EVALUATION OF SORGHUM GENOTYPES FOR VARIATION IN CANOPY TEMPERATURE AND DROUGHT TOLERANCE

by

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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most economically important cereal crop grown worldwide and adapted to a wide range of climatic conditions. Drought stress has been ranked as one of the most significant causes of crop yield loss with its effects on yield and yield components. Conservative water use by plants is one of the strategies that can be used as a drought coping mechanism. The slow wilting trait has been associated with conservative water use and has been found in some sorghum genotypes. The purpose of this study was to use canopy temperature to screen for drought tolerance in sorghum, evaluate water use efficiency for slow wilting sorghum genotypes and determine variability in root morphology and response to drought among sorghum genotypes. Canopy temperature studies were conducted under field conditions using infrared (IR) sensors while water use efficiency and root studies were conducted under greenhouse conditions. Our results showed a distinct separation in canopy temperature among genotypes under field conditions at 2:00 pm to 6:00 pm. Midday canopy temperature depression (CTD) was positively correlated to yield (R^2 = 0.19) and harvest index (R^2 = 0.11). CTD was also stable for all the genotypes during the period from 1:00 pm to 7:00 pm. There was a negative correlation between CTD and crop water stress index (CWSI) (R^2 = 0.34) and a positive one between canopy temperature and CWSI (R^2 = 0.50). Evaluation of genotypes for water use efficiency revealed significant variability among sorghum genotypes in the amount of water used (10.48 – 13.52 kg) and transpiration efficiency (TE) (2.64 – 7.11 g kg^{-1}) among genotypes. Slow wilting genotypes were high in TE. Rooting depth increased for some genotypes under drought stress with genotype SC1124 recording the largest increase (180%). Total root length for some genotypes increased by 11 – 113% with genotypes SC224 and

SC1019 recording the greatest increase. There was a positive correlation between water used and root length (R^2 = 0.21). These results show that there is potential for selection of drought tolerance in sorghum and that genotypes with the slow wilting traits are efficient in water use.

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Major Professor
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Dedication

For Peninah K. Mutava, in memoriam,

If I could have a lifetime wish

a dream that would come true

I'd pray to God with all my heart for yesterday and you

To tell you that I loved you

To say what now must be one long unbroken cry of pain

To tell you what a joy it would have been

to be the one to attend to you in your need

a burden that would have been a gift

for giving does not burden one who loves

I wish I could have been with you

when you perhaps aware, perhaps not

turned towards death alone

with no one there to share your fear, your hand, your one last breath

I wish, I wish, I wish . . . but it is done

And now that you are departed

I must surrender what is gone

Although we miss you, you will always be loved

Chapter 1 - General Introduction and Review of Literature

1.1. General introduction

1.1.1. Sorghum

Sorghum (*Sorghum bicolor* (L.) Moench), a C_4 grass, is the fifth most economically important cereal crop grown worldwide behind wheat (*Triticum aestivum*), rice (*Oryza sativa*), maize (*Zea mays*), and barley (*Hordeum vulgare*) (CGIAR, 2009). It is a photoperiod sensitive plant that can grow in rainy as well as semi-arid areas making it an important crop in areas too dry for maize production. C_4 cereals, like sorghum, originated from the tropics and are generally more heat and drought tolerant than C_3 plants, like wheat, which originated from temperate regions (Blum et al., 1990). The mean optimum temperature range for grain sorghum is 21 to 35°C for seed germination, 26 to 34°C for vegetative growth and development, and 25 to 28°C for reproductive growth (Maiti, 1996). Sorghum-producing regions often experience daytime/nighttime temperatures of >32/22°C (Prasad et al., 2006).

Sorghum bicolor is diverse and has been classified into five races (bicolor, guinea, caudatum, kafir, and durra) based on spikelet morphology (Harlan and deWet, 1972). Due to within race variability and the existence of race intermediates, Dahlberg et al. (2004) established a classification with working groups (sub-races) which integrates Harlan and deWet's classification. These working groups explain the variation that exist within a given race.

1.1.2. Sorghum production and food security

In 2010 the leading world producers of sorghum included Nigeria (19.3%), United States (16.3%), India (11.7%) and Mexico (10.5%) (Agricultural Marketing Resource Center, 2011). According to the U.S Grains Council (2012), world sorghum production has risen slightly from 60 million metric tons (2.4 billion bushels) to 65 million metric tons (2.6 billion bushels) over the past decade.

With prediction of less water available for crop production as a result of climate change, sorghum's adaptability to dry environments suggests that it may play a larger role in global food security. Sorghum has a variety of uses including food for human consumption, feed grain for livestock and industrial applications such as ethanol production. Sorghum grain is a staple diet in Africa, the Middle East, Asia and Central America. China and India account for almost all of the food use of sorghum in Asia. Several million tonnes of sorghum are used across Africa for traditional beer brewing. Since sorghum does not contain gluten, it is considered a safe food for people with celiac disease (Ciacci et al., 2007).

In other parts of the world, sorghum grain is used mainly as an animal feed. Such use is concentrated in Mexico, many South American countries, the United States, Japan, and the Commonwealth of Independent States (CIS) which include Azerbaijan, Armenia, Belarus, Georgia, Kazakhstan, Kyrgyzstan, Moldova, Russia, Tajikistan, Turkmenistan, Uzbekistan and Ukraine. The stover of sorghum also is used as fodder for animals. Brown midrib (BMR) varieties of sorghum have been developed for use as forage sources for livestock because of their reduced lignin content and higher digestibility of the stover (Aydin et al., 1999; Oliver et al., 2004). With the current focus and demand to generate a large and sustainable supply of biomass to make biofuels generation from lignocellulose profitable, there is a need to develop crops that will be grown specifically for bioenergy production. Sorghum is one of several different species that can be used as dedicated bioenergy crops.

1.2. Review of Literature

1.2.1 Effects of environmental stress on crop production

1.2.1.1 Drought stress

Taiz and Zeiger (2006) defined stress as an external factor that exerts a disadvantageous influence on a plant and its effects are measured on the basis of the plant's survival, growth (biomass accumulation), yield, or the primary assimilation processes, which are related to overall growth. The performance of a plant under a given

stress will be influenced by the stress and plant characteristics. Stress characteristics that will influence the performance of a given plant include severity, duration, frequency and combination of stresses, while plant characteristics will include the organ or tissue in question, stage of development and the genotype.

Drought stress has been ranked as one of the most significant causes of crop yield loss (Boyer and Westgate, 2004). Some of the vital roles played by water in a plant include acting as a solvent, a transport medium, biochemical reactant, creating turgor pressure and an evaporative coolant. Water limitation will therefore result in decreases in whole plant growth and photosynthesis, wilting, stomatal closure as well as be associated with changes in carbon and nitrogen metabolism (Sanchez et al., 2002). General mechanisms used by plants to cope with drought stress include avoidance, tolerance, and escape. Throughout this document the term "drought" will be used to refer to situations of limited soil moisture or when water supply to a plant is stopped.

1.2.1.2 Impacts of drought stress on sorghum

1.2.1.2.1 Growth and development

Two primary processes that are involved in plant growth are cell division and cell growth with cell division being less sensitive to drought. Leaves are the centers of photosynthesis and therefore have a major role in plant growth rates and plant development. Leaf expansion is among growth processes most sensitive to drought (Alves and Setter, 2004). A decline in leaf area expansion due to drought stress will result in a decrease in the expansion and development of the transpiration surface. This sensitivity is expressed in terms of smaller cells and reductions in the number of cells produced by leaf meristems (Randall and Sinclair, 1988; Tardieu et al., 2000). Alves and Setter (2004) showed that both cell expansion and production of cells contributed to a loss in leaf area depending on the developmental stage at which the leaf was stressed. In leaves that were no longer engaged in cell division, diminished cell expansion affected leaf area by reducing mature cell size, whereas, in younger leaves, inhibition of cell division resulted in fewer cells per leaf (Alves and Setter, 2004). The general effects of mild drought on leaves are a reduction in leaf numbers, rate of leaf expansion, and final leaf size while under severe stress, the rate of leaf elongation decreases and leaf growth can cease. Drought stress can also influence total leaf area through its effect on initiation of new leaves, which is decreased under drought

stress. Continued drought stress can accelerate leaf senescence (de Souza et al., 1997) and lead to death of leaf tissue, resulting in leaf drop, particularly old and mature leaves. Decreased leaf senescence under drought stress is often termed as a tolerance mechanism, particularly to post flowering drought that occurs during grain-filling stages while loss of leaf area can serve as a drought-avoidance mechanism as reduction in leaf area can help limit further water loss.

1.2.1.2.2 Yield and yield components

The effects of drought stress on grain yield and its components will depend, among other factors, on the stage of development in which the stress occurs. Lewis et al. (1974) found grain yield reductions of l7, 34 and l0% in sorghum when drought stress occurred before the boot stage, from the boot stage to anthesis, and from the milk dough stage to the soft dough stage, respectively while Inuyama (1978) reported a 61% reduction in grain yield when drought occurred at the boot stage. Work done by Manjarrez et al. (1989) suggested that microsporogenesis emerged as the most susceptible stage of sorghum development to drought stress, because in this stage drought stress caused collapse and death of the whole panicle. Similarly, microsporogenesis has been detected as a very critical stage under stress conditions in other cereal crops (Saini and Aspinall, 1981; Saini et al. 1984). Reductions of sorghum grain yield due to drought stress before anthesis are related to decreases in grain number, while a smaller grain size is responsible for yield losses when water deficits occur after anthesis (Eastin et al., 1983). However, compensation between grain number and grain size makes it difficult to assess the specific effects in several cases. In wheat, some drought treatments have been found to affect pollen viability, which in turn reduces seed number (Saini and Aspinall, 1981).

1.2.1.2.3 Physiological traits: photosynthesis, transpiration and stomatal conductance

Drought stress induces several changes in various physiological, biochemical, and molecular components of photosynthesis. Drought stress can influence photosynthesis through pathway regulation by stomatal closure and decreasing flow of CO_2 into mesophyll tissue (Chaves et al., 2003; Flexas et al., 2004), and

also by directly impairing metabolic activities (Farquhar et al., 1989). This will lead to a decline in regeneration of ribulose bisphosphate (RuBP) and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein content (Bota et al., 2004), decreased Rubisco activity (Parry et al., 2002), impairment of ATP synthesis, and photophosphorylation or decreased inorganic phosphorus.

Initial effects at the onset of drought stress are decreased stomatal conductance resulting in a decline in photosynthetic rate (Cornic, 2000). Increased severity will cause tissue dehydration and lead to metabolic impairment. Drought stress has been shown to cause increases in intercellular CO_2 concentration (C_i) (Siddique et al., 1999; Kicheva et al., 1994). Zhou et al. (2007) suggested that both diffusive limitation through stomatal closure and non-stomatal limitation (such as oxidative damage to chloroplasts) are responsible for decline in photosynthesis under drought stress.

Photosynthetic resilience to drought varies with the age of the leaf (Chaves, 1991). Younger leaves tend to be more resistant to drought than older leaves. Tolerance to drought stress is of importance in plants where a severe reduction in the size of the leaf canopy occurs due to shedding of older leaves as this allows a fast recovery following rehydration (Pereira and Chaves, 1993). In addition to a plant's ability to avoid and/or tolerate drought stress, photosynthetic recovery following rehydration plays an important role in dictating a plant's resistance to drought as well as preventing dramatic declines in crop yield (Chaves et al., 2009). Recovery from a severe stress can be divided into a two stage process with the first stage occurring during the first hours or days upon re-watering, corresponding to the improvement of leaf water status and stomatal re-opening (Pinheiro et al., 2005; Antonio et al., 2008) and the second stage lasting several days and requires synthesis of fresh photosynthetic proteins (Kirschbaum, 1988). Previous stress intensity and/or duration are crucial factors affecting both the rate and the extent of recovery of photosynthesis (Miyashita et al., 2005). Long-term down-regulation of stomatal conductance (g_s) after re-watering may be derived from limited recovery of leaf-specific hydraulic conductivity (Galmes et al., 2007). From the molecular point of view, the

comparison between susceptible and tolerant genotypes suggests that drought tolerance is associated with a rapid modulation of genes from different transcription factor gene families during recovery.

Under mild drought stress, stomatal closure to reduce water efflux simultaneously decreases the CO_2 influx, which limits photosynthesis. Decreased stomatal conductance is accompanied by a reduction in internal CO_2 concentration (c_i) and decreased diffusion of CO_2 via mesophyll cell walls, membranes, cytoplasm, and chloroplast envelope, leading to decreased chloroplastic CO_2 concentration (Terashima and Ono, 2002). When stomatal limitation occurs, there is often a linear dependence of photosynthesis on the internal to ambient CO_2 concentration ratio (c_i/c_a). To confirm that photosynthetic limitation is exclusively stomatal in nature, it is necessary to raise the CO_2 concentration (to 1–5% CO_2) around the leaves of drought stressed plants, and show that this overcomes any limitation of photosynthesis. More severe water deficit directly affects the photosynthetic capacity of mesophyll causing decreases in carboxylation as well as in electron transport chain activities, and induces ultrastructural changes in chloroplasts.

1.2.1.3 Mechanisms of coping with drought stress

1.2.1.3.1 Drought avoidance

Drought avoidance involves rapid phenological development, leaf rolling and leaf shedding, reduced leaf area and increased stomatal and cuticular resistance (Morgan, 1984; Turner, 1986). Drought avoidance mechanisms allow the plant to maintain cell turgor and cell water content under water-limiting conditions. This is accomplished by maintaining water uptake by the roots and/or reduction of water loss from transpiration and other non-stomatal pathways such as the cuticle. Most sorghum genotypes have a thick waxy cuticle that limits water loss during periods of water deficit. A deep, extensive root system, with the ability to penetrate hard soil layers, is often associated with plants that are able to maintain water supply during periods of low available moisture. C_4 vs. C_3 photosynthesis also improves water use efficiency, especially at high temperatures where oxygenase activity of rubisco is favored over carboxylation activity. C_4 plants have the ability to concentrate

CO_2 in bundle sheath cells, thus reducing photorespiration and allowing these plants to decrease stomatal conductance and to conserve water without decreasing carbon fixation rates. Other types of avoidance mechanisms are based on leaf abscission, dormancy, and leaf angle/rolling that reduce water loss from transpiration. Reducing the evaporative surface area of the leaf is an effective means of decreasing transpiration.

1.2.1.3.2 Drought escape

Drought escape refers to early completion of the plant's life cycle, essentially flowering prior to the onset of drought. Early maturing varieties of sorghum avoid water deficit that often occurs later in the growing season in some regions. Drought induced changes in gene expression and genes that are induced by drought stress encode proteins involved in protection and signal transduction (Mundree et al., 2002). Abscisic acid (ABA) is an important hormone in signaling of water deficit (ABA). Most drought-responsive genes are induced by exogenous ABA treatment, and are included in the ABA-dependent signal transduction. Mundree et al. (2002) showed that an additional gene set is induced by drought, providing evidence for a second, ABA-independent signal transduction pathway. Promoters of ABA inducible genes contain sequence-specific ABA-responsive cis-elements (ABRE's) with the sequence ACGTGGC (Mundree et al., 2002). These same cis-elements are found in sorghum genes that respond to ABA (Buchanan et al., 2005). Genes in the ABA-independent pathway contain a characteristic nine base pair sequence termed the dehydration responsive element (DRE). Proteins that bind the DRE include the ethylene-responsive element binding proteins, AP2 proteins, and DRE binding factor 1 and 2. These factors bind and activate transcription of genes containing the DRE sequence (Mundree et al., 2002).

1.2.1.3.3 Drought tolerance

Drought tolerance mechanisms allow the plant to maintain metabolic activity during drought and under conditions of reduced plant water potential by osmotic adjustment and antioxidant capacity. At a slightly higher water potential, the cells will survive or function metabolically although the tissues are desiccated. This leads to

the assumption that the greater the desiccation tolerance, the less the direct effects of reduced water potential will be on metabolic activity and cellular injury, other than that resulting in killing of the cells. Many plants can accumulate compatible solutes including sugars, organic acids, amino acids, sugar alcohols, or ions which accumulate in the cytosol, lowering the osmotic potential and maintaining turgor of both shoots and roots. Sorghum, for example, is known to accumulate glycine betaine and proline in response to water deficit (Buchanan et al., 2005). Antioxidant capacity is the ability of plants to detoxify reactive oxygen species (ROS) (Scandalios, 2005). These reactive oxygen species can cause cell injury by lipid peroxidation or protein and nucleic acid modification. Some plants have therefore evolved mechanisms to prevent damage from free radicals by using antioxidant enzymes such as superoxide dismutase, catalases, and peroxidases and by using free radical scavengers such as carotenoids, ascorbate, proline, tocopherols, and glutathione (Mundreee et al., 2002). The prevention of oxidative stress and reduction of the number of reactive oxygen species enhances the plant's tolerance to abiotic stresses such as drought.

Plants tolerate drought by maintaining sufficient cell turgor to allow metabolism to continue under increasing water deficits. Lowering of the osmotic potential of the cells by accumulating solutes is considered to be due to osmotic adjustment if the buildup of compounds is not merely the result of tissue dehydration (Morgan, 1984; Turner, 1979; Turner and Jones, 1980). Osmotic adjustment enables water uptake to continue under increasing soil water tension in many species and, in some cases, is associated with maintenance of growth and stable yield under drought (Gunasekera and Berkowitz, 1992; Morgan, 1984). Osmotic adjustment is important in the drought resistance of many C_4 plants in arid environments and may enable sorghum to grow when leaf water potential is low (Craufurd and Peacock, 1993).

1.2.1.4 Screening for drought tolerance in sorghum

Season-long increases in average temperature as well as periodic episodes of stress intensify the effect on many aspects of crop growth and development, resulting in reduced grain/seed numbers, quality and yield

(Prasad et al., 2008). Since different physiological mechanisms may contribute to drought tolerance, the first and foremost requirement in any crop improvement program is to identify the suitable stock that can be used. It is therefore important to efficiently and reliably measurement the various ecophysiological, morphological, and reproductive traits from available germplasm.

Several methods in both field and controlled-environment facilities are commonly being used for screening drought tolerance. Even through field studies have more advantages than a controlled environment because they represent the true nature of the farmer's and breeder's field conditions, a major limitation is the lack of control of the environment making screening processes difficult. In this document the focus will be on canopy temperature, canopy temperature depression and water use efficiency as drought tolerance screening tools.

1.2.1.4.1 Canopy temperature and canopy temperature depression

The difference between air and foliage temperature is referred to canopy temperature depression (CTD). The ability of the plant to decrease temperature through transpirational cooling will keep the plant cool and benefits plants at above optimal temperatures. As much as 10°C difference between air and leaf temperatures have been reported in cotton (Burke and Upchurch, 1989). Hatfield et al. (1987) demonstrated that canopy temperature of field grown cotton tracked air temperature at night and became cooler than air temperature each morning when the leaf temperature approached 27.5°C. This temperature was approximately midpoint of an identified thermal kinetic window (TKW) (Burke and Upchurch, 1989). Mahan et al. (1995) reported that various factors, including leaf area, root to leaf ratio, leaf orientation, leaf size and shape, leaf surface characteristics (e.g., pubescence), leaf thickness and size, and distribution of stomata, are known to affect transpiration. Accordingly, a small leaf has a thinner boundary layer that is more conducive to sensible and latent heat transfers and as a consequence is often cooler than bigger leaves in similar environments.

CTD has been used to quantify stress within a given species (Idso et al., 1981; Jackson et al., 1981). In cotton, the relationships among canopy temperature, vapor pressure deficit (VPD) have allowed the development of crop stress indices. Amani et al. (1996) reported that for a given genotype, CTD is a function of a number of environmental factors, principally soil water status, air temperature, relative humidity, and incident radiation . Moreover, they have also demonstrated that the trait is best expressed at high VPD conditions associated with low relative humidity and war m air temperature. The relative importance of the characteristic of individual leaves decreases as the plant canopy becomes denser. Under these conditions the aerodynamic characteristics of the canopy play a major role in the energy transfer between the plant and environment.

1.2.1.4.2 Crop water stress index

Initially stress degree day (SDD) was defined as the difference in foliage and air temperature (Idso et al., 1977; Jackson et al., 1977) to quantify drought stress of crops. Later, Idso et al. (1981) incorporated VPD to account for differences among environments and the concept of crop water stress index (CWSI) was refined to include this parameter. Ehrler et al. (1978) demonstrated that the difference in leaf and air temperature of well-irrigated cotton and wheat was linearly related to VPD of the atmosphere 1 m above the crop canopy. Idso et al. (1981) and Idso (1982) confirmed this observation at four different locations in the United States and further illustrated that a unique linear relationship between canopy-air temperature (Tc - Ta) and VPD could be found for 26 agricultural crop species. The relationship between canopy temperature, air temperature, and transpiration is not simple and involves atmospheric conditions (VPD, air temperature, and wind velocity), soil (soil moisture), and plant morpho-physiological characteristics (canopy size, canopy architecture, and leaf adjustment to water deficit). These variables are considered when canopy temperature is used to develop the CWSI. The CWSI is a measure of the relative transpiration rate occurring from a plant at the time of measurement using a measure of plant temperature and VPD (refers to dryness of the air). Jackson et al. (1981) presented the theory behind the energy balance that separates net radiation from the sun into sensible heat that

heats the air, and latent heat that is used for transpiration. The CWSI incorporates midday values of net radiation, canopy and air temperature, VPD, aerodynamic resistance, and canopy resistance into an energy balance for a crop surface. The CWSI has been related to yield in cotton (Burke et al., 1990). When a plant is transpiring fully the leaf temperature is 1 to 4°C below the air temperature and CWSI is zero. As the transpiration decreases, the leaf temperature rises and can reach to 4 to 6°C above the air temperature. When the plant is no longer transpiring the CWSI is 1. However, O'Toole and Hatfield (1983) found that wind speed influenced the canopy to air temperature difference. Some researchers (Hatfield, 1985; Wanjura et al., 1984) demonstrated that the unstressed baseline of cotton for the CWSI varies slightly from those initially defined by Idso et al. (1981). Keener and Kircher (1983) studied the effectiveness of stress degree days (SDD), drought stress index, and CWSI which were developed for arid or semi-arid regions. These authors demonstrated that CWSI would be of limited utility under humid conditions. Jackson et al. (1981) also acknowledge the potential problems in humid environments and pointed out that the occurrence of leaf temperature warmer than air temperature presents a limitation for any of the current canopy temperature-based stress indices.

1.2.1.4.3 Slow-wilting

A limit on maximum transpiration rate could be particularly important in non-irrigated crop production. Sinclair et al. (2005) showed that imposition of limited maximum transpiration rates increased sorghum yields in 76–90% of seasons in a semi-arid environment. This outcome was due both to the water savings associated with reduced transpiration and to increased transpiration use efficiency (TUE). The systems analysis of Sinclair and Muchow (2001) showed that decreased radiation use efficiency (RUE) could increase maize yields in water-limited environments. However, when water was not limiting, the associated lower growth rate resulted in yield penalties (Sinclair and Muchow, 2001; Sinclair et al., 2005). Crop photosynthesis is proportional to the transpiration rate multiplied by VPD divided by a transpiration efficiency coefficient (Tanner and Sinclair, 1983). A limited maximum transpiration rate that is reached when atmospheric VPD exceeds ~2.0 kPa would

result in an afternoon depression of photosynthesis (Hirasawa and Hsiao, 1999; Pettigrew et al., 1990). The correlation of the decrease in corn and sorghum RUE with increasing VPD reported by Stockle and Kiniry (1990) and Kiniry et al. (1998) is consistent with such a response.

Limiting transpiration rate to a maximum would restrict evaporative cooling of leaves resulting in a corresponding increase in leaf temperature. This would typically be greatest at maximum atmospheric VPD, which typically is greatest at about 15:00. Under rainfed conditions water-deficit is a major yield limiting factor, and in crops like soybean considerable efforts have been focused on identifying traits that will limit the yield reduction under drought (Purcell and Specht, 2004). In a drying soil the slow-wilting soybean genotypes PI416937 (Hudak and Patterson, 1995; Sloane et al., 1990) and PI471938 were found to wilt 3 to 4 days later than commercial genotypes. The mechanism of the slow wilting trait remains unresolved (Sinclair, 2004). It has been suggested that slow-wilting genotypes may have reduced transpiration rates compared with commercial genotypes, resulting in significant soil water savings early in the season. For example, although the data of Sloane et al. (1990) were not statistically significant, under well-watered conditions leaf transpiration rates were 181 mgH_2O $m^{-2}s^{-1}$ for 'Forrest'(not slow wilting), and 168 mg H_2O $m^{-2}s^{-1}$ for PI 416937 (slow wilting).

Gholipoor et al., (2010) documented marked variation among sorghum genotypes in transpiration rate (TR) response to VPD. Their study identified seventeen genotypes which exhibited a breakpoint (BP) in their VPD response ranging from 1.6 to 2.7 kPa, above which there was little or no further increase in TR. The study suggested that these genotypes with a breakpoint may conserve soil water when VPD during the midday cycle exceeds the breakpoint VPD. The slow wilting trait would be desirable in less humid environments for increasing yields in water-deficit seasons and therefore can be potentially used to develop genotypes with BP appropriate for specific environments. The expression of the slow-wilting phenotype under water-deficit conditions may be a result of restricted water use resulting in soil water conservation.

Fletcher et al. (2007) identified this slow wilting phenomenon in soybean, noting that there was no further increase in TR once a VPD threshold of about 2 kPa was exceeded. Sinclair et al. (2008) demonstrated that the source of the maximum TR response in PI416937 was associated with a limitation in hydraulic conductance for water flow from the leaf xylem into the guard cells, which was not observed in two other genotypes studied. A hypothesis based on these results was that there was a lower symplastic conductance, possibly aquaporin (AQP) mediated water transport, in the leaf hydraulic pathway of PI 416937 as compared to the other genotypes. Further investigation by Sadok and Sinclair (2010) found that decreases in TR of soybean genotype (N01-11136) following treatment with inhibitors were up to 60% for CHX, 82% for $HgCl_2$, and 42% for $AgNO_3$. These results indicate that the symplastic pathway terminating in the guard cells of these soybean leaves may be at least as important as the apoplastic pathway for water flow in the leaf under high *VPD*. While the decrease in TR for PI 416937 was similar to that of N01-11136 following exposure to CHX and $HgCl_2$, TR of PI 416937 was insensitive to $AgNO_3$ exposure. This suggests the possibility of a lack of a Ag-sensitive leaf AQP population in the slow-wilting line, PI 416937, and the presence of such a population in the commercial line, N01-11136. Although previous studies indicated the importance of the symplastic pathway in the leaf hydraulics for different species under different developmental and environmental conditions (Nardini et al., 2005; Tyree et al., 2005; Cochard et al., 2007; Ye et al., 2008), this study offers direct evidence that the symplastic pathway could be involved in the response of TR to high VPD. Given the fact that PI 416937 has restricted hydraulic flow at high VPD (Sinclair et al., 2008) and displays slow-wilting capability in the field, the current results indicate that this may be the result of a lack of Ag-sensitive symplastic pathway in PI 416937.

1.2.1.4.4 Water use efficiency

Water-use efficiency (WUE) is the ratio of biomass accumulation, expressed as carbon dioxide assimilation (A), total crop biomass (B), or crop grain yield (G), to water consumed, expressed as transpiration (T), evapotranspiration (ET), or total water input to the system (I). The time-scale for defining water-use

efficiency can be instantaneous (i), daily (d), or seasonal (s). From an agronomic perspective WUE can be divided into a biological component that expresses the amount of dry matter produced per unit of transpiration (sometimes referred to as the transpiration efficiency) and a management component that captures the amount of output produced (dry matter) for a unit of input into the system. Transpiration efficiency is affected by the saturation deficit of the atmosphere and the biochemical pathway used in photosynthesis. Therefore genetic variation in WUE exists in both natural and agricultural populations as demonstrated by studies on various species, accessions, ecotypes, cultivars, and hybrids (Takeda and Matsuoka, 2008).

According to Richards (1991) WUE can be expressed in the following terms:

$$WUE_{biomass} = \frac{TE}{\left(1 + \frac{E_s}{T}\right)}$$

where TE is the transpiration efficiency (above ground dry weight/transpiration), E_S is the water lost by evaporation from the soil surface, and T is water lost through transpiration by the crop. Based on this expression crop WUE can be increased either by increasing TE or decreasing the magnitude of E_S. The relative importance of each of these components of WUE will vary according to rainfall distribution. Therefore, for a crop that is relying on water stored in the soil where rainfall during the growing season is low, then increasing TE provides the greatest opportunity to increase WUE.

There are opportunities to improve WUE without substantial limitation of CO_2 assimilation. It has been shown that at high stomatal conductance levels, net CO_2 assimilation is nearly saturated, while the increase in transpiration rate is still linear (Martin and Ruiz-Torres, 1992; Avola et al., 2008). Consequently, WUE can be improved by reducing water use through the mechanisms that regulate or determine stomatal conductance to water vapor, which involves stomatal number (density), stomatal aperture, cuticle thickness and composition, and the boundary layer characteristics.

1.2.2 Plant roots

The capacity of plant roots to access available soil water is critical to crop adaptation in water-limited environments (Ludlow and Muchow, 1990). This is especially important in sorghum because it is a crop that is frequently grown in such environments. The importance of root system attributes in sorghum also has been implicated in modeling studies that quantified the relative adaptive advantage of sorghum over maize in water-limited conditions (Sinclair and Muchow, 2001). Extensive genetic variation has been observed in sorghum root systems (Jordan et al., 1979) and studies on other species have highlighted the critical role of root system architecture in crop adaptation.

The temporal development of root system architecture is determined by the nature of the root system and its rate of progression into the soil. In cereals, such as sorghum and maize, the root system is formed from the seminal roots that appear at germination and the nodal, crown or adventitious roots that arise later from nodes of the shoot. Seminal roots play an important part in initial water and nutrient uptake and establishment of seedlings, whereas nodal roots dominate during the later stages of growth.

Water supplied to the plant by the root contributes to the overall water balance of the shoot. There is much evidence that the force driving water into roots usually is provided by the tension (negative pressure) created by transpiration from the shoot and extending to root xylem (Steudle, 1997). Despite the important functions performed by roots, relatively little is known about the processes that govern root water uptake.

1.2.2.1 Root structure and physiology

Root morphology and development will be influenced physical, chemical and biological factors (Yamauchi et al., 1996; Schiefelbein and Benfey, 1991; McMichael and Quisenberry, 1993; Robinson, 1994). Morphology will vary with soil moisture content, sowing depth, soil physical, chemical, and biological properties as well as genotype. Monocots have a fibrous root system which consists of seminal, nodal and lateral roots whereas dicots have a taproot system. Functional roots are roots that emerge from stem nodes,

enter the soil, and develop lateral roots and/or root hairs while nonfunctional nodal roots emerge from above ground stem nodes and do not enter the soil or produce lateral roots. Seminal roots develop from primordial within seeds while mesocotyl roots grow from the axis between the node of the coleoptile and the base of the radical (mesocotyl). Mesocotyl roots are usually not coarse and seldom have lateral or branch roots. Nodal roots are post embryonic roots, which arise from nodes at the base of the main stem and tillers and may be functional or nonfunctional. Functionally, nodal roots elongate deeply into soil and form the basic framework for the whole root system. During development, when root length exceeds a certain size, branching starts by initiation, emergence, and growth of lateral roots from the root pericycle and epidermis (Morita and Yamazaki, 1993). Lateral roots, which comprise a greater proportion of the root system in total length and number (Harada and Yamazaki, 1993), are responsible for the greatest amount of water and nutrient absorption (Yoshida and Hasegawa, 1982). The different types of lateral roots vary in anatomy, developmental characteristics, carbon and nitrogen dynamics, developmental responses to various soil environments (Yamauchi et al.,1996), and genetic regulation of their development (Wang et al., 2006).

1.2.2.2 Root architecture

Root system architecture refers to the spatial configuration of plant roots in the soil and is used to describe the shape and structure of root systems (Dorlodot et al., 2007). This is an important parameter because major soil resources are heterogeneously distributed in the soil and therefore the spatial deployment of roots determines the ability of a plant to secure edaphic resources (Dorlodot et al., 2007). Root architecture will include parameters such as root length, root diameter, and root hairs. Root architecture influences plant growth and development because of its role in absorption of water and nutrients (Lynch, 1995).

Root parameters that have various functional significance and have been used to express root growth and distribution are given in table 1-1 (Gowda et al. 2011, Wang et al, 2006,). Some studies have hypothesized that coarse roots have a direct role in drought resistance because larger diameter roots are related to penetration

ability (Materechera et al., 1992; Nguyen et al., 1997; Clark et al., 2008), branching (Fitter, 1991; Ingram et al., 1994), and have greater xylem vessel radii and lower axial resistance to water flux (Yambao et al., 1992). Fine roots, which represent a very small portion of the total root weight, are the most active in absorption of water and nutrients. Root length can be used to predict root response to changes in the growing environment. The ratio of length to mass (specific root length) is an important indicator of fine root morphology and also is a good parameter to use in relation to root absorption of water and nutrients.

1.2.2.3 Root development

Root development varies with stages of plant growth and development. In maize, rapid development has been shown to occur during the first eight weeks after planting (Anderson, 1987) and as the plant ages the growth of roots increase at a slower rate than shoots (Baligar, 1986). Slaton et al. (1990) found that, in lowland rice, maximum root growth rates were reached between active tillering and panicle initiation while maximum root length was reached by early booting.

The impact of drought is a function of duration, intensity, frequency, crop growth stage, crop species or cultivar within species, soil type, and adopted management practices. Under drought conditions, the soil starts drying from the surface while deeper horizons may remain moist and continue supplying the plant with water and therefore deep rooting systems may be more adapted to soil drying than shallow root systems.

Even though roots form an important part of the plant and are key in water and nutrient uptake, they have not been studied as extensively as the above ground part of the plant. This could be attributed to the complexities and difficulties encountered in root studies. Some research has been done on maize roots (Sharp and Davies., 1979; LeNoble et al., 2004; Saab et al., 1992; Wu et al., 1996; Zhu et al., 2006) and wheat (Asseng et al., 1998). Studies in wheat (Manschadi et al., 2006, 2008) and maize (Hammer et al., 2009) have shown an association between drought tolerance and root system architecture. Very little information is available on sorghum roots and hence the need to generate information on this crop, which is drought tolerant. Information

on the response of sorghum roots to drought stress will help us explore mechanisms involved at the root level that will help plants cope with drought. Potential traits that may improve exploration of water and drought tolerance include increased lateral root production, deep rooting, increased root elongation rates, and increased root biomass. Increased osmotic regulation at the root level also will play an important role.

Although rooting depth is genetically determined and differs substantially between cultivars grown under identical conditions, it is also affected by environmental conditions in the field (Yoshida and Hasegawa, 1982). Maximum root depth of a particular genotype is achieved only when roots do not encounter a physical barrier or limit to growth. The quantity of root length (or weight) in layers within the soil profile is usually expressed in terms of root length (or weight) per unit volume of soil, referred to as root length (or weight) density. Water absorption by roots is passive and therefore root length density, which also reflects the development of lateral roots, can be directly related to water uptake ability of the plant. As root length density increases, water uptake usually increases, but up to a given length only, which is termed critical root length density. In rice, the critical root length density depends on soil conditions, especially moisture (Siopongco et al., 2005). Roots are distributed in such a way that their length and mass will decrease exponentially with depth. Root density at depth determines the exploitation of water present at deeper levels.

1.2.2.4 Root distribution in soil

The supply and availability of nutrients and other resources in the soil is heterogeneous and therefore a major source of variation in the distribution of roots in the soil in the vertical as well as horizontal dimensions. Roots will tend to be concentrated in parts of the soil where resources are abundant (Fitter, 1994) and because the concentration of essential resources in the soil often decreases with depth, root biomass and length per unit volume of soil usually decreases with depth. Differences in root length, dry weight of roots at different soil depths, and extent of rooting at the seedling stage among wheat cultivars have been related to differences in yield as well as drought tolerance (Hurd, 1974). In bean cultivars, deep rooting was shown to be positively

associated with crop growth, yield, cooler canopy temperature, and soil water extraction (Sponchiado et al., 1989). The extensiveness of a root system can be quantified by root-length density (RLD) defined as root length per unit soil volume (cm root cm^{-3} soil) (Taylor, 1980). Water uptake rate of root systems and plant drought resistance may be highly correlated with RLD since plants with a greater RLD in the deep soil layer are better able to maintain water status and stomatal conductance when the soil becomes drier than those with lower RLD (Huang et al., 2000).

1.2.2.5 Root to shoot ratio

Root to shoot ratio is a measure of the allocation of resources between different plant components. The allocation of resources to the roots is high at early vegetative stages but decreases markedly at flowering and is almost negligible after anthesis (Gregory et al., 1996). Increases in shoot-root ratio indicate that shoots had a higher priority for photosynthates accumulation than the roots while a decline of this ratio overtime indicates that roots had preferential utilization of photosynthates. Asch et al. (2004) reported that the proportion of total dry matter allocated to parts of the roots or shoot was dependent on the rate of soil dry down. Genetic variation in root–shoot ratio among *Oryza* species has been reported, and was seen among subspecies groups (Kondo et al., 2003). Root to shoot ratio also varies with tillage system; a response that may be due to mechanical impedance.

1.2.2.6 Root growth under drought stress conditions

Root growth is reduced by soil drying in many cases, but usually not as much as shoot growth and there are times when it may even be promoted resulting in an increase in root to shoot ratio under limited soil water content or at low soil water potentials (Wilson, 1988; Sharp and Davies, 1989; Banoc et al., 2000). Studies have shown that both root and shoot growth will be reduced at water potentials lower than -1.5 MPa (Spollen et al., 1993; Sharp, et al., 2004). There are spatial and temporal variations in growth rates within different parts of the root (Sharp et al., 1988; Saab et al., 1992). In a study with maize (*Zea mays*) seedling Liang et al. (1997)

found that elongation rate was unaffected by low water potential in the apical 3 mm of the primary root but it was progressively inhibited at more basal locations compared with those of well watered roots, increasing to a peak at 4.5 mm and then declining beyond 11 mm. Drought stressed roots also have been found to become substantially thinner than well-watered roots, suggesting that root thinning is an adaptive response to drought stress (Sharp et al., 1988; 2004; Liang et al., 1997).

Other important responses of root systems to soil drying include enhanced geotropism, increased branching, and deep rooting, which facilitate plants to exploit larger soil volumes (Sharp and Davies, 1985; Davies and Bacon, 2003). Serraj et al. (2004) suggested an association between deep and prolific root systems and enhanced avoidance of drought stress in chick pea (*Cicer arietinum*). In fact many field studies with various crops have shown that dense root systems that can extract more water in upper soil layers, together with longer root systems for soil moisture extraction from deeper soil horizons are both important to plant adaptation to drought stress (Ludlow and Muchow, 1990; Turner et al., 2001). In rice (*Oryza sativa*), traits such as deep and fine roots have been associated with increased water extraction during progressive drought stress (Fukai and Cooper, 1995; Azhiri-Sigari et al., 2000; Kamoshita et al., 2000). A high ratio of deep root to shoot weight also was found to be associated with higher plant water potentials and have a positive effect on yield under drought stress (Mambani et al., 1983).

1.3 Dissertation hypothesis

Even though the world collections of sorghum contain over 35,000 accessions, the genetic base currently used in breeding programs is very small (about 13% of the world collection). Thus, it is important to evaluate the existing germplasm for lines that can be used in improving drought tolerance. Selection for drought tolerance in sorghum has been mainly on the basis of staygreen. But this trait does not necessarily mean that staygreen lines will perform well under increased VPD conditions and gives no indication of conservative water use. Since there are mechanisms for coping with drought stress, research contained in this dissertation looked at ways of screening

sorghum genotypes for drought tolerance. The basis for the research was based on the identification of two different drought mechanisms (tolerance and escape) in sorghum (Mutava et al., 2011; Gholipoor et al., 2010). The hypotheses for this research were that (i) canopy temperature can be used as a screening tool for drought tolerance in sorghum, (ii) slow wilting genotypes will be high in transpiration efficiency (TE), and (iii) there is genetic diversity in root morphology and response to drought among sorghum genotypes.

1.4 Dissertation objectives

The general objectives for this research were to (i) quantify variation in canopy temperature and canopy temperature depression using infrared (IR) sensors in sorghum genotypes under field conditions, (ii) evaluate slow wilting genotypes for TE under controlled environment, and (iii) evaluate sorghum roots response to drought stress under controlled environment. These general objectives are further broken down to specific objectives in succeeding chapters. Each general objective forms a chapter in the thesis and therefore will be further explored.

1.5 Figures and Tables

Table 1-1: Root traits and their functional characteristics that are commonly characterized in root quantitative trait loci (QTL) mapping studies.

Root trait	Functional characteristics
Maximum root depth	Potential for absorption of soil moisture and nutrients in deeper soil layer
Root to shoot ratio	Assimilate allocation
Root volume	The ability to permeate a large volume of soil
Root number	Physical strength, potential for root system architecture
Root diameter	Potential for penetration ability, branching, hydraulic conductivity
Deep root to shoot ratio	Vertical root growth, potential for absorption of soil moisture and nutrients in deeper soil layers
Root length/weight density	Rate of water and nutrient uptake
Root branching	Power of soil exploration (the major contribution to total root length
Total root length/surface area	The total system size: the size of contact with soil (major determinant of water and nutrient uptake as an entire root system)
Specific root length	Degree of branching, density of root materials, porosity due to aerenchyma development
Hardpan penetration ability	Ability to penetrate subsurface hardpans

1.6 References

Agricultural Marketing Resource Center. 2011. http://www.agmrc.org/commodities_products/grains_oilseeds/sorghum/sorghum_profile.cfm

Alves, A.A.C., and T.L. Setter. 2004. Response of cassava leaf area expansion to water deficit: Cell proliferation, cell expansion and delayed development. Ann. Bot. (London) 94:605 – 613.

Amani, I., R.A. Fischer, and M.P. Reynolds. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. J. Agron. Crop Sci. 176: 119 – 129

Anderson, E.L. 1987. Corn root growth and distribution as influenced by tillage and nitrogen fertilization. Agron. J. 79: 544 – 549.

Antonio C, C. Pinheiro, M.M. Chaves, C.P. Ricardo, M.F. Ortuno, and J. Thomas-Oates. 2008. Analysis of carbohydrates in Lupinus albus stems on imposition of water deficit, using porous graphitic carbon liquid chromatography-electrospray ionization mass spectrometry. J. Chromat. 1187: 111 – 118.

Asch, F., M. Dingkuhn, A. Sow, and A. Audebert. 2004. Drought-induced changes in rooting patterns and assimilate partitioning between root and shoot in upland rice. Field Crops Res. 93: 223 – 236.

Asseng, S., J.T. Ritchie, A.J.M. Smucker, and M.J. Robertson. 1998. Root growth and water uptake during water deficit and recovering in wheat. Plant Soil 201: 265 – 273.

Avola, G., V. Cavallaro, C. Patane, and E. Riggi. 2008. Gas exchange and photosynthetic water use efficiency in response to light, CO_2 concentration and temperature in *Vicia faba*. J. Plant Physiol. 165: 796 – 804.

Aydin, G., R. J. Grant, and J. O'Rear. 1999: Brown midrib sorghum in diets for lactating dairy cows. J. Dairy Sci. 82: 2127 – 2135.

Azhiri-Sigari, T.A., A. Yamauchi, A. Kamoshita, and L.J. Wade. 2000. Genotypic variation in response of rainfed lowland rice to drought and re-watering. II. Root growth. Plant Prod. Sci. 3: 180 – 188.

