staygreen
2007	Group	NS	NS	NS	NS	NS	NS
2008	Group	NS	NS	NS	NS	NS	NS
	Planting	NS	**	***	***	NS	***
	Group*Planting	NS	NS	NS	NS	NS	NS

- *,**, *** denotes significant effect or interaction at the p<0.05, <0.01 ad <0.001 levels, respectively

- NS denotes no significant effect or interaction

Table 4.6 Least significant differences between maize groups for yield components (2007 and 2008)

Year		Yield Traits					
		Ear Length	Rows Ear^{-1}	Seeds Row^{-1}	Seed Weight Plot^{-1}	Seed Number Plant^{-1}	Staygreen
2007	Group 1- Non-Staygreen	20.49	17.23	39.60	1301.00	612.6	3.2
	Group 2- Staygreen	20.51	16.47	38.97	1480.70	587.5	3.5
	LSD	NS	NS	NS	NS	NS	NS
2008	Group 1- Non-Staygreen	19.63	16.11	33.81	1374.85	557.3	3.6
	Group 2- Staygreen	19.13	16.37	33.19	1301.11	584.1	3.6
	LSD	NS	NS	NS	NS	NS	NS
	Planting Date 1	19.39	16.59^{a}	31.91^{b}	1053.96^{b}	580.1	3.3^{b}
	Planting Date 2	19.36	15.93^{b}	34.93^{a}	1599.77^{a}	562.5	3.9^{a}
	LSD	NS	0.49	1.40	153.03	NS	0.3

*LSD values were tested at the P = 0.05 level

Table 4.7. Spearman correlation results between cell viability assay rankings and maize line performance traits.

Trait	Spearman Coefficient	P-Value
Chlorophyll Content	-0.018	0.609
Leaf Temperature	-0.019	0.679
Fo	0.066	0.161
Fv / Fm	-0.013	0.788
Ear Length	0.092	0.256
Rows Ear^{-1}	0.126	0.122
Seeds Row^{-1}	-0.014	0.863
Seed Weight $Plot^{-1}$ (g)	-0.066	0.420
Seed Number $Plant^{-1}$	0.124	0.128
Staygreen	-0.035	0.673

Table 4.8. Pearson correlation results for group, time and planting fixed effects on performance for yield and physiological traits among the 20 maize lines.

			Pearson Coefficients			
Trait	Group	P-Value	Time	P-Value	Planting	P-Value
Chlorophyll Content	-0.001	0.988	-0.589	< 0.001	0.217	< 0.001
Leaf Temperature	-0.021	0.654	-0.451	< 0.001	-0.514	< 0.001
Fo	0.051	0.277	-0.086	0.067	0.088	0.059
Fv / Fm	0.020	0.677	-0.585	< 0.001	0.055	0.244
Ear Length	-0.050	0.563	NA	NA	-0.003	0.969
Rows Ear^{-1}	0.085	0.296	NA	NA	-0.213	0.008
Seeds Row^{-1}	-0.063	0.438	NA	NA	0.309	< 0.001
Seed Weight $Plot^{-1}$ (g)	-0.066	0.417	NA	NA	0.485	< 0.001
Seed Number $Plant^{-1}$	0.062	0.448	NA	NA	-0.041	0.619
Staygreen	-0.024	0.774	NA	NA	0.365	< 0.001

Table 4.9. Mean values for yield components and staygreen scores compared between groups of sorghum.

	Group	Seed Number Plant^{-1}	Seed Weight Plot^{-1}	Staygreen Score
1	NSG	1719.9 a	413.4 a	3.2 ab
1	SG	1448.9 ab	334.3 a	3.9 a
3	NSG	1398.9 ab	433.8 a	3.2 ab
4	SG	1236.2 b	276.0 a	2.7 b

*LSD computed at $\alpha = 0.1$

Table 4.10. Mean values for yield components and staygreen scores compared between groups of maize across planting dates.

Planting	Group	Ear Length	Rows Ear^{-1}	Kernels Row^{-1}	Seed Number Plant^{-1}	Seed Weight Plot^{-1}	Staygreen Score	
1	NSG	19.9 a	16.5 a	31.9 b	551.3 a	1065.7 b	3.3 b	
1	SG	18.9 a	16.6 a	31.9 b	606.7 a	1043.1 b	3.3 b	
2	NSG	19.4 a	15.7 b	35.4 a	562.5 a	1645.3 a	4.0 a	
2	SG	19.3 a	16.1 ab	34.5 a	562.6 a	1546.2 a	3.9 a	*

LSD computed at $\alpha = 0.1$

Figure 4.1. Response of relative chlorophyll content (SPAD values) among groups over time for 30 sorghum lines.

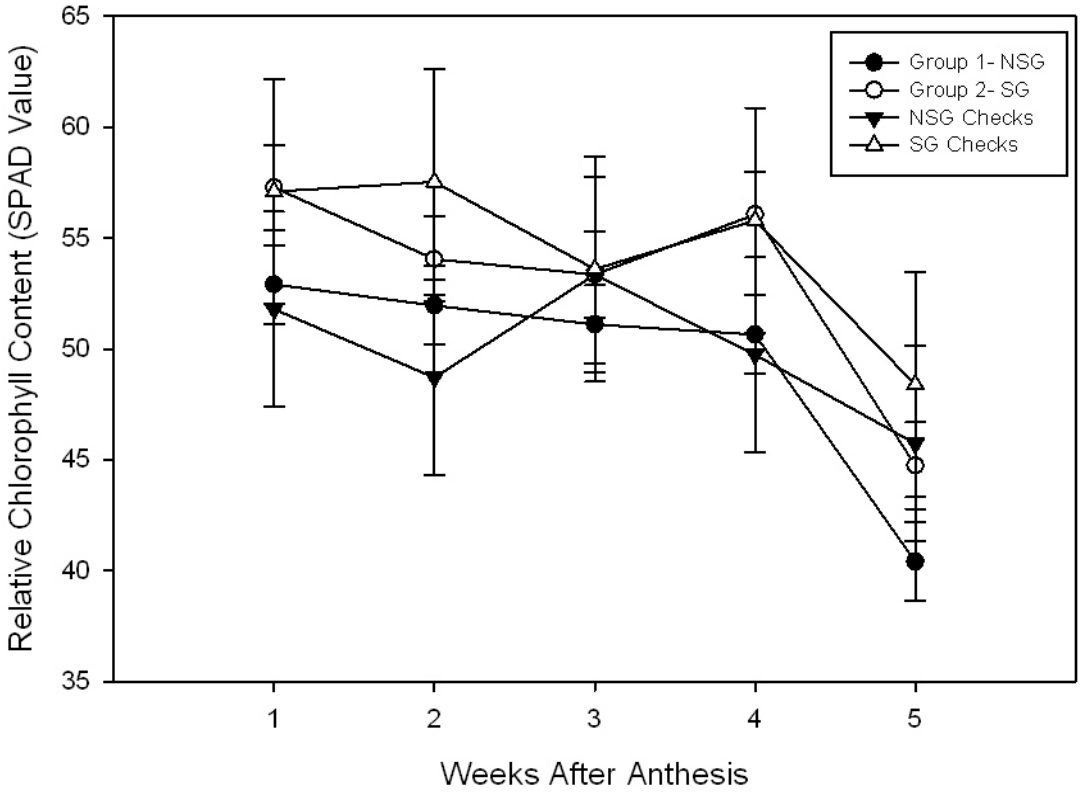
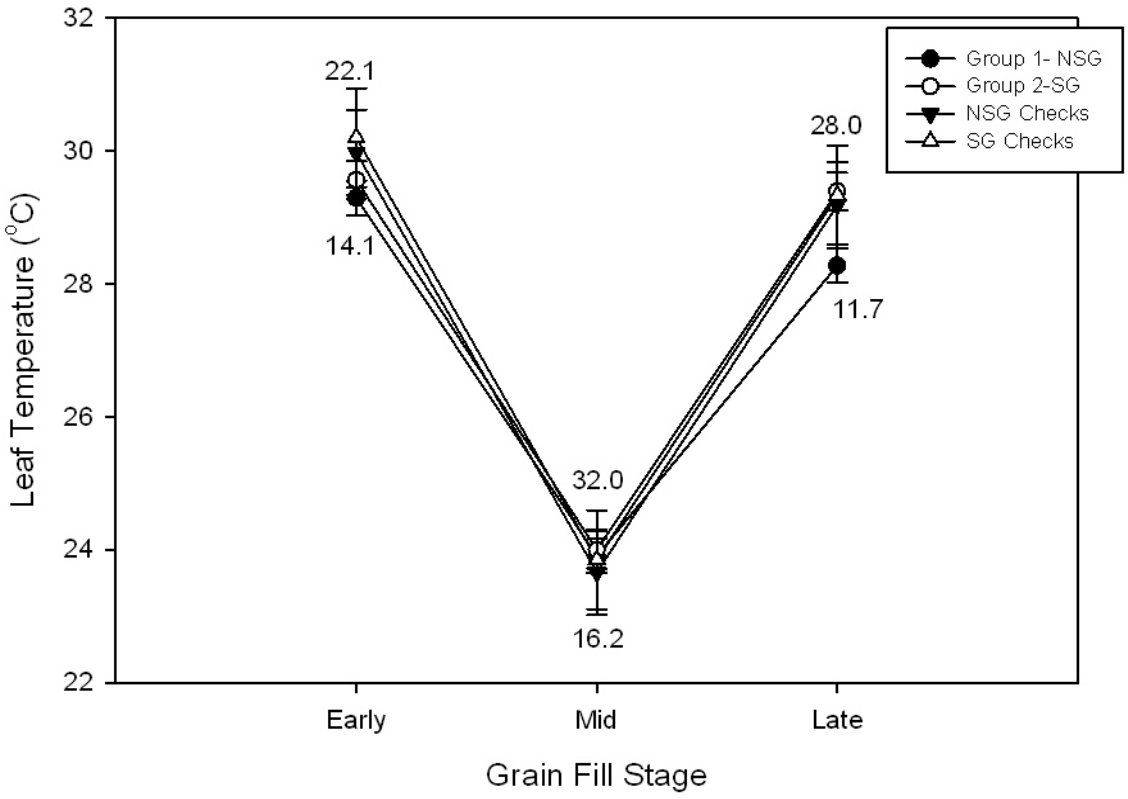


Figure 4.2. Leaf temperature response among groups at three different periods of grain filling for 30 lines of sorghum.



*Values on graph represent daily maximum and minimum temeratures (°C).

Figure 4.3. Response of minimal chlorophyll fluorescence (Fo) over among groups over three different periods of grain filling for 30 sorghum lines.

