

**Physiological characterization of parents of sorghum mapping populations exposed to
water-deficit stress**

by

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Abstract

Changing climate presents new challenges to agricultural production and global climate models project increased intensity and magnitude in water-deficit stress conditions in the future. This is especially challenging for the arid and semi-arid regions of the world, where sorghum forms an important component of the cropping system. The research objective was to characterize eleven genetically and geographically diverse sorghum Nested Association Mapping parental lines (Ajabsido, SC1103, SC265, P898012, SC35, Macia, SC283, SC1345, Segalane, SC971 and RTx430 - the common parent) for shoot and root related physiological parameters during the vegetative and grain filling stages. Using a lysimeter based experiment in the greenhouse, water-deficit stress (55 % to 60 % field capacity) imposed for 15 days during the vegetative stage recorded significant variation between water-deficit stress and well-watered treatments for all physiological and agronomic traits regardless of the genotypic variance, except for rooting depth. The genotype by treatment interaction indicated stem height to be not only under genetic control but was subject to complex effects of the watering regimes. Transpiration efficiency and carbon isotope discrimination increased for water-deficit stressed plants. A significantly higher biomass partitioning to the roots was detected under water-deficit stress compared with the well-watered plants. In Ghana, another experiment focusing on the vegetative stage with two levels of water-deficit stress imposed on selected set of sorghum genotypes, recorded a greater decrease for shoot and root related traits under the more severe stress (50 % to 55 % FC; lasting for 21 days) than the moderate water-deficit condition (60 % to 65 % FC; lasting for 15 days). The mean cumulative water transpired (liters) during the 21 days period was 2.32 for the severe water-deficit stress, 6.88 for the moderate water-deficit stress, and 10.7 for the well-watered condition. In the grain filling experiments conducted in both the greenhouse and on the field, water-deficit stress induced

variations in grain number and grain weight along different positions on the panicle among the tested genotypes. In this regard, differences in panicle positional grain number accounted for differences in panicle positional grain weight as the positional individual grain weight was not affected by the stress. Regardless of the watering treatments however, differences in grain numbers and grain weight among genotypes resulted from the diversity in panicle architecture other than grain filling dynamics. In both the greenhouse and field experiments, SC1103 did not record any significant difference between the watering treatment for all measured growth and yield traits. Spearman's rank correlations indicated the ability to select for water-deficit tolerance traits in the greenhouse that would partially represent rankings on the field. Generally, performance of genotypes such as SC35 portrayed a higher level of tolerance to water-deficit stress whereas other genotypes such as SC971 depicted significantly higher level of susceptibility. Findings from this research is helpful for providing pathways to map genomic regions responsible for increased resilience to water-deficit stress.

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Dedication

I dedicate this work to

the late Rev. Dr. Godfred Kojo Ngorli Zormelo

of the A.M.E.Zion Church

who believed in me at a tender age and invested in my education up to the tertiary

Chapter 1 - Literature Review

1.1 Overview of sorghum production

Sorghum is the fifth most economically important cereal in the world and serves as a staple food in some Asian and many African nations. It is also used as feed for animals and employed for industrial purposes such as biofuels and beer (Felch et al., 2006). In arid and semi-arid regions where some crops cannot grow due to harsh environmental conditions, crops such as sorghum and millet have acclimatized to survive and produce grain. In the toughest environments of West Africa, where no other crop can be cultivated on a steady basis, sorghum and millet are dependable crops (Callo-concha et al., 2013). With 22 % of total land coverage, sorghum is the second most important cereal after maize (about 34 % of total land coverage) in Africa, followed by millets, mainly finger and pearl millets, with 19 % of the total area (Macauley, 2015).

Sorghum is believed to have originated in Africa and was first domesticated in Ethiopia (Smith and Frederiksen, 2000). Historically, genetic improvements in sorghum have involved increasing both the aboveground (leaf mass) biomass production as well as decreasing peduncle length and increasing panicle length (Assefa and Staggenborg, 2011). Sorghum improvements in the past six decades in the USA have included germplasm development and a range of management practices including irrigation, tillage practices, and fertilization rates (Ciampitti and Prasad, 2016). About 65 % of yield increase achieved as a result of these improvements can be attributed to the management-by-environment interaction and the other 35 % through genetic improvement (Assefa et al., 2010). The final output of sorghum can thus be concluded to be a product of the interaction between management, genotype, and the environment, with environmental factors such as temperature and water greatly influencing sorghum's productivity (Roozeboom and Prasad, 2016).