Baligar, V.C. 1986. Interrelationships between growth and nutrient uptake in alfalfa and corn. J. Plant Nutri. 9: 1391 – 1404.

Banoc, D.M., A. Yamauchi, A. Kamoshita, L.J. Wade, and J.R. Pardales Jr. 2000. Genotypic variations in response of lateral root development to fluctuating soil moisture in rice. Plant Prod. Sci. 3: 335 – 343.

Blum A., S. Ramaiah, E.T. Kanemasu, and G.M. Paulsen. 1990. The physiology of heterosis in sorghum with respect to environmental stress. Ann. Bot. 65: 149 – 158.

Bota, J., H. Medrano, and J. Flexas. 2004. Is photosynthesis limited by decreased rubisco acivity and RuBP content under progressive drought stress? New Phytol. 162: 671 – 681.

Boyer, J.S., and M.E. Westgate. 2004. Grain yield with limited water. J. Exp. Bot. 55: 2385 – 2394.

Buchanan, C., S. Lim, R.A. Salzman, I. Kagiampakis, R.R. Klein, L.H. Pratt, M.M. Cordonnier-Pratt, P.E. Klein, and J.E. Mullet. 2005. Sorghum bicolor's transcriptome response to dehydration, high salinity and ABA. Plant Mol. Biol. 58: 699 – 720.

Burke, J.J., and D.R. Upchurch. 1989. Leaf temperature and transpirational control in cotton. Environ. Exp. Bot. 29: 487 – 492.

Burke, J.J., J.L. Hatfield, and D.F. Wanjura. 1990. A thermal stress index for cotton. Agron. J. 82: 526 – 530

Chaves, M.M. 1991. Effects of water deficits on carbon assimilation. J. Exp. Bot. 42: 1 – 16.

Chaves, M.M., J. Flexas, and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. (London). 103: 551 – 560.

Chaves, M.M., J.P. Marococ, and J.S. Pereira. 2003. Understanding plant responses to drought: from genes to the whole plant. Funct. Plant Biol. 30: 239 – 264.

Ciacci, C. L. Maiuri, N. Caporaso, C. Bucci, L. Del Giudice, D.R. Massardo, P. Pontieri, N. Di Fonzo, S.R. Bean, B. Ioerger, and M. Londei. 2007. Celiac disease: *In vitro* and *in vivo* safety and palatability of wheat-free sorghum food products. Clin. Nutri. 26: 799 – 805.

Clark, L.J., A.H. Price, K.A. Steele, and R.R. Whalley. 2008. Evidence from near-isogenic lines that root penetration increases with root diameter and bending stiffness in rice. Funct. Plant Biol. 35: 1163 – 1171.

Cochard, H., J.S. Venisse, T.S. Barigah, N. Brunel, S. Herbette, A. Guilliot, M.T. Tyree, and S. Sakr. 2007. Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. Plant Physiol. 143: 122 – 133.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal aperture: Not by affecting ATP synthesis. Trend Plant Sci. 5: 187 – 188.

Craufurd, P.Q., and J.M. Peacock. 1993. Effect of heat and drought stress on sorghum. Exp. Agric. 29: 77–86.

Dahlberg, J. A., J. J. Burke and D. T. Rosenow. 2004. Development of a sorghum core collection: refinement and evaluation of a subset from Sudan. Econ. Bot. 58: 556 – 567.

Davies W.J., and M.A. Bacon. 2003. Adaptation of roots to drought. In: Root Ecology, H. de Kroon, and E.J.W. Visser (Eds)., Springer Verlag, Berlin, Heidelberg.

de Souza, P.I., D.B. Egli, and W.P. Bruening. 1997. Drought stress during seed filing and leaf senescence in soybean. Agron. J. 89: 807 – 812.

Dorlodot, S., B. Forster, L. Pages, A. Price, R. Tuberosa, and X. Draye. 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. Trends Plant Sci. 12: 474 – 481.

Eastin, J. D., R.M. Castleberry, T.J. Gerik, J.H. Hultquist, V. Mahalakshmi, V.B. Ogunlela, and J.R. Rice. 1983. Physiological aspects of high temperature and drought stress. pp. 91 – 112. In: Crop reactions to water and temperature stresses in humid, temperature climates C. D. Raper, Jr. and P. J. Kramer, (Eds.). Westview Press, Boulder, CO., USA.

Ehrler, W.L., S.D. Idso, R.D. Jackson, and R.J. Reginato. 1978. Wheat canopy temperature: relation to plant water potential. Agron. J. 70: 251 – 256.

Farquhar, G.D., K.T. Hubick, A.G. Codon, and R.A. Richards. 1989. Carbon isotope fractionation and plant water use efficiency. pp. 220 – 240. In: P.W. Rundell et al. (ed.) Stable isotopes in ecological research. Springer-Verlag, New York.

Fitter, A.H. 1991. The ecological significance of root system architecture: an economic approach. In: D. Atkinson (Ed.). Plant Root Growth: An Ecological Perspective. Blackwell Scientific Publishers, London.

Fitter, A. H. 1994. Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. In: Exploitation of environmental heterogeneity of plants: Ecophysiological processes above and below ground. pp. 305 – 323. M.M. Caldwell and R.W. Pearcy (Eds.). Academic Press. San Diego

Fletcher, A.L., T.R. Sinclair, and L.H. Allen Jr. 2007. Transpiration responses to vapor pressure deficit in well watered 'slow-wilting' and commercial soybean. Environ. Exp. Bot. 61: 145 – 151.

Flexas, J., J. Bota, F. Loreta, G. Cornic, and T.D. Sharkey. 2004. Diffusive and metabolic limitation to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6: 269 – 279.

Fukai, S., and M. Cooper. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res. 40: 67 – 86.

Galmes J, J. Flexas, R. Save and H. Medrano. 2007. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to drought stress and recovery. Plant Soil 290: 139 – 155.

Gholipoor, M, P.V.V. Prasad, R.N. Mutava, and T.R. Sinclair. 2010. Genetic variability of transpiration response to vapor pressure deficit among sorghum genotypes. Field Crops Res. 119: 85 – 90.

Gowda,V.R.P., H. Amelia, A.Yamauchic, H.E.Shashidhar, R. Serraj. 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crops Res. 122: 1 – 13.

Gregory, P.J., J.A. Palta, and G.R. Batts. 1996. Root systems and root:mass ratio - carbon allocation under current and projected atmospheric conditions in arable crops. Plant Soil 187: 221 – 228.

Gunasekera, D., and G.A. Berkowitz. 1992. Evaluation of contrasting cellular-level acclimation responses to leaf water deficits in three wheat genotypes. Plant Sci. 86: 1 – 12.

Hammer, G.L., Z. Dong, G. McLean, A. Doherty, C. Messina, J. Schussler, C. Zinselmeier, A. Paszkiewicz, and M. Cooper. 2009. Can changes in canopy and/or root system architecture explain historical maize yield trends in the U.S. corn belt? Crop Sci. 49: 299 – 312.

Harlan, J.R., and J.M.J. de Wet. 1972. A simplified classification of cultivated sorghum. Crop Science 12: 172 – 176.

Harada, J., and K. Yamazaki. 1993. Roots. In: Science of the Rice Plant. Vol. 1: Morphology. T Matsuo, and K. Hoshikawa. (Eds.). Food and Agriculture Policy Research Centre, Tokyo.

Hirasawa, T.,and T.C. Hsiao. 1999. Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. Field Crops Res. 62: 53 – 62.

Hatfield, J.L. 1985. Wheat canopy resistance determined by energy balance techniques. Agron. J. 77: 279 – 283.

Hatfield, J. L., J.E. Quisenberry, and R.E. Dilbeck. 1987. Use of canopy temperatures to identify water conservation in cotton germplasm. Crop Sci. 27: 269 – 273.

Huang, B.R. 2000. Role of root morphological and physiological characteristics in drought resistance of plants. In: Plant-Environment Interactions, 2nd. Ed. Wilkinson, R.E. (Ed.), Marcel Dekker, New York.

Hudak, C.M., and R.P. Patterson. 1995. Vegetative growth analysis of a drought resistant soybean plant introduction. Crop Sci. 35: 464 – 471.

Hurd, E.A. 1974. Phenotype and drought tolerance in wheat. Agric. Meterol. 14: 39 – 55.

Idso, S.B., R.D. Jackson, R.J. Reginato.1977. Remote sensing of crop yields. Sci. 196: 19 – 25.

Idso, S.B. 1982. Non-water-stressed baselines: a key to measuring and interpreting crop water stress. Agric. Meteorol. 27: 59 – 70.

Idso SB, R.D. Jackson, P.J. Pinter, and J.L. Hatfield. 1981. Normalizing the stress-degree-day parameter for environmental variability. Agric. Meteorol. 24: 45 – 55.

Ingram, K.T., F.D. Bueno, O.S. Namuco, E.B. Yambao, and C.A. Beyrouty. 1994. Rice root traits for drought resistance and their genetic variation. In: G.J.D Kirk (Ed.), Rice Roots: Nutrient and Water Use. International Rice Research Institute, Manila, Philippines.

Inuyama, S. 1978. Varietal differences in leaf water potential, leaf diffusive resistance and grain yield of grain sorghum affected by drought stress. Jpn. J. Crop Sci. 47: 255 – 261.

Jackson R.D., S.B. Idso, R.J. Reginato, and P.J. Pinter. 1981. Canopy temperature as a crop water stress index. Water Resour. Res. 17: 1133 – 1138.

Jackson, R.D., R.J. Reginato, and S.D. Idso. 1977. Wheat canopy temperature: a practical tool for evaluating water requirements. Water Resour. Res. 13: 651 – 656.

Jordan, W.R., F.R. Miller, and D.E. Morris. 1979. Genetic variation in root and shoot growth of sorghum in hydroponics. Crop Sci. 19: 465 – 472.

Kamoshita, A., L.J. Wade, and A. Yamauchi. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. III. Water extraction during drought period. Plant Prod. Sci. 3: 189 – 196.

Keener, M.E., and P.L. Kircher. 1983. The use of canopy temperature as an indicator of drought stress in humid regions. Agric. Meteorol. 28: 339 – 349.

Kicheva, M.I., T.D. Tsonev, and L.P. Popova. 1994. Stomatal and non-stomatal limitations to photosynthesis in two wheat cultivars subjected to drought stress. Photosynthetica 30: 107 – 116.

Kiniry, J.R., J.A. Landivar, M. Witt, T.J. Gerik, J. Cavero, and L.J. Wade. 1998. Radiation-use efficiency response to vapor pressure deficit for maize and sorghum. Field Crops Res. 56: 265 – 270.

Kirschbaum, M.U.F. 1988. Recovery of photosynthesis from drought stress in *Eucalyptus pauciflora* - a process in two stages. Plant Cell Environ. 11: 685 – 694.

Kondo, M., P.P. Pablico, D.V. Aragones, R. Agbisit, J. Abe, and S. Morita. 2003. Genotypic and environmental variations in root morphology in rice genotypes under upland field conditions. Plant Soil 255: 189 – 200.

LeNoble, M.E., W.G. Spollen, and R.E. Sharp. 2004. Maintenance of shoot growth by ABA: genetic assessment of the role of ethylene suppression. J. Exp. Bot. 55: 237 – 245.

Lewis, R. B., E.A. Hiler, and W.R. Jordan. 1974. Susceptibility of grain sorghum to water deficit at three growth stages. Agron. J. 66: 589 – 591.

Liang, B.M., R.E. Sharp and T.I. Baskin. 1997. Regulation of growth anisotropy in well-watered and water-stressed maize roots. I. Spatial distribution of longitudinal, radial and tangential expansion rates. Plant Physiol. 115: 101 – 111.

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

Lynch, J.P. 1995. Root architecture and plant productivity. Plant Physiol. 109: 7 – 13

Mahan, J.R., B.L. McMichael, and D.F. Wanjura. 1995. Methods of reducing the adverse effects of temperature stress on plants: a review. Environ. Exp. Bot. 35: 251 – 258.

Maiti, R. 1996. Sorghum science. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi, India. pp. 352.

Mambani, B., and R. Lal. 1983. Response of upland rice varieties to drought stress. 1. Relation between the root system development and leaf water potential. Plant Soil 73: 59 – 72.

Manjarrez, S.P., H.V.A. Gonzales, O.L.E. Mendoza, and E.M. Engleman. 1989. Drought stress effects on the grain yield and panicle development of sorghum. Can. J. Plant Sci. 69: 631 – 641.

Manschadi, A.M., J. Christopher, P. deVoil, and G.L. Hammer. 2008. Genotypic variation in seedling root architectural traits and implications for drought adaptation in wheat (*Triticum aestivum* L.). Plant Soil 303: 115 – 129.

Manschadi, A.M., J. Christopher, P. deVoil, and G.L. Hammer. 2006. The role of root architectural traits in adaptation of wheat to water-limited environments. Funct. Plant Biol. 33: 823 – 837.

Martin, B., and R.A. Ruiz-Torres, 1992. Effects of water deficit stress on photosynthesis, its components and component limitations, and on water use efficiency in wheat (*Triticum aestivum* L.). Plant Physiol. 100: 733 – 739.

Materechera, S.A., A.M. Alston, J.M. Kirby, and A.R. Dexter. 1992. Influence of root diameter on the penetration of seminal roots into a compacted subsoil. Plant Soil 144: 297 – 303.

McMichael, B. L., and J. E. Quisenberry. 1993. The impact of the soil environment on the growth of root systems. Environ. Exp. Bot. 33: 53 – 61.

Miyashita K, S. Tanakamaru, T. Maitani, and K. Kimura. 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ. Exp. Bot. 53: 205 – 214.

Morita, S., and K. Yamazaki. 1993. Root system. In: Science of the Rice Plant. T. Matsuo and K. Hoshikawa (Eds.). Food and Agriculture Policy Research Center, Tokyo, Japan.

Morgan, J.M. 1984 Osmoregulation and drought stress in higher plants. Annu. Rev. Plant Physiol. 35: 299 – 319.

Mundree, S.G., B. Baker, S. Mowla, S. Peters, S. Marais, C. Vander Willigen, K. Govender, A. Maredza, J.M. Farrant, and J.A. Thomson. 2002. Physiological and molecular insights into drought tolerance. Afri. J. Biotech. 1: 28 – 38.

Mutava, R.N., P.V.V. Prasad, M.R. Tuinstra, K.D. Kofoid, and J. Yu. 2011. Characterization of sorghum genotypes for traits related to drought tolerance. Field Crops Res. 123: 10 – 18.

Nardini A., S. Salleo, and S. Andri. 2005. Circadian regulation of leaf hydraulic conductance in sunflower (*Helianthus annuus* L. cv. Margot). Plant Cell Environ. 28: 750 – 759.

Nguyen, H.T., R.C. Babu, and A. Blum. 1997. Breeding for drought resistance in rice: physiological and molecular genetics considerations. Crop Sci. 37: 1426 – 1434.

Oliver, A. L., R. J. Grant, J. F. Pedersen, and J. O'Rear. 2004. Comparison of brown midrib-6 and -18 forage sorghum with conventional sorghum and corn silage in diets of lactating dairy cows. J. Dairy Sci. 87: 637 – 644.

O'Toole, J.C., and J.L. Hatfield. 1983. Effect of wind on the crop water stress index derived by infrared thermometry. Agron. J. 75: 811 – 817.

Parry, M.A.J., P.J. Androlojc, S. Khan, P.J. Lea, and A.J. Keys. 2002. Rubisco activity: Effects of drought stress. Ann. Bot. (London) 89: 833 – 839.

Pereira J.S., and M.M. Chaves. 1993. Plant water deficits in Mediterranean ecosystems. In: Plant responses to water deficits: From cell to community. J.A.C. Smith, and H. Griffiths (Eds.). Oxford: BIOS Scientific, pp. 237 – 251.

Pettigrew, W.T., J.D. Hesketh, D.B. Peters, and J.T. Woolley. 1990. A vapor pressure deficit effect on crop canopy photosynthesis. Photosyn. Res. 24: 27 – 34.

Pinheiro C, J. Kehr, and C.P. Ricardo. 2005. Effect of drought stress on lupin stem protein analysed by two-dimensional gel electrophoresis. Planta 221: 716 – 728.

Prasad, P.V.V., S.R. Pisipati, R.N. Mutava, and M.R. Tuinstra. 2008. Sensitivity of sorghum to high temperature stress during reproductive development. Crop Sci. 48: 1911 – 1917.

Prasad, P.V.V., K.J. Boote, and L.H. Allen, Jr. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain sorghum are more severe at elevated carbon dioxide due to higher tissue temperatures. Agric. For. Meteorol. 139: 237 – 251.

Purcell, L.C., and J.E Specht. 2004. Physiological traits for ameliorating drought stress. In: Soybeans: Improvements, Production and Uses. Agronomy Monograph No. 16 (3rd ed.). H.R. Boerma, and J.E. Specht (Eds.). ASA, CSSA, SSSA, Madison WI, pp. 569–520.

Randall, H.C., and T.R. Sinclair. 1988. Sensitivity of soybean leaf development to water deficit. Plant Cell Environ. 11: 835 – 839.

Richards, R.A. 1991. Crop improvement for temperate Australia: future opportunities. Field. Crops Res. 26: 141 – 169.

Robinson, D. 1994. The responses of plants to non-uniform supplies of nutrients. New Phytol. 127: 635 – 674.

Saab, I.M., R.E. Sharp, and J. Pritchard. 1992. Effect of inhibition of abscisic acid accumulation on the spatial distribution of elongation in the primary root and mesocotyl of maize at low water potentials. Plant Physiol. 99: 26 – 33.

Sadok, W., and T. R. Sinclair. 2010. Transpiration response of 'slow-wilting' and commercial soybean (Glycine max [L.]) Merr.) genotypes to three aquaporin inhibitors under high evaporative demand. J. Exp. Bot. 61: 821 – 829.

Saini, H. S., and D. Aspinall. 1981. Effect of water deficit on sporogenesis in wheat (*Triticum aestivum* L.). Ann. Bot. (London) 48: 623 – 633.

Saini, H. S., M. Sedgley, and D. Aspinall. 1984. Developmental anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid. Aust. J. Plant Physiol. 11: 243 – 253.

Sanchez, A.C., P.K. Subudhi, D.T. Rosenow, and H.T. Nguyen. 2002. Mapping QTLs associated with drought resistance in sorghum (*Sorghum bicolor* L. Moench). Plant Mol. Biol. 48: 713 – 726.

Scandalios, J.G. 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz. J. Med. Biol. Res. 38: 995 – 1014.

Schiefelbein, J.W., and P.N. Benfey. 1991. The development of plant roots: New approaches to understand problems. Plant Cell. 3: 1147 – 1154.

Serraj, R., L. Krishnamurthy, J.W. Kashiwagi, J. Kumar, S. Chandra, and J.H. Crouch. 2004. Variation in root traits of chick pea (*Cicer arietinum* L.) grown under terminal drought. Field Crops Res. 88: 115 – 127.

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

Sharp, R.E. and W.J. Davies. 1989. Regulation of growth and development of plants growing with a restricted supply of water. In: Plant Under Stress. H.G. Jones, T.L. Flowers, and M.B. Jones (Eds.). Cambridge University Press, Cambridge, pp. 71 – 93.

Sharp, R.E., W.K. Silk, and T.C. Hsiao. 1988. Growth of the maize primary root at low water potentials. I. Spatial distribution of expansive growth. Plant Physiol. 87: 50 – 57.

Sharp, R.E., and W.J. Davies. 1985. Root growth and water uptake by maize plants in drying soil. J. Exp. Bot. 36: 1441 – 1456.

Sharp, R.E., V. Poroyko, G. Lindsey, L. Hejlek1, W.G. Spollen, G.K. Springer, H.J. Bohnert, and H.T. Nguyen. 2004. Root growth maintenance during water deficits: physiology to functional genomics. J. Exp. Bot. 55: 2343 – 2351.

Siddique, M.R.B., A. Hamid, and M.S. Islam. 1999. Drought stress effects on photosynthetic rates and leaf gas exchange of wheat. Bot. Bull. Acad. Sin. 40: 141 – 145.

Sinclair, T.R., and R.C. Muchow. 2001. System analysis of plant traits to increase grain yield on limited water supply. Agron. J. 93: 263 – 270.

Sinclair, T.R., G.L. Hammer, and E.J. van Oosterom. 2005. Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Funct. Plant Biol. 32: 945 – 952.

Sinclair, T.R. 2004. Physiological traits for ameliorating drought stress. In: Proceedings of the VII World Soybean Research Conference, Foz do Iguassu, PR, Brazil, pp. 245 – 252.

Sinclair, T.R., M.A. Zwieniecki, and N.M. Holbrook. 2008. Low leaf hydraulic conductance associated with drought tolerance in soybean. Physiol. Plant. 132: 446 – 451.

Siopongco, J.D.L.C., A. Yamauchi, H. Salekdeh, J. Bennett, and L.J. Wade. 2005. Root growth and water extraction response of double-haploid rice lines to drought and rewatering during the vegetative stage. Plant Prod. Sci. 8: 497 – 508.

Slaton, N.A., C.A. Beyrouty, B.R. Wells, R.J. Norman, and E.E. Gbur. 1990. Root growth and distribution of two short-season rice genotypes. Plant Soil 121: 269 – 278.

Sloane, R.J., R.P. Patterson, and T.E.Carter. 1990. Field drought tolerance of a soybean plant introduction. Crop Sci. 30: 118 – 123.

Spollen, W.G, R.E. Sharp, I.N. Saab, and Y. Wu. 1993. Regulation of cell expansion in roots and shoots at low water potentials. In: Water deficits: Plant responses from cell to community J.A.C. Smith, H. Griffiths (Eds.). Oxford: Bios Scientific Publishers.

Sponchiado, B.N., J.W. White, J.A. Castillo, and P.G. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp. Agri. 25: 249 – 257.

Steudle, E. 1997. Water transport across plant tissue: role of water channels. Biol. Cell. 89: 259 – 273.

Stockle, C.O., and J.R. Kiniry. 1990. Variability in radiation-use efficiency associated with vapor pressure deficit. Field Crops Res. 21: 171 – 181.

Taiz, L., and E. Zeiger. 2006. Stress physiology. 4th ed. Sinauer Asssociaes, Inc., MA, USA. pp. 671–702.

Takeda, S., and M. Matsuoka. 2008. Genetic approaches to crop improvement: responding to environmental and population changes. Nat. Rev. Genet. 9: 444 – 457.

Tanner, C.B. and T.R. Sinclair. 1983. Efficient water use in crop production: research or re-search. In: Limitations to Efficient Water Use in Crop Production. H.M. Taylor, W.R. Jordan and T.R. Sinclair (Eds.). ASA, Madison, WI, pp. 1 – 27.

Tardieu, F., M. Reymond, P. Hamard, C. Granier, and B. Muller. 2000. Spatial distribution of expansion rate, cell division rate and cell size in maize leaves: A synthesis of the effects of soil water status, evaporative demand and temperature. J. Exp. Bot. 51: 1505 – 1514.

Taylor, H.M. 1980. Modifying root system of cotton and soybean to increase water absorption. In: Adaptation of plants to water and high temperature stress. N.C. Turner and P.J. Kramer (Eds). pp. 75 – 84. Wiley & Sons, New York.

Terashima I, and K. Ono. 2002. Effects of $HgCl_2$ on CO_2 dependence of leaf photosynthesis: evidence indicating involvement of aquaporins in CO_2 diffusion across the plasma membrane. Plant Cell Physiol. 43: 70 – 78.

Turner, N.C., G.C. Wright, and K.H.M. Siddique. 2001. Adaptation of grain legumes (pulses) to water limited environment. Adv. Agron. 71: 193 – 2191.

Turner, N.C. 1979. Drought resistance and adaptation to water deficits in crop plants. In: Stress Physiology in Crop Plants. H. Mussel and R.C. Staples (Eds.). pp. 181 – 194. Wiley-Interscience Publ., New York, NY.

Turner, N, C, and M.M. Jones. 1980. Turgor maintenance by osmotic adjustment: A review and evaluation. In Adaptation of Plants to Water and High Temperature Stress. (Eds.) N.C Turner and P.J. Kramer. pp. 87 – 103. John Wiley & Sons, New York, NY.

Turner, N.C. 1986. Crop water deficits: a decade of progress. Adv. Agron. 39: 1 – 51.

Tyree, M.T., A. Nardini, S. Salleo, L. Sack, and B. El Omari. 2005. The dependence of leaf hydraulic conductance on irradiance during HPFM measurements: any role for stomatal response? J. Exp. Bot. 56: 737 – 744.

U.S. Grains Council. 2012. World Sorghum Production and Exports.

Wang, H., Y. Inukai, and A. Yamauchi. 2006. Root development and nutrient uptake. Crit. Rev. Plant Sci. 25: 279 – 301.

Wanjura, D. F., C.A. Kelly, C.W. Wendt, and J.L. Hatfield. 1984. Canopy temperature and water stress of cotton crops with complete and partial ground cover. Irrig. Sci. 5: 37 – 46.

Wilson, J.B. 1988. A review of evidence on the control of shoot, root ratio in relation to models. Ann. Bot. (London) 61: 433 – 449.

Wu, Y., R.E. Sharp, D.M. Durachko, and D.J. Cosgrove. 1996. Growth maintenance of the maize primary root at low water potentials involves increases in cell wall extension properties, expansin activity and wall susceptibility to expansins. Plant Physiol. 111: 765 – 772.

Yamauchi, Y., J.R. Pardales, and Y. Kono. 1996. Root system structure and its relation to stress tolerance. In: Roots and Nitrogen in Cropping Systems of the Semi-Arid Tropics. O. Ito, C. Johansen, J.J. Adu-Gyamfi, K. Katayama, J.V.D.K. Kumar Rao, T.J. Rego. (Eds.). JIRCAS Publication, Tsukuba, Japan.

Yambao, E.B., K.T. Ingram, and J.G. Real. 1992. Root xylem influence on the water relations and drought resistance of rice. J. Exp. Bot. 43: 925 – 932.

Ye, Q., N.M. Holbrook, and M.A. Zwieniecki. 2008. Cell-to-cell pathway dominates xylem–epidermis hydraulic connection in *Tradescantia fluminensis* (Vell. Conc.) leaves. Planta 227: 1311 – 1317.

Yoshida, S., and S. Hasegawa. 1982. The rice root system: its development and function. In: Drought Resistance in Crops, with Emphasis on Rice. International Rice Research Institute, Manila, Philippines.

Zhou J, X. Wang, Y. Jiao, Y. Qin, X. Liu, and K. He. 2007. Global genome expression analysis of rice in response to drought and high-salinity stresses in shoot, flag leaf, and panicle. Plant Mol. Biol. 63: 591 – 608.

Zhu, J., S. Chen, S. Alvarez, V.S. Asirvatham, D.P. Schachtman, Y. Wu, and R.E. Sharp. 2006. Cell wall proteome in the maize primary root elongation zone. I. Extraction and identification of water-soluble and lightly ionically bound proteins. Plant Physiol. 140: 311 – 325.

Chapter 2 - Evaluation of slow wilting sorghum genotypes for transpiration efficiency and water use

Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop in semiarid regions due to its adaptation to hot and dry environments. Limiting transpiration rate (TR) at increasing levels of vapor pressure deficit (VPD) is a strategy that will help a plant conserve soil water and therefore allow sustained growth during dry periods in the later part of the growing season. Research has shown that sorghum genotypes vary in their response to changes in VPD with certain genotypes manifesting a breakpoint (BP) in transpiration rate at increased VPD and therefore inherently using water conservatively. With projections of continued decline in the amount of water available for crop production, there is a pressing need to improve the water-use efficiency of rain-fed and irrigated crop production systems. Breeding crop varieties with higher water-use efficiency is seen as providing part of the solution. In this research we hypothesized that sorghum genotypes with a BP in their transpiration rate under increased VPD will also be efficient in water use. The objectives were to (i) evaluate the water use of some selected sorghum genotypes, and (ii) quantify variability in transpiration efficiency (TE). Two controlled environment experiments were conducted. Eight genotypes were evaluated for water use over the plant growing period (117 days) in one experiment, and thirty one genotypes were evaluated for TE over a period of 23 days. Transpiration efficiency was determined using a gravimetric method. Our results showed significant variability in biomass produced by plants grown for 23 days (1.4 – 12 g) and also those grown till maturity (18.8 – 27.9 g), the amount of water used by plants grown till maturity (10.5 – 13.5 kg) and those grown for 23 days (0.5 – 1.7 kg) and TE (2.6 – 7.1 g DM $kg^{-1}$of water used) among genotypes. TE was highly correlated to biomass (R^2 = 0.84) but had a low correlation with water used (R^2 = 0.16). Genotypes that had a breakpoint in their transpiration rate used less water than those without a breakpoint and were also high in TE.

2.1 Introduction

Agricultural production consumes over 70% of global fresh water resources used annually (Bacon, 2004). Future projections indicate that the greatest challenge to agriculture is a combination of a rapid decline in available fresh water resources and the demand for increased food production to meet global population growth. Terminal drought stress affects crop yields in many regions. Strategies for increasing water availability during post-anthesis stages will involve the management of the soil moisture profile in a way that leaves more water available for grain filling. Ways to achieve this will involve minimizing water use before anthesis (Kholova et al. 2010a, and b), enhancing transpiration efficiency (TE) or using plants with a deeper and/or more profuse rooting system to access water from the soil profile.

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop in the semiarid regions due to its tolerance of hot and dry environments. Even though sorghum is a drought tolerant crop, drought stress experienced either at pre- or post-flowering stages can significantly affect crop performance and decrease grain yield (Prasad et al., 2008). Traits associated with pre-flowering drought tolerance include greater leaf photosynthetic rates (Lawlor and Cornic, 2002), greater canopy temperature depression, improved panicle exsertion (Ayeneh et al., 2002; Lawlor and Cornic, 2002) and increased pollen viability. Improved rooting depth (Sharp et al., 2004), stay-green (Borrell et al., 2000), longer seed filling duration, increased seed filling rate and increased individual seed weight are associated with post flowering drought tolerance (Tuinstra et al., 1997; Borrell et al., 2000).

Because plant biomass production is a function of water used, it is important to look for traits that will help reduce the amount of water used while minimizing the associated reduction in production. Reduced plant size, leaf area, and leaf area index (LAI) are major mechanisms for moderating water use and reducing injury under drought stress (Mitchell et al., 1998).

Transpiration efficiency (TE), which is water use efficiency (WUE) at the leaf level, is determined by the delicate interplay between transient photosystem activity, sub-stomatal cavity CO_2 concentration (C_i) and stomatal conductance (Farquhar et al., 1989). Other important processes that will influence plant transpiration and water use at the whole plant and crop level include cuticular transpiration, boundary layer conductance, nighttime transpiration (Yoo et al., 2009). Where drought stress is the key issue, the goal should be plant water-use for stomatal transpiration at a given soil moisture content.

When breeding for water-limited environments it is important to match the crop growth duration with the expected or the predicted seasonal soil moisture supply. To achieve this, two important considerations are that short growth duration dictates moderate water-use and the escape of terminal (reproductive stage) drought stress while long duration genotypes generally have a greater water-use and larger and deeper root systems that allow deep soil moisture extraction where deep soil moisture is available (Mitchell et al., 1996). The improvement of biomass production under moisture limited conditions can be achieved primarily by maximizing soil water capture, through an extensive rooting system, while diverting the largest part of the available soil moisture towards stomatal transpiration. Strategies that can be used to achieve this will include breeding for extensive rooting system to maximize water capture, high plant vigor to ensure soil surface shading by crop canopy early in the season and therefore reduce water loss through evaporation and higher epicuticular wax deposition to minimize cuticular transpiration.

The reproductive growth stage is the most sensitive to drought stress (O'Toole, 1982). It is also recognized that drought stress at the reproductive stage is the most prevalent problem in rainfed drought prone agriculture. Therefore, irrespective of biomass production up to flowering, sustained water use and transpiration into the reproductive growth stage is crucial for reproductive success (Merah, 2001; Kato et al., 2008). Crop yields under water-deficit conditions also can be enhanced through conservative water use by crop plants (Richards and Passioura, 1989). Even though atmospheric VPD and transpiration rates follow a diurnal pattern

(Hirasawa and Hsiao, 1999), increase in transpiration has limits and a limiting maximum transpiration rate is commonly reached at a VPD of ~2.0 kPa (Comstock and Ehrleringer, 1993). Sinclair et al. (2005) hypothesized that conservation of water by some plants also can be achieved by limiting transpiration. Through a mechanism of limiting transpiration rate (TR) at increasing levels of VPD, a plant can conserve soil water and therefore allow sustained crop growth during dry periods in the later part of the growing season. Simulation studies have demonstrated that the existence of a limitation on maximum TR in water-limited conditions could result in significant yield increases (Sinclair et al., 2005); an outcome that was attributed to water savings associated with reduced transpiration and therefore increased WUE. Studies with different crops have shown variability in TR response to increase in VPD; soybean (*Glycine max* (L.) Merr.) (Fletcher et al., 2007), peanut (*Arachis hypogaea* L.) (Devi et al., 2010), pearl millet (*Pennisetum glaucum* (L.) R.Br.) (Kholova et al., 2010b), and rice (*Oryza sativa* L.) (Morison and Gifford, 1983). Fletcher et al. (2007) found the limited-TR trait in the soybean genotype PI 416927, which had been identified in the field as "slow-wilting", at VPD> 2.1 kPa. A study by Kholova et al. (2010b) found that genotypes tolerant of terminal drought exhibited a decreased rate of TR increase at high VPD (>2.0 kPa) while Devi et al. (2010) found that in peanut the threshold of VPD above which there was restricted TR varied from 2.0 to 2.6 kPa. A study with grain sorghum identified some lines that exhibited high leaf temperature and high yields under field conditions (Mutava et al., 2011). Further investigation of these lines showed that the majority of them had a breakpoint in their transpiration rate (Gholipoor et al., 2010), an indication of a limitation in TR in response to increasing VPD.

Even though sorghum genotypes that will impose a limitation in their transpiration rate with an increase in VPD have been identified, no information is available on their TE, which may be an indication of their conservative water use. In this study we therefore hypothesized that sorghum genotypes that exhibited the slow wilting trait also will have a high TE. Our objectives were (i) to evaluate the water use of some selected sorghum genotypes, and (ii) to quantify variability in transpiration efficiency rates.

2.2 Materials and Methods

This research was conducted in controlled environment facilities (greenhouse) in the Department of Agronomy at Kansas State University in Manhattan, Kansas and at USDA-ARS Plant Stress and Germplasm Development Unit in Lubbock, Texas. Two independent experiments were conducted to determine water use when plants were grown to maturity (experiment 1conducted at Manhattan, Kansas) and TE with plants harvested at 23 days after planting (experiment 2 conducted at Lubbock, Texas). The selection of these genotypes was based on a grouping by Mutava et al. (2011) of genotypes that showed the slow wilting trait characterized by high canopy temperature. Transpiration efficiency was determined using a high throughput gravimetric method (Xin et al., 2008).

2.2.1 Experiment 1: Determination of transpiration efficiency

This experiment was conducted at the USDA ARS greenhouses in Lubbock, Texas, USA with 31 genotypes (Table 2-1). Plastic pots (14 cm diameter and 16 cm height) were filled with Sunshine #1 potting mix (Sun Gro Horticulture Inc., Bellevue, Washington, USA) and watered with Miracle-Gro solution (24-8-16, N-P_2O_2-K_2O; The Scotts Company LLC., Marysville, Ohio, USA), at a mixing rate of 2.5g per gallon, until dripping from the bottom. Three seeds were planted per pot and the pots were then covered with a layer of dry potting mix to reduce water loss from the soil surface. One week after emergence, the pots were thinned to one plant per pot and all the pots were covered from both ends with 2 Mil poly bags (S-3478, Uline, Waukegan, Illinois, USA), which are permeable to air but impermeable to water (Xin et al., 2008). A slit was made in the top bag to permit seedling growth. This slit was further sealed with a piece of clear adhesive tape and covered with a layer of dry potting mix to minimize water loss through the slit. The pots were bar-coded, and the initial weight recorded. The plants were then grown for another 13 days, the final pot weight was recorded and the plants were harvested. Roots were collected by washing the potting mix core on a mesh. Roots and shoots were

then dried for 72 hours at 80°C and then weighted to determine root and shoot dry biomass (M_{root} and M_{shoot} respectively). The experiment was a randomized complete block design with four replications.

2.2.2 Experiment 2: Determination of water use

Eight genotypes were used in this experiment (Table 2-1), which was conducted in a greenhouse at the Kansas State University in Manhattan, Kansas, USA. PVC pipes (14 cm diameter, 0.8 cm thick and 50 cm height) (United States Plastic Corporation, Lima, Ohio, USA) were sealed at the bottom with 2 Mil poly bags (S-3478, Uline, Waukegan, Illinois, USA) and then filled with a clay-loam soil and sand mixture (3:1). Osmocote controlled release fertilizer (19-6-12) (N-P_2O_5-K_2O; Osmocote® Classic, The Scotts Company LLC, Marysville, Ohio, USA) was used as a nutrient source at a rate of 8 grams per column. Three seeds were planted per column and then thinned to one plant at 10 days after emergence. The tops of the columns were then covered with 2 Mil poly bags with a slit to permit seedling growth. The slit was further sealed with a piece of clear adhesive tape and covered with a layer of dry soil to minimize water loss through the slit. The columns were labeled and the initial weight recorded. After 10 days the columns were weighed in the evening, the top seal opened and soaked with water to saturation. The columns were then left to drain off excess water and in the morning they were weighed again and sealed. This difference in weight was recorded as the amount of water added. This was repeated after every seven to ten days until maturity when the plants were harvested. Water used ($W_{transpiration}$) was calculated by subtracting the final pot weight from the initial weight. Shoots were harvested three times (56, 83 and 118 days after planting (DAP)), dried for 3 days at 80°C and weighed to determine shoot dry mass (M_{shoot}). Data on photosynthetic rate, TR and stomatal conductance were collected on the top most fully expanded leaf at 3 and 7 days after watering (DAW) using LICOR 6400 (Licor Inc., Lincoln, Nebraska, USA). The experiment was a randomized complete block design with three replications.

2.2.3 Data analyses

From both experiments, water used ($W_{transpiration}$) was calculated by subtracting the final pot weight from the initial weight. In experiment 1, cumulative water used was calculated based on water used from water added. Total biomass (M_{total}) was calculated as the sum of root and shoot mass. TE was calculated on a shoot basis (TE_{shoot}) as the ratio (M_{shoot}) to ($W_{transpiration}$), and on a total dry weight basis (TE_{total}) as the ratio (M_{total}) to ($W_{transpiration}$). All data were statistically analyzed using PROC GLM and PROC CORR procedures in SAS (SAS 9.2, SAS Institute Inc., Cary, NC, USA). Standard errors were used to show estimate of variability, $LSD_{(\alpha = 0.05)}$ was used to compare means and P-values were used to show significance levels.

2.3 Results

There was significant genotypic variation ($P < 0.0001$) for all growth traits and TE ($P = 0.0003$, and 0.0005 for TE_{Shoot} and $TE_{Total\ Biomass}$ respectively) in experiment 1, while in experiment 2 plant height varied significantly ($P < 0.0001$) among genotypes (Table 2-2). There was significant genotypic variation in leaf area ($P = 0.046$), total biomass ($P = 0.039$) and water use efficiency ($P = 0.0532$) (Table 2-2, experiment 2). Physiological traits (photosynthetic rate, transpiration rate and stomatal conductance) did not vary significantly among genotypes or with the date of measurement (3 and 7 DAW), but leaf temperature variation was significant ($P = 0.0386$ and 0.0026) (Table 2-2, experiment 2). There was significant ($P<0.0001$) influence of date of harvest on growth plant height, leaf area and biomass.

2.3.1. Experiment 1: Transpiration efficiency and biomass production

There was significant variation in total biomass and TE among the genotypes (Figure 2-1). Biomass ranged from 1.47 – 12.03 g and TE from 2.64 – 7.11 g DM kg^{-1} of water used. Genotypes that recorded high biomass include; SC1345 (12.0 g), Pioneer Hybrid 84G62 (10.8 g), B.TxaRG-1 (9.4 g), SC1047 (9.2 g) and SC1074 (9.0 g) while TX7078, SC982, B.Tx615 and SC224 had the lowest biomass values (3.1, 2.4, 2.4, and

1.4g respectively) (Figure 2-1A). Genotype Liang Tang Ai, which was used as a check for high TE, recorded 7.1 g of biomass. Genotypes showed significant variability in TE with genotype SC1345 recording the highest TE (7.11 g DM kg^{-1} of water used) and SC224 the lowest TE (2.64 g DM kg^{-1} of water used) (Figure 2-1B). When compared to Liang Tang Ai, genotype SC1345 had significantly greater TE. TX7078 (a check for low TE) had significantly less biomass. Transpiration efficiency had a strong positive relationship with total dry biomass (Figure 2-2A and B) but not with water use (Figure 2-2B).

Shoot and root dry weights varied significantly among the 31 genotypes (Table 2-3) with genotype SC1345 and Pioneer Hybrid 84G62 having the largest values, and genotype SC224 the smallest values followed by genotype SC982 (1.8 g) for shoot and BTx615 (0.6 g) for roots. Genotype Liang Tang Ai and TX7078 recorded 5.3 g and 2.0 g for shoot dry weight and 1.8 g and 1.1 g for root dry weight respectively.

The amount of water used also varied by genotype and again genotypes SC1345 and Pioneer 84G62 used the largest amounts of water (Table 2-3). Genotypes BTx615 and SC982 used the least amount of water. When ranked based on TE_{Total}, the top five genotypes with high TE were SC1345, Pioneer Hybrid 84G62, B.TxARG-1, SC663 and SC1019 with TE_{Total} ranging from 6.3 – 7.1 g DM kg^{-1} of water used. The bottom five genotypes were SC224, Macia, B.Tx615, SC982 and SC60 with values ranging from 2.6 – 4.3 g DM kg^{-1} of water used. Genotype Liang Tang Ai ranked 16^{th} in TE_{Total}, and TX7078 ranked 24^{th}. Five of the genotypes that were selected for the slow wilting trait ranked higher than Liang Tang Ai for TE_{Total}. These were SC1019 (4^{th}), SC720 (9^{th}), SC1047 (10^{th}), SC1074 (12^{th}) and SC979 (14^{th}). Selected genotypes without a breakpoint (BP) in their transpiration rate under increasing VPD (Table 2-3) used more water (629.9 – 1692.7 g) and recorded greater biomass (2.1 – 7.9 g) while water used by genotypes with a BP ranged from 566.9 – 1545.2 g and biomass produced from 1.8 – 5.8 g.

2.3.2. Experiment 2: Water use

There were significant genotypic differences in the amount of water used over the growing period (118 days) (Figure 2-3). Hegari used the greatest amount of water from 56 DAP to 118 DAP (1.58 kg and 13.52 kg respectively) and therefore had the greatest cumulative water used, while genotype TX7078 used the least amount of water (10.43 kg) followed by genotypes SC1019 and B35 (11.13 kg). Genotypes SA5330, Liang Tang Ai, SC1124, and Pioneer Hybrid 85G46 were similar in amount of water used at 118 DAP.

2.3.2.1 Physiological traits

Data on photosynthetic rate, transpiration rate and stomatal conductance collected at 3 and 7 days after watering (DAW) did not show significant variation among genotypes, (Figure 2-4). Leaf temperature differed among genotypes ($P = 0.0433$) and at 3 and 7 DAW, ($P = 0.0016$) respectively.