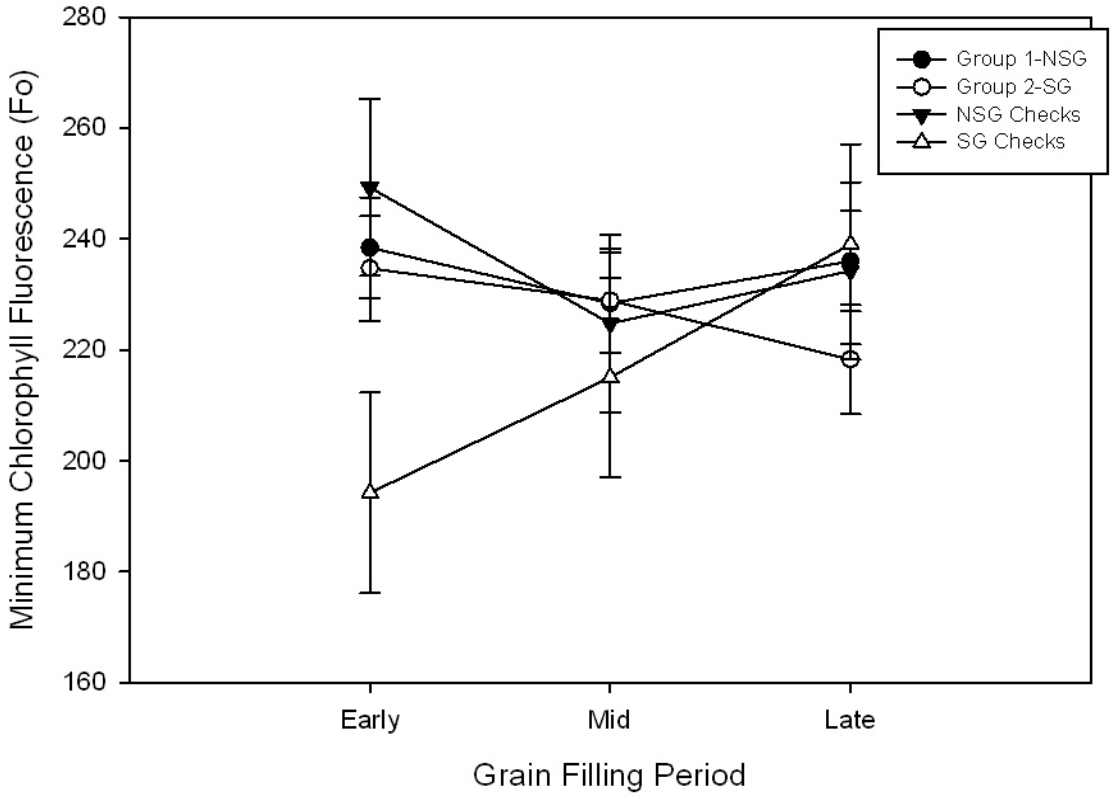


Figure 4.4. Response of chlorophyll fluorescence among groups over three periods of grain filling for 30 sorghum lines.

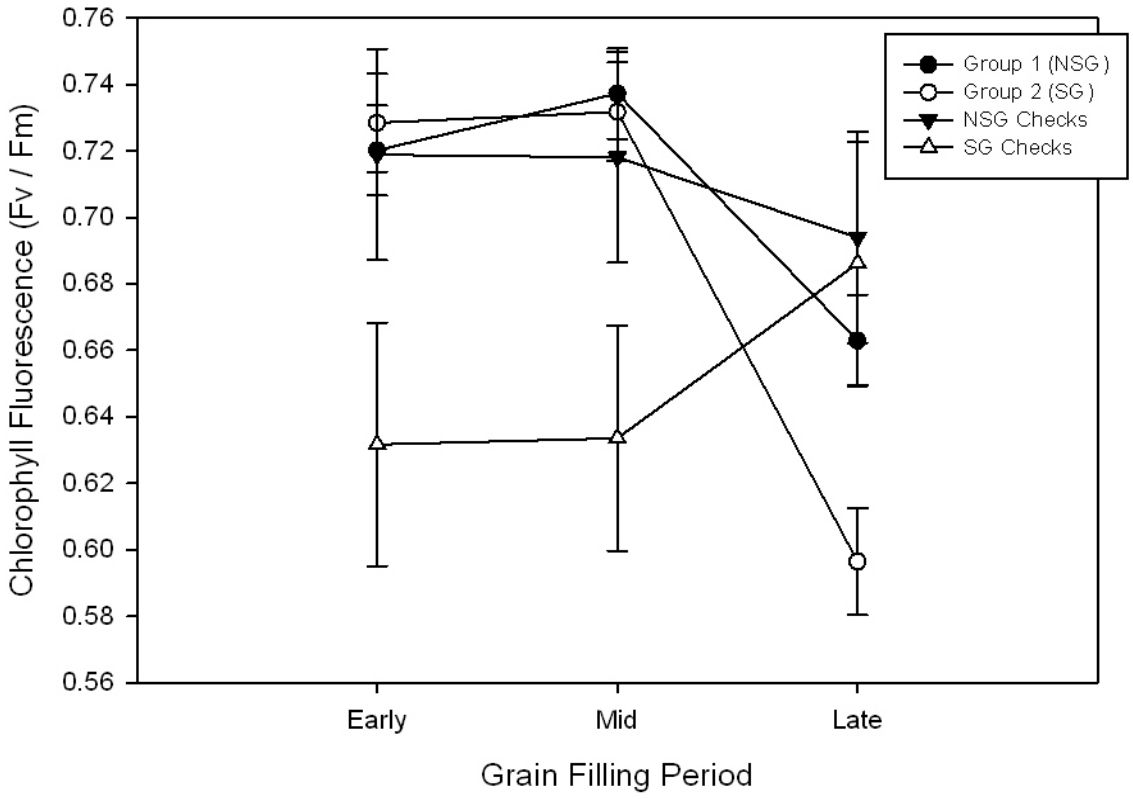
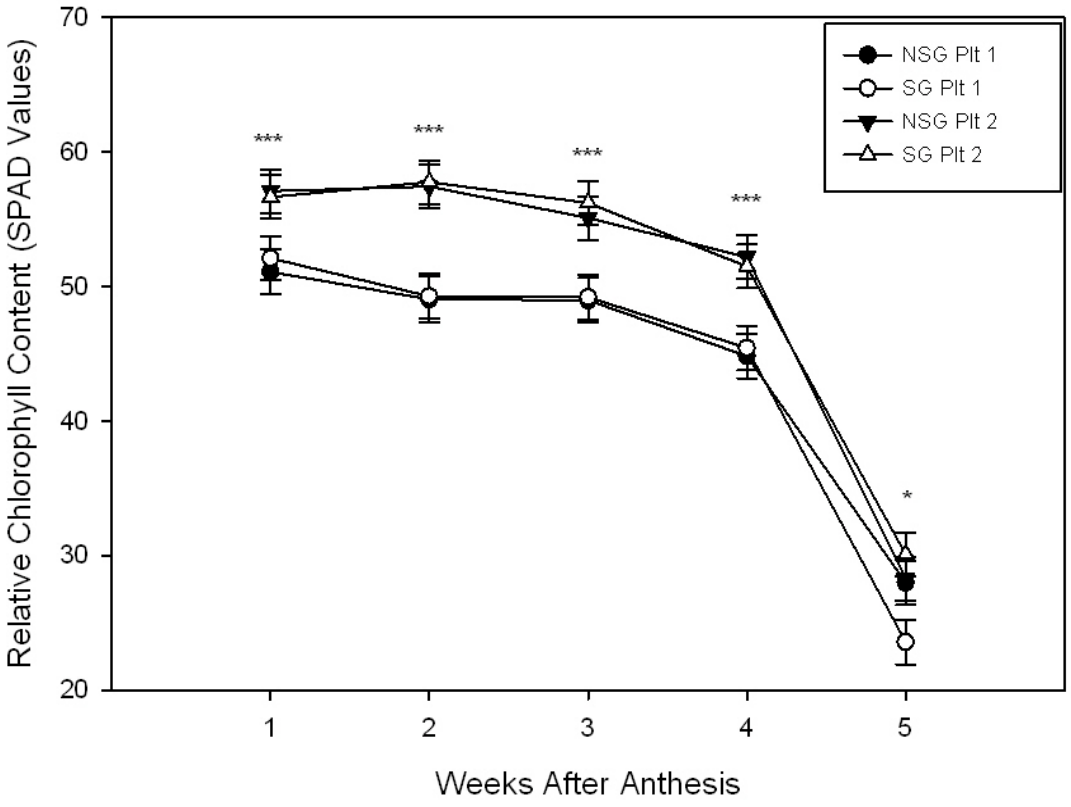
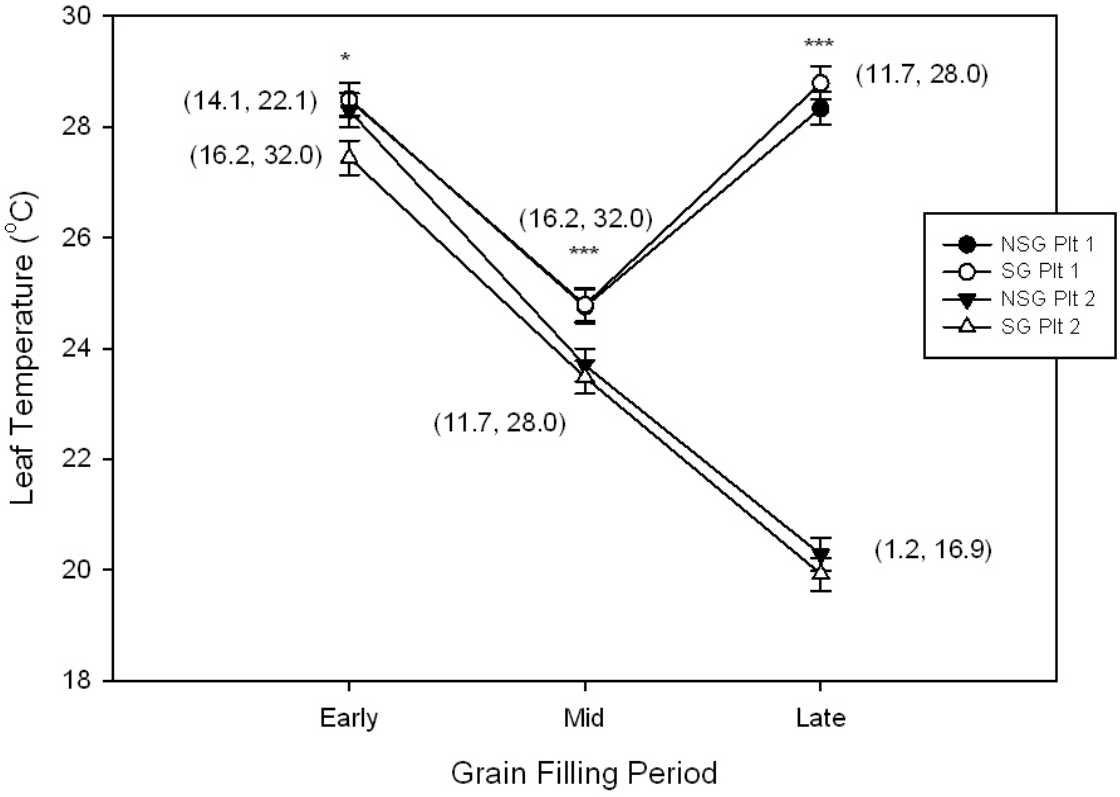


Figure 4.5. Relative chlorophyll content (SPAD values) response over five weeks after anthesis among groups of maize compared between two different plantings.