1.2 Growth and development of sorghum

Plant growth involves incremental changes in the plant's biomass overtime, while plant development is the seasonal or phenological change that occurs in a plant's lifetime such as fruiting, tiller emergence, flowering, and leaf emergence. Growth is characterized by an increase in size or mass of the whole plant or its part and would include quantifiable measures such as increase in plant height, volume, cell size, and a plant's dry and fresh weights (Bareja, 2014). An understanding of how a sorghum plant grows through its developmental stages and how it accumulates and partitions dry matter serves as a baseline in understanding the impact of environment and management interactions (Roozeboom and Prasad, 2016). It is also helpful in determining deviations from normal growth and development.

Sorghum has developmental stages labelled from zero to nine, with the vegetative period spanning from stage zero to stage four and the reproductive period from stage five to stage nine (Ciampitti and Prasad, 2016; Besancon et al., 2005). However, Roozeboom and Prasad (2016) categorized sorghum's developmental stages into three, namely the vegetative, reproductive, and grain-filling stages, where about one-third of the life cycle is spent at each stage. The vegetative stage includes the phases of emergence, appearance of third leaf collar and fifth leaf collar, while phases of the reproductive stage include growing point differentiation, flag leaf appearance, booting, and half bloom (Roozeboom and Prasad, 2016; Gerik et al., 2003). The reproductive stage, which marks the transition from the vegetative to the spikelet number determination and subsequently grain producing period, commences at the growing point differentiation where the terminal meristem forms panicle structures instead of initiating new leaves. There is a potential for yield reduction from stress or direct injury to the panicle at this phase (Vanderlip, 1993). When blooming, anthers first appear at the top of the panicle with newer appearances continuing down the panicle in circular

bands until bands form at the base, and they appear an average of four days later than when the first band appeared. Later phases of grain development (soft dough and hard dough) follows similar pattern. The half bloom phase marks the transition from reproductive to the grain-filling stage, and it is a phase where plant height and leaf area have reached the maximum (Besancon et al., 2005). The soft dough phase, hard dough phase, and the phase of physiological maturity constitute the grain-filling stage (Gerik et al., 2003).

According to Gambin et al. (2008), the grain-filling stage can be divided into three periods: (i) the lag phase (synonymous for the soft dough phase), which is the period right after embryo formation and is crucial for determining the potential grain size. There is active cell division and a fast increase in grain water content with no deposition of dry matter, although sites for subsequent reserve deposition are being established; (ii) effective grain-filling period (synonymous for the hard dough phase), where the water content of the grain continues to increase until a maximum point at mid-grain filling, after which it declines. Deposition rate of carbohydrates, proteins, and other nutrients also increases rapidly, and this accounts for loss in stem weight; (iii) physiological maturity commences when assimilate deposition is completed with a visual indicator of an abscission or black layer at the base of the grain, opposite the embryo (Gambin et al., 2008). With maximum accumulation of dry matter achieved in the grain at this point (Gerik et al., 2003), there is continual loss of water while the grains dry weight stabilizes.

1.3 Water-deficit stress

Water is an indispensable substance required for many biological processes (e.g., physiological processes), and its deficiency is detrimental to living organisms, such as plants. Under field conditions, cyclic changes and unpredictable climatic conditions affect the availability of water

(Devnarain et al., 2016). In the context of agronomy, drought can be defined as “any occurrence of plant water stress at levels that are sufficient to affect plant growth rates” (Lobell et al., 2015). According to (Taiz et al., 2015), “drought is a meteorological term for a period of insufficient precipitation that results in plant water deficit”. Nevertheless, because some plants can capture water from the soil during periods of less rainfall (Taiz et al., 2015), the term “water-deficit stress” may be more appropriate in describing conditions where precipitation is restricting crop growth. In this review, these two terms (“drought” and “water-deficit stress”) are used interchangeably.

Drought is an intricate environmental stress that limits crop productivity and thus affects global food security. Factors that influence the occurrence and severity of drought include vapor pressure deficit (VPD), available soil moisture, wind speed, atmospheric carbon dioxide (CO₂) levels, and solar radiation fluxes, which regulate potential photosynthetic rates (Lobell et al., 2015). Temperature drives water-deficit stress through its relation with VPD. Vapor pressure deficit increases nonlinearly as air temperature warms, which induces water-deficit stress by increasing plant water use (Lobell et al., 2015).