Photosynthetic rate ranged from 42.6 – 51.2 μmol m^{-2} s^{-1} at 3 DAW and 40.9 – 51.9 μmol m^{-2} s^{-1} at 7 DAW, stomatal conductance from 0.015 – 0.019 mmol m^{-2} s^{-1} and 0.009 – 0.021 mmol m^{-2} s^{-1} and transpiration rate from 0.67 – 0.87 and 0.43 – 0.95 mmol m^{-2} s^{-1} respectively (Figure 2-4A, B, C). Genotype SC1019 had the low leaf temperature (29.98°C and 29.83) at 3 and 7 DAW respectively while genotypes SA5330 and TX7078 had high leaf temperature (30.48°C, 30.07°C and 30.45°C, 30.19°C respectively) at 3 and 7 DAW (Figure 2-4D).

Both photosynthetic rate and transpiration rate depended on stomatal conductance ($R^2 = 0.46$, $P = 0.0080$ and $R^2 = 0.98$, $P < 0.0001$ respectively) (Figure 2.5A and B). The relationship between leaf temperature and transpiration rate and also between leaf temperature and stomatal conductance were similar but quadratic and not linear (Figure 2-6A and B).

2.3.2.2 Growth traits and biomass

For plants harvested at 56 and 83 DAP, there was significant genotypic variation in plant height, biomass, leaf area and TE (Table 2-4). At 56 and 83 DAP, plant height ranged from 31.7 – 41.2 cm and 40.8 –

101.1 cm, biomass from 3.6 – 6.1 g and 18.6 – 27.9 g, TE from 3.6 – 4.8 g kg^{-1} and 3.8 – 5.4 g kg^{-1} and leaf area from 778 – 1097 cm^2 and 1342 – 2893 cm^2 respectively. Genotype Hegari had significantly greater TE (4.8 g DM kg^{-1} of water used) than all other genotypes at 56 DAP, but Liang Tang Ai had the greatest TE at 83 DAP. Genotype Liang Tang Ai recorded the largest values for plant height, biomass and TE (101.7 cm, 27.9 g and 5.4 g kg^{-1} respectively) at 83 DAP compared to other genotypes while genotypes with the lowest values were TX7078 (40.8 cm) for plant height, SC1124 and TX7078 (18.6 g) for biomass and TX7078 (3.8 g DM kg^{-1} of water used) for TE. Genotype SC1019 had the largest value for leaf area at 83 DAP but genotype Liang Tang Ai had the smallest value.

2.4 Discussion

In this research we looked at how some key physiological and growth traits varied for different genotypes and how these related to water use and TE. Significant genotypic variability was noted for most of the traits monitored.

2.4.1 Physiological traits

For plants that were grown through maturity there were positive relationships between stomatal conductance and photosynthetic rate as well as between stomatal conductance and transpiration rate (Figure 2-5A and B). Stomatal opening, which is determined primarily by the plant water balance, is an important factor controlling the rate of entry of CO_2 into the plant. An increase in stomatal conductance results in greater photosynthetic activity and therefore increased CO_2 assimilation. Transpiration is more dependent than photosynthesis on stomatal opening and closing because transpiration depends on the total resistances in the stomates and the surrounding air, whereas CO_2 entry depends both on these and on the resistance of the mesophyll (Rosenberg et al., 1983; Timlin et al., 2008). This is evident from the strong relationship between stomatal conductance and transpiration (R^2 = 0.98, P <0.0001) (Figure 2-5B) as -compared to photosynthesis (R^2 = 0.46, P = 0.008) (Figure 2-5A). Studies have shown that transpiration increases linearly with stomatal

conductance assuming that VPD does not vary and boundary layer resistance is nearly zero (Jones 1976; Timlin et al., 2008).

2.4.2 Transpiration efficiency

TE varied significantly among the genotypes (Table 2-2 and Figure 2-1). TE determined by biomass accumulation per unit water transpired was positively correlated with biomass production while there was low correlation between TE and total water used (Figure 2-2A and B). This suggests that high TE lines selected based on biomass accumulation may be superior in productivity rather than conservative in water use. At the same time there are genotypes whose high TE is water use based (Table 2-3), and there are genotypes that manifest a BP in their transpiration rate. Even though biomass for the water use based TE may not be as high as those genotypes with transpiration based, studies have shown no penalty in yields (Mutava et al., 2011). Sinclair et al. (2005) demonstrated that imposition of limited maximum transpiration rates increased sorghum yields in 76–90% of seasons in a semi-arid environment. This outcome was due both to the water savings associated with reduced transpiration and to increased transpiration use efficiency.

2.4.3 Water use

There was a positive relationship between the amount of water used and biomass produced (Figures 2-3, 2-4 and Table 2-3). More water was used as the plants progressed in growth, and this corresponded to an increase in the amount of biomass produced. As a plant increases in size (height and leaf area), the surface area that needs cooling increases, and therefore more water will be needed for transpirational cooling. Plant biomass accumulation is closely linked to transpiration and therefore water use. In our results, selected genotypes that did not have a BP in their transpiration rate with increasing VPD used more water as compared to those that had a BP (629 – 1693 g vs. 567 – 1545 g) and also accumulated more biomass (2.1 – 7.9 g vs. 1.8 – 5.8 g) (Table 2-3). Xin et al. (2009) found that sorghum that exhibited restrictive stomata, i.e., reduced CO_2 concentration in sub-stomatal cavity (C_i) and greater normalized transpiration efficiency (nTE), did not exhibit high TE based on

biomass production. This suggests that lines with putative TE traits at the leaf level can exhibit superior TE, evaluated at the whole-plant level; but not in every case. Results from Xin et al. (2009) suggest that other physiological processes in addition to nTE at the leaf level also contribute significantly to TE based on integrated biomass production. Limitation of crop water use has been demonstrated as an approach to conserve water when atmospheric VPD is high (Sinclair et al., 2005; Fletcher et al., 2007; Kholova et al., 2010b). A plant-imposed limit on maximum transpiration rate could be particularly important in non-irrigated crop production.

2.5 Conclusions

Conservative water use is a trait that can be used to increase production in water limited environments. Limitation in transpiration rate will result in reduction in the amount of water used by a plant and this may lead to the availability of water over a longer period of time particularly during seed filling. Genotypes that were selected based on the presence of a BP in their transpiration had also high TE values but differed in the amount of water used and the amount of biomass accumulated. These results can be used in breeding for improving drought tolerance in crops because of the conservative nature of genotypes. There is need though to look at how these genotypes will differ in extraction of water under field condition and their yield.

2.6 Figures and Tables

Table 2-1: Genotypes used in experiment 1 and 2 and their respective grouping based on selection criterion.

Experiment 2 Transpiration Efficiency	Experiment 1 Water Use	Criteria
SC1345	-	Normal leaf temperature, high grain yield
84G62	84G62	-
B.TxARG-1	-	Normal leaf temperature, high grain yield
SC663	-	Normal leaf temperature, high grain yield
SC1019	SC1019	High leaf temperature, high grain yield
B.Tx3197	-	Low leaf temperature, high grain yield
B.Tx2752	-	Low leaf temperature, high grain yield
SN149	-	Normal leaf temperature, low grain yield
SC720	-	High leaf temperature, high grain yield
SC1047	-	High leaf temperature, high grain yield
DK28-05	-	-
SC1074	-	High leaf temperature, high grain yield
B.Tx399	-	Normal leaf temperature, high grain yield
SA5330	SA5330	Low
SC979	-	High leaf temperature, high grain yield
SC1124	SC1124	High leaf temperature, low grain yield
Liang Tang Ai	Liang Tang Ai	-
RTx430	-	Normal leaf temperature, high grain yield
B.Tx623	-	Low leaf temperature, high grain yield
SC630	-	Normal leaf temperature, low grain yield
SC532	-	Normal leaf temperature, low grain yield
DK54-00	-	-
Hegari	Hegari	Low leaf temperature, high grain yield
R.Tx436	-	Low leaf temperature, low grain yield
Tx7078	Tx7078	-
SC803	-	High leaf temperature, high grain yield
SC60	-	Low leaf temperature, high grain yield
SC982	-	Normal leaf temperature, low grain yield
B.Tx615	-	Low leaf temperature, low grain yield
Macia	-	Low leaf temperature, high grain yield
SC224	-	Low leaf temperature, low grain yield
-	B35	-

Table 2-2: Analyses of variance of transpiration efficiency (TE) based on shoot and total biomass and growth and physiological traits (G – genotype, HD – harvest date, and DAW – days after watering).

Experiment 1: Transpiration efficiency and biomass production

			Significance level
			Genotype
Trait	**Mean(CV)**	**LSD**	**(G)**
Shoot weight	3.9(20.7)	2.28	<0.0001
Root weight	1.9(24.1)	1.52	<0.0001
Total Biomass	6.0(21.7)	3.55	<0.0001
TE_{Shoot}	3.6(20.4)	1.03	0.0003
$TE_{Total\ Biomass}$	5.4(21.9)	1.68	0.0005

Experiment 2: Water use

			Genotype	Time of	Days after	G x HT	G x DAW
	Mean(CV)	**LSD**	**(G)**	**Harvest (HT)**	**watering (DAW)**		
Plant height	49.46(17.2)	9.9	<0.0001	<0.0001	-	0.0006	-
Leaf area	1534(26.5)	480	0.0455	<0.0001	-	0.1875	-
Total biomass	13.1(18.7)	2.89	0.0393	<0.0001	-	0.0713	-
Water use efficiency	4.1(22.2)	1.39	0.0532	0.1901	-	0.7708	-
Photosynthetic rate	38.4(22.4)	9.5	0.8220	-	0.4475	-	0.9666
Transpiration rate	0.84(21.8)	0.22	0.5141	-	0.0805	-	0.1875
Stomatal conductance	0.02(21.4)	0.005	0.4711	-	0.1800	-	0.7292
Leaf temperature	30.2(2.0)	0.35	0.0386	-	0.0026	-	0.8486

LSD was at $\alpha = 0.05$, amount of variability is shown by (CV).

Figure 2-1: Genotypic variation in total biomass and transpiration efficiency in plants grown for 23 days.

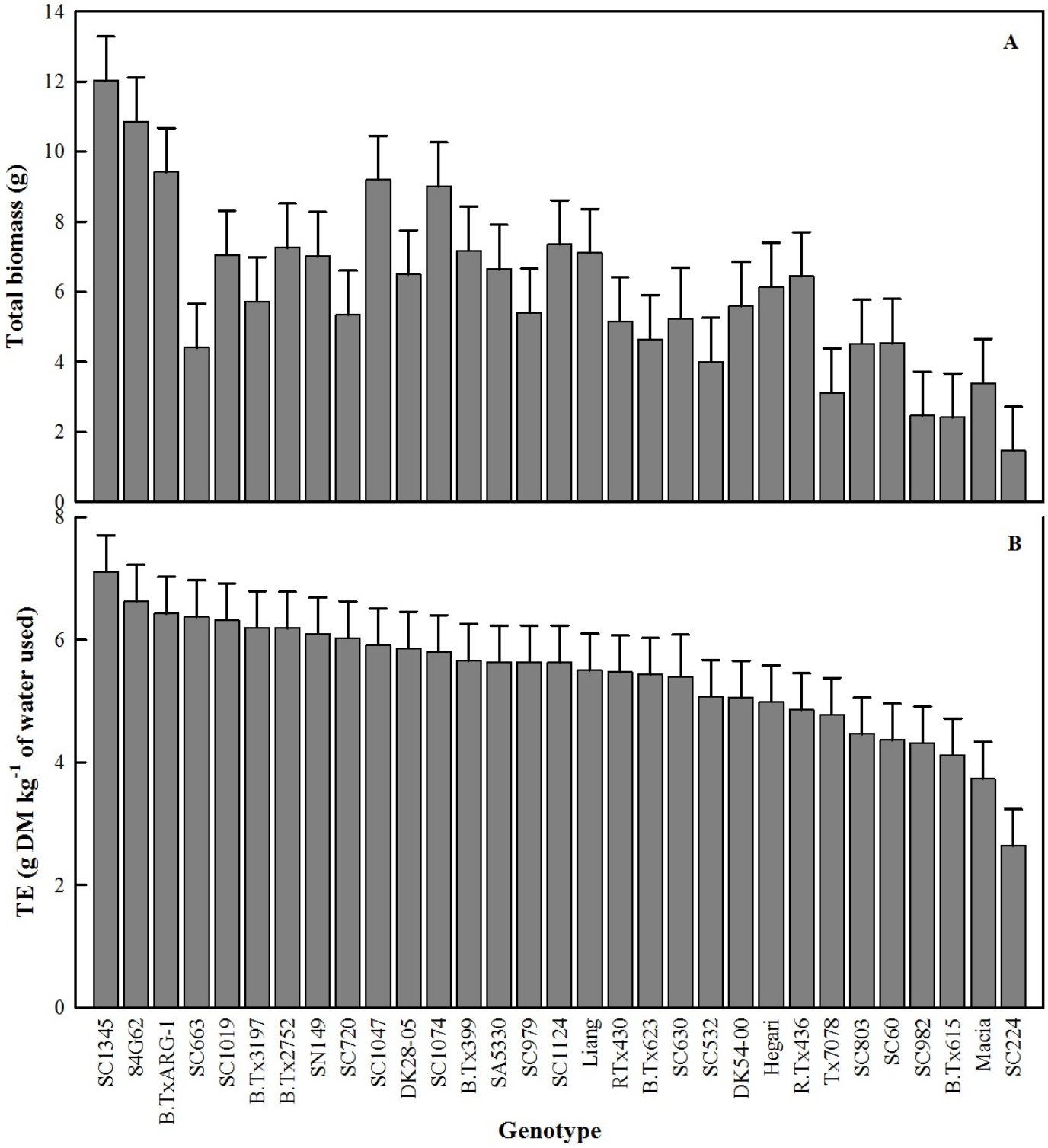


Figure 2-2: Relationship between transpiration efficiency based on total dry weight and (A) biomass and (B) total water used among various genotypes.

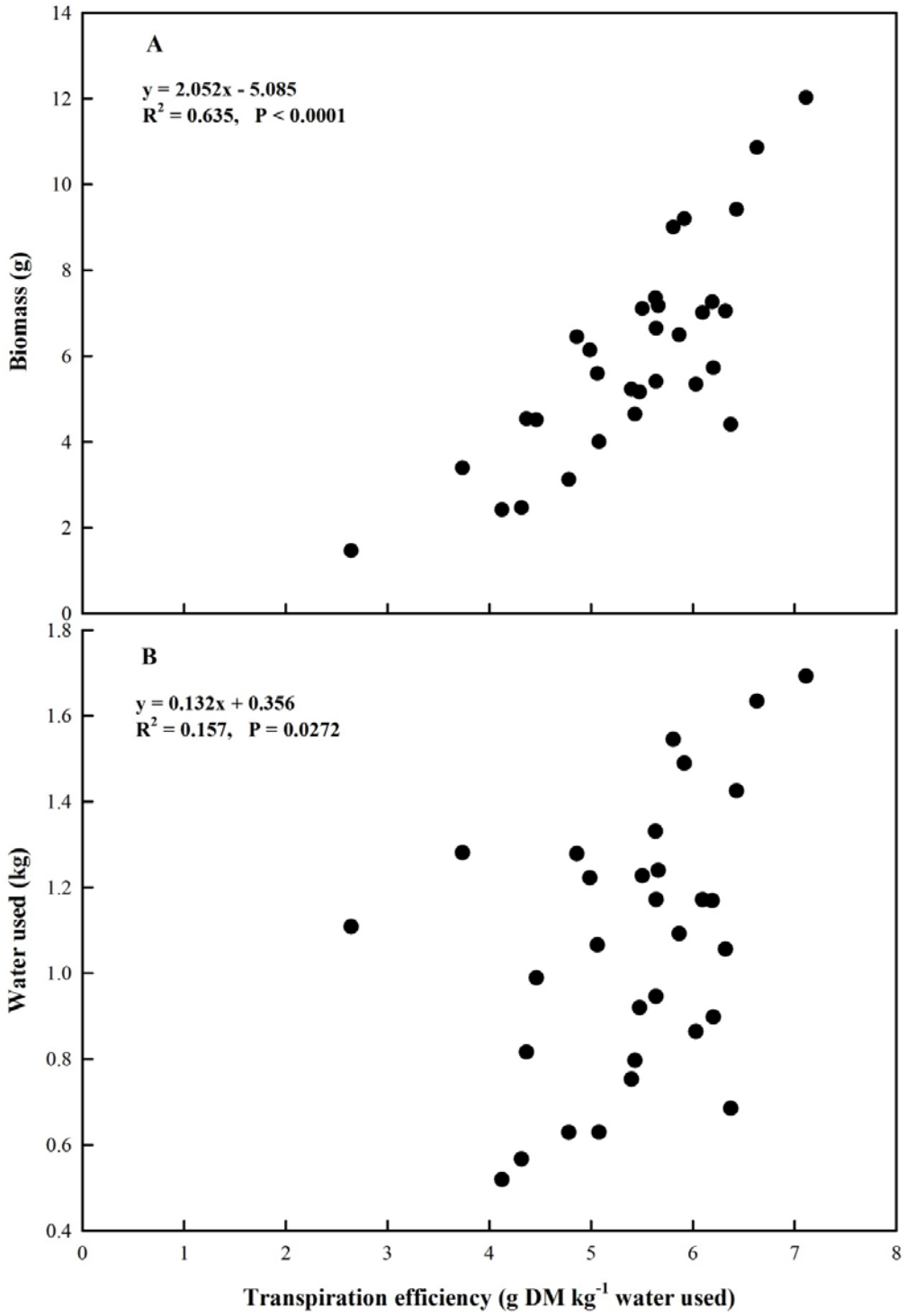


Table 2-3: Variation of transpiration efficiency based on shoot dry weight (TE_{Shoot}) and total dry weight (TE_{Total}) in thirty one (31) selected sorghum genotypes.

	Selection basis								
Genotype	**Leaf temperature**	**Grain Yield**	**BP in TR**	**Shoot DW (g)**	**Root DW (g)**	**Water used (g)**	**TE_{Shoot} (g DM kg^{-1} of water used)**	**TE_{Total} (g DM kg^{-1} of water used)**	**Ranking based on TE_{Total}**
SC1345	Normal	High	None	7.9	4.1	1692.7	4.6	7.1	1
84G62	-	-	-	6.8	4.0	1634.2	4.2	6.6	2
B.TxARG-1	Normal	High	None	6.0	3.4	1425.1	4.2	6.4	3
SC663	Normal	High	-	2.7	1.7	685.2	4.1	6.4	4
SC1019	High	High	Yes	4.4	2.7	1056.3	4.1	6.3	5
B.Tx3197	Low	High	Yes	3.8	1.9	897.9	4.1	6.2	6
B.Tx2752	Low	High	Yes	4.9	2.4	1169.4	4.0	6.2	7
SN149	Normal	Low	Yes	4.7	2.3	1171.7	4.0	6.1	8
SC720	High	High	-	3.4	1.9	864.2	3.9	6.0	9
SC1047	High	High	Yes	5.8	3.4	1489.6	3.9	5.9	10
DK28-05	-	-	None	4.3	2.1	1092.2	3.9	5.9	11
SC1074	High	High	Yes	5.8	3.2	1545.2	3.9	5.8	12
B.Tx399	Normal	High	-	4.5	2.6	1240.0	3.8	5.7	13
SA5330	Low	Low	-	4.7	1.9	1171.8	3.8	5.6	14
SC979	High	High	Yes	3.7	1.7	946.2	3.8	5.6	15
SC1124	High	Low	-	5.2	2.2	1330.8	3.8	5.6	16
Liang Tang Ai	-	-	-	5.3	1.8	1227.4	3.8	5.5	17
RTx430	Normal	High	None	3.7	1.5	919.8	3.8	5.5	18
B.Tx623	Low	High	Yes	3.4	1.2	796.8	3.6	5.4	19
SC630	Normal	Low	Yes	3.1	1.1	753.1	3.6	5.4	20
SC532	Normal	Low	None	2.1	1.9	629.6	3.4	5.1	21
DK54-00	-	-	None	3.6	2.0	1066.2	3.3	5.1	22
Hegari	Low	High	None	4.1	2.0	1222.5	3.3	5.0	23
R.Tx436	Low	Low	-	4.7	1.7	1278.7	3.1	4.9	24
Tx7078	-	-	Yes	2.0	1.1	629.3	3.1	4.8	25
SC803	High	High	-	3.1	1.4	989.3	3.0	4.5	26
SC60	Low	High	-	3.0	1.5	816.4	2.9	4.4	27
SC982	Normal	Low	Yes	1.8	0.7	566.9	2.9	4.3	28
B.Tx615	Low	Low	-	1.8	0.6	519.4	2.9	4.1	29
Macia	Low	High	Yes	2.3	1.0	1281.1	2.6	3.7	30
SC224	Low	Low	-	1.0	0.4	1108.6	1.8	2.6	31
LSD				**2.29**	**1.53**	**241.17**	**1.03**	**1.68**	

Figure 2-3: Variation in cumulative water use among genotypes during the growing period (29 – 118 DAS)

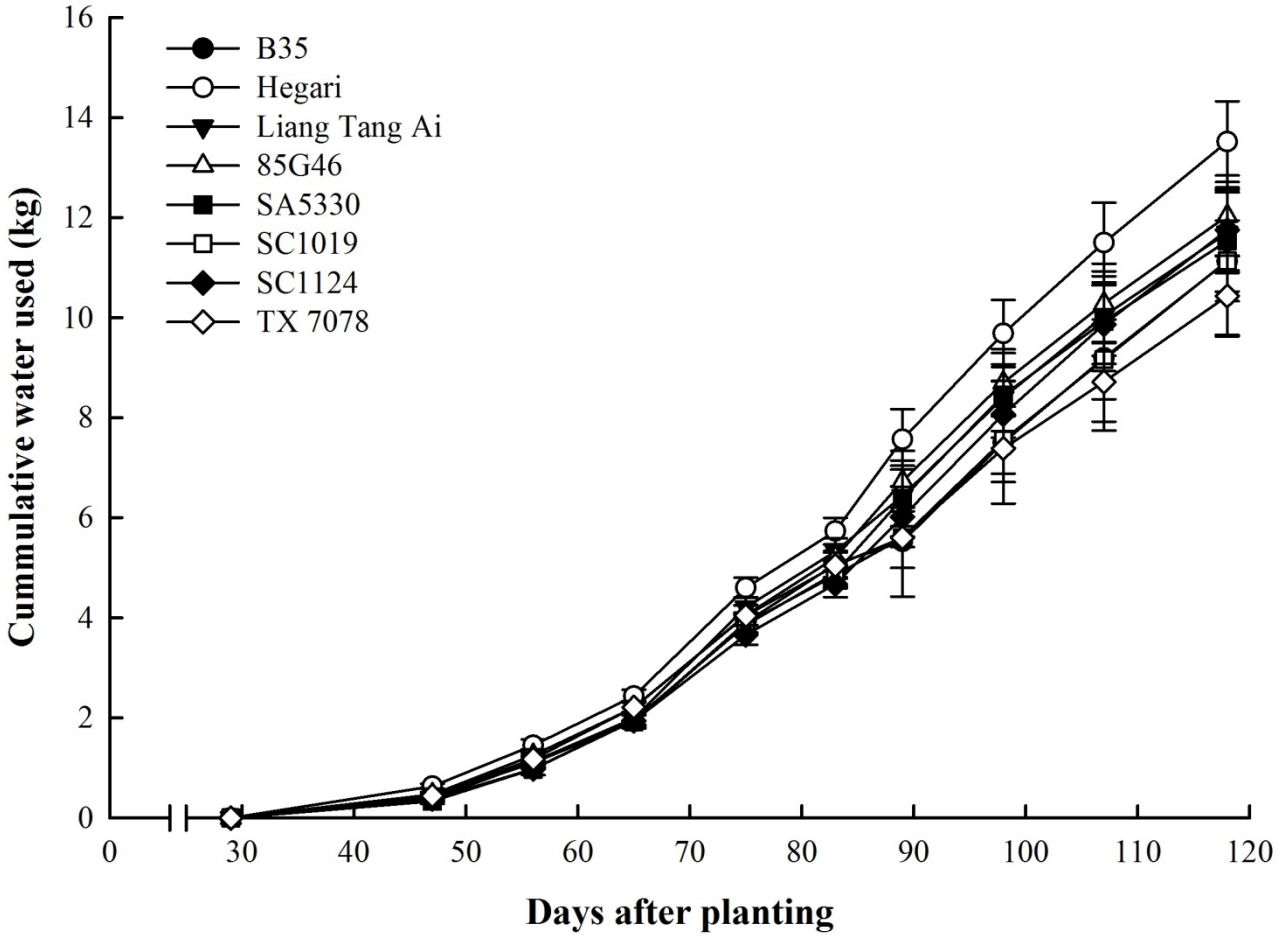


Figure 2-4: Photosynthetic rate, stomatal conductance, transpiration rate and leaf temperature of various genotypes at three and seven days after watering.

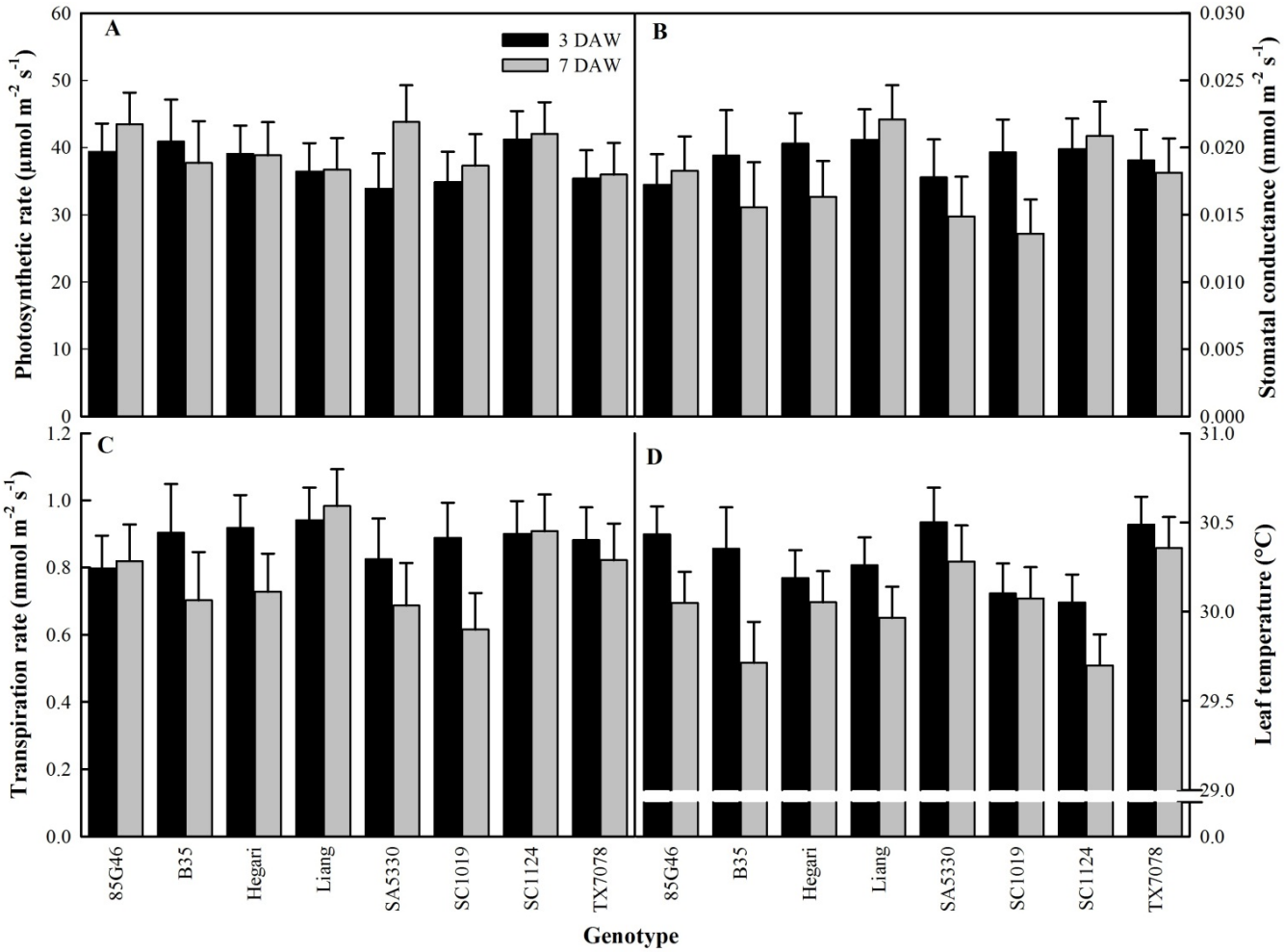


Figure 2-5: Relationship between stomatal conductance and (A) photosynthetic rate and (B) transpiration rate among various sorghum genotypes

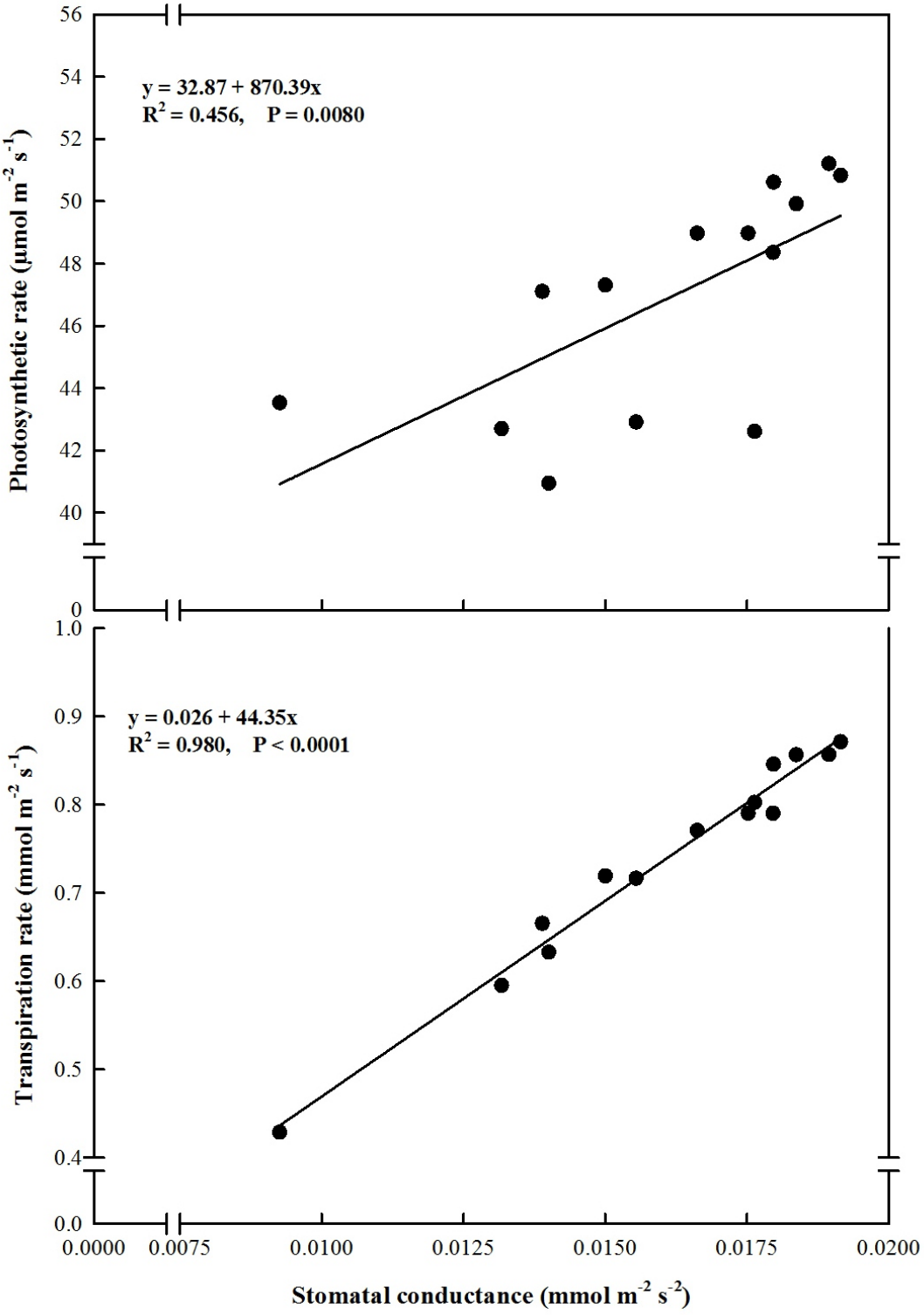


Figure 2-6: Relationship between leaf temperature and (A) transpiration rate and (B) stomatal conductance of various sorghum genotypes.

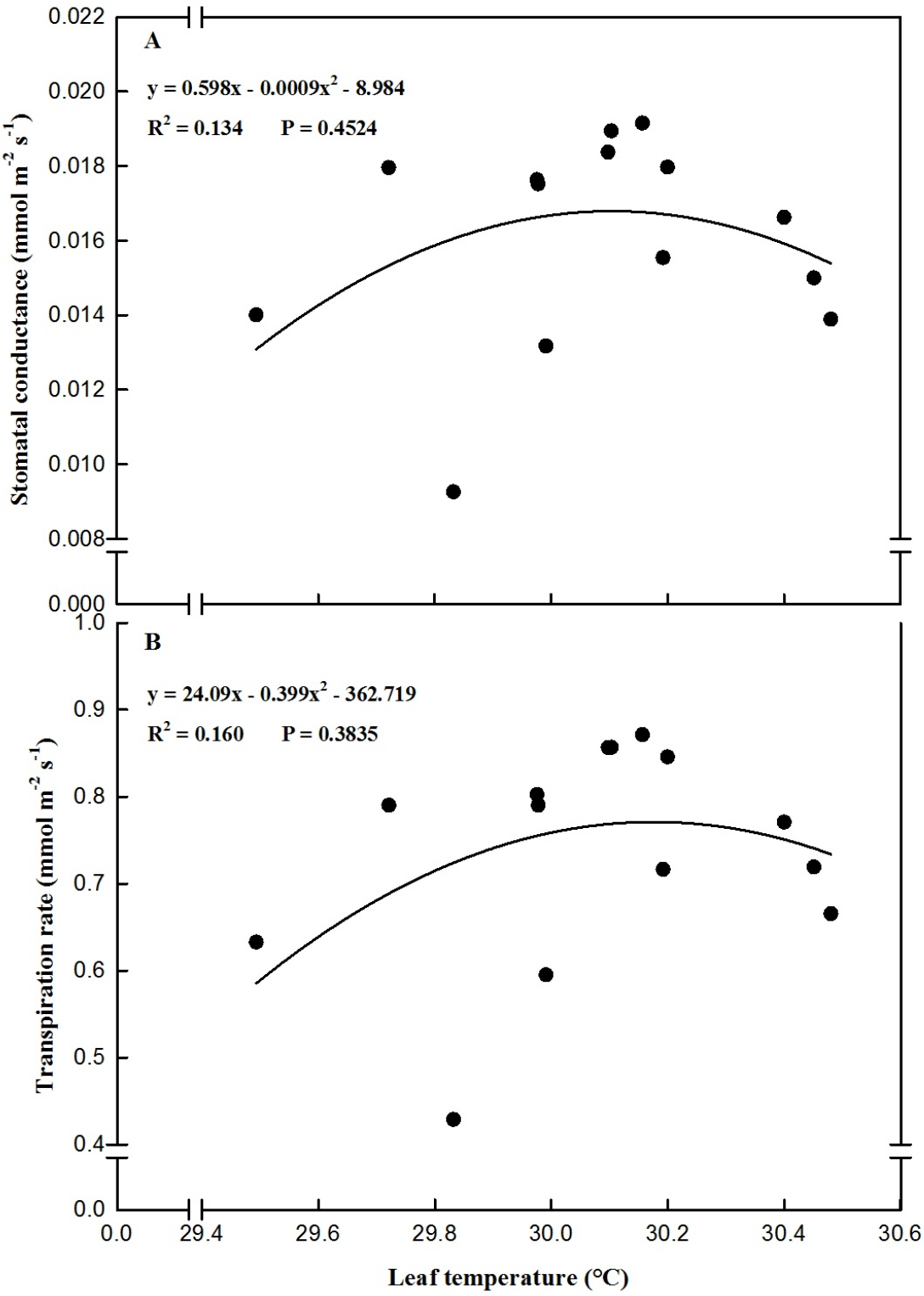


Table 2-4: Mean values showing in leaf area, plant height, biomass and transpiration efficiency at 56 and 83 days after planting. Standard errors (SE) are provided in parenthesis

	Leaf Area (cm^2) (SE = 235.2)		**Plant Height (cm)** (SE = 4.9)		**Biomass (g DM)** (SE = 1.4)		**Transpiration Efficiency (g DM kg^{-1} of water used)** (SE = 0.7)	
Genotype	**56 DAP**	**83 DAP**	**56 DAP**	**83 DAP**	**56 DAP**	**83 DAP**	**56 DAP**	**83 DAP**
B35	788.1	2488.8	36.9	55.3	4.2	22.8	3.6	4.6
Hegari	1097.0	2341.0	37.3	73.5	6.1	22.8	4.8	4.1
Liang	882.1	1342.1	41.2	101.7	4.2	27.9	3.8	5.4
85G46	778.2	2006.8	31.8	64.2	4.8	20.9	4.0	4.1
SA5330	781.2	2206.2	34.5	54.0	3.6	21.8	3.6	4.6
SC1019	1069.6	2893.1	29.6	53.5	4.1	21.5	4.1	4.6
SC1124	850.7	1949.2	31.8	65.8	3.9	18.6	4.2	4.1
TX 7078	877.6	2195.5	32.0	40.8	4.1	18.6	3.6	3.8

2.7 References

Ayeneh, A., M. Ginkel, M.P. Reynolds, and K. Ammar. 2002. Comparison of leaf, spike, peduncle, and canopy temperature depression in wheat under heat stress. Field Crops Res. 79: 173 – 184.

Bacon, M.A. 2004. Water Use Efficiency in Plant Biology. Blackwell Publishing Ltd., Boca Raton, FL.

Borrell, A.K., G.L. Hammer, and A.C.L. Douglas. 2000. Does maintaining green leaf area in sorghum improve yield under drought? I. Leaf growth and senescence. Crop Sci. 40: 1026 – 1037.

Comstock, J., and J. Ehrleringer. 1993. Stomatal response to humidity in common bean (*Phaseolus vulgaris*): implications for maximum transpiration rate, water-use efficiency and productivity. Aust. J. Plant Physiol. 20: 669 – 691.

Devi, M.J., T.R. Sinclair, and V. Vadez. 2010. Genotypic variation in peanut for transpiration response to vapor pressure deficit. Crop Sci. 50: 191 – 196.

Farquhar, G.D., J.R. Ehleringer, and K. Hubick 1989. Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 40: 503 – 537.

Fletcher, A.L., T.R. Sinclair, and L.H. Allen Jr. 2007. Transpiration responses to vapor pressure deficit in well watered 'slow-wilting' and commercial soybean. Environ. Exp. Bot. 61: 145 – 151.

Gholipoor, M, P.V.V. Prasad, R.N. Mutava, and T.R. Sinclair. 2010. Genetic variability of transpiration response to vapor pressure deficit among sorghum genotypes. Field Crops Res. 119: 85 – 90.

Hirasawa, T., and T.C. Hsiao. 1999. Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. Field Crops Res. 62: 53 – 62.

Jones, H. G. 1976. Crop characteristics and the ratio between assimilation and transpiration. J. Appl. Ecol. 13: 605 – 622.

Kato, Y., A. Kamoshita, and J. Yamagishi. 2008. Preflowering abortion reduces spikelet number in upland rice (*Oryza sativa* L.) under drought stress. Crop Sci. 48: 2389 – 2395.

Kholova, J., C.T. Hash, A. Kakkera, M. Kocova, and V. Vadez. 2010a. Constitutive water-conserving mechanisms are correlated with the terminal drought tolerance of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. J. Exp. Bot. 61: 369 – 377.

Kholova, J., C.T. Hash, P.L. Kumar, R.S. Yadav, M. Kocova, and V. Vadez. 2010b. Terminal drought-tolerant pearl millet [*Pennisetum glaucum* (L.) R. Br.] have high leaf ABA and limit transpiration at high vapour pressure deficit. J. Exp. Bot. 61: 1431 – 1440.

Lawlor, D.W., and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 25: 275 – 294.

Merah, O. 2001. Potential importance of water status traits for durum wheat improvement under Mediterranean conditions. J. Agric. Sci. 137: 139 – 145.

Mitchell, J.H., S. Fukai, and M. Cooper, 1996. Influence of phenology on grain yield variation among barley cultivars grown under terminal drought. Aust. J. Agric. Res. 47: 757 – 774.

Mitchell, J.H., D. Siamhan, M.H. Wamala, J.B. Risimeri, E. Chinyamakobvu, S.A. Henderson, and S. Fukai. 1998. The use of seedling leaf death score for evaluation of drought resistance of rice. Field Crops Res. 55:129–139.

Morison, J.I.L., and R.M. Gifford. 1983. Stomatal sensitivity to carbon dioxide and air humidity: A comparison of two C_3 and two C_4 grass species. Plant Physiol. 71: 789 – 96.

Mutava, R.N., P.V.V. Prasad, M.R. Tuinstra, K.D. Kofoid, and J. Yu. 2011. Characterization of sorghum genotypes for traits related to drought tolerance. Field Crops Res. 123: 10 – 18.

O'Toole, J.C. 1982. Adaptation of rice to drought-prone environments. *In*: Drought Resistance in Crops With Emphasis on Rice, IRRI, International Rice Research Institute, Los Banos, Philippines, pp. 195 – 213.

Prasad, P.V.V., S.A. Staggenborg, and Z. Ristic. 2008. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: Response of Crops to Limited Water:

Understanding and Modeling Drought stress Effects on Plant Growth Processes. L.R Ahuja, V.R. Reddy, S.A. Saseendran, and Qiang Yu. (Eds). American Society of Agronomy. Madison, WI, USA. pp. 301–356.

Richards, R.A., and J.B. Passioura. 1989. A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. Aust. J. Agric. Res. 40: 943 – 950.

Rosenberg, N.J., B.L. Blad, and S.B. Verma. 1983. Microclimate: The Biological Environment. 2nd ed., Wiley-Interscience, Hoboken, NJ.

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

Sinclair, T.R., G.L. Hammer, and E.J. van Oosterom. 2005. Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Funct. Plant Biol. 32: 945 – 952.

Timlin, D., J. Bunce, D. Fleisher, V.R. Reddy, Y. Yang, S.H. Kim, S.A. Saseendran, and B. Quebedeaux. 2008. Simulation of the effects of limited water on photosynthesis and transpiration in field crops: Can we advance our modeling approaches? In: Responses of Crops to Limited Water: Understanding and Modeling Drought stress Effects on Plant Growth Processes. L.R. Ahuja, V.R. Reddy, S.A. Saseendran, and Q.Yu. (Eds.). pp. 105 – 144. ASA-CSSA-SSSA, Madison, WI.

Tuinstra, M.R., G. Ejeta, and P.B. Goldsbrough. 1997. Heterogeneous inbred family (HIF) analysis: An approach for developing near-isogenic lines that differ at quantitative trait loci. Theor. Appl. Genet. 95: 1005 – 1011.

Xin, Z., C. Franks, P. Payton, and J.J. Burke. 2008. A simple method to determine transpiration efficiency in sorghum. Field Crops Research 107: 180 – 183.

Xin, Z., R. Aiken, and J.J. Burke. 2009. Genetic diversity of transpiration efficiency in sorghum. Field Crops Res. 111: 74 – 80.

Yoo, C.Y., H.E. Pence, P.M. Hasegawa, and M.V. Mickelbart. 2009. Regulation of Transpiration to Improve Crop Water Use. Crit. Rev. Plant Sci. 28: 410 – 431.