*, **, *** denotes significant differences between plantings at the P = 0.05, 0.01 and 0.001 levels respectively.

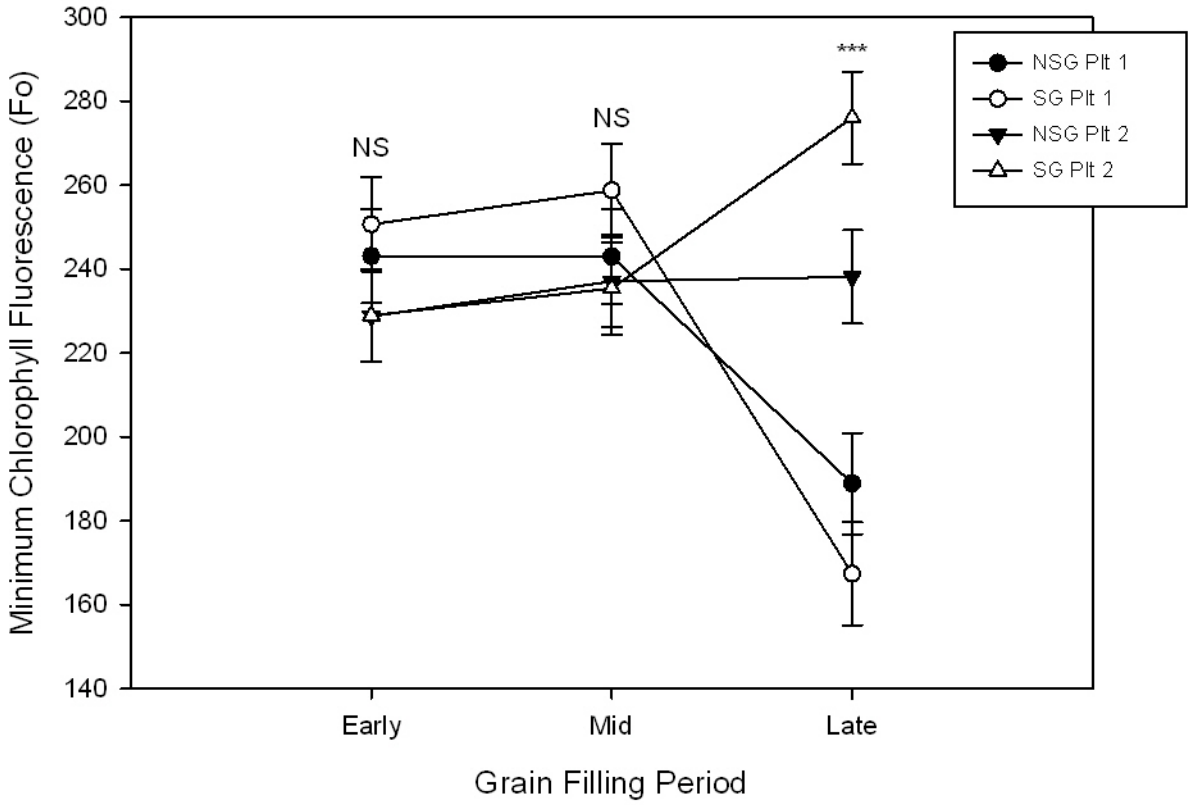
Figure 4.6. Leaf temperature response over three different periods of grain filling among groups of maize compared between two different planting dates.



*, **, *** denotes significant differences between plantings at the P = 0.05, 0.01 and 0.001 levels respectively.

- Values listed at different points on the line represent daily minimum and maximum temperature.

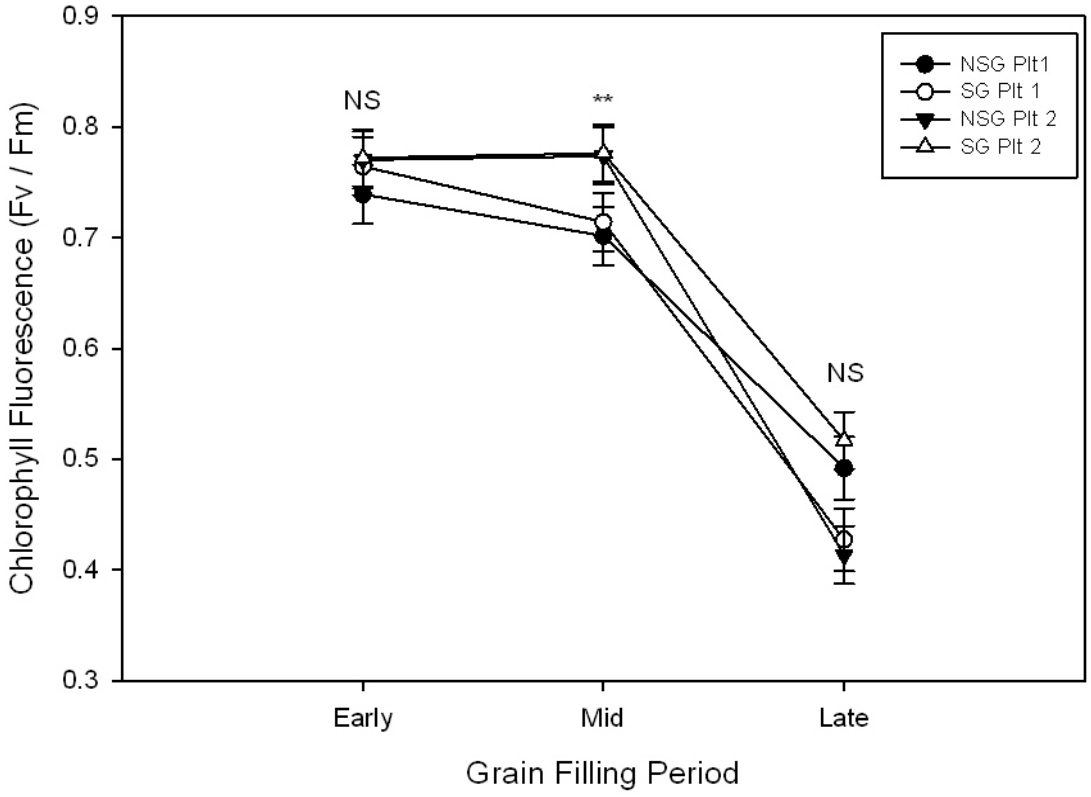
Figure 4.7. Minimum chlorophyll fluorescence (Fo) response over three different periods of grain filling among groups of maize compared between two plantings.



*, **, *** denotes significant differences between plantings at the P = 0.05, 0.01 and 0.001 levels respectively.

NS denotes no significant differences.

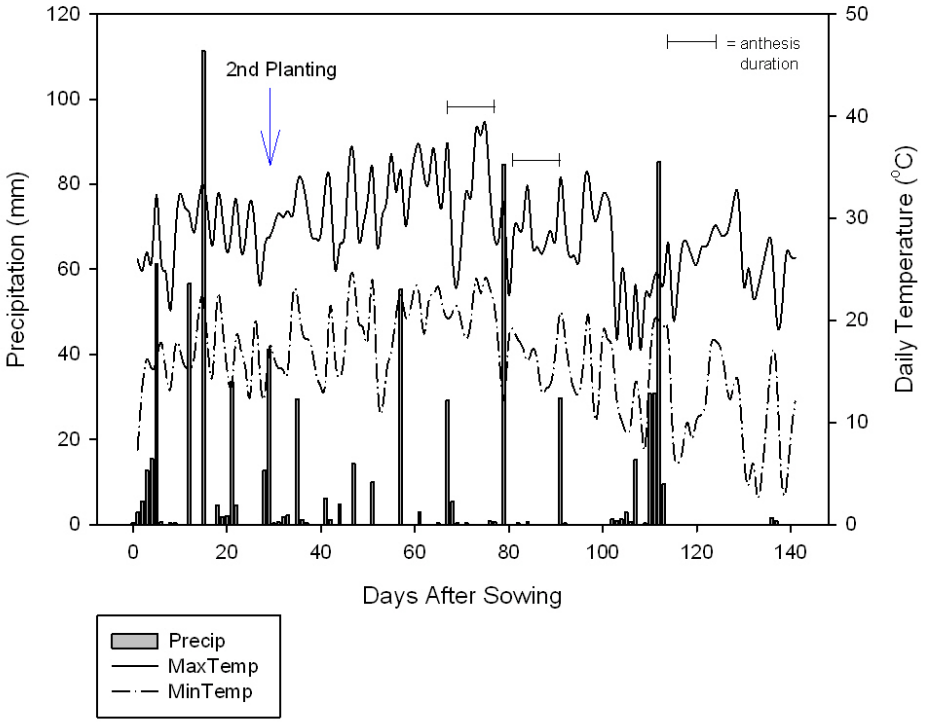
Figure 4.8. Chlorophyll fluorescence (Fv / Fm) response over three different periods of grain filling among groups compared between two plantings.



*, **, *** denotes significant differences between plantings at the P = 0.05, 0.01 and 0.001 levels respectively.

NS denotes no significant differences.

Figure 4.9. Daily values for precipitation and air temperature over the growing season.