Water-deficit stress generates many physiological and biochemical changes, and responses of sorghum to water-deficit stress is dependent on the severity and duration of the stress, age of the plant, and phase of development, type of organ, cell type, and sub-cellular compartment (Barnabas et al., 2008; Prasad et al., 2008). Irrespective of sorghum’s general resiliency to water-deficit stress, phases within its life cycle can be vulnerable to stress from water deficit. The early phase of the vegetative growth and the reproductive stage of sorghum are susceptible to water-deficit stress (Devnarain et al., 2016). Water-deficit stress considerably slows tissue expansion before any visual symptoms, such as wilting and leaf rolling are noticeable (Lobell et al., 2015). A

genotype is considered “drought tolerant” when it has the ability to yield significantly better than another genotype during severe water-deficit stress (Hasan et al., 2017; Mai et al., 2012).

1.3.1 Drought coping mechanisms

Coping with drought includes mechanisms that are associated with either efficient usage of limited water supply or efficient conversion of photosynthates into reproductive organs (Liu et al., 2005). The mechanisms employed to deal with water-deficit stress can be categorized as drought escape, tolerance, and avoidance (Gowda et al., 2011; Lopes et al., 2011; Araus et al., 2002; Levitt, 1972). They may work synergistically to ensure that the sorghum plant copes with stress from water-deficit conditions. A crop may inherently possess characteristics that allow it to be tolerant to drought, while another crop develops adaptive properties that allows it to acquire resilience following exposure to drought conditions.

1.3.1.1 Avoidance

The avoidance mechanism is when there is maintenance of a high plant water status irrespective of the stress, either through maximal uptake of water or through minimized loss of water by programmed senescence of older leaves, stomatal closure, or reduced leaf area (Barnabás et al., 2008). Features that enable plants to reduce the quantity of water loss are considered as drought avoidance mechanisms (Devnarain et al., 2016). One classical drought avoidance mechanism is the preferential allocation of carbon to developing deep roots at the expense of shoot growth during periods of water-deficit stress (Munamava and Riddoch, 2001). This feature ensures a high water absorption to enhance survival during subsequent drought exposures (Munamava and Riddoch, 2001).

1.3.1.2 Escape

The escape mechanism is when the reproduction and early grain formation are completed before the onset of severe stress which includes higher growth rate, shortened life cycle, and proficient use of storage reserves (Barnabás et al., 2008). Plants employ the drought escape mechanism in order to complete their life cycle before soil moisture becomes limiting (Devnarain et al., 2016). Plants that are drought escapers tend to be dedicated to seed production and thus accelerate maturity because of the water-deficit stress (Munamava and Riddoch, 2001). The sorghum genotype ‘Segaolane’ has been identified as a good drought escaper ((Munamava and Riddoch, 2001).

1.3.1.3 Tolerance

The tolerance mechanisms occurs when the plant is able to undergo its life processes under limited water availability through osmotic adjustment or scavenging reactive oxygen species (ROS) (Barnabás et al., 2008). Drought tolerance includes stabilizing mechanisms that protect cellular integrity and maintaining metabolic activity (Devnarain et al., 2016). These mechanisms may include harnessing the potassium ion for osmotic adjustment, production of osmolytes (such as glycine betaine, proline, organic acids, polyols, and other amino acids), production of plant growth regulators (which include salicylic acid, auxins, gibberellins, cytokinins, and abscisic acid) to modulate response to stress, and production of enzymes that act as antioxidants (Farooq et al., 2009).

1.4 Physiological traits and their responses to water-deficit stress in sorghum

Manifestation of a single or combined physiological, morphological, and biochemical response at the molecular, cellular, tissue, and whole-plant level determines the ability of the plant to sustain itself under stress (Farooq et al., 2009). An increase or decrease in any of these responses are crucial in conferring some type of tolerance to stress, either just for survival or for survival plus higher productivity.

1.4.1 Photosynthesis and stomatal conductance

Generally, the amount of CO₂ entering a leaf is reduced at low water potential as a result of stomatal closure. Water-deficit stress stimulates the accumulation of abscisic acid, which triggers stomatal closure, and water is conserved through reduction in transpiration. This process regulates gas exchange and inhibits photosynthesis (Taiz et al., 2015). A sufficient supply of photosynthates is crucial during reproduction and any form of water-deficit stress that impairs photosynthesis will alter the development of reproductive structures (Ali et al., 2009). When photosynthesis is reduced during stress, there is conversion and mobilization of the stem reserves into soluble sugars for the grain-filling process (Barnabas et al., 2008; Prasad et al., 2008).