Chapter 3 - Canopy temperature as an indicator of crop water stress in grain sorghum genotypes under field conditions

Abstract

Canopy temperature measurements have been widely used to study response to drought in various crops. Studies with various crops including sorghum have identified genotypes that will impose a limitation on transpiration rate (TR) under high evaporative conditions and therefore use water conservatively, ensuring availability of water for a longer period of time during the grain filling stages. Evaluation of canopy temperature and its derivative, canopy temperature depression (CTD), in sorghum under field conditions may provide more insight into its drought tolerance mechanisms. The objectives of this study were: (i) to evaluate variability in canopy temperature of selected sorghum genotypes under field conditions over the growing season, (ii) to determine optimal CTD sampling conditions in terms of time of day and, (iii) to determine the relationships between CTD, yield and HI among sorghum genotypes. Twenty genotypes were selected based leaf temperature and grain yield and studied under irrigated and rainfed conditions. Infrared (IR) sensors were mounted to monitor canopy temperature during the growing season. Our results showed significant variation in mean CTD diurnal patterns under both rainfed and irrigated conditions with most negative CTD occurring at 8:00 am to 11:00 am and most positive at 4:00 pm to 7:00 pm. There were also genotypic differences in midday and predawn CTD with variation among the genotypes ranging from 0.89 to 1.96. There was a positive relationship between midday CTD and yield ($R^2 = 0.19$) and harvest index ($R^2 = 0.11$). CTD was stable for all the genotypes during the period between 1:00 pm and 7:00 pm and also 2:00 am to 8:00 am. The relationship between CTD and CWSI was negative ($R^2 = 0.34$) but positive between canopy temperature and CWSI ($R^2 = 0.50$). Yield and harvest index were significantly affected by moisture stress with decreases ranging from 35.6% to 93.5% for yield and 0.4% to 86.5% for harvest index.

3.1 Introduction

Projections for future climate changes indicate that water availability for crops in some regions may be decreased due to more infrequent rain events, long intervals between rain events, and less rainfall during the crop growing season (Allen et al., 2010). Crops with traits that are generally associated with drought tolerance may become more important in future climates and therefore specific plant traits that enhance tolerance to water-deficits are of interest. One of the key approaches that has been used to enhance crop yields during late-season water deficit conditions is conservation of water in the soil. Studies with various crops, soybean (*Glycine max* (L.) Merr.; Fletcher et al., 2007), peanut (*Arachis hypogaea* L.; Devi et al., 2010), pearl millet (*Pennisetum glaucum* (L.) R.Br.; Kholova et al., 2010), sorghum (*Sorghum bicolor* (L.) Moench; Mutava et al., 2011, Gholipoor et al., 2010), have shown genotypes that will impose a limitation on transpiration rate (TR) under high evaporative conditions and therefore use water conservatively. When this happens, canopies will tend to be warmer as the plants are not using asmuch water from the soil for transpirational cooling. A simulation study with sorghum to examine the putative benefits of restricted transpiration rate during high vapor pressure deficit (VPD) conditions showed yield gains with the largest benefit seen in dry, low yielding seasons (Sinclair et al., 2005).

A key role of transpiration in plants is leaf cooling, and therefore changes in canopy temperature relative to air temperature are an indication of the capability of transpiration in cooling plant leaves under a demanding environmental load. Atmospheric evaporative demand and consequently crop transpiration increase with increasing atmospheric VPD (Sinclair and Bennett, 1998). Atmospheric VPD and transpiration rates follow a diurnal pattern, being lowest at sunrise and increasing to a maximum at around 3:00 pm (Hirasawa and Hsiao, 1999). There are limits to increase in transpiration rate and a maximum rate is commonly reached at a VPD of ~2.0 kPa (Turner et al., 1984; Comstock and Ehrleringer, 1993). Bunce (1981) also showed the existence of decreased stomatal conductance (limitation on transpiration) in a number of crop species, including soybean, at

VPD between 1.0 and 2.5 kPa. A limit on maximum transpiration rate could be particularly important in rain-fed crop production systems where water could be limited. Continuous measurement of plant canopy temperature can therefore be used to provide a better understanding of the physiological processes related to canopy temperature and in turn drought stress.

Canopy temperature measurements have been widely used to study response to drought in various crops (Blum et al., 1989; Chaudhuri et al., 1986; Hatfield et al., 1987; Singh and Kanemasu, 1983). This approach is based upon the close, inverse relationship between leaf temperature and transpirational cooling (Jackson, 1982). Blum et al. (1989) used canopy temperatures of drought stressed wheat genotypes to characterize yield stability under various moisture conditions and found a positive correlation between drought susceptibility and canopy temperature in stressed environments.

Canopy temperature depression (CTD), the difference between air temperature (Ta) and canopy temperature (Tc), is positive when the canopy is cooler than the air (CTD = Ta – Tc). Various practical applications where CTD have been used include evaluation of plant response to environmental stress (Ehrler et al., 1978; Idso, 1982; Howell et al., 1986; Jackson et al., 1981; Singh and Kanemasu, 1983), irrigation scheduling (Hatfield, 1982; Pinter and Reginato, 1982; Evett et al., 1996; Wanjura et al., 1995), and the evaluation of cultivars for water use (Pinter et al., 1990; Hatfield et al., 1987), tolerance to heat (Amani et al., 1996; Reynolds et al., 1994), and drought (Blum et al., 1989; Royo et al., 2002; Rashid et al., 1999). CTD represents an overall, integrated physiological response to drought and high temperature (Blum, 1988; Amani et al., 1996) and has therefore been used generally to assess plant water status. Leaf orientation was shown to have a major role in soybean canopy temperature (Stevenson and Shaw, 1971). Soil water availability also affects canopy temperature (Campbell and Norman, 1998), and is influenced by root morphology and activity, especially under dryland conditions, e.g., for upland rice (*Oryza sativa* L.) (O'Toole et al., 1998). Overall, the existing literature suggests that the dominant mechanisms that increase CTD vary with environment and crop

species. Greater CTD has been associated with increased wheat yield under irrigated, hot environments (Amani et al., 1996; Reynolds et al., 1994; Reynolds, 2002; Fischer et al., 1998) and also under dryland environments (Blum 1988, Balota et al., 2007). High CTD has been used as a selection criterion to improve tolerance to drought and heat (Amani et al., 1996; Ayeneh et al., 2002; Blum, 1988; Blum et al., 1989; Pinter et al., 1990; Rashid et al., 1999; Reynolds et al., 1994; Fischer et al., 1998, Balota et al., 2007).

The crop water stress index (CWSI) has been used to quantify crop water stress based on canopy surface temperature. The calculation of this index is based on two baselines: the non-water-stressed baseline, which represents a fully watered crop, and the maximum stressed baseline, which corresponds to a non-transpiring crop (stomata fully closed). One method used in determining the CWSI is based on the definition by Idso (Idso et al., 1981), which is a derivative from the empirical relationship between the canopy–air temperature differences ($T_c - T_a$) and the air vapor pressure deficit (VPD) for a well-watered crop.

Variations in canopy temperature and CTD are part of the drought stress coping mechanisms in plants. Plants that will impose a limitation on their transpiration rate and therefore maintain a warmer canopy are conservative in the way they use available water and therefore will be drought tolerant. On the other hand there are plants that use more water for transpirational cooling (higher stomatal conductance) and therefore will have cooler canopies and higher CTD. This is a short-term drought escaping mechanism. In a study with 300 sorghum genotypes (Mutava et al., 2011) we found indications of these two mechanisms in sorghum. There were sorghum genotypes that had high leaf temperature (tolerance mechanism) with high yield and others that had low leaf temperature (escape mechanism) and high yields. A further analyses of these genotypes revealed that those with high leaf temperature had a breakpoint (BP) in their transpiration rate with increased VPD but this was absent in genotypes with low leaf temperature (Gholipoor et al., 2010).

Even though canopy temperature monitoring has been used as a tool to screen for drought tolerance in various crops, no studies have been done on sorghum. Sorghum is a drought tolerant crop, and evaluation of

canopy temperature and its derivatives under field conditions may provide more insight into its drought tolerance mechanisms. Our hypothesis in this study was that continuous measurement of canopy temperature can be used to quantify differences in CTD and provide an understanding of how canopy temperature relates to yield and water use in sorghum. The objectives were: (i) to evaluate variability in canopy temperature of some selected sorghum genotypes under field conditions over periods of the growing season (ii) to determine optimal time of day for canopy temperature sampling and CTD, and (iii) to determine the relationship between canopy temperature, CTD and yield and HI among sorghum genotypes.

3.2 Materials and methods

3.2.1 Location and crop husbandry

This experiment was conducted at the Kansas State University, Department of Agronomy farm at Ashland Bottoms (Manhattan, Kansas, USA) in 2010 and 2011. In 2010 we had a rainfed plot and an irrigated plot, while in 2011 we had only a rainfed plot. In 2010 and 2011, the plots were chisel plowed in the fall and field cultivated in the spring. Dates of planting were June 23 and June 7 for the rainfed plots in 2010 and 2011, respectively, and May 25 for the irrigated plot in 2010. Fertilizer application was applied at a rate of 90 kg ha^{-1} N in both years. Herbicides used for pre-emergence weed control for both years were: Calisto® (active ingredients: Mesotrione 40% [0.48 kg ai L^{-1}], other ingredients 60%) at a rate of 0.37 Lha^{-1} and Bicep II Magnum® (active ingredients: Atrazine 33.0%, Atrazine related compounds 0.7% [0.37 kg ai L^{-1}], S-metolachlor 26.1% [0.29 kg ai L^{-1}], other ingredients 40.2%) (Syngenta Crop Protection Inc., Greensboro, North Carolina, USA) at a rate of 2.75 Lha^{-1} in the irrigated plot and Lumax® (active ingredients: S-metolachlor, 29.4% [0.32 kg ai L^{-1}], Atrazine, 11.0% [0.032 kg ai L^{-1}], Mesotrione, 2.94% [0.12 kg ai L^{-1}], other ingredients, 56.66%) (Syngenta Crop Protection Inc., Greensboro, North Carolina, USA) at a rate of 2.9 Lha^{-1} in the rainfed plots. Hand weeding was performed as necessary to keep plots free of weeds.

3.2.2 Plant material and data collection

Twenty genotypes were selected based on leaf temperature and grain yield as identified in Mutava et al. (2011) (Table 3-1). The genotypes were planted in four rows of 6.1 m long with 0.75m spacing between rows. There were 120 seeds sown per row for each genotype providing a seeding density of about 250,000 seeds per ha. The experimental design was randomized complete block design (RCBD) with three replications.

3.2.2.1 Growth measurements

In 2010 and 2011, five plants from each genotype were harvested at vegetative (8 leaf stage), booting/flowering and seed filling (hard dough) stages for biomass determination. These plants were dried at 60˚C for seven days and weighed. Photosynthetically active radiation (PAR), leaf area index (LAI) and extinction coefficient (τ) were measured using a Ceptometer (AccuPAR LP-80, Decagon Devices Inc., Pullman, Washington, USA) at the same growth stages. These measurements were taken at 12:00 pm – 4:30 pm on clear, sunny days.

3.2.2.2 Physiological traits

At early vegetative development in 2011, a single representative plant from each genotype was tagged in each replication from which measurements were made on photosynthesis, transpiration, stomatal conductance and leaf temperature using a handheld photosynthesis system (CI-340, CID Bio-Science, Inc. Camas, Washington, USA) and also chlorophyll content using a chlorophyll meter (Model 502, Spectrum Technologies, Plainfield, Illinois, USA). Data on these parameters was collected three times (July 27, August 9 and August 23). Measurements were taken on the tagged plants from the top most fully expanded leaf. This was done within a single day for each plot. All measurements were taken between 12:00 pm and 4:00 pm on a clear sky day.

3.2.2.3 Canopy temperature and soil moisture

When the canopy was fully developed, Smartcrop® IR sensors (Smartfield, Lubbock, Texas, USA) were installed in the field at a height of 30 cm above the canopy and through a base station ambient temperature,

canopy temperature and relative humidity were monitored continuously throughout the remainder of the growing season. The sensor height was regularly adjusted throughout the growing season so as to maintain it at a 30 cm above the canopy. Soil moisture was monitored throughout the growing season at 30, 60, and 100 cm depths using watermark soil moisture sensors (Spectrum Technologies Inc. Plainfield, Illinios, USA).

3.2.2.4 Yield and harvest index

At maturity in 2010 five plants were hand harvested from each plot, the stem and leaves were dried at 60˚C for seven days and weighed. Panicles were oven dried at 40˚C for three days, threshed and seeds were weighed and counted to determine yield and yield components. The middle two rows were machine harvested for yield. In 2011 five plants, including the tagged plant were harvested and above ground biomass (stem and leaves) was oven dried at 60˚C for seven days and weighed. Harvested panicles from the 5 plants were oven dried at 40˚C for three days, threshed and seeds were weighed. Data were used to calculate the ratio of grain produced to the total above ground biomass (harvest index) and also compute yield expressed as kg ha^{-1}.

3.2.3 Data analyses

Data analyses were done using PROC GLM, PROC REG and PROC GLM with repeated measures in SAS (SAS Inc., Version 9.2). Analysis was based on an average of data from the three measurements. Means, SE and LSDs were determined for all traits. Analysis of variance (ANOVA) was used to compare genotype means based on P-value at $\alpha = 0.05$.

Vapor pressure deficit was calculated as the difference between the saturation vapor pressure (e_s) and the actual vapor pressure (e_a); (e_s - e_a). Variables that were used to compute VPD include the ambient air temperature and relative humidity. The saturation vapor pressure at a desired temperature was calculated using equation (1) (Prenger and Ling, 2001):

$$VP_{sat} = e^{A/T+B+CT+DT^2+ET^3+FlnT} \quad (1)$$

where $A = -1.88x10^4$, $B = -13.1$, $C = -1.5x10^{-2}$, $D = 8x10^{-7}$, $E = -1.69x10^{-11}$, $F = 6.456$, T – Temperature of the air in K, $T(°K) = T(°C) + 273.15$. The saturated vapor pressure can be computed for both the ambient and canopy temperatures. Actual partial pressure of the water vapor in the air was calculated using equation (2) and VPD using equation (3):

$$VP_{air} = VP_{sat} \times \frac{RH}{100} \quad (2)$$

$$VPD = VP_{sat} - VP_{air} \quad (3)$$

The calculation of CWSI was based on Idso's definition and the parameters used were crop canopy temperature (T_c), air temperature (T_a), and air VPD (Eq. 4). According to the Idso's definition (Idso et al., 1981), the CWSI can be expressed:

$$CWSI = \frac{(T_C - T_a) - D_2}{D_1 - D_2} \quad (4)$$

where D_1 is the maximum canopy and air temperature difference for a stressed crop (the maximum stressed baseline), D_2 is the lower limit canopy and air temperature difference for a well-watered crop (the non-water-stressed baseline), Tc the measured canopy surface temperature (°C), and Ta the air temperature (°C).

3.3 Results

3.3.1 Growing conditions

Precipitation data show that there was more rainfall in 2010 than 2011 (Table 3-2). In 2011 the distribution of the total rainfall received was poor, about 45% (121 mm) was received in June, as compared to 2010 when 38% was received in the same month (Table 3-2 and Figure 3-1.2B). In 2011 maximum air temperature were higher in July and August when as compared to the same period in 2010 (Figure 3-1 and Figure 3-2A). In 2011, most of July and some parts of August can be described as dry and hot. This is a critical

time for sorghum because it coincides with flowering and seed set for most of the genotypes used in this study. In 2010, rainfall distribution was good (Table 3-2 and Figure 3-1) with adequate rainfall in June, July and August. Plants in 2010 therefore did not experience the moisture and heat stress that was experienced in 2011.

Volumetric soil water content was low during the first and second week of July a duration that corresponds to the mid to late stages of vegetative growth (Figure 3-2B). This can be attributed to high evaporation rates because of more bare ground because the crop canopy was not fully developed. Even though we had a substantial amount of rainfall in the first week of July, recharging of soil water was gradual at the 30, 60 and 100 cm depths where measurements were made.

3.3.2 Physiological traits

There was significant variation at the genotype and date of measurement for the physiological traits except intercellular carbon dioxide concentration (Ci). Leaf temperature, photosynthetic rate, transpiration rate, stomatal conductance and Ci differed with date of measurement. Leaf temperature, transpiration rate, stomatal conductance and photosynthetic rate varied significantly among genotypes (Table 3-3). The interaction between genotypes and dates of measurement was significant only for photosynthetic rate and stomatal conductance.

Genotypes SC720 and SC60 were among those that recorded highest mean leaf temperature readings while the lowest were in DeKalb 54-00, Pioneer Hybrid 84G62 and B.Tx2752. Pioneer 85G46 and SC1124 had the highest value in photosynthetic rate, transpiration rate and stomatal conductance while genotype Hegari recorded low values for photosynthetic rate, transpiration rate and stomatal conductance. SC701 was low in photosynthetic rate and B.Tx2752 had low values for transpiration rate and stomatal conductance.

Ci was positively related to stomatal conductance, transpiration rate and photosynthetic rate (Figure 3-3). This relationship was stronger for stomatal conductance and transpiration rate than for photosynthetic rate (Figure 3-3C, B and A, respectively).

3.3.3 Growth and yield traits

Analysis of variance showed that growth and yield traits varied significantly both at the genotype level and growth stage at which they were taken but the interaction between genotype and growth stage was not significant (Table 3-4). Genotype differences in all growth traits; extinction coefficient (τ), leaf area index (LAI), intercepted photosynthetically active radiation (IPAR) and biomass were highly significant at all growth stages. LAI and τ were also both highly significant among genotypes, while IPAR and biomass also differed among genotypes. Genotypic variation was significant for yield and harvest index.

Genotypes Tx7078, PI584085 and B.Tx2752 were among those with high values for τ while hybrids Pioneer 85G46, 84G62 and DeKalb 54-00 had the lowest values. LAI ranged from 2.45 – 4.64 m^2 m^{-2} with hybrids Pioneer 85G46, 84G62 and DeKalb 54-00 recording the highest values and Tx7078, PI584085 and SC701 had the lowest values (Table 3-4). Genotypes with the highest values for IPAR were R.Tx436, SC60, DeKalb 2829, Liang tang ai and SC1019 while Pioneer 84G62 and B.Tx2752 had the lowest values. Biomass, based on 5 plants, ranged from 175 – 337g. Genotypes with the highest biomass were Pioneer 85G46, SC1019 and Hegari while those with the lowest biomass were Tx7078 and DeKalb 2829.

Harvest index and yield varied significantly among the genotypes. Genotypes with the highest HI and also yields were Pioneer 84G62 and 85G46 and PI584085 (Table 3-4). Other genotypes that were also high yielding included. Genotypes with low HI and poor yields were Hegari, TX7078 and SC663.

For all the genotypes LAI reached maximum in August with a decline in October (Table 3-5). IPAR was at a maximum in July with some decline in August and significant decline in October. Genotypes with significant decline in LAI include DeKalb 2829, Liang Tang Ai and SC60. Biomass, based on five plants per genotype, increased with time and was highest in October for all the genotypes (measurements for October are total biomass – leaves, stems and panicles). In July genotypes with the highest biomass were SC1124, SC1019, 85G46 and SC701 while genotypes with the lowest included B.Tx2752, DeKalb 2829 and TX7078. Pioneer

hybrid 85G46, Liang Tang Ai, SC60 and SC701 had the highest biomass in August while DeKalb 2829, TX7078 and B.Tx615 had the lowest biomass. Towards the end of the season (October) highest biomass was recorded by Hegari, DeKalb 54-00, 85G46 and SC1019 while SC701, DeKalb 2829 and TX7078 recorded the lowest values.

Yield and HI were affected by low soil moisture conditions under rainfed conditions in 2011 when compared to 2010 (Figure 3-4). Reduction in yield from 2010 to 2011 ranged from 36% to 93% with genotypes that suffered the greatest reduction being Hegari, TX7078, DeKalb 2829 and SC720. Genotypes SC1124, SC60, SC1019 and PI584085 had the lowest reductions in yield while genotype SC701 did not suffer a reduction but recorded an increase of 26%. It should be noted though that SC701 yields were very low for both years. HI was also affected the same way with reduction ranging from 0.4% to 86%. Genotypes with the smallest reduction in HI were SC1019, Pioneer hybrid 85G46 and DeKalb 2829 and those with the highest reduction included SC720, SC663 and Hegari. Some genotypes that recorded an increase in HI included R.Tx436, SC224, PI584085 and SC60.

3.3.4 Canopy temperature

Canopy temperature varied during the course of 24 h with the same trend in 2010 rainfed and irrigated and 2011 rainfed plots. Temperatures were lowest between 5:00 am and 7:00 am followed by a steady increase till about 3:00 pm when they peaked and then decreased during the night hours (Figure 3-5). Significant differences were observed among genotypes in the afternoon hours (12:00 – 7:00 pm).

The irrigated plot recorded higher air temperatures and consequently higher canopy temperature than the rainfed plot in 2010 (39.5°C and 32.4°C respectively at 3:00 pm) (Figure 3-5 and supplementary Tables 3-1 and 3-2). Air temperatures were also higher in 2011 rainfed plot (33.0°C) when compared to 2010 rainfed plot (32.4°C) (Figure 3-5 and supplementary Tables 3-1 and 3-3). In 2010 most genotypes in the irrigated and rainfed plots reached maximum canopy temperature during the time between 1:00 and 3:00 pm while in 2011

this was between 3:00 and 5:00 pm in rainfed plots. Minimum canopy temperature was at the hours 5:00 to 7:00 am during the two years for the irrigated and rainfed plots.

3.3.5 Canopy temperature and yield

Canopy temperature varied among genotypes. In 2010, DeKalb 2829 and Pioneer 85G46 recorded high canopy temperature with high yields (Figure 3-6A). Inbred lines that showed higher canopy temperature and also high yields include B.Tx399, PI584085, R.Tx436 and SC1019. Genotypes TX7078 and Hegari had low canopy temperatures and low yields. SC701 and SC1124 had high canopy temperature but with low yield Figure 3-6A.

In 2011, genotypes that recorded high canopy temperature were SC701, SC1019, SA5330 and TX7078 with SC1019 also recording high yield (Figure 3-6B). Low canopy temperature genotypes were Pioneer hybrids 85G46, 84G62 as well as SC1124 and SC663. Among these the hybrids had high yields.

3.3.6 Canopy temperature and vapor pressure deficit (VPD)

Canopy temperature increased with increases in VPD for all the genotypes in 2010 and 2011 (Figure 3-7). The increase was curvilinear with canopy temperature almost reaching a plateau at about 3 kPa in irrigated plots in 2010 (Figure 3-7C). Although this did not reach a plateau as such in the rainfed plots in both 2010 and 2011, the trend was similar (Figure 3-7A & B). There was a decrease in the rate of increase in canopy temperature with increasing VPD after about 1 kPa in the rainfed plots. Graph plots for the different genotypes are provided in the supplementary figures (Supplementary Figure 3-1, 3-2 & 3-3). Also there was a wider range in genotype canopy temperature response to increase in VPD in the irrigated plots than in the rainfed plots (Figure 3-7 - Inset graphs).

3.3.7 Canopy temperature depression (CTD)

Mean CTD diurnal patterns measurement periods varied under both rainfed and irrigated conditions (Figure 3.8). In all environments, CTD was most negative at 8:00 to 11:00 am and most positive at 4:00 to 7:00

pm. The most positive CTD values recorded in 2010 irrigated plot were for Pioneer 84G62 and B.Tx2752 in the rainfed plot both at 6:00 pm while the most negative values were for SC224 at 8:00 am and Pioneer 85G46 at 9:00 am in the irrigated and rainfed plots respectively. In 2011 rainfed plots, the most positive value was for Pioneer 84G62 at 7:00 pm and most negative was for TX7078 at 1:00 pm and also PI584085 at 10:00 am (Figure. 3-8, Suppl. Tables 4-6). In 2010 irrigated plots CTD at 8:00 am ranged from -0.18 to 2.70 while at 7:00 pm the range was from 1.42 to 5.75 (Figure 3-8C). For the rainfed plots in 2010, the ranges were from -0.29 to 1.96 at 10:00 am and 1.78 to 3.15 at 7:00 pm (Figure 3-8B). In 2011 rainfed plots CTD ranges were -0.74 to 0.26 at10:00 am and 0.35 to 3.08 at 6:00 pm (Figure 3-8A).

Midday and predawn CTD varied between the genotype based on 2011 data (Figure 3-9). Midday CTD values seemed to be stable in their increase with time. Genotypes that had high values at 6:00 pm were Pioneer hybrids 84G62, 85G46 and also SC1124 and SC663, while SC701 and SA5330 had the lowest values. The trend was similar for predawn CTD where genotypes with high values included Pioneer hybrid 85G46 and Hegari while Pioneer 84G62 had the lowest value throughout the predawn hours.

Midday CTD was positively related to yield and HI (Figure 3-10) in 2011. Mid-day canopy temperature was negatively correlated to yield and harvest index in 2010 (Figure 3-11A & B) but in 2011 this did not exist for both traits (Figure 3-11C & D).

3.3.8 Crop water stress index (CWSI)

Figure 3-12A shows the variation in average CTD among the genotypes ranging from 0.89 (TX7078) to 1.96 (Pioneer 85G46). CWSI also varied significantly among the genotypes (Figure 3-12B). Genotypes with high CWSI being TX7078, PI584085, B.Tx615, B.Tx399 and B.Tx2752 while Pioneer 85G62 had the lowest CWSI value. There was a linear relationship between CTD and CWSI as well as canopy temperature and CWSI (Figure 3-13). This relationship was negative between CTD and CWSI (Figure 3-13A) and positive between canopy temperature and CWSI (Figure 3-13B).

3.4 Discussion

This study identified significant variation among sorghum genotypes for physiological, growth and yield traits and also demonstrated that IR sensors can be used as a tool to evaluate changes in canopy temperature and help determine how this relates to changes in VPD over the course of the day. This information is important in crop production and more so when we are looking at water use and mechanisms involved in drought tolerance.

3.4.1 Physiological traits

Leaf temperature, photosynthetic rate, transpiration rate, stomatal conductance and intercellular carbon dioxide concentration (C*i*) are interrelated physiological processes, and in our study they differed ($P < 0.0001$) with the date on which measurements were made and with genotypes ($P<0.0001$) in photosynthetic rate (Table 3-3). Genotype x environment interaction was significant for photosynthetic rate and stomatal conductance ($P = 0.0150$ and 0.0015 respectively). Different dates corresponded to different environmental conditions and crop growth stages, therefore the variability in physiological traits. Intercellular carbon dioxide concentration values ranged from 100 – 155 μmol mol^{-1} and were positively correlated to stomatal conductance, transpiration rate and photosynthetic rate (Figure 3-3). The regression between C*i* and stomatal conductance (Adj. $R^2 = 0.550$) and also transpiration rate (Adj. $R^2 = 0.419$) were higher than with photosynthetic rate (Adj. $R^2 = 0.210$) (Figure 3-3C, B and A respectively). These measurements taken under field conditions agree with what is known about C_4 plants where the high affinity of phosphoenolpyruvate (PEP) carboxylase for CO_2 allows assimilation rates to be saturated at lower concentrations than in C_3 species (Laisk and Edwards, 1998). Although there are exceptions, photosynthesis in C_4 plants often is saturated at C*i* of 100–150 μmol mol^{-1} (Laisk and Edwards, 1998). This explains the lower adjusted R^2 value for the regression between C*i* and photosynthetic rate. Stomatal conductance levels resulting in C*i* values above the saturation level would therefore lead to more water used by the plant without increasing net photosynthetic rates. In our study some genotypes, SC1019, SC224 and PI584085, had stomatal conductance values that were statistically lower than Pioneer hybrid 85G46 (Table 3-3)

yet there was no difference in their yields (Table 3-4). This implies that it is possible to regulate stomatal conductance and transpiration rate in C_4 crops such as sorghum without reducing net photosynthetic rate and therefore increase their WUE without sacrificing yield potential. Lower stomatal conductance may result in lower transpirational cooling and hence warmer canopies which would be an indication of conservative water use which is a drought tolerance mechanism that has been identified in sorghum.

3.4.2 Growth and yield traits

Yield and HI were affected by low soil moisture in 2011 when compared to 2010 with reductions ranging from 36% to 93% for yield and 0.4% to 86% for HI (Figure 3-5). This reduction in yields is indicative of stress conditions during 2011. The periods of high temperature and low water moisture during the vegetative development stages and also high temperatures at flowering stages had negative impacts on the performance of all genotypes. Drought and heat stress have been shown to result in decreased leaf size, stem growth and plant height (Prasad et al., 2006). Uninterrupted vegetative and reproductive development often is necessary to improve reproductive potential and also to maintain leaves and tillers that act as a source supplying assimilate during grain filling in cereal crops. Studies also have shown that decreased leaf area due to drought before anthesis is correlated with reductions in the number of kernels per spike (Frederick and Camberato, 1995). Drought and heat stress during panicle or flower development will cause a decrease in seed numbers due to abortion (Wheeler et al., 2000; Prasad et al., 2008). Grain yield formation is most often sink limited, rather than source limited, as observed for maize (*Zea mays* L.), wheat and soybean (Borras et al., 2004). Even though biomass production and potential yield in sorghum tends to be source limited (Gambin and Borras, 2007), grain numbers and therefore yield tend to be sensitive to stress due to moisture limitation. Grain yield is influenced by a complex of different morphological and physiological traits which are in turn influenced by water availability. Environmental factors that influence yield will also affect HI in a similar manner.

3.4.3 Canopy temperature as a drought tolerance mechanism

Mean air and canopy temperature values were higher in the rainfed plots for the year 2011 than for 2010 (Figure 3-6A & B), an indication that plants were potentially under more stress in 2011 than 2010. This was confirmed by the reductions in yield when the two years were compared (Figure 3-5). In 2010, under rainfed conditions, Pioneer Hybrid 85G46 and DeKalb 2829 recorded elevated canopy temperatures and also high yields as well as genotypes B.Tx399, PI584085, R.Tx436 and SC1019 (Figure 3-7A). In 2011 Genotype SC1019 recorded high canopy temperature and also high yields under rainfed conditions (Figure 3-7B). This genotype had been selected for high leaf temperature and high yields, and in this study it proved to be drought tolerant in 2011, a year with limited moisture and high temperatures.

Warmer canopies are associated with low stomatal conductance and high-transpiration efficiency, attributes that are favorable for drought adaptation. Pinter et al. (1990) found that wheat cultivars with the warmest canopy temperatures under well watered conditions not only had the lowest leaf conductance and the lowest seasonal water use under normal irrigation practices but also had the most favorable yield response when subjected to water deficit conditions. Genotype SC1019 had warmer canopy and produced high yields in 2011. Therefore we can conclude that it may have water conserving ability because the warmer canopy could be due to reduced transpirational cooling under well-watered conditions. The low crop water use will allow this genotype to conserve more water during periods of drought. Based on these observations we can conclude that this is a drought tolerant genotype. Genotype TX7078 is a case whereby, under moisture stressed conditions in 2011, elevated canopy temperature was accompanied by yield reduction. This is because this genotype was not able to maintain adequate transpiration rates and therefore transpirational cooling was reduced resulting in a reduction in photosynthetic rates and therefore reduction in yields Similar results have been reported by Chaudhuri and Kanemasu (1982) and McKinney et al. (1989) for soybean and Blum et al. (1989) for wheat.

3.4.4 Canopy temperature depression (CTD) as a drought escape mechanism

This study showed significant variation in mean CTD diurnal patterns under both rainfed and irrigated conditions (Figure 3-9) with most negative occurring at 8:00 am to 11:00 am and most positive at 4:00 pm to 7:00 pm. There were also genotypic differences in midday and predawn CTD (Figure 3-10). In our study measurements were taken over a period of 24 hours and consistent differences were noted starting from 11:00 am to 6:00 pm (Figure 3.10). Nighttime measurements may provide more stable conditions for CTD comparison among genotypes because we also saw consistent differences among genotypes between 2:00 am and 8:00 am. Other studies comparing genotypes measured CTD anywhere from 10:00 am to 4:00 pm (Amani et al., 1996; Pinter et al., 1990; Reynolds et al., 1994). This suggests that CTD measurements must be taken during relatively short intervals during the day to differentiate genotypes, effectively reducing the number of entries that can be compared.

Differences seen in our study in different environments concur with the suggestion by Balota et al. (2007) that CTD must be determined for individual environments. Some suggestions have been for CTD measurements under drought stress conditions to identify cooler canopies because higher associated transpiration rates indicate greater growth and yield (Blum et al., 1982; Gardner et al., 1986; Mtui et al., 1981; Sojka et al., 1981), while others have suggested measuring CTD under well-watered conditions to identify warmer canopies because smaller associated transpiration rates indicate greater water conservation and therefore more water for growth and reproduction later in the season (Chaudhuri et al., 1986; Kirkham et al., 1984; Pinter et al., 1990). Based on these suggestions, selection of drought tolerant genotypes based on the positive correlation between CTD and yield may leave out slow wilting genotypes that will have warmer canopies (lower CTD) but are conservative in the way they use water. Even though our study showed a positive relationship between CTD and yield and also harvest index (Figure 3-11), the R^2 values were low (0.19 and 0.11 respectively). Canopy temperature also showed a weak negative correlation with yield and HI in 2010 but

the stress conditions in 2011 did not display this relationship. This could be explained by that fact that the genotypes in this study had been selected based on leaf temperature and yield (Table 3-1).

3.5 Conclusions

Our study demonstrated that there were significant genotypic differences in growth and yield traits. Moisture and heat stress in 2011 reduced yield and HI in most of the genotypes. Physiological and growth traits (except Ci which was only significant for date of measurement) varied significantly among genotypes and date of measurement. There was distinct separation of genotypes based on canopy temperature and CTD. The IR sensors used were able to detect differences in canopy temperature among genotypes, enabling us to determine variation in mean CTD diurnal patterns under both rainfed and irrigated conditions. The study also showed that the period between 1:00 pm and 7:00 pm and also 2:00 am to 8:00 am CTD was stable for all the genotypes and therefore these are the best times to take CTD readings for sorghum. CTD behaved differently under different environmental conditions and therefore this parameter should be determined for individual environments. Even though midday CTD was positively correlated to yield and HI it should be noted that there are genotypes that will have low CTD resulting in warmers canopies because they are conservative in the way they use water. Use of CTD when screening for drought tolerance needs to take this into consideration so as not to leave out these genotypes since they are also drought tolerant.

3.6 Figures and Tables

Table 3-1: List of genotypes used and their selection criteria

Genotype	Group
84G62†	-
SC663	Normal leaf temperature, high grain yield
SC1019	High leaf temperature, high grain yield
B.Tx2752	Low leaf temperature, high grain yield
SC720	High leaf temperature, high grain yield
Dekalb 2829	-
B.Tx399	Normal leaf temperature, high grain yield
SA5330	Low leaf temperature, low grain yield
SC1124	High leaf temperature, low yields
Liang Tang Ai†	-
DeKalb 54-00†	-
Hegari	Low leaf temperature, high grain yield
R.Tx436	Low leaf temperature, low grain yield
Tx7078	-
SC60	Low leaf temperature, high grain yield
B.Tx615	Low leaf temperature, low grain yield
SC224	Low leaf temperature, low grain yield
PI584085†	-
85G46†	-
SC701	Low leaf temperature, high grain yield

†These genotypes were not in the initial study and therefore their group was not known during this study.

Table 3-2: Summary of rainfall and temperature conditions in Manhattan 2010 and 2011 (June to September)

	Maximum air temperature (°C)		Minimum air temperature (°C)		Total Precipitation (mm)	
Month	**2010**	**2011**	**2010**	**2011**	**2010**	**2011**
June	31.2	31.5	19.3	18.8	168.1	121.2
July	32.4	36.5	21.8	23.2	106.4	52.8
August	33.9	34.5	19.9	20.2	81.3	59.2
September	29.1	26.7	14.9	11.6	76.2	37.1
				Total	**432.1**	**270.3**

Figure 3-1: Weather conditions (temperature and precipitation) at Manhattan: 2010

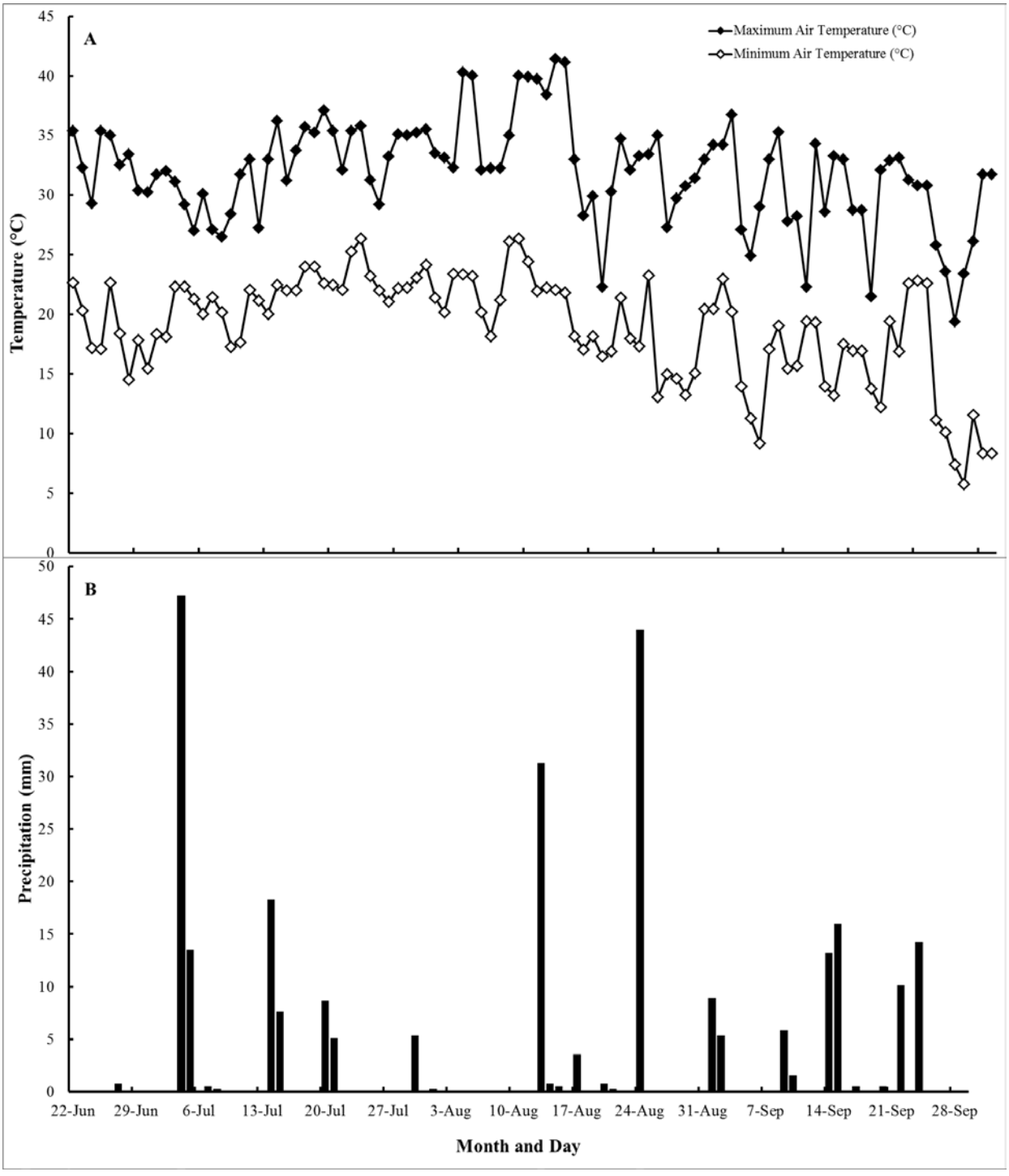


Figure 3-2: Weather conditions (precipitation and temperature) and soil water moisture at Manhattan: 2011

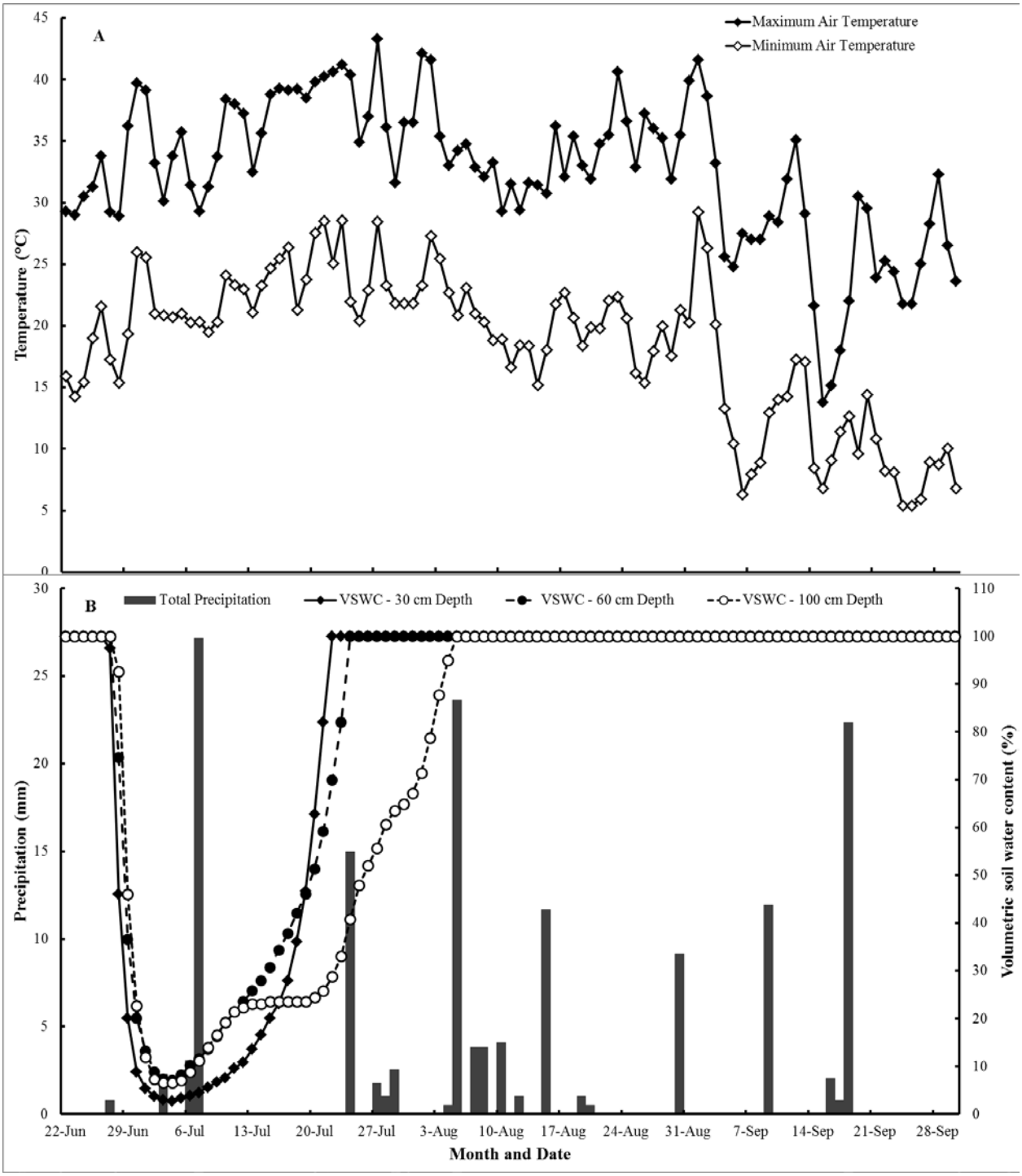


Table 3-3: Means for physiological traits showing significance among genotypes and dates of measurement

Genotype	Leaf temperature† (°C)	Photosynthetic rate (µmol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Stomatal conductance (mmol m^{-2} s^{-1})	Intercellular CO_2 concentration (Ci) (µmol mol^{-1})
85G46	41.4	39.67	8.28	264.2	119.3
SC1124	41.1	40.19	8.13	248.6	125.0
Dekalb 54-00	40.4	36.94	7.37	229.1	134.1
84G62	40.4	35.59	6.96	203.3	109.7
SC663	41.5	34.34	7.97	221.8	144.2
DeKalb 2829	40.6	33.00	6.88	194.4	128.2
B.Tx399	40.3	32.11	6.61	191.5	99.6
Liang Tang Ai	40.6	32.31	7.11	214.8	103.4
SC1019	41.9	32.09	7.11	195.1	115.9
PI584085	41.2	30.35	6.87	187.2	117.3
R.Tx436	41.4	30.19	6.87	176.5	119.7
SC224	42.0	28.88	6.60	169.7	145.4
SC720	42.3	29.95	7.17	174.0	107.5
TX7078	42.0	28.17	6.09	170.7	135.1
SC60	42.1	26.53	6.63	166.7	131.8
B.Tx2752	39.7	25.16	4.58	132.3	105.0
SA5330	41.7	26.04	6.12	165.1	129.6
B.Tx615	40.8	24.43	5.97	157.0	155.0
Hegari	41.6	20.40	5.07	139.4	134.2
SC701	42.0	18.34	6.25	154.7	126.3
LSD $_{(\alpha = 0.05)}$	**1.67**	**8.25**	**1.48**	**57.65**	**52.08**
P-Value					
Genotype	**0.0246**	**<0.0001**	**0.0005**	**0.0015**	**0.7018**
Date	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Genotype*Date	**0.9989**	**0.0150**	**0.0917**	**0.0015**	**0.3757**

†This was leaf temperature taken at the leaf level using a CID photosynthesis system.