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Appendix A - Sorghum Diversity Panel Screened by the Cell Viability Assay

			Fv/Fm				
Pedigree	**Initial**	**Hours2**	**Hours4**	**Hours6**	**Delta2h**	**Delta4h**	**Delta6h***
B.Tx2752	0.742	0.339	0.283	0.160	0.403	0.459	0.583
B.Tx3042	0.748	0.504	0.242	0.137	0.244	0.506	0.611
B.Tx3197	0.756	0.483	0.206	0.085	0.273	0.549	0.671
B.Tx378	0.764	0.531	0.277	0.188	0.234	0.488	0.576
B.Tx399	0.746	0.401	0.233	0.091	0.345	0.513	0.655
B.Tx615	0.763	0.452	0.083	0.026	0.311	0.680	0.737
B.Tx623	0.754	0.409	0.175	0.076	0.345	0.579	0.678
B.Tx641	0.753	0.458	0.283	0.203	0.295	0.469	0.550
B.Tx642	0.760	0.437	0.147	0.061	0.323	0.613	0.699
B.Tx643	0.752	0.568	0.235	0.136	0.184	0.517	0.616
B.Tx645	0.754	0.363	0.230	0.139	0.391	0.524	0.615
B.TxARG-1	0.763	0.447	0.213	0.152	0.316	0.549	0.611
B.OK11	0.761	0.500	0.326	0.250	0.262	0.436	0.512
P9517	0.762	0.349	0.160	0.116	0.413	0.602	0.647
B.QL41	0.757	0.439	0.169	0.107	0.318	0.589	0.650
Segaolane	0.761	0.465	0.156	0.049	0.296	0.605	0.712
Shan Qui Red	0.766	0.368	0.143	0.037	0.398	0.623	0.729
Ajabsido	0.754	0.510	0.217	0.109	0.244	0.537	0.645
Macia	0.753	0.473	0.105	0.035	0.280	0.649	0.718
SURENO	0.760	0.414	0.210	0.123	0.345	0.550	0.637
Malisor 84-7	0.764	0.482	0.288	0.163	0.281	0.475	0.601
El Mota	0.757	0.589	0.427	0.337	0.168	0.330	0.420
SRN39	0.752	0.502	0.246	0.116	0.250	0.506	0.635
Feterita Gishesh	0.763	0.510	0.224	0.073	0.253	0.540	0.690
MR732	0.756	0.419	0.238	0.121	0.337	0.517	0.635
San Chi San	0.763	0.451	0.252	0.078	0.313	0.512	0.685
KS19	0.763	0.541	0.273	0.154	0.222	0.490	0.609
KS115	0.751	0.517	0.227	0.039	0.234	0.524	0.712
Tx2911	0.752	0.293	0.187	0.093	0.459	0.565	0.660

Day	0.768	0.361	0.152	0.079	0.407	0.616	0.689
HEGARI	0.762	0.412	0.177	0.057	0.350	0.585	0.705
SA5330/Martin	0.758	0.442	0.237	0.122	0.316	0.521	0.636
(SN149)SA7000 CAPROCK	0.765	0.311	0.068	0.065	0.454	0.697	0.700
(SN147)SA7078 COMBINE 7078	0.763	0.498	0.266	0.131	0.265	0.497	0.632
(SN142)SA386 REDBINE-60	0.766	0.558	0.379	0.233	0.208	0.387	0.533
Dorado	0.762	0.505	0.308	0.195	0.257	0.455	0.568
R.TAM2566	0.762	0.465	0.194	0.107	0.297	0.568	0.655
R.TX2536	0.762	0.471	0.215	0.026	0.291	0.547	0.736
R.TX2737	0.763	0.353	0.084	0.057	0.411	0.679	0.706
R.Tx2917	0.746	0.415	0.216	0.121	0.331	0.530	0.626
R.Tx430	0.759	0.469	0.212	0.158	0.291	0.548	0.602
R.Tx436	0.764	0.454	0.172	0.056	0.310	0.593	0.709
R.Tx437	0.761	0.449	0.305	0.145	0.312	0.456	0.616
R.TAM428	0.768	0.367	0.176	0.112	0.401	0.592	0.656
Tx2741	0.771	0.405	0.203	0.102	0.366	0.568	0.669
R.Tx2783	0.761	0.383	0.393	0.172	0.378	0.369	0.590
SC6	0.768	0.464	0.293	0.222	0.304	0.476	0.547
SC13	0.766	0.424	0.238	0.063	0.342	0.528	0.703
SC15	0.771	0.415	0.214	0.130	0.356	0.557	0.641
SC17	0.766	0.535	0.303	0.218	0.231	0.463	0.548
SC21	0.756	0.529	0.200	0.041	0.228	0.556	0.715
SC22	0.774	0.402	0.242	0.107	0.372	0.532	0.667
SC23	0.774	0.520	0.269	0.117	0.255	0.506	0.657
SC25	0.761	0.533	0.315	0.115	0.228	0.446	0.646
SC33	0.766	0.461	0.266	0.223	0.305	0.500	0.543
SC35	0.755	0.544	0.294	0.155	0.211	0.461	0.600
SC38	0.765	0.551	0.236	0.183	0.215	0.530	0.582
SC42	0.764	0.476	0.289	0.163	0.288	0.475	0.600
SC49	0.770	0.496	0.373	0.182	0.274	0.397	0.588
SC51	0.760	0.392	0.264	0.144	0.368	0.496	0.616
SC52	0.753	0.420	0.102	0.025	0.333	0.652	0.728
SC53	0.753	0.516	0.248	0.127	0.237	0.505	0.626
SC55	0.750	0.410	0.297	0.140	0.339	0.452	0.609

SC56	0.765	0.514	0.340	0.171	0.252	0.425	0.595
SC57	0.755	0.505	0.201	0.100	0.250	0.554	0.655
SC58	0.747	0.450	0.300	0.262	0.297	0.447	0.485
SC59	0.759	0.367	0.230	0.113	0.392	0.529	0.647
SC60	0.740	0.385	0.230	0.095	0.355	0.510	0.644
SC62	0.758	0.422	0.147	0.073	0.336	0.611	0.685
SC63	0.757	0.482	0.161	0.056	0.276	0.596	0.701
SC64	0.761	0.520	0.262	0.159	0.241	0.499	0.602
SC66	0.758	0.348	0.139	0.069	0.410	0.619	0.689
SC67	0.762	0.537	0.129	0.060	0.226	0.634	0.703
SC79	0.769	0.327	0.124	0.050	0.441	0.645	0.718
SC84	0.756	0.487	0.275	0.189	0.269	0.482	0.568
SC91	0.764	0.332	0.311	0.168	0.432	0.453	0.596
SC103	0.772	0.537	0.241	0.205	0.235	0.531	0.567
SC108	0.760	0.381	0.088	0.033	0.379	0.672	0.728
SC110	0.764	0.456	0.314	0.263	0.308	0.450	0.502
SC115	0.754	0.398	0.145	0.053	0.357	0.609	0.701
SC118	0.759	0.485	0.136	0.050	0.274	0.623	0.709
SC121	0.754	0.489	0.149	0.066	0.265	0.605	0.688
SC124	0.760	0.437	0.407	0.370	0.323	0.353	0.390
SC132	0.759	0.505	0.388	0.238	0.255	0.371	0.521
SC134	0.761	0.531	0.332	0.188	0.230	0.429	0.573
SC135	0.770	0.486	0.212	0.110	0.285	0.558	0.660
SC145	0.764	0.591	0.283	0.130	0.174	0.482	0.634
SC155	0.771	0.373	0.223	0.110	0.398	0.548	0.661
SC170	0.759	0.387	0.247	0.117	0.373	0.512	0.642
SC172	0.765	0.472	0.164	0.099	0.293	0.601	0.667
SC173	0.756	0.376	0.174	0.083	0.379	0.582	0.673
SC175	0.765	0.405	0.145	0.108	0.360	0.621	0.658
SC192	0.742	0.508	0.251	0.132	0.234	0.491	0.610
SC199	0.756	0.417	0.118	0.047	0.339	0.638	0.708
SC206	0.752	0.468	0.206	0.116	0.284	0.546	0.636
SC209	0.765	0.576	0.308	0.173	0.189	0.458	0.592
SC213	0.758	0.527	0.424	0.289	0.231	0.334	0.469
SC214	0.754	0.418	0.223	0.172	0.336	0.530	0.582
SC223	0.760	0.342	0.287	0.166	0.419	0.473	0.594

SC224	0.746	0.384	0.273	0.174	0.363	0.474	0.572
SC240	0.759	0.485	0.243	0.127	0.274	0.516	0.632
SC241	0.757	0.486	0.207	0.035	0.271	0.551	0.722
SC243	0.761	0.452	0.144	0.048	0.309	0.617	0.712
SC261	0.754	0.470	0.200	0.173	0.285	0.555	0.581
SC265	0.764	0.395	0.278	0.235	0.369	0.486	0.529
SC279	0.755	0.441	0.234	0.209	0.314	0.520	0.546
SC283	0.755	0.330	0.200	0.104	0.425	0.555	0.651
SC295	0.762	0.441	0.255	0.179	0.321	0.508	0.583
SC299	0.757	0.489	0.311	0.170	0.268	0.446	0.587
SC301	0.753	0.384	0.202	0.073	0.369	0.551	0.681
SC303	0.761	0.515	0.220	0.152	0.246	0.541	0.609
SC305	0.750	0.513	0.320	0.283	0.237	0.430	0.467
SC309	0.755	0.517	0.251	0.179	0.238	0.504	0.576
SC317	0.763	0.497	0.321	0.220	0.265	0.442	0.543
SC319	0.766	0.483	0.270	0.078	0.283	0.496	0.688
SC322	0.765	0.428	0.290	0.142	0.337	0.475	0.623
SC323	0.751	0.445	0.223	0.069	0.306	0.528	0.682
SC324	0.758	0.534	0.298	0.100	0.224	0.460	0.658
SC325	0.765	0.518	0.311	0.178	0.248	0.454	0.588
SC328	0.760	0.464	0.288	0.171	0.296	0.472	0.589
SC329	0.762	0.294	0.238	0.244	0.468	0.524	0.518
SC331	0.757	0.501	0.278	0.123	0.257	0.480	0.634
SC332	0.754	0.488	0.271	0.175	0.266	0.483	0.579
SC333	0.754	0.437	0.138	0.025	0.317	0.617	0.730
SC334	0.763	0.527	0.337	0.179	0.236	0.426	0.584
SC370	0.753	0.466	0.151	0.086	0.287	0.602	0.667
SC372	0.751	0.357	0.282	0.184	0.395	0.470	0.568
SC373	0.759	0.496	0.309	0.187	0.264	0.450	0.572
SC382	0.748	0.464	0.122	0.064	0.284	0.626	0.684
SC386	0.768	0.457	0.223	0.144	0.312	0.546	0.625
SC391	0.750	0.494	0.300	0.203	0.256	0.450	0.547
SC396	0.744	0.476	0.148	0.062	0.268	0.596	0.683
SC411	0.758	0.502	0.161	0.067	0.256	0.597	0.691
SC413	0.744	0.410	0.180	0.088	0.334	0.564	0.656
SC414	0.764	0.464	0.101	0.026	0.300	0.664	0.739