As reported by Munamava and Riddoch (2001), the ability of stomata to remain open at low leaf water potentials varies by genotypes. Some sorghum genotypes are able to maintain a higher water uptake at low water potential through osmotic adjustment and this ensures higher rates of photosynthesis (Hasan et al., 2017). In a comparative experiment involving corn and sorghum, the stomata of water-stressed maize were found to close at a higher water potential than sorghum (Sanchez-Diaz and Kramer, 1971). When it comes to screening sorghum genotypes for drought tolerance, stomatal conductance can be employed, especially at the vegetative growth stage.

Munamava and Riddoch (2001) found that stomatal conductance of sorghum stressed plants were lower within each growth stage but all varieties fully recovered upon resumption of normal watering after the vegetative stage stress.

1.4.2 Chlorophyll content

Chlorophyll content influences a plant's ability to absorb light for photosynthesis and serves as an indicator of overall plant health (Devnarain et al., 2016). Water-deficit stress has been noted to reduce photosynthetic pigmentation with varying degrees, dependent on the tolerance level of the genotype. In comparison to its control, chlorophyll content of the sorghum genotype P898012 subjected to water-deficit stress was reduced significantly by 33.8 % (Devnarain et al., 2016). For sorghum growing in moisture limited soil, a significantly lower chlorophyll index was observed when compared with well-watered plants (Mutava et al., 2011). Similarly, Takele (2010) found a decrease in chlorophyll content in both pre- and post-flowering dehydrated sorghum and maize, with the latter being significantly lower compared with the former. Within a range of 95-51% relative water content, 98-96 % of chlorophyll was found to be retained in leaves during pre-flowering dehydration, while it gradually decreased during a post-flowering dehydration period (Takele, 2010). Sorghum genotypes significantly varied in total chlorophyll content, not only under water-deficit treatments but also under well-watered treatments (Nxele et al., 2017; Devnarain et al., 2016; Stout et al., 1977).

The imbalance between carbon and nitrogen ratio, as a consequence of water-deficit stress, contributes to induction of leaf senescence, due to an increased C:N ratio of mature leaves (Chen et al., 2015). When chlorophyll is lost, which leads to leaf senescence, there is a high tendency of losing yield. What if a plant could maintain chlorophyll content even with limited moisture supply?

That would involve undisrupted enzymatic reactions and maintenance of photosynthetic machinery under stress. Alternatively, the plant would exhibit the stay-green trait. Retention of green leaves during the grain-filling stage usually leads to higher grain yields (Souza et al., 2015; Borrell et al., 2014b). Devnarain et al. (2016) reported a 23 % and 75 % reduction in total chlorophyll content for stay green and non-stay green sorghum genotypes, respectively, exposed to water-deficit stress. Opposite to chlorophyll and carotenoid contents under water-deficit stress, anthocyanin contents was increased during pre- and post-flowering dehydration in both sorghum and maize, where dehydration was achieved by withholding watering for 20 days in each stage (Takele, 2010).

1.4.2.1 Stay green

Leaf senescence usually results during grain filling, because a significant amount of nitrogen needed by the grains are obtained by remobilized nitrogen from leaves (Prasad et al., 2008). Stay green is the phenotypic persistence of green leaves during the grain-filling phase in limited-water environments, to ensure high yields (Thomas and Howarth, 2000). Mechanisms have been outlined to be responsible for the stay green trait of which “senescence delay” appears to be the operating mechanism in sorghum (Thomas and Howarth, 2000). There is either a constraint on transpiration or reduced canopy/tillering to ensure decreased water usage early in the growing season, which contribute to senescence delay later in the season (Choudhary et al., 2013a). Details of other mechanistic activities that culminate in the stay green expression are illustrated in Figure 1.1.

Enhanced sensitivity to drying soil, which leads to regulating transpiration, appears to be factored in plants exhibiting the stay-green trait. One trait for obtaining higher yield is water conservation as it ensures water availability during crucial stages of crop development (Gholipour et al., 2012).

Plants decrease transpiration when growing on a drying soil which has reached a threshold of extractable water (in accordance to the concept of FTSW - fraction of transpirable soil water by Sinclair and Ludlow, 1986). Genotypes with high thresholds have tendencies to conserve more soil water (Choudhary et al., 2013a). While FTSW generally ranges from 0.25 to 0.40, Gholipour et al. (2012) found the FTSW threshold involving 16 sorghum genotypes to range from 0.32 to 0.48. Although the stay-green trait is phenotypically similar in genotypes, the physiological and genetic paths differ (Thomas and Howarth, 2000). Apart from the senescence delay that is more associated with sorghum, the other ways to stay green could be explored to determine their relations with sorghum.