Figure 3-3: Relationships between photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration.

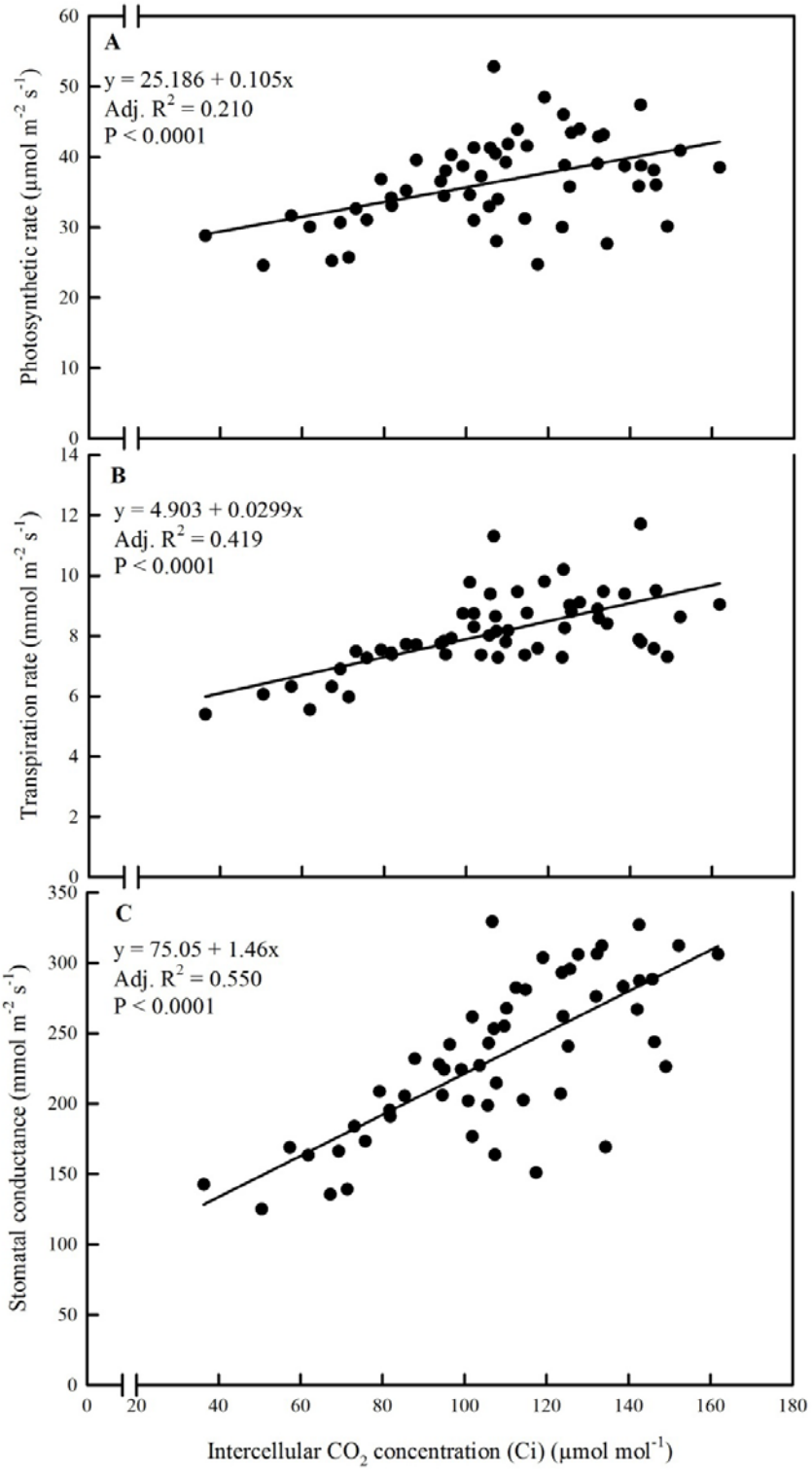


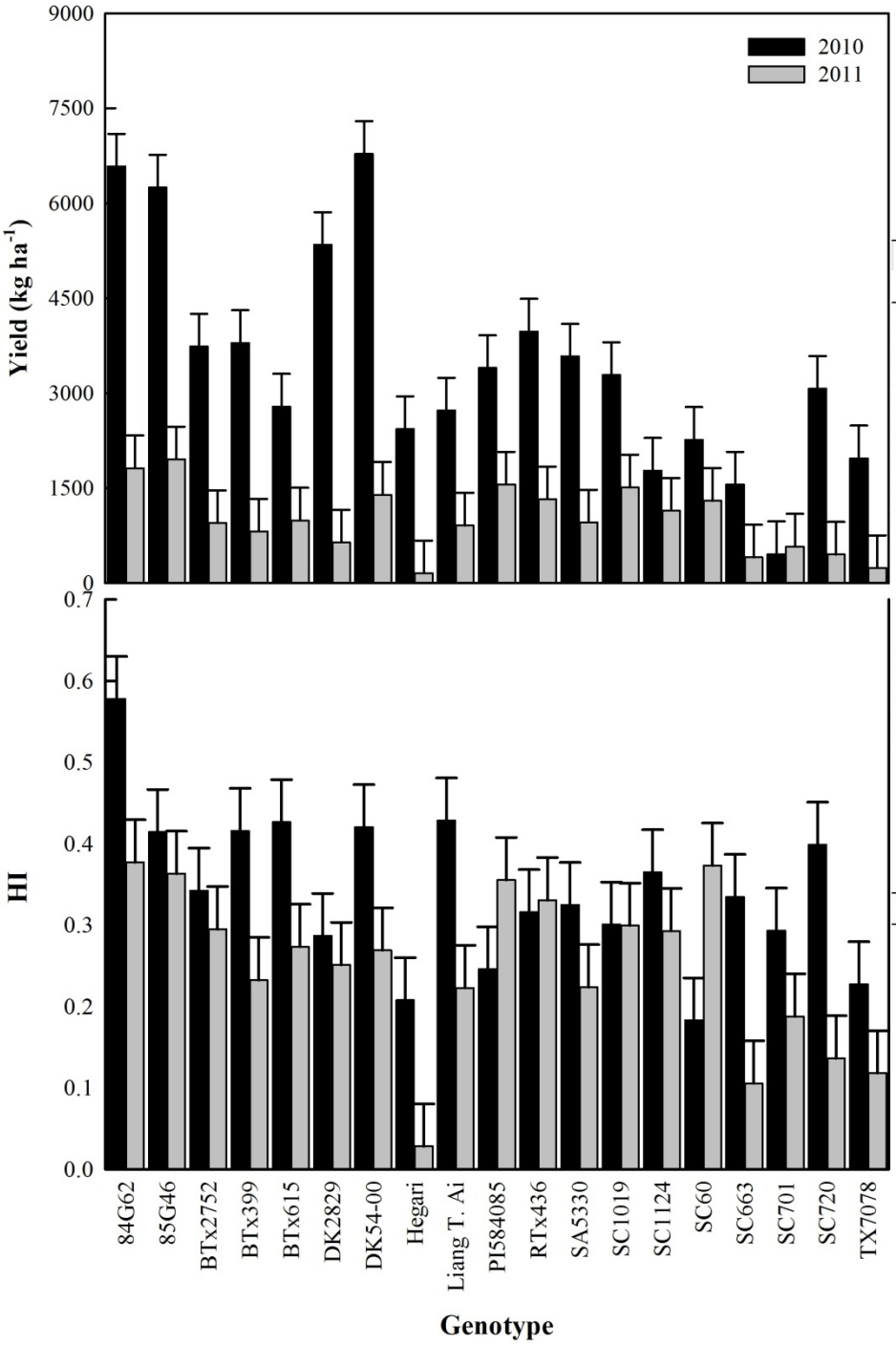
Table 3-4: Means for growth traits of various genotypes along with LSD and P-values: 2011

Genotype	Extinction coefficient (τ)	Leaf area index	Intercepted PAR	Biomass (5 plants) g	Harvest Index	Yield (kg ha^{-1})
85G46	0.12	4.64	573.7	337.5	0.36	1954.7
84G62	0.16	4.53	494.3	285.6	0.38	1814.7
B.Tx2752	0.34	2.67	519.3	213.2	0.29	948.0
B.Tx399	0.26	3.12	586.9	232.9	0.23	815.3
B.Tx615	0.33	2.66	602.3	226.3	0.27	989.0
DeKalb 2829	0.20	3.93	661.0	188.6	0.25	643.3
Dekalb 54-00	0.15	4.39	541.1	306.8	0.27	1395.0
Hegari	0.21	3.48	600.3	325.2	0.03	157.0
Liang Tang Ai	0.18	3.72	650.5	283.2	0.22	911.0
PI584085	0.34	2.63	566.4	264.7	0.36	1557.7
R.Tx436	0.29	2.98	676.7	263.0	0.33	1323.7
SA5330	0.30	2.85	639.8	257.1	0.22	957.3
SC1019	0.17	4.08	651.5	310.5	0.30	1512.0
SC1124	0.24	3.31	632.8	270.9	0.29	1144.0
SC224	0.21	3.61	594.2	283.0	0.23	1019.7
SC60	0.18	3.69	657.6	266.3	0.37	1300.0
SC663	0.24	3.51	614.2	254.5	0.11	405.7
SC701	0.33	2.64	639.5	241.3	0.19	576.3
SC720	0.18	3.72	596.6	234.6	0.14	452.0
TX7078	0.36	2.45	614.0	175.5	0.12	237.3
$LSD_{(\alpha = 0.05)}$	**0.10**	**0.82**	**109.1**	**88.4**	**0.15**	**680.5**
P-Value						
Genotype	**<0.0001**	**<0.0001**	**0.0527**	**0.0042**	**0.0012**	**<0.0001**
Growth Stage	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**-**	**-**
Genotype x Stage	**0.9996**	**0.9995**	**0.2143**	**0.7925**	**-**	**-**

Table 3-5: Variation in leaf area index (LAI), intercepted photosynthetically active radiation (IPAR) and biomass accumulation among genotypes between July and October 2011

	Leaf area index ($m^2 m^{-2}$)			Intercepted PAR ($MJ m^{-2}$)			Biomass (based on 5 plants) (g)		
Genotype	**July**	**August**	**October**	**July**	**August**	**October**	**July**	**August**	**October**
B.Tx2752	1.61	3.08	3.31	468.1	591.5	498.4	104.3	209.2	326.1
B.Tx399	2.38	3.70	3.29	606.5	631.8	522.3	145.9	202.3	350.5
B.Tx615	1.90	3.14	2.95	567.2	692.9	546.8	133.3	178.7	366.8
DeKalb 2829	3.53	4.58	3.68	720.5	728.7	533.7	118.9	196.0	251.1
DeKalb 54-00	3.29	5.06	4.83	706.5	555.6	361.1	157.9	228.5	534.1
Hegari	2.79	3.83	3.81	686.3	699.9	414.7	155.8	212.9	606.9
Liang Tang Ai	3.23	4.51	3.42	752.9	728.8	469.7	176.5	269.4	403.7
PI584085	1.45	2.94	3.51	456.2	727.7	515.2	143.2	214.3	436.5
84G62	3.57	5.28	4.73	636.0	459.9	387.2	161.0	212.8	482.9
85G46	3.88	5.32	4.73	746.3	575.5	399.4	185.2	299.0	528.2
R.Tx436	2.54	3.59	2.82	703.7	759.1	567.4	173.3	218.6	397.0
SA5330	2.03	3.15	3.36	603.8	737.2	578.3	147.3	201.0	423.0
SC1019	3.29	4.50	4.45	750.1	749.1	455.1	186.8	237.8	507.0
SC1124	3.22	3.32	3.40	750.4	621.0	527.0	189.4	235.4	387.8
SC224	2.77	4.18	3.88	704.3	643.1	435.3	166.6	234.7	447.5
SC60	3.39	4.36	3.32	757.5	657.6	557.8	175.5	268.6	354.9
SC663	2.58	3.67	4.27	649.4	730.5	462.8	152.7	219.8	390.9
SC701	2.34	2.85	2.73	642.9	709.3	566.3	184.1	240.6	299.1
SC720	3.43	4.28	3.46	651.3	647.6	491.0	150.6	217.8	335.3
TX7078	1.84	2.99	2.52	558.0	724.7	559.2	128.0	195.4	203.2
$LSD_{(\alpha=0.05)}$	**1.37**	**1.62**	**1.12**	**167.8**	**195.5**	**180.8**	**38.6**	**65.0**	**39.3**

Figure 3-4: Effects of heat and drought stress on yield and harvest index (HI) at Manhattan, Kansas; a comparison between 2010 and 2011



	% Change	
Genotype	**Yield**	**HI**
Hegari	93.5	86.5
TX7078	88.0	48.0
DK2829	88.0	12.4
SC720	85.3	65.9
DK54-00	79.4	36.0
BTx399	78.5	44.1
BTx2752	74.6	13.8
SC663	74.0	68.4
SA5330	73.3	31.1
84G62	72.4	34.8
85G46	68.7	12.4
RTx436	66.7	-4.7
Liang	66.6	48.0
BTx615	64.5	35.9
PI584085	54.2	-44.6
SC1019	54.0	0.4
SC60	42.6	-104.1
SC1124	35.6	19.8
SC701	-25.6	36.0
Mean	**65.0**	**23.2**

Figure 3-5: Diurnal changes in canopy temperature under rainfed and irrigated conditions in 2011 and 2010 at Manhattan, Kansas

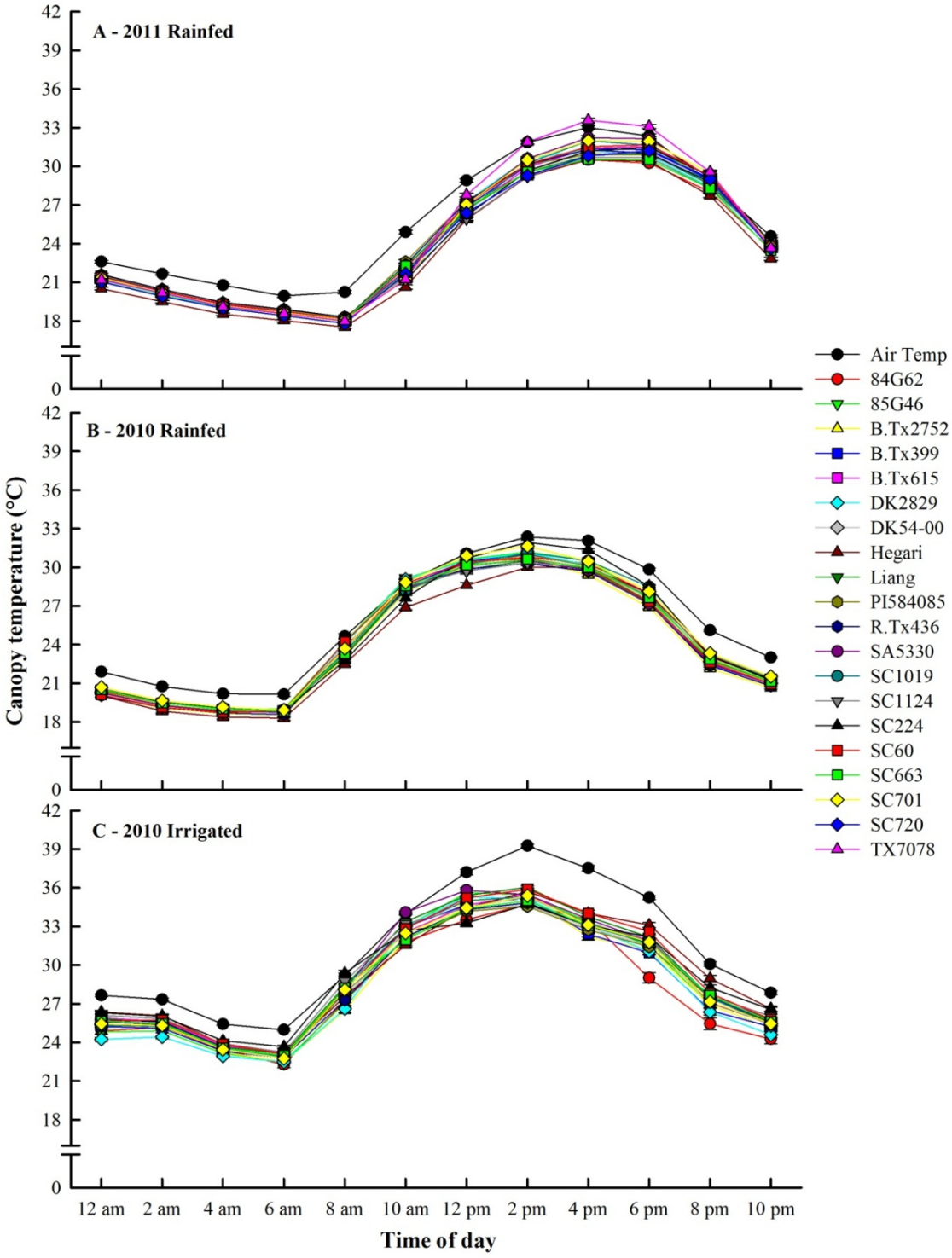


Figure 3-6: Variation in yield and canopy temperature among genotypes grown in 2010 and 2011 at Manhattan, Kansas

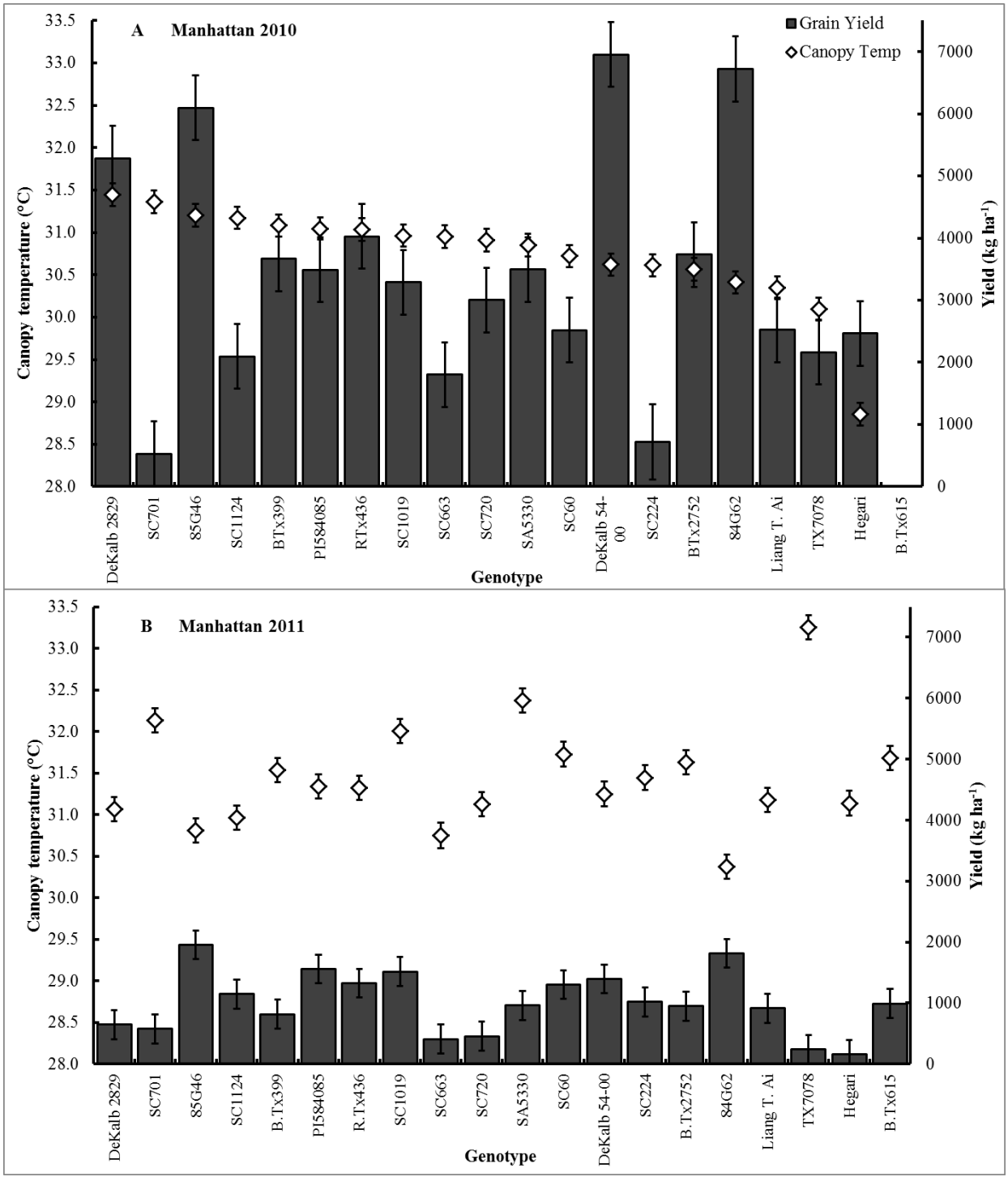


Figure 3-7: Effects of vapor pressure deficit on canopy temperature under irrigated and rainfed conditions in 2010 and 2011 at Manhattan, Kansas

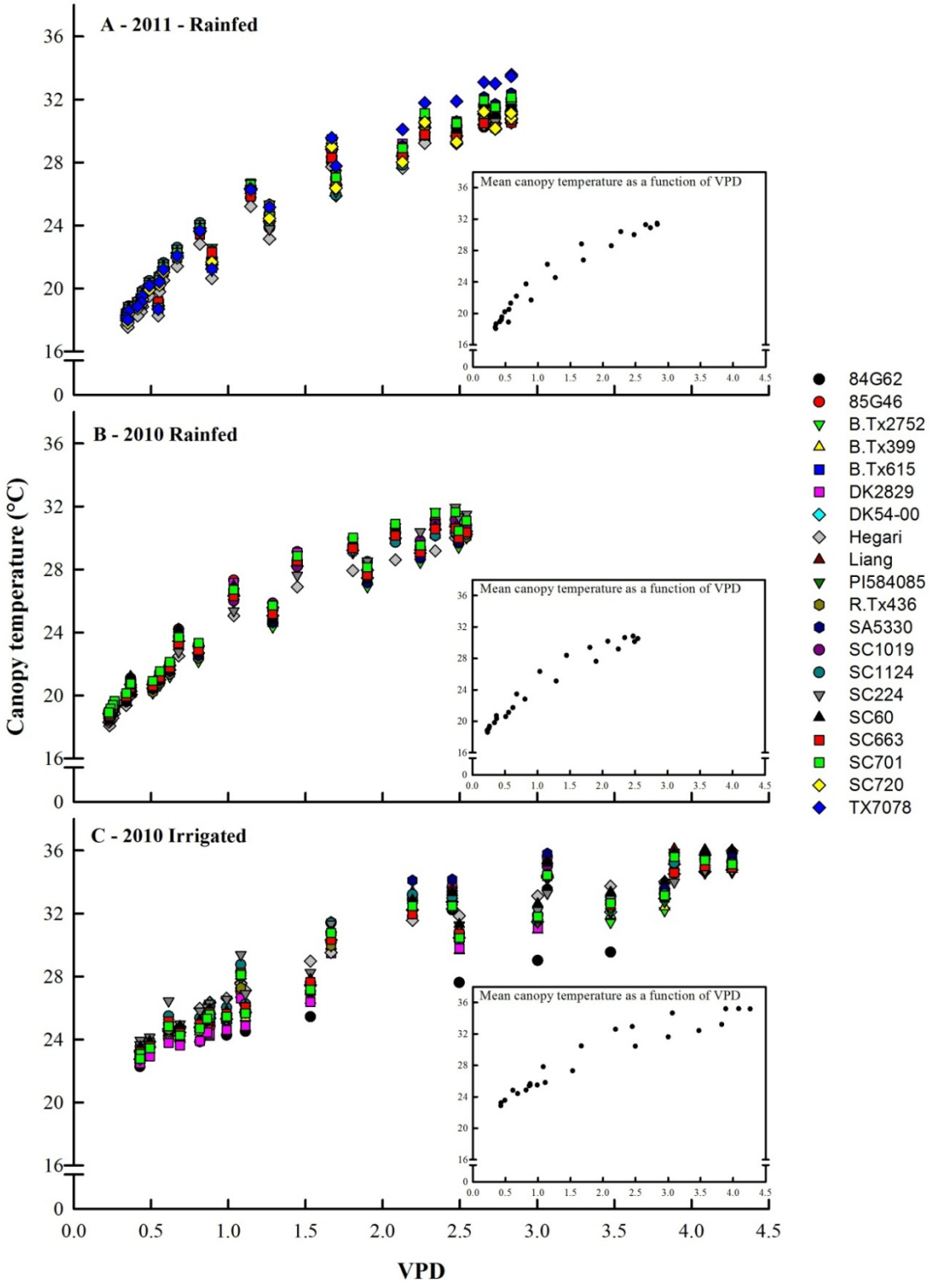


Figure 3-8: Diurnal variation in canopy temperature depression among genotypes under rainfed and irrigated conditions in 2010 at Manhattan, Kansas

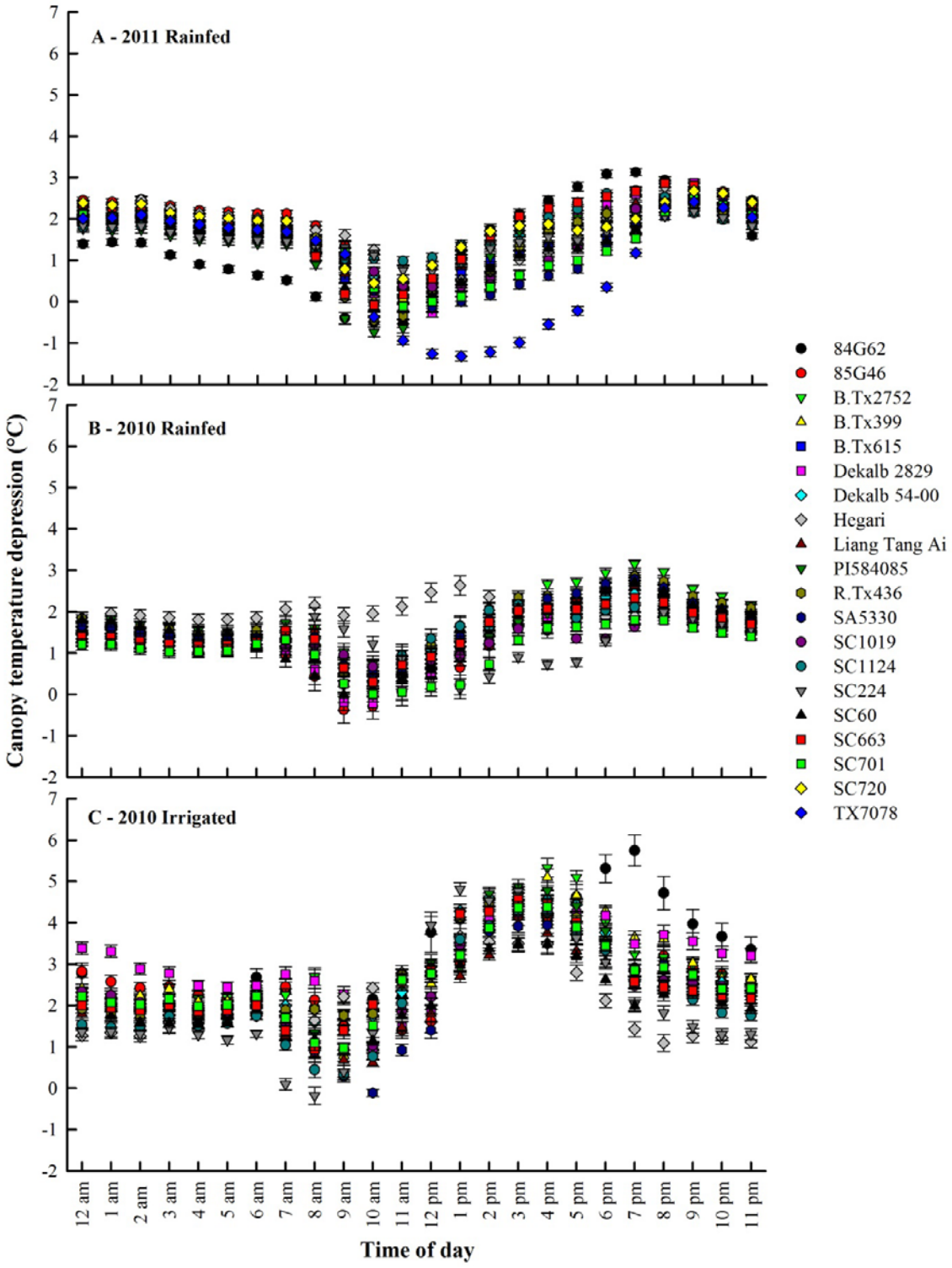


Figure 3-9: Variation in mid-day and pre-dawn canopy temperature depression among genotypes grown under rainfed conditions in 2011 at Manhattan, Kansas

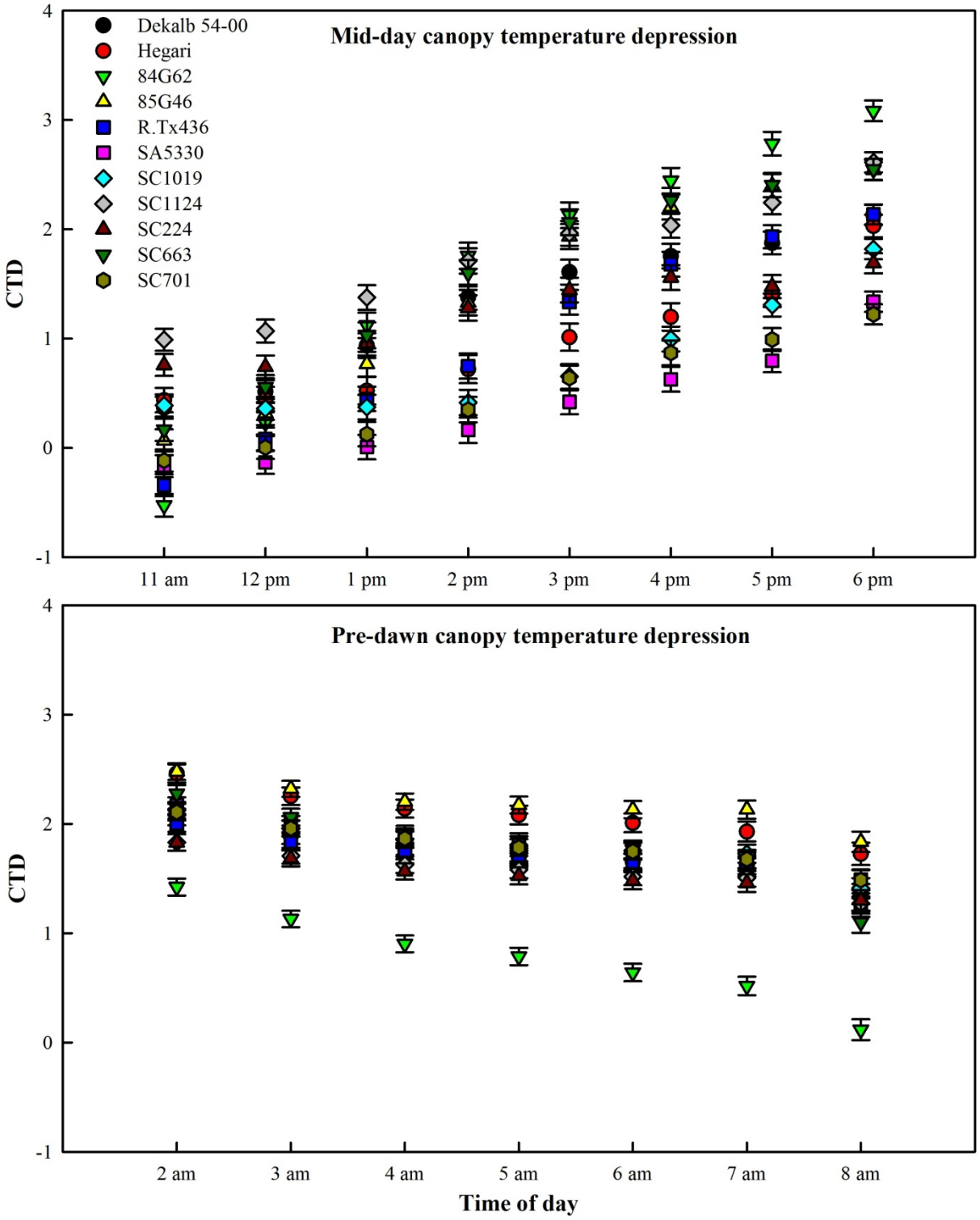


Figure 3-10: Relationship between mid-day canopy temperature depression and harvest index and yield for genotypes grown under rainfed conditions in 2011 at Manhattan, Kansas

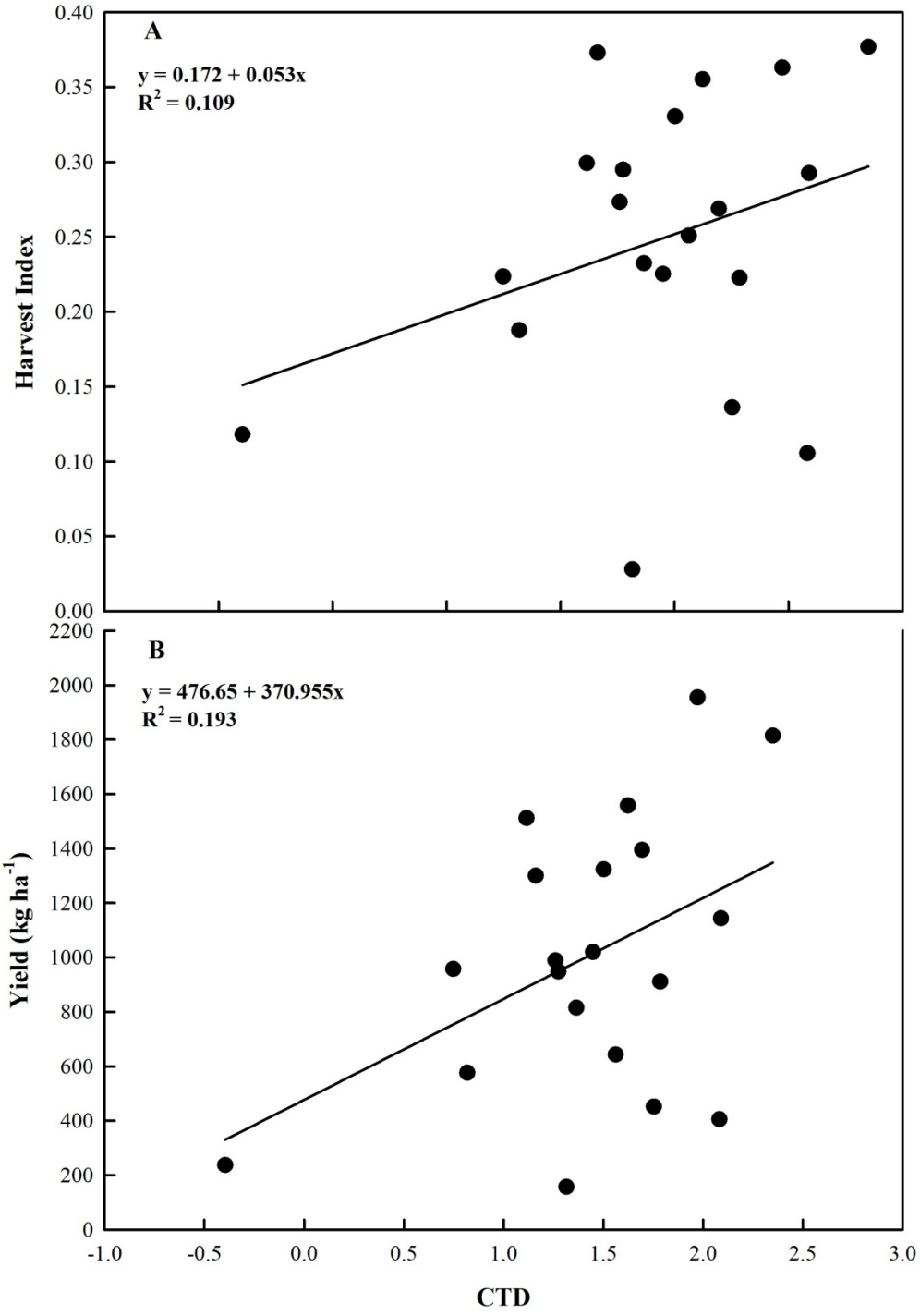


Figure 3-11: Relationship between mid-day canopy temperature and harvest index and yield for genotypes grown under rainfed conditions in 2011 at Manhattan, Kansas

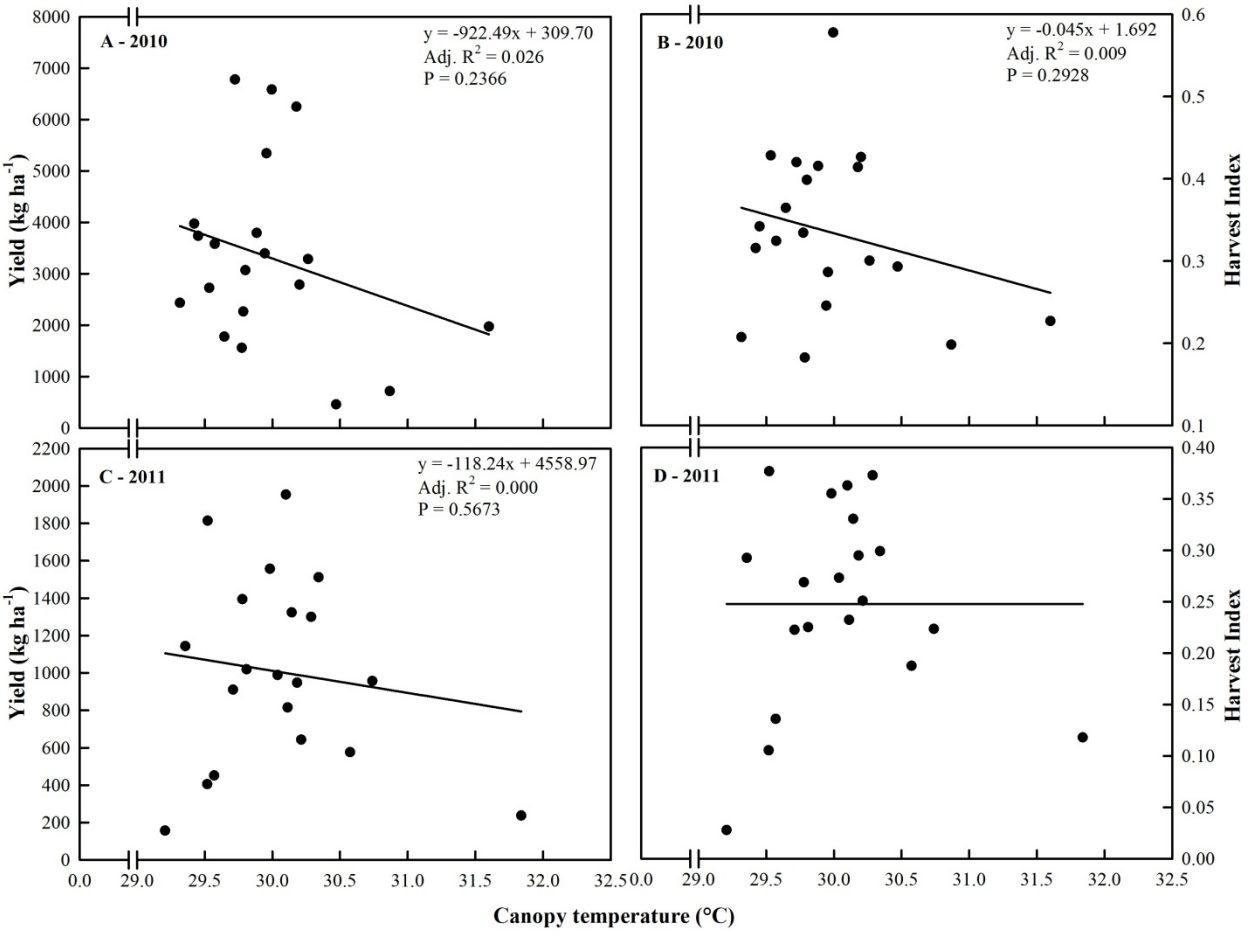


Figure 3-12: Variation in canopy temperature depression and crop water stress index among genotypes in 2011 at Manhattan, Kansas