SC418	0.759	0.374	0.179	0.103	0.385	0.581	0.657
SC420	0.760	0.500	0.232	0.112	0.260	0.528	0.648
SC423	0.764	0.503	0.212	0.088	0.261	0.552	0.676
SC424	0.775	0.464	0.332	0.231	0.311	0.443	0.544
SC425	0.761	0.503	0.320	0.146	0.258	0.441	0.615
SC441	0.749	0.418	0.289	0.130	0.330	0.460	0.618
SC449	0.758	0.546	0.272	0.115	0.212	0.486	0.643
SC450	0.747	0.410	0.196	0.107	0.338	0.551	0.641
SC465	0.764	0.469	0.188	0.118	0.295	0.576	0.646
SC467	0.762	0.407	0.256	0.082	0.355	0.506	0.680
SC473	0.754	0.450	0.176	0.038	0.304	0.578	0.716
SC480	0.760	0.350	0.109	0.030	0.410	0.651	0.730
SC489	0.758	0.446	0.184	0.112	0.313	0.575	0.647
SC498	0.769	0.367	0.139	0.048	0.401	0.630	0.721
SC500	0.751	0.393	0.114	0.072	0.358	0.637	0.679
SC502	0.766	0.519	0.373	0.213	0.248	0.393	0.554
SC525	0.770	0.449	0.144	0.084	0.321	0.625	0.686
SC532	0.766	0.458	0.208	0.115	0.308	0.558	0.651
SC553	0.767	0.338	0.196	0.120	0.429	0.571	0.647
SC557	0.755	0.489	0.228	0.150	0.266	0.527	0.606
SC558	0.760	0.453	0.138	0.061	0.307	0.622	0.699
SC562	0.770	0.396	0.223	0.110	0.374	0.547	0.661
SC563	0.774	0.502	0.274	0.123	0.271	0.500	0.651
SC564	0.764	0.428	0.200	0.054	0.336	0.564	0.711
SC566	0.763	0.429	0.193	0.099	0.335	0.570	0.664
SC574	0.774	0.450	0.281	0.137	0.324	0.493	0.637
SC587	0.760	0.402	0.221	0.101	0.358	0.539	0.660
SC599	0.767	0.374	0.146	0.109	0.394	0.621	0.658
SC605	0.759	0.436	0.166	0.078	0.323	0.594	0.682
SC606	0.766	0.403	0.242	0.147	0.363	0.524	0.619
SC609	0.761	0.499	0.224	0.122	0.263	0.537	0.640
SC610	0.775	0.318	0.142	0.046	0.457	0.633	0.729
SC614	0.773	0.432	0.330	0.162	0.340	0.443	0.611
SC621	0.767	0.436	0.243	0.132	0.331	0.524	0.635
SC623	0.759	0.451	0.212	0.114	0.308	0.547	0.645
SC624	0.739	0.536	0.308	0.267	0.203	0.431	0.472

SC625	0.753	0.437	0.141	0.121	0.317	0.612	0.633
SC627	0.751	0.469	0.161	0.066	0.282	0.590	0.685
SC628	0.768	0.363	0.157	0.097	0.405	0.611	0.672
SC630	0.770	0.335	0.192	0.063	0.435	0.578	0.707
SC637	0.761	0.285	0.221	0.104	0.476	0.540	0.657
SC639	0.765	0.454	0.325	0.077	0.311	0.440	0.688
SC641	0.765	0.324	0.106	0.048	0.441	0.659	0.717
SC645	0.764	0.397	0.140	0.055	0.367	0.624	0.709
SC648	0.763	0.319	0.084	0.038	0.444	0.679	0.726
SC650	0.751	0.404	0.113	0.059	0.347	0.638	0.693
SC655	0.744	0.339	0.183	0.075	0.405	0.561	0.669
SC659	0.751	0.348	0.202	0.123	0.403	0.549	0.628
SC663	0.764	0.430	0.291	0.170	0.333	0.473	0.594
SC671	0.754	0.400	0.098	0.063	0.354	0.656	0.691
SC672	0.769	0.388	0.098	0.047	0.381	0.671	0.722
SC673	0.750	0.505	0.283	0.159	0.245	0.467	0.591
SC679	0.769	0.360	0.275	0.169	0.409	0.494	0.601
SC695	0.767	0.444	0.302	0.255	0.323	0.465	0.512
SC701	0.761	0.549	0.273	0.138	0.212	0.488	0.623
SC702	0.759	0.479	0.235	0.158	0.279	0.524	0.601
SC704	0.766	0.340	0.255	0.127	0.426	0.511	0.639
SC708	0.768	0.512	0.243	0.204	0.256	0.525	0.564
SC720	0.767	0.431	0.252	0.108	0.336	0.515	0.659
SC725	0.761	0.260	0.226	0.111	0.501	0.535	0.649
SC734	0.749	0.524	0.217	0.123	0.225	0.532	0.626
SC738	0.765	0.413	0.100	0.036	0.352	0.665	0.729
SC748	0.751	0.406	0.205	0.097	0.346	0.546	0.654
SC749	0.766	0.505	0.309	0.227	0.261	0.457	0.539
SC755	0.762	0.477	0.144	0.057	0.286	0.619	0.706
SC757	0.755	0.559	0.279	0.138	0.197	0.477	0.617
SC760	0.757	0.471	0.363	0.278	0.285	0.394	0.479
SC782	0.758	0.426	0.219	0.092	0.332	0.539	0.666
SC790	0.762	0.485	0.259	0.141	0.278	0.504	0.622
SC798	0.765	0.326	0.239	0.112	0.439	0.526	0.652
SC803	0.772	0.371	0.092	0.065	0.402	0.680	0.707
SC805	0.753	0.461	0.108	0.028	0.292	0.645	0.725

SC833	0.764	0.366	0.203	0.109	0.398	0.561	0.655
SC855	0.754	0.294	0.069	0.018	0.460	0.685	0.736
SC888	0.763	0.266	0.068	0.051	0.498	0.696	0.712
SC929	0.775	0.360	0.076	0.018	0.415	0.700	0.757
SC937	0.767	0.380	0.264	0.132	0.387	0.503	0.636
SC941	0.761	0.324	0.184	0.095	0.438	0.577	0.667
SC942	0.768	0.531	0.246	0.122	0.237	0.522	0.647
SC947	0.765	0.446	0.190	0.099	0.319	0.575	0.666
SC949	0.760	0.457	0.224	0.088	0.303	0.536	0.673
SC964	0.762	0.314	0.231	0.176	0.448	0.531	0.586
SC968	0.760	0.443	0.243	0.052	0.317	0.517	0.708
SC970	0.756	0.459	0.236	0.123	0.297	0.520	0.632
SC971	0.749	0.397	0.181	0.153	0.353	0.569	0.596
SC979	0.766	0.384	0.139	0.085	0.382	0.627	0.681
SC982	0.768	0.460	0.432	0.352	0.308	0.336	0.416
SC987	0.777	0.381	0.219	0.135	0.397	0.559	0.642
SC991	0.775	0.340	0.256	0.122	0.435	0.519	0.653
SC998	0.761	0.480	0.220	0.075	0.281	0.542	0.686
SC1014	0.772	0.320	0.124	0.048	0.452	0.648	0.724
SC1017	0.761	0.454	0.265	0.114	0.307	0.496	0.647
SC1019	0.769	0.473	0.288	0.211	0.296	0.481	0.557
SC1033	0.769	0.457	0.170	0.105	0.313	0.599	0.664
SC1038	0.772	0.429	0.273	0.178	0.343	0.499	0.594
SC1047	0.759	0.478	0.285	0.232	0.282	0.475	0.527
SC1055	0.763	0.327	0.274	0.181	0.436	0.489	0.582
SC1056	0.767	0.354	0.234	0.068	0.413	0.533	0.699
SC1057	0.771	0.520	0.191	0.068	0.251	0.580	0.703
SC1070	0.773	0.416	0.190	0.080	0.356	0.583	0.693
SC1074	0.771	0.345	0.231	0.093	0.427	0.540	0.678
SC1076	0.769	0.457	0.236	0.151	0.312	0.533	0.617
SC1077	0.770	0.367	0.151	0.130	0.403	0.619	0.640
SC1079	0.765	0.358	0.200	0.090	0.407	0.565	0.675
SC1080	0.754	0.407	0.169	0.113	0.346	0.585	0.641
SC1085	0.760	0.527	0.343	0.182	0.233	0.417	0.578
SC1103	0.768	0.384	0.111	0.017	0.384	0.657	0.751
SC1104	0.766	0.386	0.203	0.082	0.381	0.563	0.684

SC1124	0.765	0.551	0.240	0.143	0.213	0.525	0.621
SC1154	0.766	0.320	0.137	0.068	0.446	0.629	0.698
SC1155	0.766	0.385	0.230	0.110	0.381	0.536	0.656
SC1158	0.767	0.532	0.303	0.152	0.236	0.465	0.615
SC1201	0.769	0.447	0.116	0.020	0.322	0.653	0.749
SC1203	0.761	0.440	0.262	0.125	0.320	0.498	0.636
SC1205	0.772	0.528	0.319	0.125	0.244	0.454	0.647
SC1211	0.762	0.484	0.320	0.179	0.279	0.442	0.584
SC1212	0.764	0.429	0.190	0.075	0.335	0.575	0.690
SC1214	0.753	0.282	0.272	0.324	0.471	0.481	0.429
SC1215	0.762	0.381	0.167	0.072	0.381	0.595	0.690
SC1218	0.760	0.328	0.218	0.094	0.432	0.542	0.666
SC1246	0.760	0.473	0.221	0.089	0.287	0.539	0.670
SC1251	0.762	0.377	0.260	0.083	0.385	0.502	0.679
SC1271	0.748	0.429	0.166	0.037	0.319	0.581	0.711
SC1277	0.766	0.483	0.180	0.068	0.283	0.586	0.698
SC1319	0.768	0.348	0.282	0.234	0.420	0.486	0.534
SC1320	0.753	0.376	0.186	0.069	0.377	0.566	0.683
SC1322	0.764	0.429	0.169	0.037	0.335	0.595	0.727
SC1328	0.767	0.442	0.248	0.060	0.325	0.519	0.707
SC1329	0.762	0.451	0.281	0.212	0.311	0.481	0.550
SC1330	0.764	0.498	0.275	0.148	0.266	0.490	0.616
SC1337	0.751	0.447	0.166	0.104	0.304	0.585	0.648
SC1345	0.766	0.376	0.187	0.041	0.390	0.579	0.725
SC1356	0.768	0.468	0.246	0.093	0.300	0.523	0.676
SC1416	0.753	0.475	0.206	0.087	0.279	0.547	0.666
SC1424	0.763	0.343	0.218	0.107	0.420	0.545	0.656
SC1429	0.768	0.538	0.297	0.140	0.230	0.471	0.628
SC1439	0.768	0.518	0.311	0.161	0.250	0.457	0.607
SC1440	0.767	0.447	0.174	0.133	0.320	0.593	0.634
SC1451	0.769	0.354	0.213	0.046	0.415	0.556	0.723
SC1471	0.757	0.281	0.099	0.014	0.476	0.658	0.743
SC1484	0.756	0.508	0.273	0.098	0.248	0.483	0.658
SC1489	0.769	0.350	0.155	0.076	0.420	0.615	0.694
SC1494	0.772	0.362	0.201	0.051	0.410	0.571	0.721
P898012	0.759	0.446	0.172	0.096	0.313	0.587	0.663