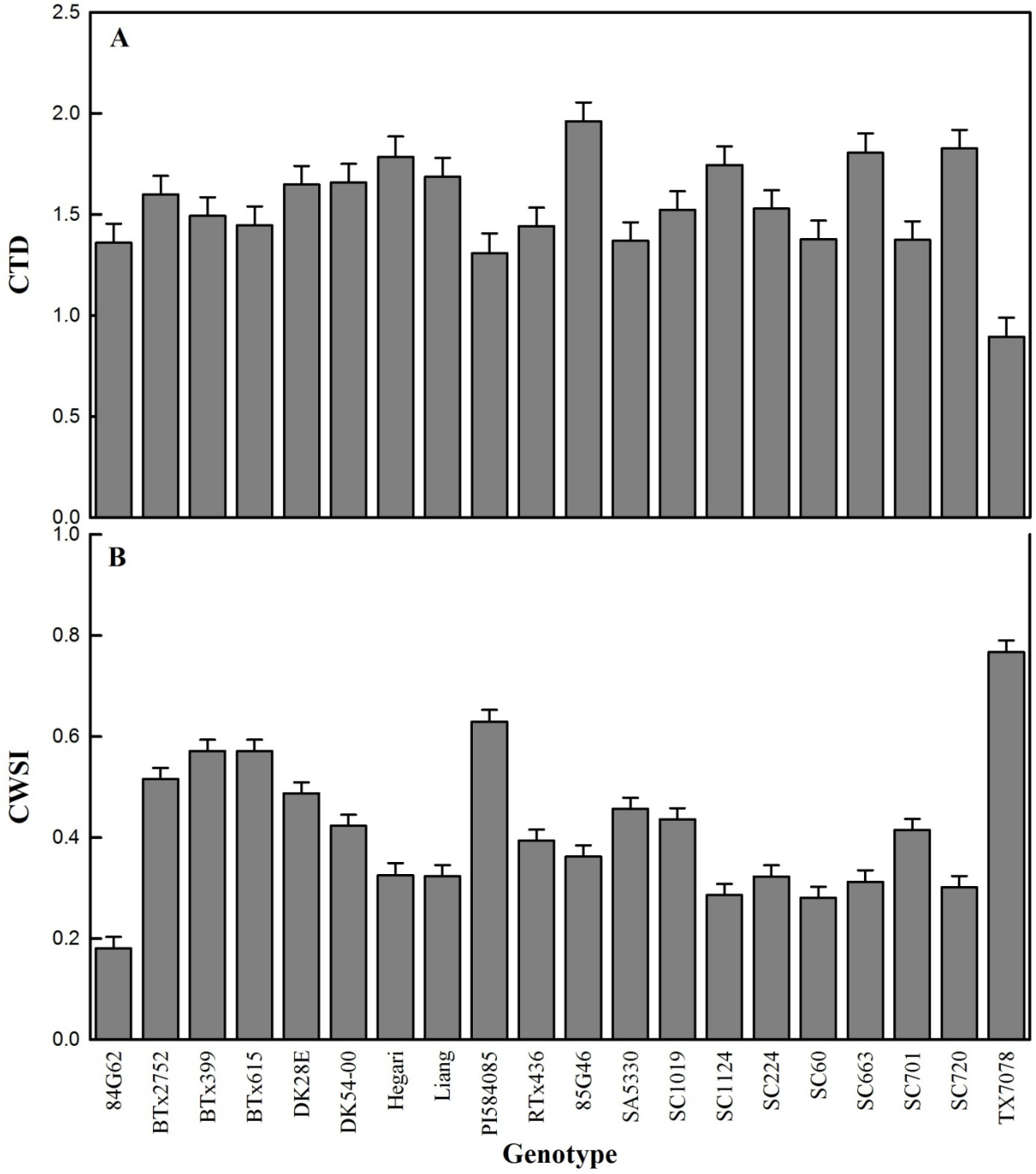
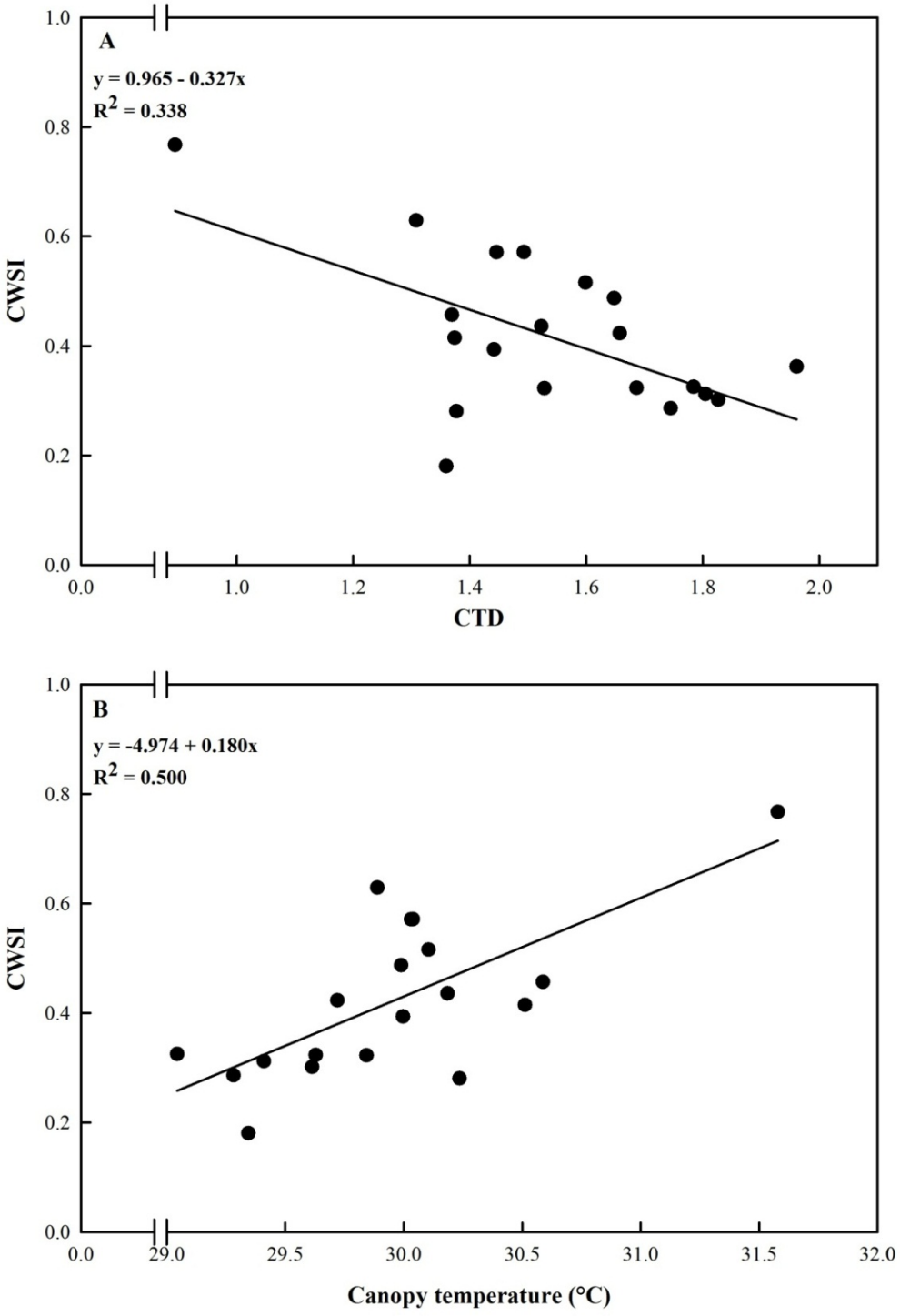


Figure 3-13: Crop water stress index as a function of canopy temperature depression and canopy temperature for genotypes grown in 2011 at Manhattan, Kansas



Supplementary Table 3-1: Air and genotype canopy temperature for rainfed plots at Manhattan in 2011

Time	AirTemp	84G62	B.Tx2752	B.Tx399	B.Tx615	DK28E	DK54	Hegari	Liang	PI584085	R.Tx436	SA 5330	SC1019	SC1124	SC224	SC60	SC663	SC701	SC720	TX7078
12:00 AM	22.6	20.6	20.4	20.6	20.6	20.4	20.6	19.8	20.5	20.8	20.6	20.5	20.5	20.8	20.8	20.6	20.2	20.5	20.2	20.4
1:00 AM	22.3	20.3	20.0	20.3	20.3	20.1	20.2	19.5	20.2	20.5	20.3	20.2	20.2	20.5	20.4	20.3	20.0	20.2	19.9	20.2
2:00 AM	21.7	19.6	19.4	19.6	19.7	19.5	19.6	18.8	19.5	19.8	19.7	19.5	19.5	19.8	19.8	19.6	19.4	19.5	19.3	19.5
3:00 AM	21.1	19.3	19.0	19.2	19.3	19.1	19.2	18.5	19.2	19.4	19.3	19.1	19.2	19.4	19.4	19.3	19.1	19.2	19.0	19.1
4:00 AM	20.8	19.2	18.7	18.9	19.0	18.9	19.0	18.3	18.9	19.2	19.0	18.9	18.9	19.2	19.2	19.0	18.9	18.9	18.7	18.9
5:00 AM	20.4	18.9	18.5	18.7	18.7	18.6	18.7	18.0	18.6	18.8	18.7	18.6	18.6	18.8	18.9	18.7	18.6	18.6	18.4	18.6
6:00 AM	19.9	18.4	18.0	18.2	18.3	18.1	18.3	17.7	18.2	18.3	18.3	18.2	18.2	18.4	18.5	18.3	18.2	18.2	18.0	18.1
7:00 AM	19.8	18.3	17.9	18.1	18.1	18.0	18.1	17.5	18.0	18.1	18.2	18.1	18.0	18.2	18.3	18.1	18.1	18.1	17.8	18.0
8:00 AM	20.2	19.1	18.8	18.9	19.2	18.7	18.9	18.3	19.0	19.1	19.0	18.8	18.8	19.0	18.9	19.1	19.1	18.8	18.7	18.7
9:00 AM	22.5	21.8	21.7	22.1	21.9	21.4	21.5	20.6	21.8	22.6	22.4	21.6	21.5	21.4	21.2	22.1	22.3	21.3	21.7	21.2
10:00 AM	24.9	24.6	24.5	24.9	24.4	24.7	24.3	23.1	24.5	25.4	25.3	24.6	24.2	23.8	23.8	25.0	25.0	24.6	24.4	25.2
11:00 AM	26.9	27.0	26.7	26.8	26.5	27.3	26.6	25.9	26.7	27.3	27.3	27.1	26.5	25.9	26.2	27.1	26.8	27.0	26.4	27.8
12:00 PM	28.9	28.5	28.7	28.7	28.4	29.2	28.4	27.6	28.4	28.9	28.8	29.0	28.5	27.8	28.2	28.8	28.3	28.9	28.0	30.1
1:00 PM	30.6	29.6	30.2	30.1	29.9	30.6	29.7	29.2	29.6	30.1	30.2	30.6	30.2	29.2	29.7	30.2	29.6	30.5	29.3	31.9
2:00 PM	31.9	30.2	31.1	31.0	30.8	31.3	30.5	30.1	30.3	30.7	31.1	31.7	31.4	30.1	30.6	31.1	30.2	31.5	30.2	33.0
3:00 PM	32.6	30.5	31.4	31.4	31.4	31.3	31.0	30.5	30.8	30.9	31.3	32.2	32.0	30.7	31.2	31.5	30.5	32.0	30.8	33.6
4:00 PM	33.0	30.6	31.6	31.5	31.6	31.1	31.2	30.7	31.2	31.0	31.3	32.4	32.0	31.0	31.4	31.7	30.7	32.1	31.1	33.5
5:00 PM	32.9	30.3	31.5	31.3	31.5	30.8	31.1	30.4	31.0	30.9	31.0	32.1	31.6	30.7	31.5	31.7	30.5	31.9	31.2	33.1
6:00 PM	32.3	29.4	30.6	30.5	30.8	30.0	30.2	29.2	30.1	30.2	30.2	31.0	30.5	29.7	30.7	30.9	29.8	31.1	30.5	31.8
7:00 PM	31.0	28.0	29.0	29.1	29.2	28.4	28.8	27.7	28.6	28.9	28.8	29.1	28.8	28.3	29.3	29.2	28.3	29.5	29.0	29.6
8:00 PM	28.7	25.8	26.3	26.4	26.7	25.9	26.3	25.2	26.2	26.3	26.3	26.3	26.2	26.2	26.7	26.5	25.9	26.6	26.3	26.3
9:00 PM	26.3	23.6	23.7	23.8	24.0	23.4	23.8	22.8	23.8	24.0	23.9	23.7	23.8	24.1	24.1	23.9	23.5	23.8	23.6	23.7
10:00 PM	24.6	22.1	22.1	22.3	22.4	22.0	22.2	21.4	22.2	22.5	22.4	22.1	22.2	22.6	22.5	22.3	21.9	22.2	21.9	22.1
11:00 PM	23.4	21.3	21.1	21.3	21.4	21.2	21.3	20.5	21.3	21.6	21.5	21.3	21.3	21.6	21.6	21.4	21.0	21.3	21.0	21.2

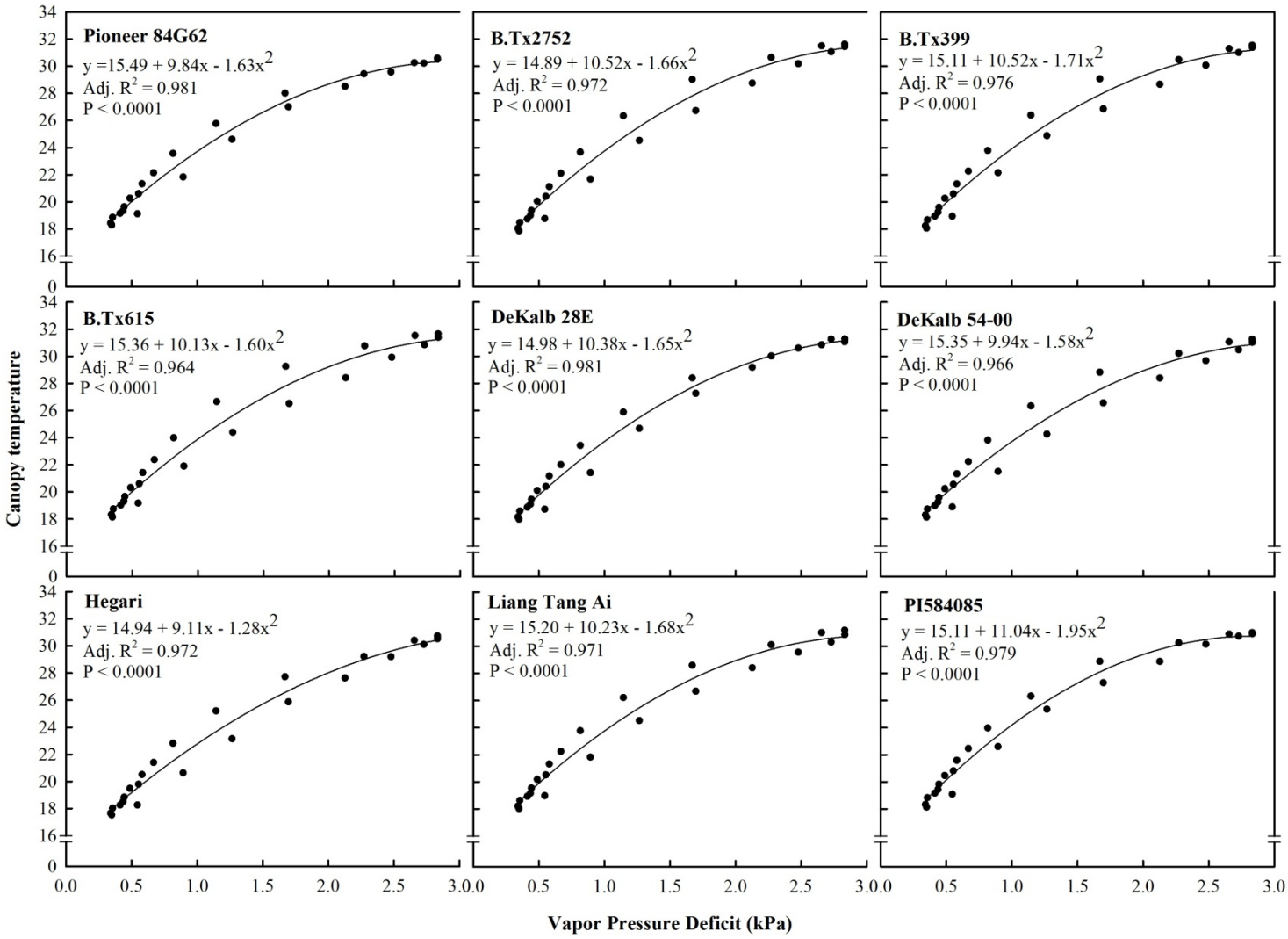
Supplementary Table 3-2: Air temperature and genotype canopy temperature for irrigated plots at Manhattan in 2010

Time	Air Temp	84G62	85G46	BTx2752	BTx399	BTx615	DK2829	DK54-00	Hegari	Liang	PI584085	RTx436	SA5330	SC1019	SC1124	SC224	SC60	SC663	SC701
12:00 AM	27.6	24.9	24.8	25.3	25.2	25.9	24.2	25.3	26.4	25.8	25.4	25.7	25.6	25.3	26.1	26.3	25.6	25.6	25.4
1:00 AM	27.7	25.7	25.1	25.5	25.4	25.9	24.3	25.6	26.3	25.8	25.6	25.8	25.7	25.4	26.2	26.3	25.9	25.7	25.6
2:00 AM	27.3	25.2	24.9	25.2	25.1	25.6	24.4	25.4	26.1	25.5	25.3	25.6	25.4	25.3	25.8	26.0	25.7	25.4	25.3
3:00 AM	26.4	24.7	23.9	24.0	24.0	24.5	23.6	24.4	24.8	24.4	24.5	24.5	24.3	24.4	24.7	25.0	24.8	24.4	24.2
4:00 AM	25.4	23.4	23.1	23.3	23.3	23.8	22.9	23.5	23.9	23.7	23.6	23.6	23.6	23.6	23.9	24.1	23.9	23.6	23.4
5:00 AM	25.1	23.2	22.9	22.9	23.0	23.4	22.7	23.2	23.3	23.3	23.3	23.2	23.2	23.2	23.5	23.9	23.5	23.2	23.1
6:00 AM	25.0	22.3	22.5	22.5	22.8	23.2	22.5	22.7	23.2	23.1	22.9	22.9	22.9	22.9	23.2	23.7	23.1	23.0	22.8
7:00 AM	26.5	24.2	24.1	24.3	24.9	25.4	23.8	24.5	24.7	25.3	25.1	24.6	24.9	24.9	25.5	26.4	24.9	25.1	24.8
8:00 AM	29.2	27.2	27.1	26.5	28.0	28.1	26.6	27.6	27.5	28.4	27.9	27.3	28.1	28.3	28.7	29.4	28.0	28.2	28.1
9:00 AM	31.7	30.2	30.2	29.6	30.9	30.5	29.5	30.3	29.5	31.0	29.9	30.0	31.4	30.8	31.4	31.3	30.8	30.3	30.8
10:00 AM	34.0	31.8	33.0	32.0	33.0	32.5	33.0	32.2	31.6	33.4	32.0	32.2	34.1	32.9	33.2	32.7	32.8	32.0	32.5
11:00 AM	35.1	32.2	33.6	32.7	33.1	32.6	33.6	32.7	32.3	33.6	32.6	32.3	34.1	33.2	33.0	32.4	33.3	32.5	32.5
12:00 PM	37.2	33.5	35.6	35.0	34.7	34.6	35.3	34.3	34.4	35.4	34.2	34.3	35.8	35.0	34.5	33.3	35.2	34.4	34.4
1:00 PM	38.8	34.7	35.8	35.9	35.4	35.2	35.4	34.5	35.1	36.1	34.6	34.7	35.6	35.3	35.2	34.0	35.8	34.6	35.6
2:00 PM	39.3	34.7	35.5	35.3	35.2	35.5	35.1	34.7	35.7	36.0	34.5	34.8	35.5	35.3	35.3	34.7	35.9	35.0	35.4
3:00 PM	39.5	35.0	35.3	35.0	34.8	35.5	34.9	34.8	36.0	36.0	34.6	34.8	35.6	35.2	35.2	34.7	36.0	34.9	35.1
4:00 PM	37.5	33.5	33.4	32.2	32.4	33.1	33.0	32.9	34.0	33.8	32.7	33.1	33.6	33.2	33.3	33.1	34.0	33.1	33.1
5:00 PM	36.5	29.5	32.6	31.4	31.9	32.9	32.3	32.1	33.7	33.2	32.1	32.4	32.9	32.7	32.8	32.9	33.3	32.5	32.6
6:00 PM	35.2	29.0	31.5	31.2	30.9	32.0	31.1	31.5	33.1	32.2	31.4	31.7	31.8	31.8	31.9	32.2	32.6	31.8	31.8
7:00 PM	33.3	27.6	30.4	30.0	29.6	30.7	29.8	30.5	31.8	30.8	30.6	30.7	30.4	30.8	30.7	31.3	31.3	30.7	30.4
8:00 PM	30.1	25.4	26.9	26.9	26.5	27.5	26.4	27.5	29.0	27.6	27.4	27.5	27.1	27.5	27.6	28.2	27.8	27.6	27.2
9:00 PM	28.4	24.5	25.5	25.4	25.4	26.1	24.8	25.7	27.1	26.0	25.9	25.9	25.7	25.8	26.3	26.9	26.1	26.0	25.7
10:00 PM	27.8	24.3	25.1	25.1	25.2	25.8	24.6	25.3	26.6	25.7	25.5	25.6	25.4	25.4	26.0	26.5	25.8	25.6	25.4
11:00 PM	27.1	23.8	24.5	24.5	24.5	25.1	23.9	24.8	26.0	25.0	24.9	24.9	24.8	24.8	25.4	25.8	25.2	24.9	24.7

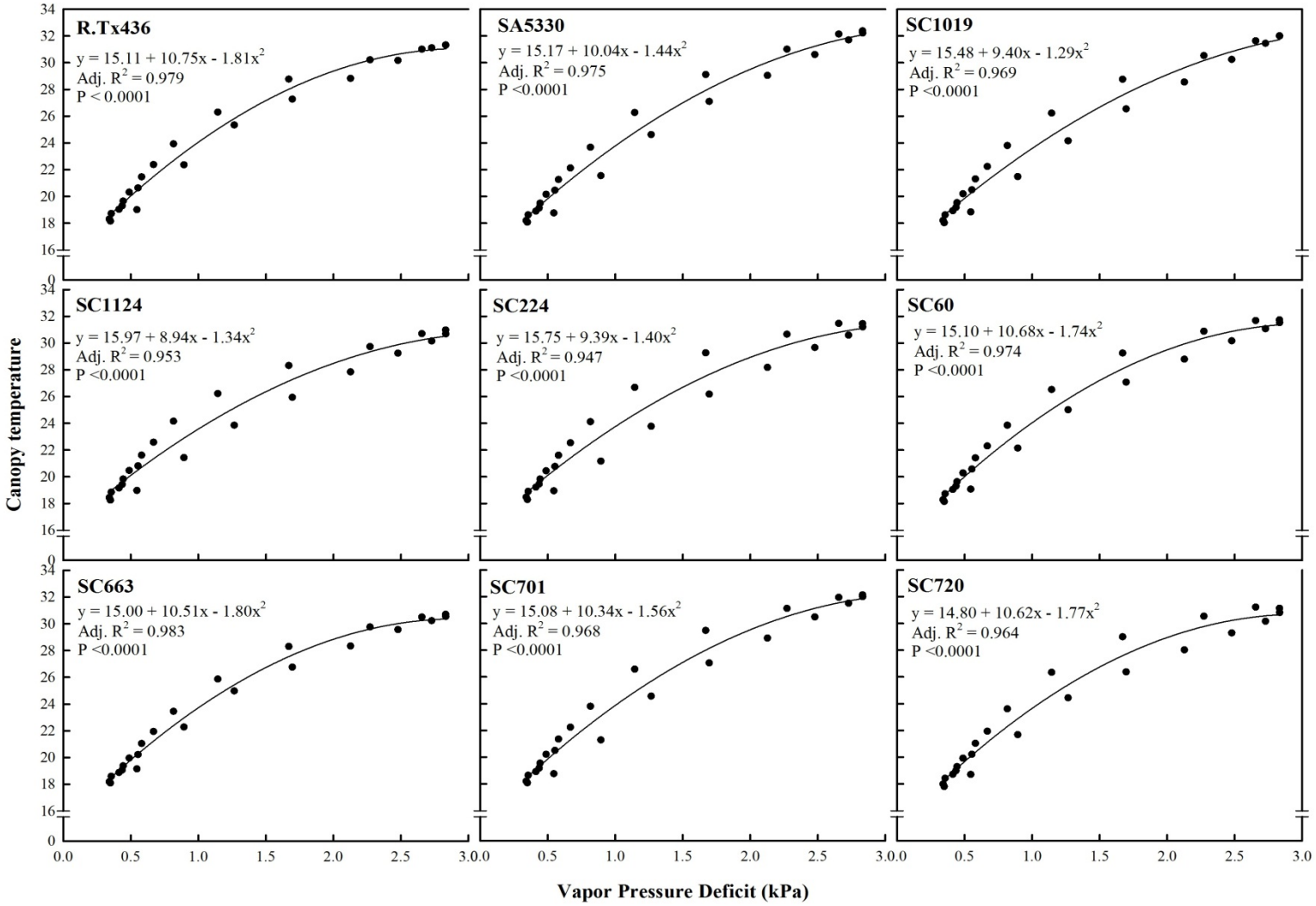
Supplementary Table 3-3: Air temperature and genotype canopy temperature for rainfed plots at Manhattan in 2010

Time	Air Temp	84G62	85G46	BTx2752	BTx399	DK2829	DK54-00	Hegari	Liang	PI584085	RTx436	SA5330	SC1019	SC1124	SC224	SC60	SC663	SC701
12:00 AM	21.9	20.4	20.5	20.0	20.3	20.3	20.3	20.0	20.5	20.3	20.0	20.2	20.6	20.6	20.5	20.1	20.5	20.7
1:00 AM	21.3	20.0	19.9	19.5	19.7	19.7	19.7	19.4	19.9	19.8	19.6	19.7	20.1	20.1	20.0	19.6	19.9	20.1
2:00 AM	20.8	19.5	19.5	19.1	19.3	19.3	19.3	18.8	19.5	19.3	19.1	19.3	19.6	19.6	19.5	19.1	19.5	19.6
3:00 AM	20.4	19.2	19.2	18.8	19.0	19.0	19.0	18.6	19.2	19.1	18.9	19.0	19.3	19.3	19.3	18.9	19.2	19.4
4:00 AM	20.2	19.0	19.0	18.6	18.9	18.9	18.9	18.4	19.1	18.9	18.7	18.8	19.1	19.1	19.1	18.7	19.0	19.1
5:00 AM	19.9	18.7	18.7	18.4	18.6	18.6	18.6	18.1	18.7	18.5	18.4	18.5	18.8	18.8	18.8	18.4	18.7	18.8
6:00 AM	20.1	18.9	19.0	18.5	18.9	18.9	18.8	18.3	18.9	18.8	18.6	18.8	18.9	18.9	18.9	18.9	18.8	18.9
7:00 AM	22.1	20.9	21.1	20.3	20.6	21.0	20.7	20.0	20.6	20.7	20.5	20.7	20.9	20.8	20.5	21.2	20.5	20.7
8:00 AM	24.6	23.8	24.2	23.1	23.3	24.0	23.4	22.5	23.1	23.5	23.4	23.3	23.4	23.7	22.8	24.2	23.3	23.7
9:00 AM	27.0	26.4	27.3	25.9	26.3	27.1	26.3	25.0	26.3	26.1	26.4	26.2	26.0	26.7	25.4	27.0	26.3	26.7
10:00 AM	28.8	28.6	29.1	28.1	28.5	29.1	28.2	26.9	28.3	28.2	28.5	28.3	28.2	28.5	27.6	28.7	28.5	28.8
11:00 AM	30.1	29.5	30.0	29.0	29.6	30.0	29.2	27.9	29.2	29.4	29.4	29.4	29.3	29.1	29.2	29.7	29.3	30.0
12:00 PM	31.1	30.3	30.6	30.1	30.5	30.5	30.0	28.6	29.8	30.2	29.9	30.2	30.2	29.7	30.7	30.5	30.2	30.9
1:00 PM	31.8	30.6	31.2	30.6	30.8	30.9	30.5	29.2	30.2	30.8	30.1	30.4	30.9	30.2	31.7	30.7	30.6	31.6
2:00 PM	32.4	30.8	31.2	30.7	31.0	31.1	30.7	30.0	30.5	31.0	30.3	30.6	31.1	30.3	31.9	30.7	30.6	31.6
3:00 PM	32.4	30.6	30.8	30.0	30.6	30.8	30.4	30.2	30.3	30.6	30.0	30.3	30.8	30.2	31.5	30.3	30.4	31.1
4:00 PM	32.1	30.3	30.5	29.4	30.1	30.2	29.9	30.0	29.8	30.1	29.7	29.8	30.5	30.0	31.3	30.0	30.0	30.5
5:00 PM	31.2	29.4	29.5	28.5	29.0	28.9	29.0	29.2	28.8	29.2	28.8	28.7	29.8	29.3	30.4	29.0	29.1	29.5
6:00 PM	29.8	28.0	27.5	26.9	27.2	27.3	27.5	28.0	27.3	27.7	27.2	27.2	28.5	27.8	28.5	27.3	27.6	28.1
7:00 PM	27.5	25.4	24.8	24.3	24.6	24.8	25.1	25.6	24.9	25.2	24.6	24.7	25.9	25.4	25.7	24.8	25.2	25.7
8:00 PM	25.1	23.0	22.6	22.1	22.5	22.7	22.7	23.1	22.8	22.8	22.4	22.5	23.2	23.2	23.1	22.6	22.9	23.3
9:00 PM	23.7	21.8	21.7	21.2	21.5	21.6	21.6	21.9	21.7	21.7	21.4	21.5	22.0	22.1	21.9	21.6	21.8	22.1
10:00 PM	23.0	21.3	21.1	20.6	20.9	21.0	21.0	21.2	21.1	21.1	20.8	20.9	21.4	21.5	21.3	20.9	21.1	21.5
11:00 PM	22.3	20.7	20.7	20.2	20.4	20.5	20.5	20.7	20.7	20.5	20.2	20.4	20.8	20.9	20.7	20.4	20.6	20.9

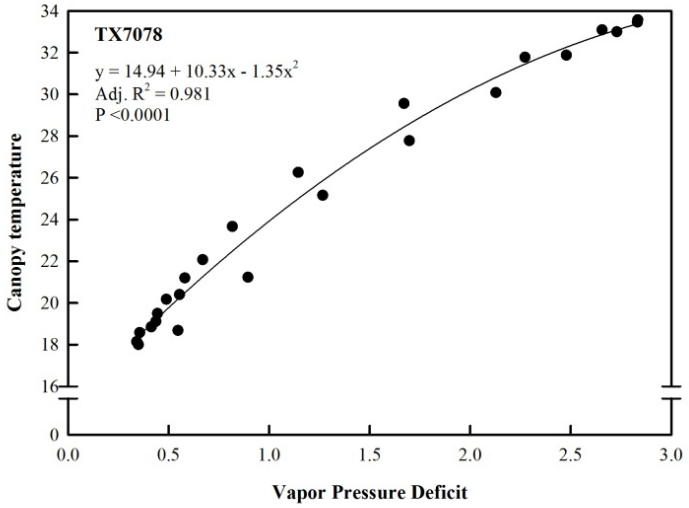
Supplementary Figure 3-1: Correlation between vapor pressure deficit and canopy temperature for genotypes grown in 2011 at Manhattan, Kansas



Supplementary Figure 3-2: Correlation between vapor pressure deficit and canopy temperature for genotypes grown in 2011 at Manhattan, Kansas



Supplementary Figure 3-3: Correlation between vapor pressure deficit and canopy temperature for TX7078 grown in 2011 at Manhattan, Kansas



3.7 References

Allen, C.D., A.K., Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier, T. Kitzberger, A. Rigling, D.D. Breshears, E.H. Hogg, P. Gonzalez, R. Fensham, Z. Zhang, J. Castro, N. Demidova, J.H. Lim, G. Allard, S.W. Running, A. Semerci, and N. Cobb. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecol. Manage. 259: 660 – 684.

Amani, I., R.A. Fischer, and M.P. Reynolds. 1996. Canopy temperature depression association with yield of irrigated spring wheat cultivars in a hot climate. J. Agron. Crop Sci. 176: 119 – 129.

Ayeneh, A., M. van Ginkel, M.P. Reynolds, and K. Ammar. 2002. Comparison of leaf, spike, peduncle and canopy temperature depression in wheat under heat stress. Field Crops Res. 79:173 – 184.

Balota, M., W.A. Payne, S.R. Evett, and M.D. Lazar. 2007. Canopy temperature depression sampling to assess grain yield and genotypic differentiation in winter wheat. Crop Sci. 47: 1518 – 1529.

Blum, A., L. Shipiler, G. Golan, and J. Mayer. 1989. Yield stability and canopy temperature of wheat genotypes under drought stress. Field Crops Res. 22: 289 – 296.

Blum, A., J. Mayer, and G. Gozlan. 1982. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Res. 5: 137 – 146.Blum, A. 1980. Drought avoidance in wheat and its rapid estimation by remote infrared thermal leaf canopy measurements. In Proc. 3rd Int. Wheat Conf., 22nd May – 3rd June, 1980. Madrid, Spain.

Blum, A. 1988. Breeding plants for dryland farming. **In**: Challenges in dryland agriculture. A global Prospective. P.W.Unger, W.R. Jordan, T.V. Sneed and R.W. Jensen (eds). Proc. Internat. Conf., 15–19 August, 1988, Bushland/Amarillo, TX.

Borras, L., G.A. Slafer, and M.E. Otegui. 2004. Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Res. 86: 131 – 146.

Bunce, J.A. 1981. Comparative responses of leaf conductance to humidity in single attached leaves. J. Exp. Bot. 32: 629 – 634.

Campbell, G.S., and J.M. Norman. 1998. An introduction to environmental biophysics. 2nd Ed. Springer-Verlag, Inc., New York.

Chaudhuri, U.N., M.L. Deaton, E.T. Kanemasu, G.W. Wall, V. Marcarian, and A.K. Dobrenz. 1986. A procedure to select drought tolerant sorghum and millet genotypes using canopy temperature and vapor pressure deficit. Agron. J. 78: 490 – 494.

Chaudhuri, U.N. and E.T. Kanemasu. 1982. Effect of water gradient on sorghum growth, water relations and yield. Can. J. Plant Sci. 62: 599 – 607.

Comstock, J., and J. Ehrleringer. 1993. Stomatal response to humidity in common bean (*Phaseolus vulgaris*): implications for maximum transpiration rate, water-use efficiency and productivity. Aust. J. Plant Physiol. 20: 669 – 691.

Devi, M.J., T.R. Sinclair, and V. Vadez. 2010. Genotypic variation in peanut for transpiration response to vapor pressure deficit. Crop Sci. 50: 191 – 196.

Ehrler, W.L., S.D. Idso, R.D. Jackson, and R.J. Reginato. 1978. Wheat canopy temperature: relation to plant water potential. Agron. J. 70: 251 – 256.

Evett, S.R., T.A. Howell, A.D. Schneider, D.R. Upchurch, and D.F. Wanjura. 1996. Canopy temperature based automated irrigation control. pp. 207 – 213. In: Evaporation and Irrigation Scheduling. C.R. Camp, E.J. Sadler, and R.E. Yoder (Eds.) Proc. Int. Conf. 3 – 6, Nov. 1996. San Antonio, TX.

Fischer, R.A., D. Rees, K.D. Sayre, Z.-M. Lu, G.A. Condon, and A. Larque Saavedra. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. Crop Sci. 38:1467 – 1475.

Fletcher, A.L., T.R. Sinclair, and L.H. Allen Jr. 2007. Transpiration responses to vapor pressure deficit in well watered 'slow-wilting' and commercial soybean. Environ. Exp. Bot. 61: 145 – 151.

Frederick, J.R., and J.J. Camberato. 1995. Water and nitrogen effects on winter wheat in the southeastern Coastal Plain: I. Grain yield and kernel traits. Agron. J. 87: 521 – 526.

Gambin, B.L., and L. Borras. 2007. Plasticity of sorghum kernel weight to increased assimilate availability. Field Crops Res. 100: 272 – 284.

Gardner, B.R., B.L. Blad, and G.D. Wilson. 1986. Characterizing corn hybrid moisture stress sensitivity using canopy temperature measurements. Remote Sens. Environ. 19: 207 – 211.

Gholipoor, M, P.V.V. Prasad, R.N. Mutava, and T.R. Sinclair. 2010. Genetic variability of transpiration response to vapor pressure deficit among sorghum genotypes. Field Crops Res. 119: 85 – 90.

Hatfield, J.L. 1982. The utilization of thermal infrared inputs from grain sorghum as methods of assessing irrigation requirements. Irrig Sci. 3: 259 – 268.

Hatfield, J. L., J.E. Quisenberry, and R.E. Dilbeck. 1987. Use of canopy temperatures to identify water conservation in cotton germplasm. Crop Sci. 27: 269 – 273.

Hirasawa, T., and T.C. Hsiao. 1999. Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. Field Crops Res. 62: 53–62.

Howell, T.A., J.T. Musick, and J.A. Talk. 1986. Canopy temperature of irrigated winter wheat. Trans ASAE. 29: 1692-1699.

Idso, S.B. 1982. Non-water-stressed baselines: a key to measuring and interpreting crop water stress. Agric. Meteorol. 27: 59 – 70.

Idso, S.B., R.D. Jackson, P.J. Pinter Jr., R.J. Reginato, and J.L. Hatfield. 1981. Normalizing the stress degree day for environmental variability. Agric. Meteorol. 24: 45 – 55.

Jackson R.D., S.B. Idso, R.J. Reginato, and P.J. Pinter. 1981. Canopy temperature as a crop water stress index. Water Resour. Res. 17: 1133 – 1138.

Jackson, R.D. 1982. Canopy temperature and crop water stress, pp. 43 – 85. *In*: D.E. Hillel (Ed.). Advances in Irrigation, Academic Press, New York, USA.

Kholova, J., C.T. Hash, P.L. Kumar, R.S. Yadav, M. Kocova, and V. Vadez. 2010. Terminal drought-tolerant pearl millet have high leaf ABA and limit transpiration at high vapor pressure deficit. J. Exp. Bot. 61: 1431 – 1440.

Kirkham, M.B., K. Suksayretrup, C.E. Wassom, and E.T. Kanemasu. 1984. Canopy temperature of drought-resistant and drought-sensitive genotypes of maize. Maydica 24: 287 – 303.

Laisk, A., and G.E. Edwards.1998. Oxygen and electron flow in C4 photosynthesis: Mehler reaction, photorespiration and CO_2 concentration in the bundle sheath. Planta 205: 632 – 645.

McKinney, N.V., W.T. Schapaugh, and E.T. Kanemasu. 1989. Canopy temperature, seed yield, and vapor pressure deficit relationships in soybean. Crop Sci. 29: 1038 – 1041

Mtui, T.A., E.T. Kanemasu, and C. Wassom. 1981. Canopy temperatures, water use, and water use efficiency of corn genotypes. Agron. J. 73: 639 – 643.

Mutava, R.N., P.V.V. Prasad, M.R. Tuinstra, K.D. Kofoid, and J. Yu. 2011. Characterization of sorghum genotypes for traits related to drought tolerance. Field Crops Res. 123: 10 – 18.

O'Toole, J., O. Ito, and B. Hardy. 1998. Genetic improvement of rice for water-limited environments. Proceedings of a workshop 1 – 3 Dec. 1998, Los Banos, Laguna, Philippines. IRRI, Los Banos, Philippines.

Pinter, P.J. Jr., G. Zipoli, R.J. Reginato, R.D. Jackson, S.B. Idso, and J.P. Hohman. 1990. Canopy temperature as an indicator of differential water use and yield performance among wheat cultivars. Agric. Water Manage. 18: 35 – 48.

Pinter, P.J., and R.J. Reginato. 1982. A thermal infrared technique for monitoring cotton water stress and scheduling irrigations. Trans. ASAE, 25: 1651 – 1655.

Prasad, P.V.V., K.J. Boote, and L.H. Allen, Jr. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain sorghum are more severe at elevated carbon dioxide due to higher tissue temperatures. Agric. For. Meteorol. 139: 237 – 251.

Prasad, P.V.V., S.R. Pisipati, R.N. Mutava, and M.R. Tuinstra. 2008. Sensitivity of sorghum to high temperature stress during reproductive development. Crop Sci. 48: 1911 – 1917.

Prenger, J.J. and P.P. Ling. 2001. Greenhouse condensation control: Understanding and using vapor pressure deficit (VPD). Fact Sheet (Series) AEX-804. Ohio State University Ext., Columbus, OH, USA.

Rashid, A., J.C. Stark, A. Tanveer, and T. Mustafa. 1999. Use of canopy temperature measurements as a screening tool for drought tolerance in spring wheat. J Agron Crop Sci. 182: 231 – 237.

Reynolds, M.P., M. Balota, M.I.B. Delgado, I. Amani, and R.A. Fischer. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust. J. Plant Physiol. 21: 717 – 730.

Reynolds, M.P. 2002. Physiological approaches to wheat breeding. **In:** Bread wheat Improvement and production. B.C. Curtis, S. Rajaram, H.G. Macpherson (eds.) FAO Plant Production and Protection Series No. 30. Food and Agricultural Organization (FAO) of the United Nations, Rome, Italy.

Royo, C., D. Villegas, L.F. Garcia Del Moral, S. Elhani, N. Aparicio, Y. Rharrabti, and J.L. Araus. 2002. Comparative performance of carbon isotope discrimination and canopy temperature depression as predictors of genotypes differences in durum wheat yield in Spain. Aust J Agric Res. 53: 561 – 569.

Sinclair, T.R., G.L. Hammer, and E.J. van Oosterom. 2005. Potential yield and water-use efficiency benefits in sorghum from limited maximum transpiration rate. Funct. Plant Biol. 32: 945 – 952.

Sinclair, T.R., and J.M. Bennett. 1998. Water. In: Principles of Ecology in Plant Production T.R. Sinclair, F.P. Gardner. (Eds.). pp. 103 – 120. CAB International.

Singh, P., and E.T. Kanemasu. 1983. Leaf and canopy temperatures of pearl millet genotypes under irrigated and non-irrigated conditions. Agron. J. 75: 497 – 501.

Sojka, R.E., H.L. Stolzy, and R.A. Fischer. 1981. Seasonal drought response of selected wheat cultivars. Agron. J. 73: 838 – 845.

Stevenson, K.R., and R.H. Shaw. 1971. Eff ect of leaf orientation on leaf resistance to water vapor diff usion in soybean (*Glycine max* L. Merr.) leaves. Agron. J. 63: 327 – 329.

Turner, N.C., E.D. Schulze, and T. Gollan. 1984. The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content. I. Species comparisons at high soil water contents. Oecologia 63: 338 – 342.

Wanjura, D.F., D.R. Upchurch, and J.R. Mahan. 1995. Control of irrigation scheduling using temperature-time thresholds. Trans. ASAE 38: 403 – 409.

Wheeler, T.R., P.Q. Craufurd, R.H. Ellis, J.R. Porter, and P.V.V. Prasad. 2000. Temperature variability and the yield of annual crops. Agric. Ecosyst. Environ. 82: 159 – 167.

Chapter 4 - Evaluation of variation in sorghum roots response to drought stress

Abstract

Root systems exhibit morphological, structural, and physiological responses to changing environmental conditions in order to maximize the acquisition of resources. Deep roots will help increase crop yields under drought conditions as they can extract moisture from deeper soil horizons. Sorghum is a drought tolerant crop, and root traits can play a key role in its drought adaptation. Although several drought related studies have been carried out with sorghum, limited work has been done on roots. The goal of this study was to look at variability in root morphology among sorghum genotype and their response to drought stress. We hypothesized that there is genetic diversity among grain sorghum genotypes in root growth and development in response to drought stress. The objectives of the study were to quantify (i) the genotypic variation in root growth and development in response to water deficits for selected grain sorghum genotypes, (ii) effects of drought stress on different root classes in these genotypes. Plants were grown under controlled environment in PVC columns 1 m tall with an inside diameter of 20 cm and subjected to drought stress for 45 days. Drought stress reduced plant height with Pioneer Hybrid 85G46 recording the greatest reduction (50%) and B.Tx2752 recording the smallest (2%). Lines PI584085 and SC224 recorded an increase in plant height (18 and 14% respectively) . Leaf area was reduced by drought stress by 2 – 76% with SC663 recorded the smallest reduction. Rooting depth increased for some genotypes under drought stress with genotype SC1124 recording the largest increase (180%). Total root length for some genotypes increased by 11 – 113% with genotypes SC224 and SC1019 recording the greatest increase. There was a positive relationship between water used and root length (R^2 = 0.21 and P = 0.493).

4.1. Introduction

Plant growth and performance depends on the ability of its root system to provide physical support and the basis for uptake of water and nutrients from the soil. The state and conditions of roots in a plant will therefore determine the condition of a plant. A plant rooting system may comprise of a taproot and/or seminal roots, produced during embryogenesis, and lateral roots, which are produced from the taproot and seminal roots during the lifetime of a plant (Malamy and Benfey, 1997). Even though plant root system development and growth is controlled by genetic factors, the soil environment (rhizosphere) within which the roots grow has a significant influence. Root systems in plants have the ability to exhibit morphological, structural, and physiological responses to changing environmental conditions in order to maximize the acquisition of resources. This ability to adapt is referred to as root developmental plasticity (Lynch and Ho, 2005) and includes responses such as changes of tap/seminal root elongation, lateral root and root hair formation, elongation and distribution, and the ability to absorb water and nutrients.

As water resources for agricultural uses become more limiting, the development of drought-tolerant lines will become increasingly important. One aspect of principal importance in this arena is the response of root growth and development to water-deficit conditions. For plants growing in soils with limited moisture, the development of the root system is usually less inhibited than shoot growth, and may even be promoted (Sharp and Davies, 1989). Therefore the maintenance of root growth during water deficits is an obvious benefit as it helps maintain a continued water supply to the plant (O'Toole and Bland, 1987; Sponchiado et al., 1989).

The functions of roots include anchorage, the absorption of water and mineral nutrients, synthesis of various essential compounds such as growth regulators, and energy storage in root crops. A casual consideration of their functions indicates that physiologically vigorous root systems are as essential as vigorous shoots for successful plant growth because root and shoot growth are so interdependent that one cannot succeed without the other. Knowledge of root structure is important because it affects the pathway and resistance to water and

solute movement, while the extent of root systems affects the volume of soil available as a source of water and mineral nutrients.

Root distribution refers to the presence of roots in a positional gradient or grid. Studies of root distribution focus on root biomass or root length as a function of factors such as depth in the soil, distance from the stem, and position between neighboring plants. Root architecture refers to the spatial configuration of the root system and important aspects of this will include the entire root system or a large subset of the root system of an individual plant. Morphological features include characteristics of the epidermis such as root hairs, root diameter, the root cap, the pattern of appearance of daughter roots, undulations of the root axis, and cortical senescence. Root to shoot ratio is a measure of the allocation of resources between different plant components. The allocation of resources toward the root is high at early vegetative stages but decreases markedly at flowering and is almost negligible after anthesis (Gregory et al., 1996). In rice (*Oryza sativa*), Asch et al. (2004) reported that the proportion of total dry matter allocated to root or shoot parts depended on the rate of soil dry-down, with root-shoot ratios averaging 0.05–0.1 at flowering in soil-filled PVC pipes. Some important parameters used to describe root growth and distribution include maximum rooting depth, total root length, root surface area, root volume, root diameter, root length density, root distribution pattern in the soil column, root to shoot ratio, root branching, root hydraulic conductance, root anatomy, root elongation rate, total plant length, and hard pan penetrability.

Deep roots will help increase crop yields under drought conditions as they can extract moisture from deeper soil horizons. Deep rooting is a major cause of differences in drought tolerance between plant species (Boyer, 1996). Yoshida and Hasegawa (1982) showed that the potential maximum rooting depth is genetically determined (although affected by environmental conditions in the field) and varies substantially between genotypes grown under identical conditions. Maximum root depth for any given genotype will be attained under conditions where roots do not encounter a physical limit to growth. The quantity of root length (or weight) in

layers within the soil profile is usually expressed in terms of root length (or weight) per unit volume of soil, referred to as root length (or weight) density. Since water is mostly absorbed passively, root length density, which reflects the development of lateral roots, can be directly related to water uptake ability of the plant. As root length density increases, water uptake usually increases, but up to a given length only, which is termed critical root length density. In rice, like other crops, the critical root length density depends on soil conditions, especially moisture (Siopongco et al., 2005), and roots are distributed in such a way that their length and mass will decrease exponentially with depth. Root density at depth determines the exploitation of water present at deeper levels. Fine roots present a large percentage of total root length in almost all conditions and thus are strongly expected to contribute greatly to water uptake by the entire root system.

When focusing on roots in breeding for improved drought tolerance, potential traits may include increasing root distribution at depth to improve deep water capture (O'Brien, 1979; Manske and Vlek, 2002), depth of rooting to extract water from full soil depth (Hurd, 1974), fast root elongation rates for deep water capture (O'Brien, 1979), reducing the diameter of the xylem vessel in the seminal roots to conserve soil water (Richards and Passioura, 1989), angle of seminal roots for extracting water from full soil depth (Nakamoto and Oyanagi, 1994; Manschadi et al., 2006) and increasing the root : shoot dry matter for improved water capture across the soil profile (Siddique et al., 1990; Reynolds et al., 2007)

Sorghum is considered as a drought tolerant crop with deep roots that are assumed to play a key role in its drought adaptation. Although, several drought-related studies have been carried out with sorghum, surprisingly limited work has been done on the roots. Studies that have presented evidence of genotypic variation for root traits (Mayaki et al., 1976; Jordan et al., 1979), focused on only a few breeding lines with a limited genetic base. Genotypic variations for root traits have been found in other studies using solution culture (Blum et al., 1977), or in small pots (Nour et al., 1978). Salih et al. (1999) showed that a drought tolerant sorghum line possessed roots at least 40 cm deeper than a drought sensitive one. Due to the important role

played by roots in plant growth, there is a need to conduct more studies on sorghum roots. The goal of this study was to look at genetic variability in sorghum roots and their response to drought stress. We hypothesized that there is genetic diversity among grain sorghum genotypes in root growth and development in response to water deficit. The objectives of the study were to quantify (i) the genetic variability exhibited in root system development in response to water deficits for selected grain sorghum genotypes, and (ii) effects of drought stress on different root classes in these genotypes.

4.2. Materials and methods

Nineteen grain sorghum genotypes comprising of inbred lines and hybrids were used in this experiment (Table 4.1). Selection of these genotypes was based on canopy temperature and grain yield for inbred lines while hybrids were selected based on maturity class. Genotypes Liang tang ai and Tx7078 were used as checks based on their TE rating..

4.2.1. Growth conditions

The experiment was conducted under greenhouse conditions with temperatures set at 32/26°C (day and night). The crops were grown in plastic columns with inside diameter of 20 cm and 1 m tall filled with Turface® MVP (PROFILE Products, LLC, Buffalo Grove, Illinois, USA). Osmocote controlled release fertilizer (19-6-12, N-P_2O_2-K_2O; Osmocote® Classic, The Scotts Company LLC, Marysville, Ohio, USA) was used as a source of nutrients. The columns were sealed at the bottom with heavy duty plastic sheeting and clamped with hose-clamps to hold the soil and prevent water loss. Moisture content was calculated based on weight basis. A given amount of turface was weighed and then soaked with water. After sitting for 24 hours to allow drainage of excess water the soil was weighed again and percent water content calculated. This was used to establish percent water content in the weighed columns. Three seeds were sown in each column and watered every day till 10 days after germination. Thinning was done to one plant per column, columns were fully watered and then weighed and the top of the columns sealed to prevent water loss through evaporation. Drought

stress was imposed by with-holding water for 30 days starting at 15 days after emergence. The controls were watered after every 4 days throughout the experiment period. Weighing was done using a hanging scale (Salter Brecknell 235-10X, Brooklyn, New York, USA). There were three replications of each genotype laid out in a randomized complete block design (RCBD).

4.2.2. Data collection

The columns were weighed once a week and changes in weight recorded for those that were under drought stress. At 30 days of drought stress, plant height was measured and tiller and leaf number (on the main stem) were counted. Plants were then harvested and rooting depth measured by laying the roots on a flat surface and measuring the length. Above and below ground biomass was separated. Leaf area was measured using a leaf area meter (LI-3100C Area Meter, LI-COR Inc., Lincoln, Nebraska, USA). Leaves and stems were dried to determine dry above ground biomass. Roots were placed between moist tissue paper and sealed in Ziploc® bags (26.8 x 27.3 cm) (S.C. Johnson & Sons, Inc. Racine, Wisconsin, USA) and kept in a cold room. The roots were cut into shorter sections, thoroughly washed, spread out in a scanning tray with water and scanned using a photo scanner (Epson perfection V700 photo scanner, Epson America Inc., Long Beach, California, USA). The scanned images were used to analyze the roots for total root length, root length for different root diameter classes using WhinRhizo (WhinRhizo Pro., Regent Instruments Inc., Quebec City, Quebec, Canada).

Data was analyzed used PROC GLM in SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA) with genotype and treatment (water stressed or fully irrigated) classed as main effects. Standard errors were used to show estimate of variability, LSD at alpha of 0.05 was used to compare means and P-values were used to show significance levels.

4.3. Results

4.3.1 Growth parameters

Genotypes varied significantly in shoot growth and root parameters and these variables were also significantly affected by drought stress (Table 4.2). Variation was highly significant ($P < 0.0001$) for all growth parameters (plant height, rooting depth, leaf area, total root length, total surface area and average root diameter among the genotypes and was significant ($P = 0.0012$) for relative water content (RWC). Effects of drought stress were also highly significant for RWC, plant height, leaf area, total root surface area, average root diameter and total root length ($P < 0.0001$ and 0.0019 respectively). Average relative water content (RWC) varied from 86% (SC663) to 92% (SC701). Plant height varied from 20.9 cm (TX7078) to 42.0 cm (Liang Tang Ai) and leaf area from 130 cm^2 (B.Tx615) to 931 cm^2 (SC60). Liang Tang Ai recorded the largest values for total root length, total surface area and average root diameter while TX7078 had the lowest values (7.4 m, 0.9 m^2 and 0.4 cm respectively). Genotype SC701 had consistently high values for most of the parameters except plant height and rooting depth (Table 4.2).

Plant height varied significantly ($P < 0.0001$) among the genotypes and also between drought stress treatments, but rooting depth was only significant among the genotypes (Table 4.2). Genotypes Liang Tang Ai and SC701 recorded highest values for plant height while TX7078 and B.Tx615 had the lowest (Figure4.1). Rooting depth was greatest for DeKalb 54-00 and DeKalb 28E and lowest values were for R.Tx436 and SC701.

4.3.2. Leaf relative water content (RWC)

RWC significantly varied among the genotypes ($P = 0.0012$) and was affected significantly affected by drought stress ($P < 0.0001$) but there was no genotype x treatment interaction (Table 4.2). Liang Tang Ai and PI584085 had significantly low RWC for the controls but for the rest of the genotypes RWC for controls ranged from 91 – 94%. The greatest reduction of 11% was seen in SC663 and SC224 a reduction of 10% while SC1124 had the smallest reduction (Figure 4.2).

4.3.3. Effects of drought stress on leaf area, plant height and rooting depth

Leaf area varied significantly among the genotypes and the effects of drought stress were also significant ($P < 0.0001$) (Table 4.2). There were significant changes in leaf area among all the genotypes as a response to drought stress. All the genotypes, except PI584085, experienced a reduction in leaf area due to imposition of drought stress ranging from 2.8 (SC663) to 76% (R.Tx436) (Figure 4.3). Other genotypes that had a reduction in leaf area of more than 50% were TX7078, SC701, SC720, SC60, Pioneer hybrid 85G46 and B.Tx615. Genotypes with the smallest reduction in leaf area included B.Tx399 and SC1124. Genotype PI584085 increased leaf area by 11% under drought stress conditions.

Plant height and rooting depth was also significantly affected by drought stress (Figure 4.4). Plant height was reduced for all the genotypes except PI584085 and SC224, which increased by 18% and 41% respectively. Height reduction was greatest for genotypes Pioneer Hybrid 85G46, TX7078 , DeKalb 28E and SC720 .Genotypes that recorded small reductions in plant height were B.Tx2752 , BT.x615 ,SC663 and B.Tx399. Rooting depth was increased for thirteen out of the nineteen genotypes used with increases ranging from 6% (Liang Tang Ai) to 180% (SC1124) (Figure 4.4). Other genotypes that had large increases in rooting depth were PI584085 , BT.x399 and SC663 . Genotypes that showed a decrease in rooting depth under drought stress conditions were SC701, R.Tx436, Pioneer Hybrid 85G64, TX7078, SC1019 and DeKalb 28E. Correlation between rooting depth and plant height was positive under well watered as well drought stressed conditions (R^2 = 0.062 and 0.314 respectively) and significant ($P = 0.021$) under drought stressed conditions only (Figure 4.5).

4.3.4. Root length and surface area

Variation in total length and surface area for different root classes, (< 0.25, 0.25 – 0.50, 0.50 – 1.00, 1.00 – 2.00 and > 2.00 mm in diameter), was highly significant among the genotypes (Table 4.3). Variation due to drought stress was also significant for all the classes. Genotypes that consistently recorded high values in total

root length and surface area were Liang Tang Ai, SC701 and SC224. These genotypes with consistently low values included TX7078 , R.Tx436 and SC663.

4.3.5. Effects of drought stress on root length and surface area

Effect of drought stress was significant for total root length and root surface area (P = 0.0019 and <0.0001 respectively) (Table 4.2). Percentage change was similar among the genotypes for these two parameters. Genotypes whose total root length increased or decreased under drought stress had also a similar increase or decrease in root surface area (Figure 4.6). Genotypes that recorded highest increase in total root length and also surface area under drought stress were SC224 and SC1019. Others genotypes that recorded an increase in total root length and root surface area included SA5330/Martin, SC663, B.Tx615 and SC1124 . Decrease of more than 50% in root length was recorded for genotypes R.Tx436, SC70, SC60 and BTx2752.

Root length and surface area of the fine roots (< 0.25 mm in diameter) were affected in a similar manner as total root length (Figure 4.7). Genotypes that experienced increased total root length and surface area under drought stress were SC224, SC1019, SA5330/Martin, SC663, SC1124 and B.Tx615. Genotypes R.Tx436, SC720, SC60 and B.Tx2752 showed a reduction in total root length . Highest increases in the rest of the root classes were seen in genotypes SC224 for 0.25 – 0.5 mm and > 2.0 mm diameter classes, SC1019 for 0.5 – 1.0 mm diameter class and TX7078 for 1.0 – 2.0 mm diameter class (Figure 4.8). Genotypes with the greatest reductions in total root length for these classes were R.Tx436 for 0.25 – 0.5 mm and 0.5 – 1.0 mm diameter classes, DeKalb 54-00 for 1.0 – 2.0 mm diameter class and DeKalb 54-00 and DeKalb 28E for > 2.0 mm diameter class.

4.3.6. Effects of drought stress on root distribution along column depth

Root distribution generally increased with depth (Figure 4.9). There were fewer roots at the top section of the column (0 – 30 cm depth) for all the genotypes except R.Tx436, which had a reduction of total root length from 12.0 m to 6.8 and 9.3 m at 30 – 60 cm and > 60 cm depths, and genotype B.Tx615 with a reduction

from 17.6 m at top section to 12.0 m at > 60 cm depth. Major increases in root length at different depths were seen in genotypes SC701, and DeKalb 28E.

Distribution of roots along the column depth varied among the genotypes and was affected by drought stress (Figure 4.10). There were genotypes whose root distribution was increased and others decreased by drought stress. Genotypes that recorded an increase in total root length at 0 – 30 cm depth with drought stress were SC224, B.Tx615, SC1019 and SA5330 while SC720 and SC60 recorded the greatest decrease at this depth. At the 30 – 60 cm depth, genotypes that had greatest increase in total root length under drought stressed conditions were SC224, TX7078 and SC663, while the largest decrease was in genotypes SC60 and R.Tx436. At a depth of more than 60 cm, genotypes with the largest increase in total root length were SC1019, SA5330, SC663, and genotypes with greatest reductions were TX7078, R.Tx436 and SC720.

4.3.7. Water used as a function of total root length and root surface area

There was a strong relationship between total root length and root surface area ($R^2 = 0.99$, $P < 0.0001$) (Figure 4.11). Total water used was positively related to both total root length and surface area. This relationship was high and significant in total root length when compared to root surface area (Figure 4.12).

4.4. Discussion

4.4.1. Effects of drought stress on growth

Our study showed that drought stress reduced plant height (2 – 50%) and leaf area (3 – 76%). This concurs with what has been reported in existing literature. Leaf area expansion will influence plant height as more leaf area means more photosynthetic activity and therefore more assimilate allocation to various parts of the plant. Leaf expansion in plants is among the most sensitive growth processes to drought stress (Alves and Setter, 2004). Effects of drought on leaf expansion will be manifested through smaller cells and a reduction in the number of cells produced by leaf meristems (Randall and Sinclair, 1988; Tardieu et al., 2000). Cell expansion and production of new cells have been shown to result in a reduction in leaf area depending on the

developmental stage at which the stressed occurred (Alves and Setter, 2004). Effects of drought on leaves at different stages in plant development or age of the leaf will vary. In older leaves that were no longer engaged in cell division, reduced cell expansion will affect leaf area by reducing mature cell size, whereas, in younger leaves, inhibition of cell division will result in fewer cells per leaf (Alves and Setter, 2004). Under mild drought stress conditions there will be a reduction in leaf numbers, rate of expansion and final leaf size, while under severe stress conditions there will be a reduction in the rate of leaf elongation and leaf growth can cease. Drought stress also can influence total leaf area through its effect on initiation of new leaves, which is decreased under drought stress. de Souza et al. (1997) showed that continued drought stress can accelerate leaf senescence and lead to death of leaf tissue, resulting in leaf drop of particularly old and mature leaves. The level of reduction in leaf area may therefore give us an indication of drought tolerance levels and therefore from this study those genotypes with low percentage decrease in leaf area (PI584085, SC663, B.Tx399, SC1124 and SC1019) may be plants that are more drought tolerant (Figure 4.3).

4.4.2. Root response to drought stress

Although drought stress decreased leaf area and plant height, our study showed significant increases in rooting depth, total root length and root surface area (Figure 4 and 6). Under drought stress conditions an increased rooting depth would contribute to better drought tolerance. Deep rooting would increase crop yield under drought stress (Jordan et al., 1983). It has been argued that an increase in the soil volume explored by roots would result in an increase in crop yield under water-limited environments (Jones and Zur, 1984). A review by Ludlow and Muchow (1990) of 16 traits that potentially contribute to drought tolerance ranked the three most important to be plant phenology, osmotic adjustment, and rooting depth. In our study, rooting depth was increased for thirteen out of the nineteen genotypes with increases ranging from 6.3% – 180% (Figure 4). Some of the genotypes with the largest increase in rooting depth were Liang Tang Ai, SC1124, PI584085, BT.x399, and SC663.

Declining plant available soil water results in a reduction in shoot growth before root growth is affected. A reduction in leaf growth may occur before a decline in photosynthetic activity due to drought stress (Boyer, 1970), resulting in a surplus of carbohydrates which are available for root growth. Sharp et al. (1990) showed that osmotic adjustment may occur in root tips prolonging root cell expansion. In cases where these two processes occur, the absolute size of the root system for stressed plants will sometimes exceed that of well-watered plants (Sharp and Davies, 1979). Our results showed significant increases in total root length for genotypes SC224, SC1019, SA5330/Martin, SC663, B.Tx615 and SC1124.

4.4.3. Root length and water use

Total water used was positively related to both total root length and surface area. This relationship was high and significant in total root length ($R^2 = 0.21$, $P = 0.0493$) when compared to root surface area ($R^2 = 0.18$, $P = 0.0676$) (Figure 12). Total root length or root surface area is a key determinant for water and nutrient uptake for a plant and therefore the correlation found in our study. Reviews by Kramer (1969), Hurd (1974) and Jackson et al. (2000) have suggested that a deep, wide-spreading and much-branched root system is essential in the design of drought-tolerant crops. On the other hand, Passioura (1983) suggested that small root systems could provide benefits in water-limited situations through improved water use efficiency.

Plants growing in moisture limited environments face major variations in water supply among environments and seasons and will experience drought stress at different stages of development. Under conditions where crops grow on soil water accumulated before sowing or early in growth, conserving water for use during the reproductive phase will provide significant benefits (Richards and Passioura, 1989; Morison et al., 2008). Plants that will do well in these conditions will be drought tolerant and will produce warmer canopies. Alternatively, for crops growing under conditions of more uniform rainfall distribution, the ability to capture water and use it quickly may be beneficial (Turner and Nicolas, 1987; Moeller et al., 2009). These are drought escaping plants due to their high transpirational cooling and will have cooler canopies. Plants with

small root systems may not fully exploit moisture at deeper soil horizons and hence experience severe drought stress while at the same time leaving substantial amounts of available water in the subsoil. However, for crops growing on stored soil water, there may be benefit if the root system expands slowly to allow soil water to be used later during the grain filling.

4.5. Conclusion

Our study has shown that there is genetic diversity among grain sorghum genotypes in root response to water deficit. There were genotypes whose root growth and development increased with drought stress. Most of the genotypes with increased rooting depth and total root length under drought stress also had an increase in plant height and leaf area. Rooting depth and increased total root length (root density) are desirable traits under water limited conditions. These traits will enable the plant to extract water at deeper levels in the soil and hence maintain a large leaf area and therefore more photosynthetic activity.

In our study there were some genotypes that responded positively to drought stress. Genotypes SC1124 and SC663 recorded increase in rooting depth and total root length and had low reduction in leaf area. Genotypes SC663 and SC1019 recorded large increases in total root length, had high total root length at > 60 cm depth and had low reduction in leaf area. These are genotypes that have desirable drought tolerance traits.

The fine roots (<0.25 mm in diameter) form the bulk of the rooting system and they were affected in the same way as total root length. Root morphology and distribution in the soil plays a major role in the way a plant will extract water from the soil. There is therefore a potential for selection of genotypes with root systems adapted to given environments. Increasing root vigor in a crop results in an increase in the total size of the root system. Greater vigor will result in early and fast rates of root extension as well as early and profuse root proliferation and this will lead to an increase in root biomass and root length density. A vigorous or large root system will contribute to adaptation in dry environments and dry seasons where crop growth depends on seasonal rainfall. However, a large root system may be of less value in environments where crop growth is

dependent on stored soil water where access to more soil water runs the risk of exhausting soil water before completing grain filling.

4.6. Figures and Tables

Table 4-1: Genotypes used in the study

Genotype	Type	Characteristics
B.Tx2752	Inbred line	Low leaf temperature, high grain yield
B.Tx399	Inbred line	Normal leaf temperature, high grain yield
B.Tx615	Inbred line	Low leaf temperature, low grain yield
Dekalb 28E	Hybrid	Early season hybrid
Dekalb 54-00	Hybrid	Medium – full season hybrid
Liang Tang Ai	Inbred line	High TE line
PI584085	Inbred line	-
Pioneer 84G62	Hybrid	Full season hybrid
Pioneer 85G46	Hybrid	Medium season hybrid
R.Tx436	Inbred line	Low leaf temperature, low grain yield
SA5330/Martin	Inbred line	Low leaf temperature, low grain yield
SC1019	Inbred line	High leaf temperature, high grain yield
SC1124	Inbred line	High leaf temperature, low grain yield
SC224	Inbred line	Low leaf temperature, low grain yield
SC60	Inbred line	Low leaf temperature, low grain yield
SC663	Inbred line	Normal leaf temperature, high grain yield
SC701	Inbred line	Low leaf temperature, high grain yield
SC720	Inbred line	High leaf temperature, high grain yield
TX7078	Inbred line	Low TE line

Table 4-2: Means showing variation among genotypes in shoot and root growth parameters

Genotype	RWC (%)	Plant height (cm)	Rooting depth (cm)	Leaf area (cm^2)	Total root length (m)	Total surface area (m^2)	Average root diameter (cm)
B.Tx2752	89.5	30.01	99.77	671.01	35.45	5.11	0.93
B.Tx399	88.5	21.13	83.02	334.21	16.25	2.19	0.56
B.Tx615	89.9	22.69	92.80	130.10	16.30	2.29	0.56
Dekalb 28E	87.1	33.01	104.27	521.85	28.47	4.12	0.76
Dekalb 54-00	86.8	26.82	106.39	573.59	15.46	2.35	0.54
Liang Tang Ai	85.9	42.01	84.14	747.80	51.45	7.59	1.12
PI584085	86.1	26.07	85.35	502.70	30.86	4.39	0.78
Pioneer 84G62	91.4	30.51	91.14	571.43	18.21	2.63	0.56
Pioneer 85G46	90.9	26.20	99.27	523.44	22.80	3.52	0.80
R.Tx436	86.7	24.20	70.77	462.87	9.39	1.48	0.50
SA5330/Martin	87.5	27.76	84.27	594.51	32.32	4.50	0.81
SC1019	90.4	23.38	78.77	478.95	17.37	2.69	0.64
SC1124	88.1	31.52	80.03	591.79	25.76	4.23	0.87
SC224	86.2	24.13	84.89	518.40	43.99	6.18	1.09
SC60	87.2	29.38	102.64	931.34	28.27	4.41	0.78
SC663	85.7	25.51	85.64	409.96	14.44	2.06	0.57
SC701	91.7	22.76	79.64	753.80	39.96	6.24	1.08
SC720	90.1	36.20	98.02	848.39	31.68	4.95	0.98
TX7078	89.7	20.95	82.10	345.72	7.40	0.90	0.37
$LSD_{(\alpha = 0.05)}$	3.40	6.37	18.19	269.08	11.19	1.72	0.239
P-Value							
Genotype	0.0012	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment	<0.0001	<0.0001	NS	<0.0001	0.0019	0.0001	0.0016
Geno x Trt	NS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 4-3: Means showing variation among genotypes for total length and surface area for different root classes based on root diameter

	Root class based on dimater									
	<0.25	**0.25 – 0.5**	**0.5 – 1.0**	**1.0 – 2.0**	**> 2.0**	**<0.25**	**0.25 – 0.5**	**0.5 – 1.0**	**1.0 – 2.0**	**> 2.0**
Genotype	**Total length (m)**					**Total surface area (m^2)**				
B.Tx2752	19.11	7.79	5.27	2.83	0.45	0.86	0.86	1.17	1.19	0.36
B.Tx399	8.51	4.09	2.49	1.08	0.07	0.40	0.45	0.56	0.44	0.06
B.Tx615	8.62	3.75	2.63	1.13	0.15	0.39	0.41	0.59	0.47	0.12
Dekalb 28E	14.89	6.35	4.78	2.11	0.33	0.67	0.71	1.05	0.88	0.27
Dekalb 54-00	7.48	4.03	2.49	1.23	0.23	0.35	0.44	0.55	0.52	0.19
Liang Tang Ai	27.33	11.44	8.05	3.78	0.84	1.20	1.28	1.77	1.60	0.73
PI584085	17.36	6.43	4.24	2.30	0.52	0.76	0.71	0.93	0.98	0.44
Pioneer 84G62	9.29	4.42	2.90	1.42	0.17	0.43	0.49	0.64	0.59	0.14
Pioneer 85G46	11.14	5.48	4.00	1.76	0.41	0.50	0.61	0.88	0.76	0.34
R.Tx436	4.38	2.25	1.84	0.79	0.13	0.21	0.25	0.41	0.34	0.10
SA5330/Martin	16.80	7.81	5.14	2.31	0.24	0.76	0.87	1.13	0.96	0.19
SC1019	8.27	4.24	3.22	1.46	0.18	0.39	0.47	0.72	0.62	0.14
SC1124	11.90	6.33	4.69	2.39	0.44	0.56	0.71	1.03	1.02	0.37
SC224	24.29	9.41	6.39	3.39	0.49	1.06	1.04	1.41	1.44	0.39
SC60	13.80	6.73	4.99	2.25	0.48	0.63	0.75	1.10	0.96	0.38
SC663	7.61	3.26	2.35	1.08	0.13	0.35	0.36	0.52	0.46	0.10
SC701	19.60	9.68	6.80	3.14	0.73	0.90	1.08	1.49	1.35	0.61
SC720	15.09	7.57	5.92	2.63	0.46	0.67	0.85	1.30	1.11	0.38
TX7078	3.67	2.28	1.20	0.28	0.03	0.18	0.25	0.26	0.10	0.03
$LSD_{(\alpha = 0.05)}$	6.20	2.35	1.74	0.94	0.33	0.27	0.26	0.38	0.41	0.29
P-Value										
Genotype	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment	0.0142	0.0006	0.0008	<0.0001	<0.0001	0.0094	0.0006	0.0009	<0.0001	0.0002
Geno x Trt	<0.0001	<0.0001	<0.0001	<0.0001	0.0232	<0.0001	<0.0001	<0.0001	<0.0001	0.0523

Figure 4-1: Variation in average plant height and rooting depth among sorghum genotypes (LSD(α = 0.05): Plant height = 6.37, Rooting depth = 18.19)

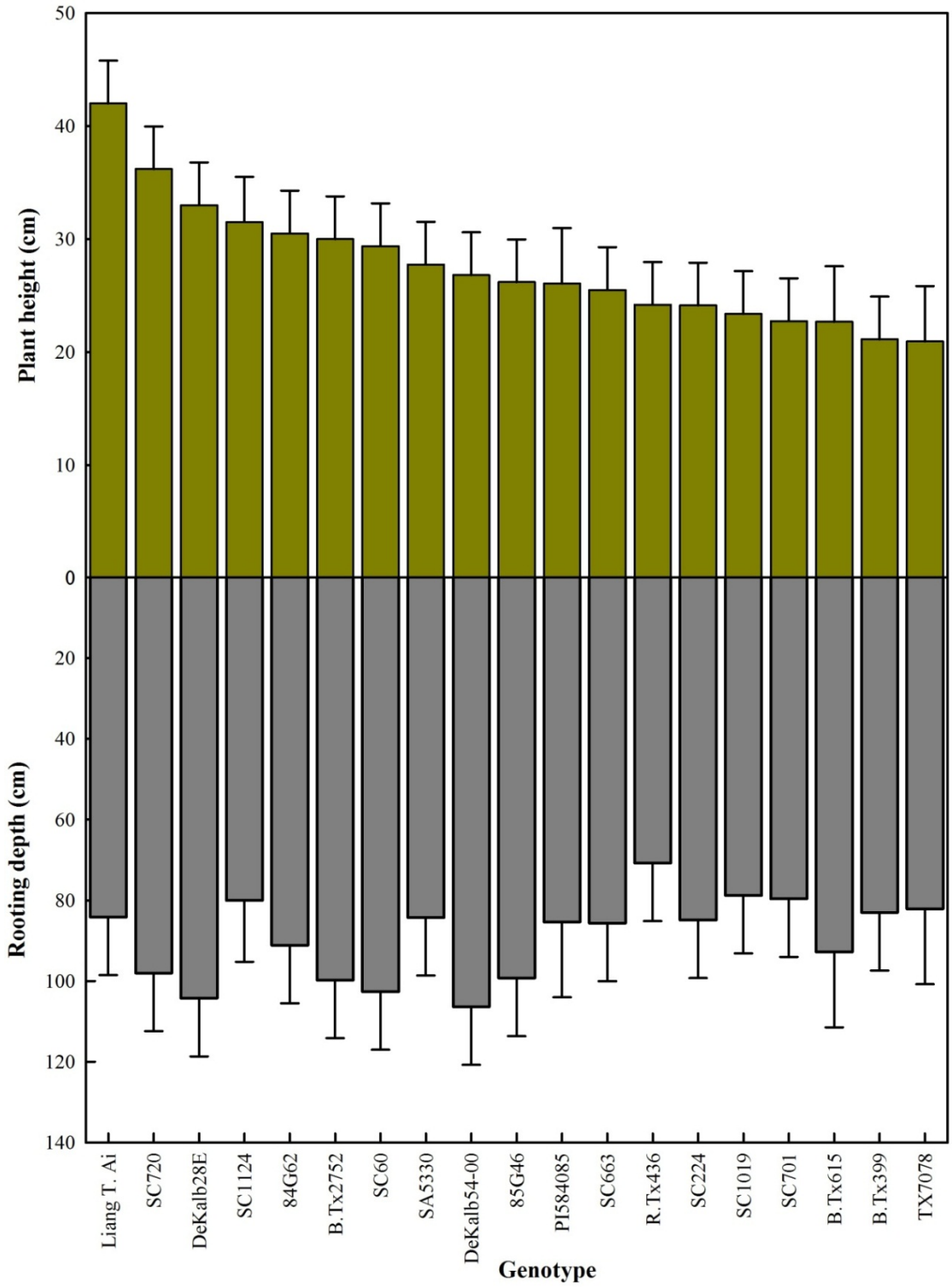
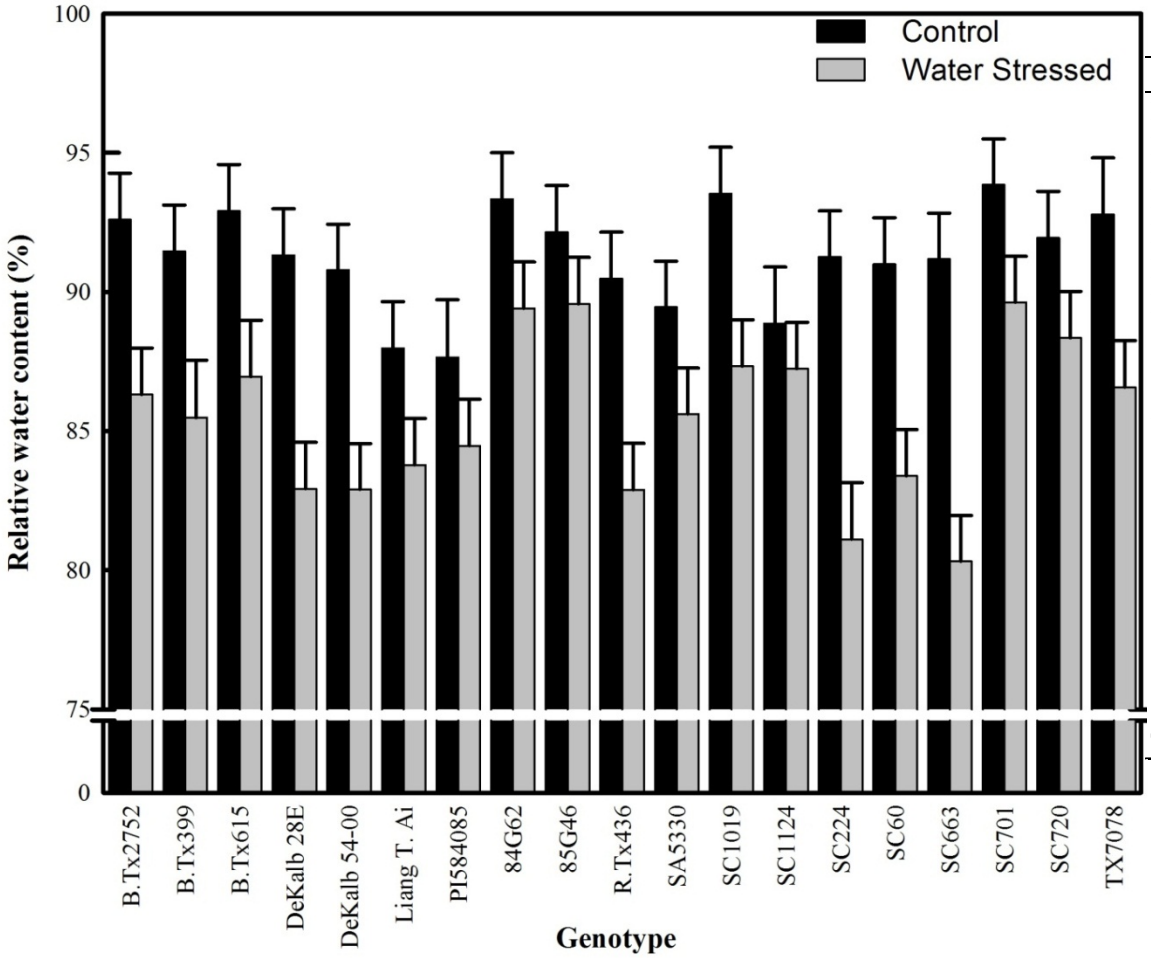
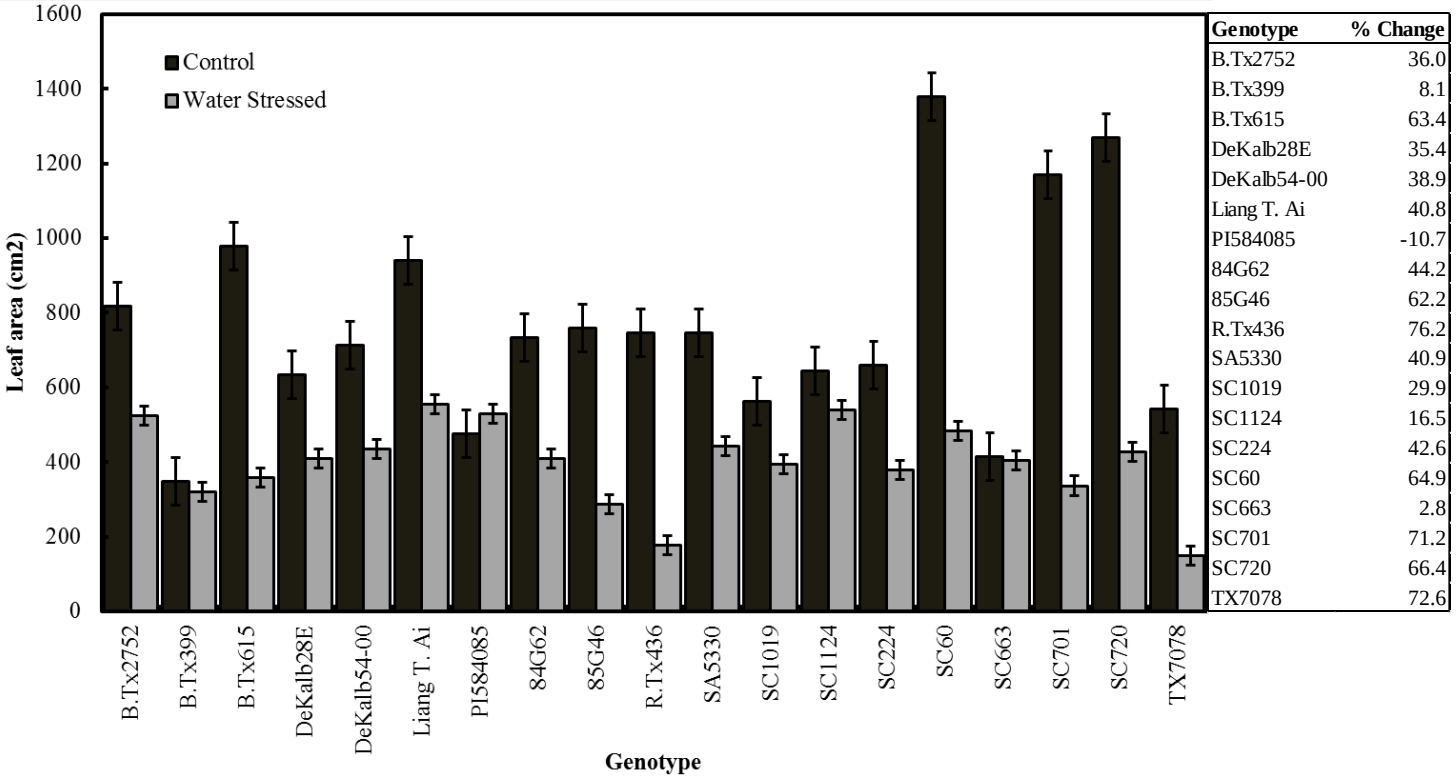


Figure 4-2: Leaf relative water content (RWC) as an indicator of drought stress level among genotypes.