00MN7645	0.766	0.367	0.076	0.040	0.399	0.690	0.726
SC141	0.768	0.553	0.268	0.099	0.216	0.501	0.670
SC142	0.769	0.475	0.162	0.034	0.294	0.607	0.736
SC146	0.761	0.459	0.259	0.117	0.302	0.503	0.644
SC202	0.753	0.444	0.216	0.122	0.309	0.537	0.631
SU629	0.765	0.459	0.169	0.084	0.306	0.597	0.681
SC284	0.767	0.390	0.132	0.065	0.378	0.635	0.703
SC330	0.772	0.291	0.176	0.107	0.481	0.596	0.665
SC336	0.749	0.361	0.317	0.229	0.388	0.432	0.520
SC337	0.760	0.421	0.138	0.025	0.339	0.623	0.735
SC346	0.756	0.438	0.230	0.086	0.318	0.526	0.670
SC348	0.762	0.437	0.202	0.069	0.325	0.560	0.693
SC367	0.759	0.486	0.143	0.034	0.272	0.616	0.725
SC405	0.764	0.300	0.154	0.091	0.465	0.611	0.674
SC452	0.763	0.464	0.136	0.021	0.299	0.627	0.742
SC471	0.764	0.512	0.271	0.125	0.252	0.493	0.639
SC477	0.759	0.419	0.222	0.092	0.340	0.537	0.667
SC499	0.766	0.395	0.297	0.177	0.371	0.469	0.589
SC504	0.767	0.453	0.123	0.067	0.314	0.643	0.700
SC520	0.767	0.427	0.162	0.081	0.340	0.604	0.686
SC575	0.757	0.458	0.142	0.052	0.299	0.616	0.706

Appendix B - Experimental Maize Lines Screened by the Cell Viability Assay

Chlorophyll Fluorescence Values For 197 Experimental Corn Lines

Entry	Initial Fv/Fm Mean	Initial Fv/Fm Stdev	2 Hours Fv/Fm Mean	2 Hours Fv/Fm Stdev	4 Hours Fv/Fm Mean	4 Hours Fv/Fm Stdev	6 Hours Fv/Fm Mean	6 Hours Fv/Fm Stdev	Δ 2h Fv/Fm Mean	Δ 2h Fv/Fm Stdev	Δ 4h Fv/Fm Mean	Δ 4h Fv/Fm Stdev	Δ 6h Fv/Fm Mean	Δ 6h Fv/Fm Stdev
EXP006	0.742	0.019	0.629	0.018	0.571	0.070	0.552	0.070	0.114	0.027	0.171	0.060	0.190	0.081
EXP156	0.747	0.019	0.613	0.032	0.586	0.050	0.540	0.066	0.134	0.032	0.160	0.049	0.207	0.082
EXP157 (B73xMO17)	0.746	0.012	0.637	0.024	0.533	0.112	0.535	0.043	0.109	0.028	0.213	0.119	0.211	0.051
EXP126	0.747	0.022	0.569	0.053	0.558	0.055	0.517	0.069	0.178	0.055	0.189	0.066	0.229	0.078
EXP180	0.745	0.015	0.588	0.059	0.536	0.072	0.514	0.028	0.157	0.058	0.208	0.068	0.230	0.032
EXP070	0.763	0.008	0.468	0.115	0.547	0.030	0.531	0.057	0.295	0.113	0.216	0.030	0.231	0.056
EXP161	0.757	0.011	0.626	0.030	0.547	0.035	0.520	0.088	0.130	0.035	0.209	0.037	0.236	0.090
EXP132	0.748	0.009	0.548	0.066	0.554	0.049	0.511	0.061	0.200	0.062	0.194	0.049	0.237	0.068
EXP020	0.753	0.013	0.640	0.026	0.533	0.095	0.516	0.094	0.112	0.030	0.220	0.100	0.237	0.103
EXP102	0.763	0.009	0.643	0.074	0.582	0.072	0.522	0.154	0.120	0.078	0.181	0.074	0.240	0.151
EXP063	0.760	0.013	0.561	0.150	0.531	0.084	0.520	0.116	0.199	0.157	0.230	0.095	0.241	0.125
EXP160	0.749	0.020	0.619	0.034	0.584	0.031	0.503	0.004	0.130	0.053	0.165	0.033	0.246	0.023
EXP193	0.741	0.019	0.529	0.064	0.494	0.059	0.493	0.045	0.213	0.063	0.248	0.062	0.249	0.054
EXP169	0.748	0.013	0.618	0.075	0.571	0.035	0.498	0.039	0.130	0.071	0.177	0.024	0.249	0.030
EXP066	0.765	0.010	0.561	0.109	0.553	0.082	0.516	0.100	0.204	0.115	0.212	0.087	0.250	0.106
EXP159 (B73xMO17)	0.752	0.009	0.570	0.047	0.519	0.088	0.499	0.112	0.182	0.049	0.233	0.093	0.253	0.117
EXP129	0.751	0.008	0.555	0.099	0.509	0.061	0.498	0.053	0.196	0.099	0.242	0.067	0.253	0.060
EXP055	0.762	0.027	0.624	0.011	0.549	0.119	0.505	0.137	0.139	0.038	0.213	0.124	0.257	0.151
EXP021	0.748	0.019	0.602	0.054	0.547	0.033	0.488	0.074	0.146	0.055	0.201	0.026	0.259	0.079
EXP083	0.754	0.007	0.584	0.133	0.537	0.094	0.494	0.159	0.170	0.128	0.217	0.096	0.260	0.164
EXP094	0.751	0.016	0.512	0.141	0.506	0.034	0.489	0.022	0.238	0.152	0.245	0.041	0.262	0.024
EXP173	0.755	0.010	0.603	0.063	0.561	0.042	0.492	0.063	0.152	0.057	0.194	0.040	0.263	0.058

EXP049	0.748	0.013	0.593	0.047	0.568	0.051	0.484	0.072	0.155	0.054	0.180	0.044	0.263	0.069
EXP188	0.751	0.008	0.584	0.064	0.519	0.054	0.485	0.057	0.167	0.070	0.233	0.058	0.266	0.056
EXP164	0.756	0.008	0.603	0.045	0.557	0.040	0.490	0.040	0.154	0.046	0.199	0.043	0.267	0.039
EXP163	0.748	0.014	0.608	0.048	0.545	0.065	0.481	0.047	0.139	0.050	0.203	0.058	0.267	0.056
EXP045	0.753	0.011	0.614	0.017	0.514	0.044	0.486	0.038	0.140	0.024	0.239	0.033	0.268	0.043
EXP091	0.750	0.009	0.604	0.030	0.547	0.021	0.481	0.034	0.146	0.034	0.203	0.028	0.269	0.041
EXP106	0.765	0.006	0.641	0.025	0.564	0.052	0.495	0.103	0.124	0.022	0.201	0.056	0.271	0.106
EXP167	0.758	0.008	0.572	0.051	0.549	0.047	0.486	0.107	0.186	0.048	0.209	0.041	0.272	0.107
EXP057	0.762	0.011	0.632	0.035	0.562	0.028	0.490	0.054	0.129	0.036	0.200	0.038	0.272	0.063
EXP195	0.758	0.016	0.621	0.019	0.515	0.090	0.483	0.176	0.136	0.031	0.243	0.089	0.275	0.170
EXP189	0.751	0.015	0.561	0.050	0.518	0.071	0.476	0.041	0.190	0.064	0.233	0.083	0.275	0.049
EXP086	0.756	0.008	0.491	0.172	0.486	0.113	0.477	0.073	0.264	0.173	0.269	0.111	0.279	0.069
EXP053	0.758	0.010	0.615	0.046	0.529	0.093	0.479	0.098	0.143	0.050	0.230	0.088	0.280	0.097
EXP048	0.751	0.016	0.602	0.062	0.505	0.138	0.470	0.166	0.149	0.070	0.246	0.137	0.281	0.171
EXP019	0.751	0.018	0.604	0.072	0.539	0.041	0.469	0.046	0.147	0.084	0.212	0.038	0.282	0.047
EXP052	0.751	0.017	0.595	0.026	0.530	0.037	0.469	0.041	0.156	0.030	0.221	0.039	0.283	0.040
EXP175	0.750	0.011	0.619	0.055	0.518	0.051	0.466	0.090	0.130	0.056	0.231	0.052	0.284	0.093
EXP068	0.759	0.024	0.610	0.025	0.553	0.037	0.475	0.049	0.149	0.030	0.206	0.028	0.284	0.039
EXP016	0.755	0.015	0.548	0.169	0.505	0.125	0.468	0.085	0.207	0.162	0.249	0.116	0.286	0.074
EXP162	0.762	0.010	0.596	0.052	0.547	0.096	0.476	0.135	0.167	0.051	0.215	0.101	0.287	0.140
EXP177	0.757	0.010	0.588	0.038	0.557	0.047	0.470	0.143	0.169	0.043	0.201	0.051	0.287	0.144
EXP191	0.755	0.017	0.582	0.058	0.516	0.053	0.467	0.104	0.174	0.064	0.240	0.044	0.289	0.111
EXP124	0.756	0.010	0.594	0.028	0.551	0.037	0.467	0.043	0.162	0.030	0.206	0.030	0.289	0.036
EXP051	0.770	0.019	0.635	0.029	0.559	0.047	0.480	0.076	0.135	0.025	0.211	0.046	0.290	0.091
EXP166	0.747	0.012	0.611	0.021	0.516	0.031	0.456	0.062	0.136	0.018	0.231	0.028	0.291	0.053
EXP144	0.751	0.019	0.640	0.043	0.532	0.141	0.459	0.208	0.111	0.052	0.219	0.149	0.292	0.217
EXP158	0.762	0.009	0.617	0.024	0.564	0.037	0.469	0.058	0.145	0.017	0.198	0.030	0.292	0.053
EXP075	0.762	0.014	0.647	0.021	0.561	0.065	0.467	0.116	0.116	0.027	0.201	0.075	0.296	0.126
EXP174	0.747	0.008	0.602	0.069	0.493	0.080	0.451	0.142	0.145	0.070	0.254	0.084	0.296	0.145
EXP178	0.755	0.007	0.581	0.028	0.529	0.039	0.458	0.035	0.173	0.024	0.226	0.041	0.297	0.037
EXP035	0.755	0.018	0.600	0.057	0.517	0.113	0.456	0.139	0.155	0.055	0.237	0.104	0.299	0.127
EXP034	0.745	0.009	0.595	0.045	0.513	0.104	0.446	0.103	0.151	0.037	0.232	0.096	0.299	0.098
EXP192	0.755	0.017	0.599	0.032	0.537	0.062	0.453	0.103	0.156	0.024	0.217	0.047	0.302	0.103
EXP181	0.751	0.003	0.604	0.044	0.515	0.057	0.445	0.064	0.148	0.043	0.236	0.054	0.306	0.065
EXP036	0.767	0.004	0.581	0.131	0.513	0.086	0.461	0.106	0.186	0.131	0.254	0.086	0.307	0.106
EXP062	0.757	0.008	0.555	0.133	0.509	0.060	0.449	0.111	0.202	0.128	0.248	0.063	0.308	0.115