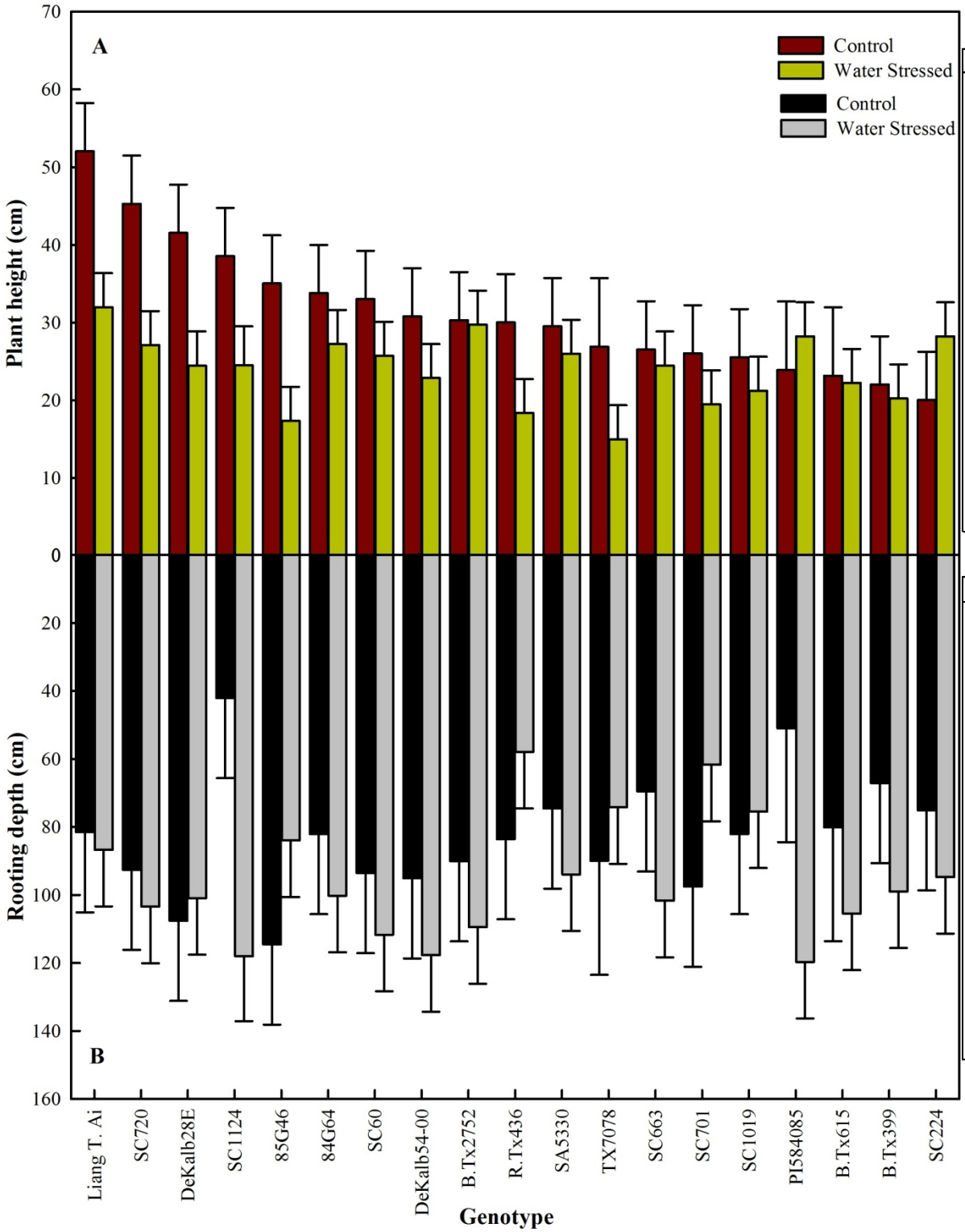
Genotype	% change
B.Tx2752	6.29
B.Tx399	5.98
B.Tx615	5.96
DeKalb 28E	8.39
DeKalb 54-00	7.88
Liang T. Ai	4.2
PI584085	3.17
84G62	3.92
85G46	2.58
R.Tx436	7.59
SA5330	3.84
SC1019	6.2
SC1124	1.62
SC224	10.15
SC60	7.61
SC663	10.87
SC701	4.22
SC720	3.6
TX7078	6.21

Figure 4-3: Effects of drought stress on leaf area of different sorghum genotypes



Genotype	% Change
B.Tx2752	36.0
B.Tx399	8.1
B.Tx615	63.4
DeKalb28E	35.4
DeKalb54-00	38.9
Liang T. Ai	40.8
PI584085	-10.7
84G62	44.2
85G46	62.2
R.Tx436	76.2
SA5330	40.9
SC1019	29.9
SC1124	16.5
SC224	42.6
SC60	64.9
SC663	2.8
SC701	71.2
SC720	66.4
TX7078	72.6

Figure 4-4: Effects of drought stress on plant height and rooting depth among genotypes



Genotype	% Change
Liang Tang Ai	38.5
SC720	40.1
Dekalb 28E	41.1
SC1124	36.4
Pioneer 85G46	50.5
Pioneer 84G62	19.4
SC60	22.1
Dekalb 54-00	25.8
B.Tx2752	1.8
R.Tx436	38.9
SA5330/Martin	12.1
TX7078	44.3
SC663	7.8
SC701	25.2
SC1019	16.9
PI584085	-18.1
B.Tx615	4.0
B.Tx399	8.2
SC224	-40.9

Genotype	% Change
Liang Tang Ai	-6.3
SC720	-11.8
Dekalb 28E	6.1
SC1124	-180.6
Pioneer 85G46	26.7
Pioneer 84G62	-22.1
SC60	-19.4
Dekalb 54-00	-23.8
B.Tx2752	-21.6
R.Tx436	30.6
SA5330/Martin	-26.0
TX7078	17.5
SC663	-46.2
SC701	36.7
SC1019	8.0
PI584085	-134.8
B.Tx615	-31.6
B.Tx399	-47.6
SC224	-26.2

Figure 4-5: Correlation between rooting depth and plant height under well watered and drought stressed conditions

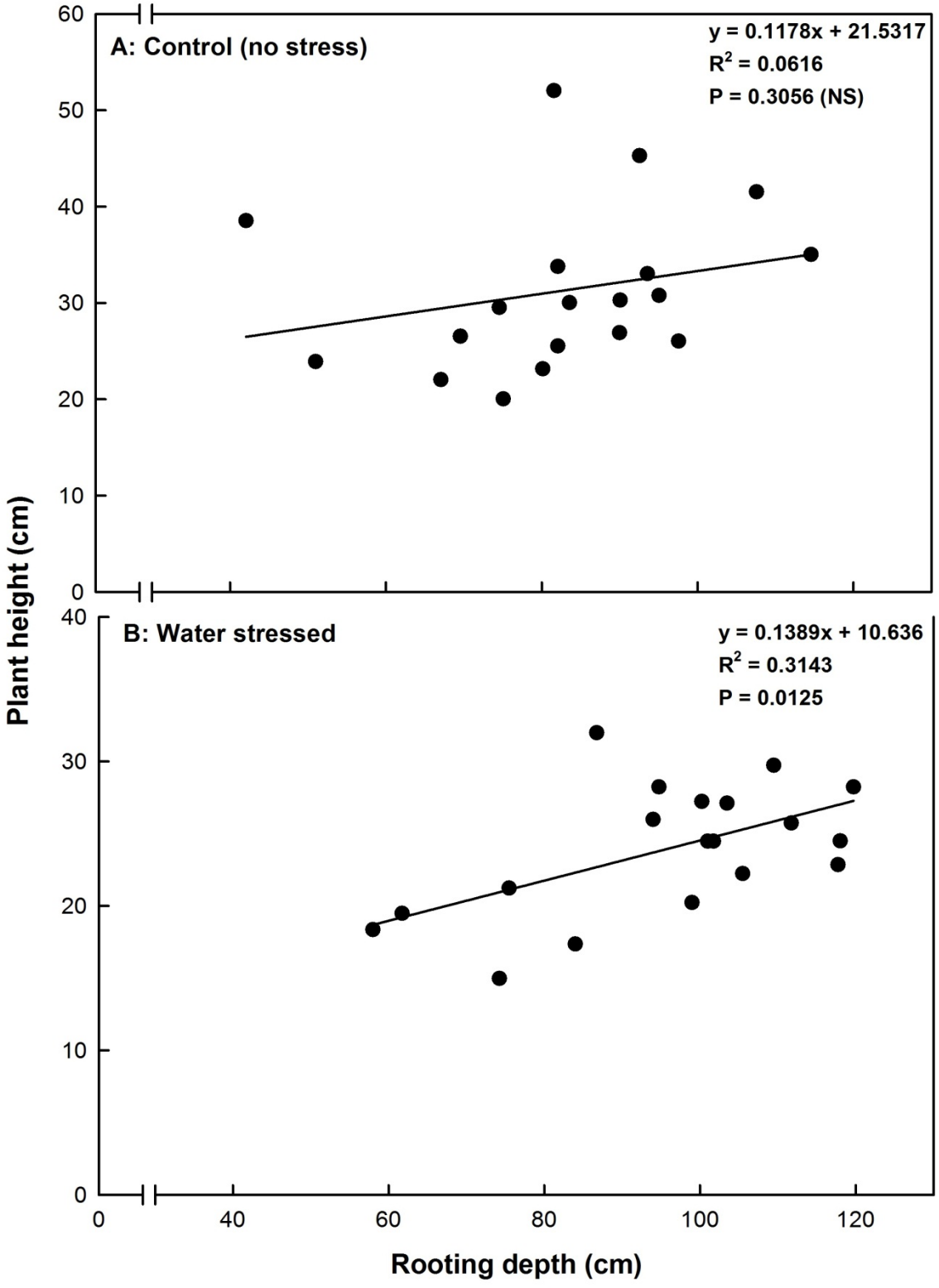
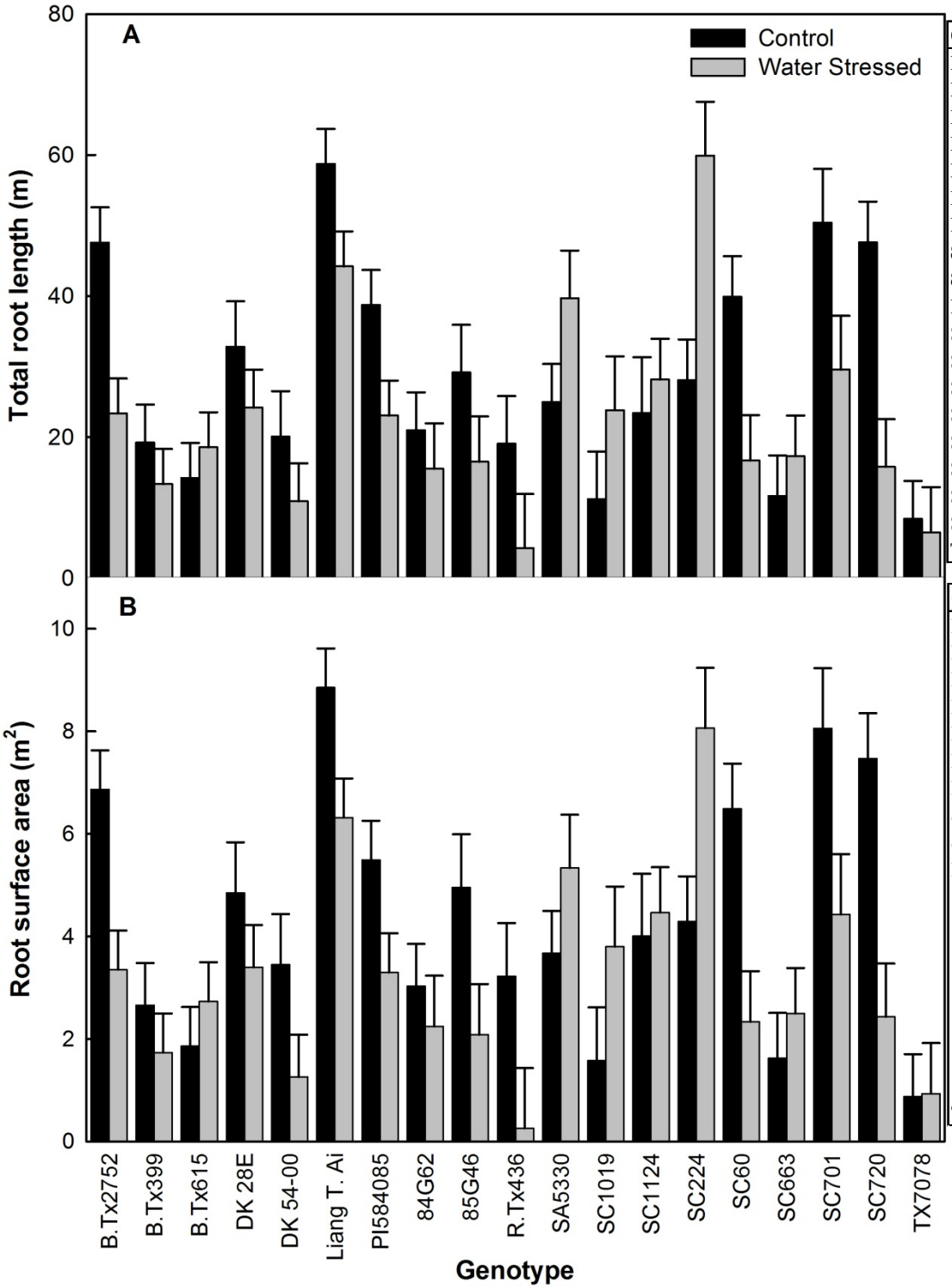


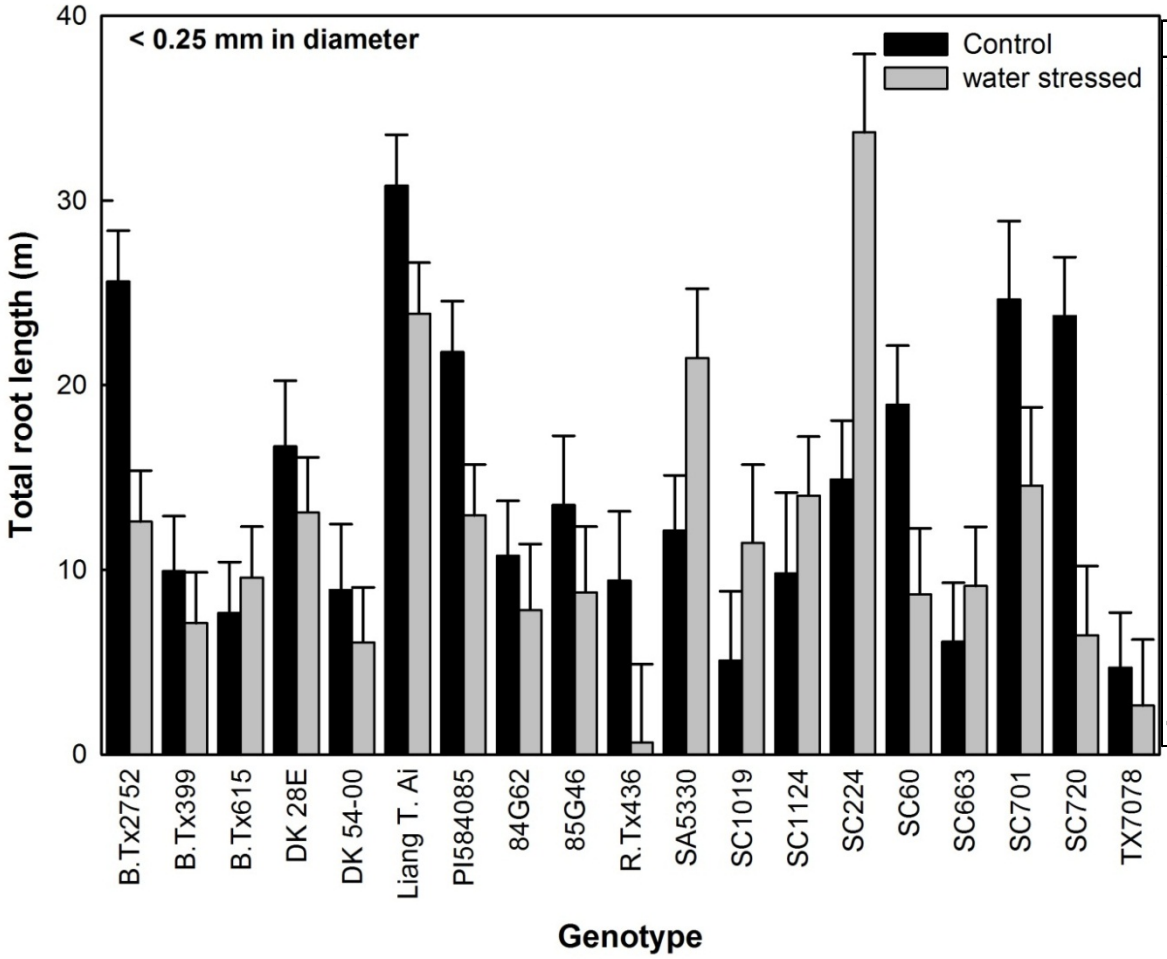
Figure 4-6: Effects of drought stress on total root length and total root surface area of different sorghum genotypes



Genotype	% Change
B.Tx2752	51.0
B.Tx399	30.7
B.Tx615	-30.6
Dekalb 28E	26.4
Dekalb 54-00	45.8
Liang Tang Ai	24.7
PI584085	40.5
84G62	26.1
85G46	43.5
R.Tx436	77.7
SA5330/Martin	-58.9
SC1019	-112.9
SC1124	-20.4
SC224	-113.4
SC60	58.2
SC663	-48.6
SC701	41.4
SC720	66.9
TX7078	23.1

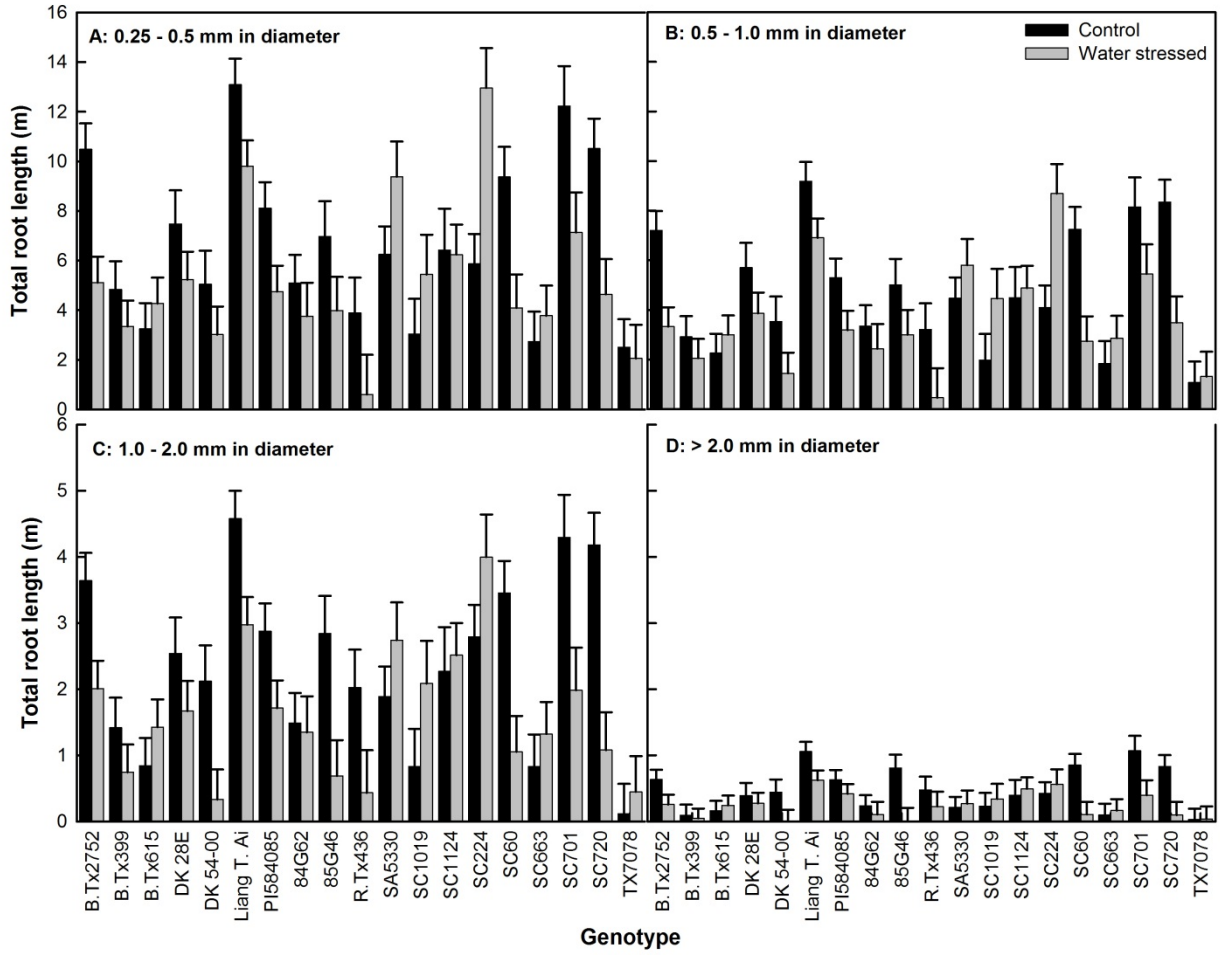
Genotype	% Change
B.Tx2752	51.2
B.Tx399	34.7
B.Tx615	-47.2
Dekalb 28E	29.9
Dekalb 54-00	63.5
Liang Tang Ai	28.6
PI584085	40.0
84G62	25.8
85G46	58.0
R.Tx436	92.1
SA5330/Martin	-45.4
SC1019	-141.1
SC1124	-11.4
SC224	-88.1
SC60	64.0
SC663	-54.0
SC701	45.0
SC720	67.5
TX7078	-6.3

Figure 4-7: Effects of drought stress on fine roots (diameter < 0.25 mm) of different sorghum genotypes



Genotype	% Change
B.Tx2752	50.75
B.Tx399	28.40
B.Tx615	-24.93
Dekalb 28E	21.44
Dekalb 54-00	31.90
Liang Tang Ai	22.49
PI584085	40.59
84G62	27.21
85G46	35.04
R.Tx436	93.11
SA5330	-76.94
SC1019	-124.95
SC1124	-43.14
SC224	-126.20
SC60	54.22
SC663	-49.57
SC701	40.91
SC720	72.83
TX7078	43.63

Figure 4-8: Effects of drought stress on different root classes (diameters: 0.25 – 0.5, 0.5 – 1.0, 1.0 – 2.0 and > 2.0 mm) of various sorghum genotypes



Genotype	% Change in total root length			
	0.25 - 0.5 mm	0.5 - 1.0 mm	1.0 - 2.0 mm	> 2.0 mm
B.Tx2752	51.3	53.8	44.8	0.0
B.Tx399	30.9	29.4	47.5	7.5
B.Tx615	-31.6	-32.7	-69.0	0.0
Dekalb 28E	30.1	32.2	34.2	16.4
Dekalb 54-00	40.2	59.2	84.3	16.5
Liang Tang Ai	25.1	24.8	35.0	0.0
PI584085	41.5	39.8	40.4	0.0
84G62	26.4	27.4	9.5	-19.7
85G46	42.8	40.1	75.8	4.8
R.Tx436	84.5	85.6	78.6	-13.1

Genotype	% Change in total root length			
	0.25 - 0.5 mm	0.5 - 1.0 mm	1.0 - 2.0 mm	> 2.0 mm
SA5330	-50.1	-29.9	-45.1	-25.7
SC1019	-79.0	-125.6	-150.5	-13.1
SC1124	2.9	-8.7	-10.7	27.3
SC224	-120.9	-112.3	-43.3	-32.7
SC60	56.4	62.2	69.5	-11.7
SC663	-38.2	-55.1	-59.2	0.0
SC701	41.7	33.2	53.8	0.0
SC720	55.9	58.2	74.1	-17.3
TX7078	17.9	-21.7	-286.7	-19.7

Figure 4-9: Total root length at different depths for different sorghum genotypes

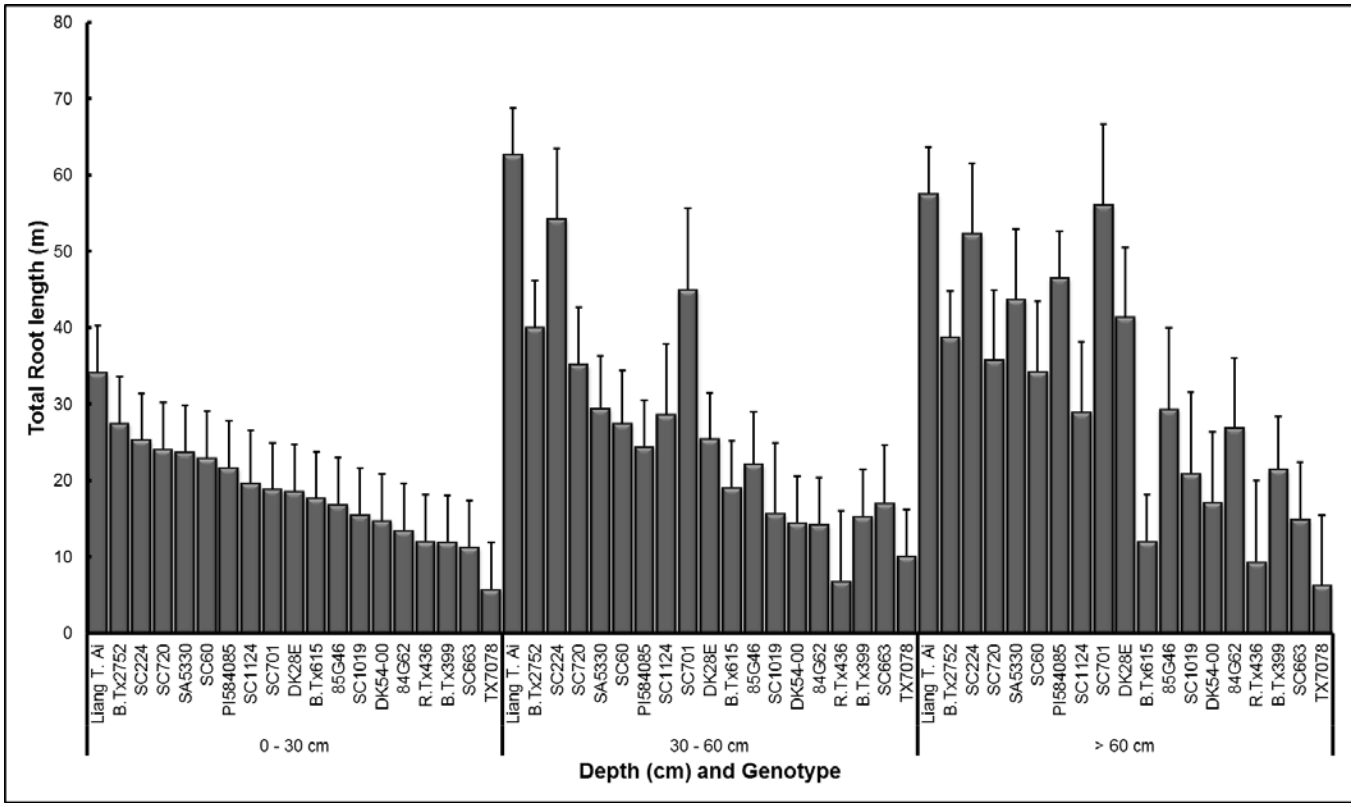


Figure 4-10: Effects of drought stress on distribution of roots at different depths among different sorghum genotypes

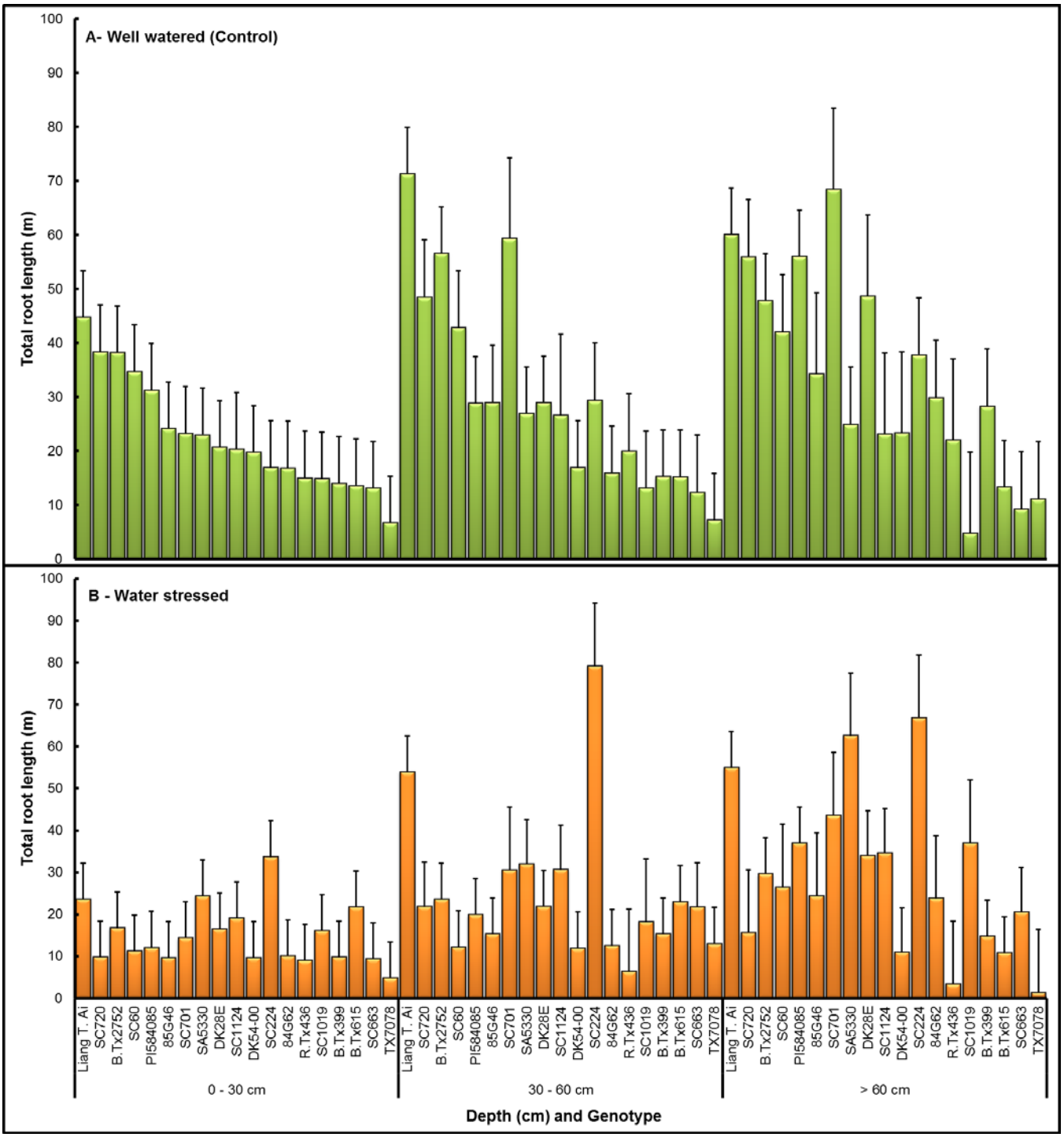


Figure 4-11: Relationship between root length and surface area

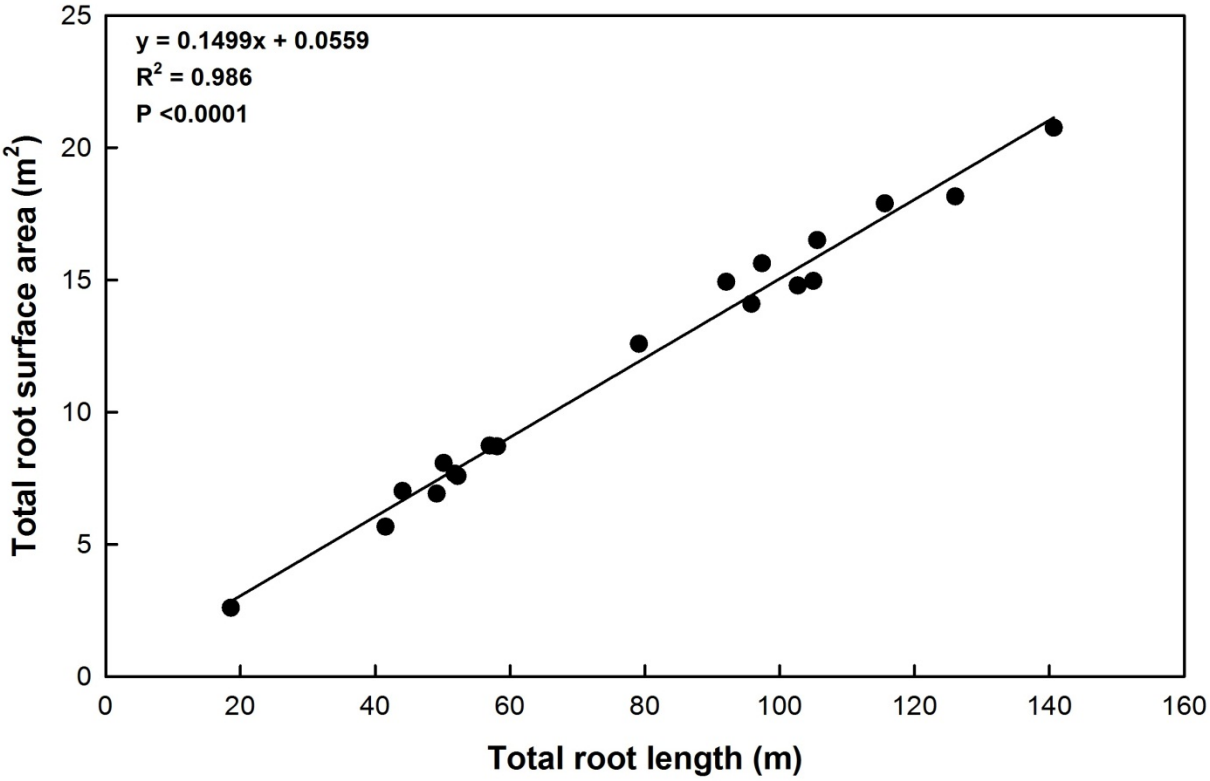
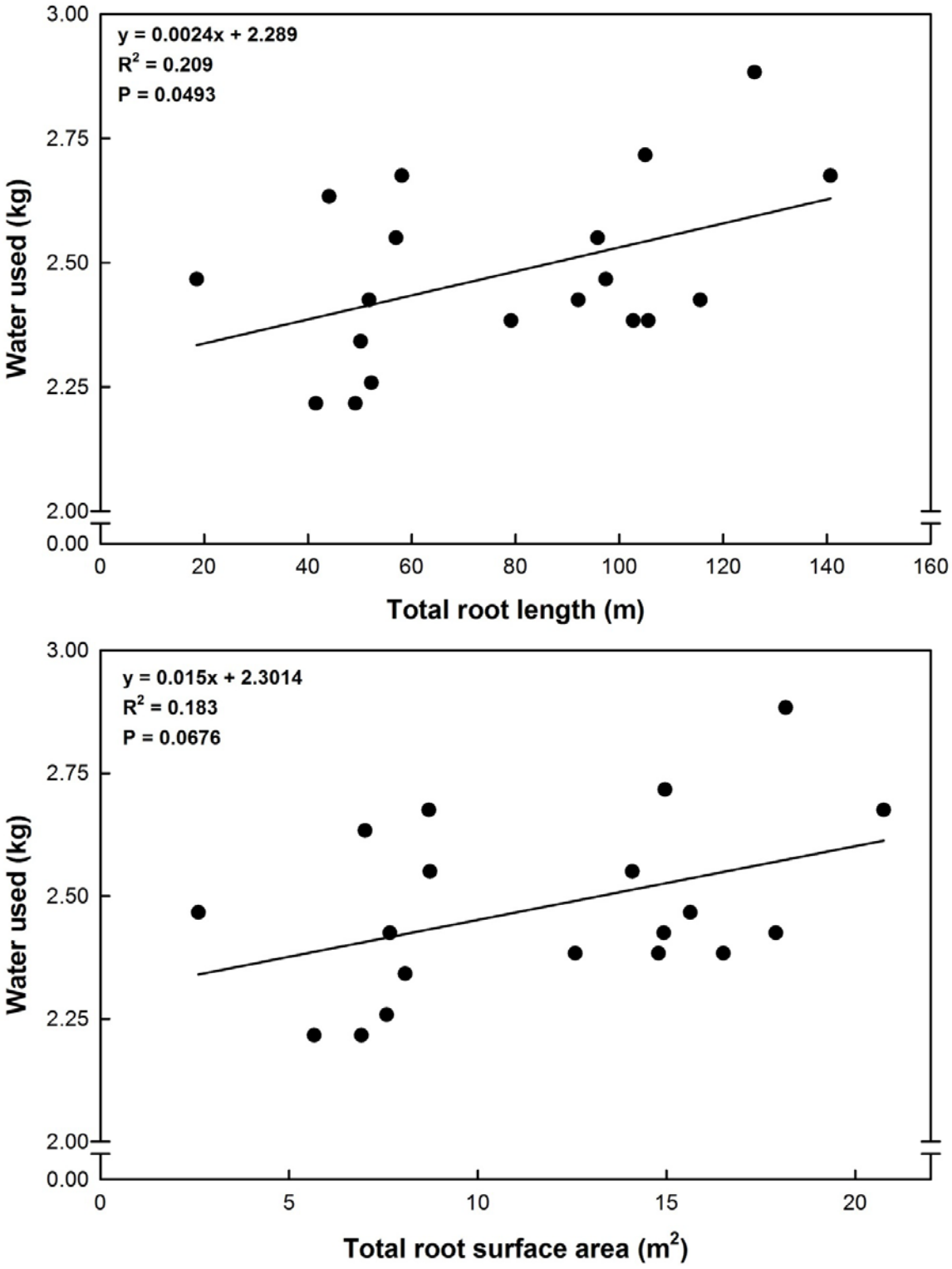


Figure 4-12: Water used as a function of total root length and total root surface area



4.7. References

Alves, A.A.C., and T.L. Setter. 2004. Response of cassava leaf area expansion to water deficit: Cell proliferation, cell expansion and delayed development. Ann. Bot. 94: 605 – 613.

Asch, F., M. Dingkuhn, A. Sow, and A. Audebert. 2004. Drought-induced changes in rooting patterns and assimilate partitioning between root and shoot in upland rice. Field Crops Res. 93: 223 – 236.

Blum, A., W.R. Jordan, and G.F. Arkin. 1977. Sorghum root morphogenesis and growth. II. Manifestations of heterosis. Crop Sci. 17: 153 – 157.

Boyer, J.S. 1996. Advances in drought tolerance in plants. Adv. Agron. 56: 187–218.

Boyer, J.S. 1970. Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. Plant Physiol. 46: 236 – 239.

de Souza, P.I., D.B. Egli, and W.P. Bruening. 1997. Drought stress during seed filing and leaf senescence in soybean. Agron. J. 89: 807–812.

Gregory, P.J., J.A. Palta, and G.R. Batts. 1996. Root systems and root:mass ratio - carbon allocation under current and projected atmospheric conditions in arable crops. Plant Soil 187: 221–228.

Hurd, E.A. 1974. Phenotype and drought tolerance in wheat. Agric. Meteor. 14: 39 – 55

Jackson, R.B., J.S. Sperry, and T.E. Dawson. 2000. Root water uptake and transport: physiological processes in global predictions. Trends Plant Sci. 5: 482 – 488.

Jones, J.W., and B. Zur. 1984. Simulation of possible adaptive mechanisms in crops subjected to drought stress. Irrig. Sci. 5: 25 – 264.

Jordan, W.R., R.L. Monk, F.R. Miller, D.T. Rosenow, L.E. Clark, and P.J. Shouse. 1983. Environmental physiology of sorghum. I. Environ-mental and genetic control of epicuticular wax load. Crop Sci. 23: 552 – 558.

Jordan, W.R., F.R. Miller, and D.E. Morris. 1979. Genetic Variation in Root and Shoot Growth of Sorghum in Hydroponics. Crop Sci. 19: 468 – 472.

Kramer, P.J. 1969. Plant and soil water relationships: a modern synthesis. McGraw-Hill: New York.

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

Lynch, J.P. and M. Ho. 2005. Rhizoeconomics: carbon costs of phosphorus acquisition. Plant Soil 269: 45 – 56.

Malamy, J.E. and P.N. Benfey. 1997. Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development 124: 33 – 44

Manschadi, A.M., J. Christopher, P. de Voil, and G.L. Hammer. 2006. The role of root architectural traits in adaptation of wheat to water-limited environments. Funct. Plant Biol. 33: 823 – 837.

Manske, G.G.B., and P.L.G. Vlek. 2002. Root architecture – wheat as a model. In: Plant roots: the hidden half. Y. Waisel, A. Eshel (Eds.). pp. 249–259. Marcel Dekker, Inc., New York.

Mayaki, W.C., L.R. Stone, and I.D. Teare. 1976. Irrigated and non-irrigated soybean, corn, and grain sorghum root systems. Agron. J. 68: 532–534.

Moeller, C., S. Asseng, J. Berger, and S.P. Milroy. 2009. Plant available soil water at sowing in Mediterranean environments – is it a useful criterion to aid nitrogen fertiliser and sowing decisions? Field Crops Res. 114: 127 – 136.

Morison, J.I.L., N.R. Baker, P.M. Mullineaux, and W.J. Davies. 2008. Improving water use in crop production. Philo. Trans. Royal Soc. of Lond. Series B, Biol. Sci. 363: 639 – 658.

Nakamoto, T. and A. Oyanagi. 1994. The direction of growth of seminal roots of *Triticum aestivum* L. an experimental modification thereof. Ann. Bot. 73: 363 – 367.

Nour, A.E.M., and D.L. Weibel. 1978. Evaluation of root characteristics in grain sorghum. Agron. J. 70: 217 – 218.

O'Brien, L. 1979. Genetic variability of root growth in wheat (*T. aestivum* L.). Aust. J. Agric. Res. 30: 587 – 595

O'Toole J.C., and W.L. Bland. 1987. Genotypic variation in crop plant root systems. Adv. Agron. 41: 91 – 145.

Passioura, J.B. 1983. Roots and drought resistance. Agric. Water Manag. 7: 265 – 280.

Randall, H.C., and T.R. Sinclair. 1988. Sensitivity of soybean leaf development to water deficit. Plant Cell Environ. 11: 835–839.

Reynolds, M.P., C. Saint Pierre, A.S.I. Saad, M. Vargas, and A.G. Condon. 2007. Evaluating potential genetic gains in wheat associated with stress adaptive trait expression in elite genetic resources under drought and heat stress. Crop Sci. 47: 172 – 189

Richards, R.A., and J.B. Passioura. 1989. A breeding program to reduce the diameter of the major xylem vessel in the seminal roots of wheat and its effect on grain yield in rain-fed environments. Aust. J. Agric. Res. 40: 943 – 950.

Salih, A.A., I.A. Ali, A. Lux, M. Luxova, Y. Cohen, Y. Sugimoto, and S. Inanaga. 1999. Rooting, water uptake, and xylem structure adaptation to drought of two sorghum cultivars. Crop Sci. 39: 168 – 173.

Sharp, R.E., T.C. Hsiao, and W.K. Silk. 1990. Growth of the maize primary root at low water potentials. II. The role of growth and deposition of hexose and potassium in osmotic adjustment. Plant Physiol. 93: 1337 – 1346.

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

Siddique, K.H.M., R.K. Belford, and D. Tennant. 1990. Growth, development and light interception of old and modern wheat cultivars in a Mediterranean type environment. Aust. J. Agric. Res. 40: 473 – 487

Siopongco, J.D.L.C., A. Yamauchi, H. Salekdeh, J. Bennett, and L.J. Wade. 2005. Root growth and water extraction response of double-haploid rice lines to drought and re-watering during the vegetative stage. Plant Prod. Sci. 8: 497 – 508.

Sponchiado, B.N., J.W. White, J.A. Castillo, P.G. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exper. Agric. 25: 249 – 257.

Tardieu, F., M. Reymond, P. Hamard, C. Granier, and B. Muller. 2000. Spatial distribution of expansion rate, cell division rate and cell size in maize leaves: A synthesis of the effects of soil water status, evaporative demand and temperature. J. Exp. Bot. 51: 1505 – 1514.

Turner, N.C. and M.E. Nicolas. 1987. Drought resistance of wheat for light-textured soils in a Mediterranean climate. In: Drought tolerance in winter cereals. J.P. Srivastava, E. Porceddu, E. Acevedo, S. Varma (Eds.). pp. 203 – 216. John Wiley and son, Chichester, UK.

Yoshida, S., and S. Hasegawa. 1982. The rice root system: its development and function. In: Drought Resistance in Crops, with Emphasis on Rice. International Rice Research Institute, Manila, Philippines.

Chapter 5 – General Conclusions and Recommendations

5.1. General conclusions

Based on the results from these studies, general conclusions that can be drawn from the different experiments are:

5.1.1. Chapter 2

Genotypes that were selected based on the presence of a breakpoint in their transpiration (Gholipoor et al., 2010) had also high TE values but differed in the amount of water used and the amount of biomass accumulated. These results can be used in breeding for improving drought tolerance in crops because of the conservative nature of genotypes. However, these genotypes must be examined for differences in extraction of water under field conditions and their yield response.

5.1.2. Chapter 3

Infrared (IR) sensors can be used to detect differences in canopy temperature among genotypes. Canopy temperature depression (CTD) was stable for all the genotypes the period between 1:00 pm and 7:00 pm and also 2:00 am to 8:00 am, indicating that these are the best times to take CTD readings for sorghum. CTD behaved differently under different environmental conditions, and therefore this parameter should be determined for individual environments. Even though midday CTD was positively correlated to yield and HI, it should be noted that genotypes exist that will have low CTD resulting in warmers canopies because they are conservative in the way they use water. Use of CTD when screening for drought tolerance must take this into consideration so as not leave out these genotypes since they are also drought tolerant.

5.1.3. Chapter 4

Rooting depth and increased total root length (root density) are desirable traits under water limited conditions because they enable the plant to extract water at deeper levels in the soil and hence maintain a large

leaf area and therefore more photosynthetic activity. Root morphology and distribution in the soil plays a major role in the way a plant will extract water from the soil. Genotypes exist that responded positively to drought stress, implying the potential for selection of genotypes with root systems adapted to given environments. There is a need to screen more sorghum genotypes for root traits that are associated with drought tolerance.

5.2. Recommendations

Conservative water use by a plant means the availability of water even under water limited conditions during late grain filling stages. There is a need monitor soil moisture levels over time using slow wilting and non-slow wilting genotypes. The heritability of the slow wilting trait needs to be evaluated so as to determine whether this can be used for increased drought tolerance in sorghum breeding programs. This study did not look at genotypes that are drought escapers (cooler canopies due to high transpirational cooling). These genotypes must be evaluated to identify environments where they will be well adapted. More genotypes need to be screened for root traits and their response to other abiotic factors since roots form an important part of the plant.