EXP028	0.752	0.018	0.581	0.102	0.516	0.099	0.443	0.150	0.171	0.107	0.235	0.093	0.309	0.146
EXP172	0.752	0.010	0.600	0.027	0.522	0.034	0.442	0.106	0.152	0.026	0.231	0.034	0.310	0.109
EXP155	0.766	0.004	0.552	0.169	0.462	0.223	0.455	0.239	0.214	0.170	0.304	0.226	0.311	0.242
EXP186	0.749	0.011	0.586	0.094	0.548	0.052	0.437	0.060	0.164	0.093	0.201	0.045	0.312	0.066
EXP017	0.735	0.037	0.573	0.095	0.495	0.097	0.423	0.100	0.162	0.074	0.240	0.093	0.312	0.131
EXP011	0.746	0.026	0.565	0.116	0.482	0.097	0.433	0.111	0.181	0.094	0.264	0.075	0.313	0.118
EXP037	0.753	0.007	0.565	0.082	0.546	0.092	0.440	0.184	0.188	0.082	0.207	0.092	0.313	0.185
EXP119	0.755	0.007	0.594	0.031	0.539	0.064	0.440	0.084	0.160	0.036	0.216	0.059	0.314	0.080
EXP025	0.755	0.012	0.538	0.146	0.511	0.072	0.440	0.092	0.216	0.148	0.244	0.077	0.315	0.100
EXP010	0.751	0.014	0.606	0.045	0.492	0.063	0.436	0.101	0.145	0.034	0.259	0.072	0.315	0.113
EXP165	0.751	0.014	0.602	0.043	0.535	0.038	0.435	0.072	0.149	0.038	0.216	0.031	0.316	0.076
EXP080	0.760	0.012	0.584	0.115	0.488	0.082	0.443	0.141	0.176	0.122	0.272	0.091	0.317	0.143
EXP107	0.768	0.019	0.612	0.075	0.528	0.086	0.451	0.154	0.156	0.077	0.241	0.079	0.318	0.143
EXP187	0.755	0.011	0.616	0.060	0.534	0.104	0.437	0.140	0.139	0.054	0.221	0.098	0.318	0.134
EXP136	0.752	0.009	0.599	0.034	0.515	0.086	0.433	0.137	0.153	0.034	0.237	0.092	0.319	0.143
EXP104	0.750	0.022	0.610	0.046	0.489	0.077	0.430	0.081	0.140	0.039	0.261	0.071	0.319	0.084
EXP190	0.758	0.008	0.593	0.084	0.515	0.059	0.438	0.144	0.165	0.086	0.243	0.063	0.319	0.147
EXP171	0.757	0.006	0.595	0.061	0.500	0.078	0.435	0.045	0.162	0.059	0.257	0.077	0.322	0.045
EXP118	0.769	0.017	0.599	0.011	0.529	0.047	0.446	0.146	0.169	0.023	0.240	0.051	0.323	0.149
EXP067	0.764	0.006	0.584	0.054	0.499	0.088	0.440	0.153	0.180	0.054	0.264	0.089	0.323	0.153
EXP120	0.743	0.007	0.639	0.016	0.513	0.192	0.419	0.186	0.104	0.016	0.230	0.188	0.324	0.182
EXP033	0.757	0.014	0.622	0.014	0.484	0.131	0.433	0.136	0.135	0.023	0.273	0.135	0.324	0.138
EXP184	0.747	0.014	0.567	0.105	0.479	0.163	0.422	0.194	0.181	0.116	0.268	0.167	0.325	0.196
EXP196	0.758	0.013	0.544	0.154	0.523	0.055	0.433	0.107	0.214	0.157	0.235	0.046	0.326	0.102
EXP108	0.758	0.015	0.593	0.036	0.515	0.055	0.431	0.106	0.165	0.030	0.243	0.054	0.327	0.108
EXP030	0.755	0.011	0.575	0.033	0.445	0.169	0.427	0.181	0.180	0.040	0.310	0.178	0.328	0.186
EXP170	0.750	0.005	0.552	0.082	0.478	0.115	0.422	0.136	0.198	0.086	0.272	0.115	0.328	0.133
EXP140	0.753	0.022	0.563	0.118	0.522	0.111	0.423	0.136	0.190	0.117	0.231	0.105	0.329	0.136
EXP023	0.748	0.010	0.624	0.013	0.509	0.082	0.417	0.119	0.124	0.009	0.239	0.083	0.331	0.124
EXP197	0.754	0.009	0.651	0.062	0.507	0.084	0.422	0.075	0.103	0.056	0.247	0.082	0.332	0.080
EXP111	0.756	0.011	0.629	0.026	0.515	0.098	0.422	0.179	0.127	0.033	0.242	0.098	0.335	0.180
EXP137	0.754	0.013	0.535	0.155	0.365	0.076	0.419	0.114	0.220	0.142	0.389	0.064	0.336	0.127
EXP005	0.753	0.012	0.607	0.036	0.491	0.131	0.417	0.155	0.146	0.039	0.262	0.139	0.336	0.164
EXP078	0.762	0.009	0.600	0.137	0.494	0.137	0.425	0.193	0.162	0.140	0.267	0.133	0.337	0.191
EXP122	0.763	0.004	0.583	0.063	0.500	0.056	0.425	0.086	0.179	0.061	0.263	0.058	0.338	0.088
EXP109	0.760	0.014	0.560	0.113	0.499	0.118	0.421	0.113	0.200	0.116	0.261	0.116	0.339	0.113

EXP040	0.770	0.022	0.628	0.029	0.547	0.060	0.432	0.107	0.143	0.048	0.224	0.081	0.339	0.126
EXP127	0.758	0.006	0.616	0.051	0.518	0.112	0.419	0.090	0.142	0.052	0.240	0.113	0.339	0.093
EXP058	0.768	0.040	0.571	0.077	0.475	0.088	0.428	0.111	0.196	0.099	0.292	0.095	0.340	0.099
EXP027	0.757	0.005	0.645	0.033	0.524	0.074	0.416	0.097	0.112	0.036	0.233	0.077	0.341	0.097
EXP029	0.755	0.012	0.594	0.013	0.446	0.118	0.412	0.151	0.161	0.020	0.309	0.121	0.343	0.158
EXP044	0.746	0.021	0.584	0.121	0.519	0.063	0.402	0.111	0.162	0.107	0.227	0.071	0.344	0.126
EXP105	0.756	0.009	0.649	0.025	0.507	0.112	0.411	0.172	0.106	0.027	0.248	0.108	0.345	0.170
EXP064	0.767	0.011	0.614	0.018	0.480	0.156	0.419	0.209	0.152	0.029	0.287	0.158	0.348	0.210
EXP123	0.758	0.006	0.582	0.043	0.469	0.065	0.410	0.135	0.176	0.045	0.289	0.061	0.348	0.134
EXP039	0.756	0.007	0.601	0.020	0.507	0.091	0.408	0.127	0.155	0.021	0.249	0.092	0.348	0.129
EXP007	0.751	0.017	0.602	0.024	0.526	0.059	0.402	0.100	0.149	0.037	0.225	0.057	0.349	0.104
EXP103	0.769	0.014	0.621	0.034	0.471	0.211	0.419	0.189	0.147	0.037	0.298	0.206	0.350	0.182
EXP135	0.760	0.022	0.602	0.050	0.443	0.116	0.410	0.125	0.158	0.056	0.317	0.119	0.351	0.129
EXP061	0.754	0.020	0.531	0.091	0.474	0.071	0.403	0.125	0.224	0.098	0.281	0.079	0.351	0.137
EXP069	0.746	0.028	0.587	0.045	0.503	0.055	0.395	0.075	0.159	0.065	0.244	0.054	0.352	0.087
EXP065	0.755	0.011	0.620	0.057	0.504	0.147	0.403	0.216	0.136	0.060	0.251	0.138	0.352	0.208
EXP076	0.762	0.007	0.634	0.042	0.505	0.114	0.408	0.157	0.128	0.039	0.257	0.106	0.354	0.150
EXP054	0.746	0.009	0.603	0.032	0.512	0.093	0.392	0.134	0.143	0.032	0.234	0.086	0.354	0.128
EXP147	0.754	0.008	0.619	0.021	0.445	0.136	0.399	0.148	0.134	0.026	0.308	0.132	0.354	0.148
EXP176	0.767	0.011	0.637	0.092	0.469	0.171	0.412	0.174	0.130	0.086	0.297	0.177	0.355	0.179
EXP095	0.759	0.011	0.615	0.014	0.474	0.112	0.403	0.110	0.144	0.013	0.284	0.118	0.356	0.115
EXP056	0.756	0.006	0.644	0.020	0.525	0.073	0.400	0.147	0.112	0.023	0.231	0.069	0.356	0.145
EXP071	0.755	0.014	0.630	0.024	0.435	0.214	0.394	0.210	0.126	0.031	0.320	0.216	0.361	0.210
EXP079	0.757	0.013	0.574	0.057	0.461	0.087	0.396	0.155	0.183	0.063	0.296	0.077	0.361	0.144
EXP112	0.759	0.012	0.634	0.031	0.512	0.061	0.397	0.120	0.125	0.041	0.247	0.066	0.362	0.122
EXP072	0.756	0.018	0.618	0.034	0.447	0.043	0.394	0.116	0.138	0.048	0.310	0.038	0.363	0.127
EXP182	0.756	0.050	0.599	0.045	0.508	0.058	0.393	0.099	0.157	0.034	0.248	0.052	0.363	0.087
EXP185	0.759	0.010	0.595	0.025	0.457	0.133	0.395	0.143	0.164	0.028	0.302	0.132	0.363	0.141
EXP145	0.767	0.017	0.589	0.036	0.479	0.132	0.403	0.140	0.178	0.038	0.288	0.135	0.364	0.146
EXP100	0.755	0.013	0.619	0.047	0.530	0.069	0.391	0.083	0.136	0.044	0.225	0.064	0.364	0.074
EXP077	0.742	0.017	0.586	0.055	0.483	0.108	0.378	0.135	0.156	0.067	0.259	0.093	0.364	0.121
EXP047	0.756	0.013	0.582	0.110	0.500	0.142	0.391	0.175	0.174	0.120	0.256	0.142	0.365	0.176
EXP014	0.758	0.021	0.526	0.129	0.400	0.144	0.393	0.151	0.232	0.116	0.358	0.141	0.365	0.156
EXP089	0.750	0.019	0.615	0.037	0.467	0.198	0.384	0.195	0.135	0.044	0.283	0.209	0.366	0.204
EXP022	0.758	0.011	0.629	0.018	0.457	0.114	0.391	0.170	0.128	0.019	0.301	0.115	0.367	0.171
EXP085	0.761	0.005	0.662	0.040	0.533	0.122	0.388	0.228	0.099	0.043	0.228	0.122	0.372	0.228

EXP116	0.760	0.009	0.648	0.018	0.500	0.065	0.386	0.080	0.112	0.017	0.260	0.066	0.374	0.088
EXP138	0.750	0.013	0.620	0.016	0.464	0.123	0.375	0.125	0.130	0.018	0.286	0.135	0.375	0.137
EXP101	0.752	0.014	0.589	0.101	0.471	0.069	0.375	0.130	0.163	0.102	0.280	0.082	0.376	0.143
EXP043	0.753	0.009	0.599	0.056	0.508	0.104	0.377	0.167	0.154	0.052	0.245	0.112	0.376	0.176
EXP150	0.757	0.010	0.551	0.102	0.441	0.135	0.380	0.075	0.206	0.104	0.316	0.140	0.377	0.076
EXP012	0.755	0.010	0.584	0.041	0.459	0.104	0.376	0.185	0.171	0.047	0.295	0.110	0.378	0.190
EXP038	0.748	0.018	0.631	0.022	0.470	0.080	0.368	0.128	0.116	0.016	0.278	0.071	0.380	0.140
EXP046	0.755	0.012	0.641	0.023	0.500	0.106	0.374	0.175	0.114	0.031	0.255	0.114	0.381	0.186
EXP114	0.763	0.020	0.582	0.022	0.465	0.134	0.380	0.157	0.181	0.022	0.298	0.134	0.383	0.158
EXP050	0.761	0.010	0.605	0.144	0.492	0.052	0.378	0.068	0.156	0.138	0.269	0.060	0.383	0.075
EXP026	0.750	0.005	0.621	0.046	0.427	0.203	0.367	0.191	0.130	0.045	0.323	0.202	0.383	0.191
EXP110	0.758	0.013	0.612	0.026	0.481	0.101	0.375	0.112	0.146	0.027	0.278	0.107	0.383	0.121
EXP096	0.759	0.009	0.557	0.088	0.401	0.126	0.374	0.096	0.202	0.093	0.358	0.133	0.385	0.098
EXP115	0.750	0.011	0.590	0.093	0.442	0.120	0.364	0.185	0.160	0.094	0.308	0.116	0.386	0.183
EXP018	0.750	0.008	0.629	0.027	0.476	0.114	0.363	0.209	0.121	0.024	0.274	0.111	0.387	0.204
EXP139	0.751	0.017	0.575	0.077	0.470	0.080	0.364	0.116	0.176	0.069	0.282	0.081	0.387	0.123
EXP042	0.756	0.011	0.602	0.102	0.433	0.096	0.369	0.175	0.154	0.105	0.323	0.104	0.387	0.178
EXP131	0.763	0.009	0.609	0.029	0.395	0.119	0.376	0.155	0.154	0.025	0.368	0.122	0.388	0.160
EXP098	0.753	0.014	0.560	0.124	0.470	0.138	0.364	0.074	0.193	0.135	0.283	0.143	0.389	0.076
EXP013	0.741	0.021	0.637	0.019	0.522	0.106	0.350	0.130	0.104	0.032	0.219	0.125	0.391	0.145
EXP146	0.759	0.011	0.613	0.045	0.440	0.137	0.367	0.091	0.146	0.047	0.319	0.143	0.392	0.101
EXP060	0.756	0.008	0.588	0.045	0.411	0.104	0.363	0.125	0.168	0.037	0.345	0.106	0.393	0.127
EXP082	0.765	0.006	0.610	0.039	0.443	0.089	0.368	0.104	0.154	0.042	0.322	0.093	0.397	0.108
EXP099	0.762	0.011	0.622	0.009	0.507	0.103	0.365	0.129	0.139	0.015	0.255	0.112	0.397	0.138
EXP059	0.755	0.014	0.587	0.054	0.441	0.215	0.358	0.210	0.167	0.060	0.313	0.229	0.397	0.224
EXP003	0.752	0.006	0.615	0.036	0.482	0.100	0.354	0.160	0.136	0.035	0.270	0.100	0.398	0.160
EXP088	0.754	0.010	0.578	0.042	0.427	0.090	0.355	0.107	0.176	0.042	0.327	0.087	0.399	0.101
EXP168	0.733	0.024	0.629	0.020	0.451	0.182	0.333	0.253	0.103	0.035	0.282	0.196	0.399	0.271
EXP152	0.752	0.017	0.611	0.023	0.458	0.104	0.352	0.190	0.141	0.012	0.294	0.117	0.400	0.202
EXP024	0.762	0.013	0.615	0.057	0.476	0.068	0.362	0.110	0.147	0.051	0.286	0.064	0.400	0.102
EXP179	0.753	0.010	0.584	0.056	0.416	0.085	0.352	0.140	0.169	0.063	0.337	0.086	0.401	0.139
EXP093	0.751	0.014	0.595	0.034	0.496	0.088	0.344	0.131	0.156	0.044	0.255	0.077	0.407	0.117
EXP117	0.761	0.006	0.651	0.009	0.496	0.061	0.352	0.112	0.110	0.015	0.265	0.066	0.409	0.118
EXP081	0.760	0.013	0.617	0.037	0.436	0.072	0.349	0.113	0.143	0.046	0.324	0.068	0.411	0.120
EXP073	0.758	0.007	0.562	0.154	0.423	0.123	0.347	0.108	0.196	0.156	0.336	0.121	0.411	0.105
EXP008	0.769	0.007	0.615	0.059	0.520	0.078	0.357	0.161	0.154	0.053	0.249	0.081	0.411	0.166

EXP097	0.749	0.014	0.623	0.037	0.456	0.201	0.338	0.184	0.126	0.037	0.293	0.206	0.411	0.185
EXP151	0.755	0.014	0.611	0.050	0.424	0.098	0.343	0.093	0.144	0.044	0.331	0.092	0.412	0.092
EXP087	0.755	0.010	0.596	0.018	0.488	0.048	0.341	0.145	0.158	0.020	0.266	0.052	0.413	0.148
EXP074	0.750	0.013	0.650	0.024	0.436	0.082	0.336	0.085	0.099	0.034	0.314	0.080	0.414	0.075
EXP183	0.764	0.009	0.613	0.037	0.468	0.144	0.348	0.148	0.151	0.035	0.296	0.142	0.416	0.146
EXP002	0.756	0.016	0.570	0.159	0.426	0.155	0.339	0.233	0.186	0.171	0.329	0.159	0.416	0.231
EXP032	0.755	0.013	0.634	0.025	0.424	0.215	0.338	0.253	0.121	0.029	0.330	0.210	0.416	0.252
EXP090	0.757	0.009	0.620	0.019	0.507	0.073	0.334	0.157	0.137	0.022	0.249	0.073	0.423	0.156
EXP113	0.754	0.015	0.617	0.026	0.498	0.046	0.330	0.142	0.138	0.030	0.256	0.034	0.424	0.141
EXP149	0.747	0.028	0.556	0.098	0.425	0.119	0.315	0.149	0.191	0.107	0.322	0.110	0.432	0.147
EXP041	0.749	0.010	0.597	0.028	0.375	0.155	0.314	0.191	0.152	0.026	0.374	0.152	0.435	0.191
EXP004	0.748	0.013	0.609	0.019	0.433	0.121	0.312	0.168	0.139	0.022	0.315	0.117	0.436	0.162
EXP084	0.753	0.006	0.612	0.034	0.441	0.099	0.311	0.190	0.140	0.036	0.311	0.099	0.442	0.191
EXP121	0.758	0.014	0.615	0.047	0.367	0.156	0.314	0.119	0.142	0.052	0.390	0.157	0.444	0.118
EXP142	0.754	0.008	0.629	0.024	0.410	0.124	0.310	0.128	0.124	0.021	0.344	0.127	0.444	0.128
EXP194	0.763	0.009	0.633	0.044	0.484	0.133	0.317	0.134	0.130	0.038	0.279	0.127	0.446	0.136
EXP143	0.755	0.012	0.584	0.047	0.418	0.137	0.307	0.142	0.171	0.046	0.337	0.143	0.448	0.145
EXP128	0.773	0.029	0.600	0.032	0.437	0.166	0.323	0.201	0.173	0.060	0.336	0.159	0.449	0.196
EXP154	0.752	0.016	0.628	0.032	0.487	0.103	0.294	0.095	0.123	0.020	0.265	0.112	0.458	0.098
EXP133	0.760	0.009	0.601	0.024	0.388	0.244	0.300	0.238	0.159	0.022	0.371	0.250	0.459	0.241
EXP134	0.752	0.010	0.616	0.031	0.403	0.147	0.290	0.141	0.137	0.032	0.349	0.155	0.462	0.149
EXP015	0.757	0.009	0.619	0.027	0.388	0.128	0.293	0.163	0.138	0.035	0.369	0.133	0.465	0.166
EXP031	0.748	0.018	0.589	0.062	0.357	0.136	0.283	0.142	0.159	0.062	0.391	0.143	0.465	0.146
EXP125	0.755	0.012	0.620	0.028	0.380	0.127	0.285	0.168	0.135	0.020	0.375	0.134	0.470	0.176
EXP092	0.758	0.008	0.621	0.031	0.376	0.147	0.280	0.153	0.137	0.033	0.381	0.151	0.477	0.158
EXP153	0.755	0.009	0.627	0.013	0.427	0.121	0.274	0.145	0.128	0.021	0.328	0.112	0.481	0.141
EXP001	0.756	0.007	0.637	0.008	0.475	0.067	0.270	0.058	0.119	0.010	0.281	0.061	0.486	0.053
EXP009	0.756	0.010	0.615	0.054	0.377	0.192	0.269	0.155	0.141	0.063	0.379	0.196	0.487	0.157
EXP141	0.764	0.011	0.626	0.008	0.345	0.196	0.273	0.222	0.138	0.014	0.420	0.203	0.492	0.229
EXP148	0.767	0.008	0.635	0.011	0.398	0.121	0.274	0.139	0.132	0.010	0.369	0.117	0.493	0.135
EXP130	0.768	0.012	0.619	0.038	0.347	0.147	0.208	0.179	0.149	0.037	0.421	0.148	0.560	0.177