Sorghum grain yield under field conditions correlates positively with the stay-green trait under terminal drought (Borrell et al., 2014a), although it was perceived initially to correlate with low grain yield due to an apparent small sink demand that caused green leaf retention (Borrell et al., 2014b). Recent research points to individual stay-green (*Stg*) quantitative trait loci (QTLs), which confer adaptation to drought in a cereal (sorghum) where pre-anthesis plant size is regulated so that water is conserved to support post-anthesis grain-filling phase (Borrell et al., 2014a). Effects of *Stg* QTL on increased grain yield appear to be connected with pre-anthesis QTL effects on leaf-area and tiller number dynamics, which cause extension of the photosynthetically active phase of the leaf and possible higher photosynthetic rates (Borrell et al., 2014b). Environmental and management conditions experienced by sorghum preceding flowering influence the extent to which *Stg* QTL affects grain yield (Borrell et al., 2014b).

1.4.3 Chlorophyll fluorescence

As light reaches a chlorophyll molecule, it may be used for photosynthesis, dissipated as heat, or re-emitted as fluorescence, i.e., chlorophyll fluorescence (Taiz et al., 2015). Only 2 to 10 % of light absorbed by the plant is directed towards chlorophyll fluorescence, but because there is competition between these three processes, the measurement of chlorophyll fluorescence gives an indication of the functionality of the photosynthetic machinery (Maarschalkerweerd and Husted, 2015).

Accumulation of reactive oxygen species (ROS), as a result of water-deficit stress, causes the blocking of the PSII reaction center and electron flow, which disrupts the photosynthetic process. This increases the F_o/F_m ratio, giving an indication of increased thylakoid membrane damage by the water-deficit stress (Djanaguiraman et al., 2010). An increase in the F_o/F_m ratio leads to a decrease in F_v/F_m ratio (chlorophyll fluorescence).

1.4.4 Transpiration efficiency

Transpiration efficiency (TE) can be defined as dry biomass produced per unit water transpired (Vadez et al., 2014). Transpiration efficiency defines water productivity at the plant level. Water use efficiency (WUE) defines water productivity at the plot/plant level, while intrinsic WUE does so at the leaf level (Vadez et al., 2014). Many physiological parameters can be associated with increased TE in sorghum but finding the actual physiological trait or trait composites that are critical for TE has not been very obvious. Impa et al. (2005) have indicated that either by improved photosynthetic capacity or reduced transpiration rate a higher TE is possible in rice. For some sorghum genotypes, high TE has been associated with low internal CO_2 concentration and greater photosynthetic capacity (Xin et al., 2009). However, when CO_2 leaks from the bundle sheath cells,

additional reducing power is needed to regenerate phosphoenol pyruvate (PEP), which can lead to lower TE (Xin et al., 2009). A high TE value could imply one of the following: an increase in biomass production for the same amount of water transpired, same biomass resulting from reduced transpiration, or a mix of these two scenarios (Xin et al., 2009).

In their investigations, Emendack et al. (2011) found variation in WUE among sorghum and millet genotypes under different watering regimes. Likewise, Xin et al. (2009) concluded that there is substantial genetic variation in TE among sorghum genotypes plus a strong environmental influence on TE. Physiological mechanisms contributing to TE alteration includes stomatal regulation of gas-exchange (Xin et al., 2009). A drought tolerant characteristic exhibited by sorghum is the lesser loss of water for a given reduction in leaf water potential compared with maize, which loses much more water before it fully closes its stomata (Sanchez-Diaz and Kramer, 1971)

Through reducing transpiration, it has been suggested that water-deficit stress can increase leaf WUE, yet the stress may hinder the accumulation of dry matter per amount of water consumed (Hasan et al., 2017). When TE was regressed against the total biomass produced and against the total amount of water transpired, a strong correlation between TE and biomass accumulation was detected while there was little correlation with water transpired (Xin et al., 2009). This indicated that genotypes identified with high TE by biomass accumulation had higher productivity rather than reduced water use. In a related investigation, Narayanan et al. (2013) evaluated eight sorghum genotypes and found a strong relation between WUE and biomass production. Increased WUE was related with increased biomass production rather than to decreased water use. As recounted by Hasan et al. (2017), high WUE does not necessarily correlate with high growth rates under

water-deficit stress, suggesting the need for more investigations into the relation between WUE and water-deficit stress.

1.4.4.1 VPD-limited transpiration

According to Truong et al. (2017), “VPD describes the difference in water vapor pressure within the leaf and the surrounding air”. In response to increasing VPD, some plants reduce stomatal conductance to prevent water loss, which adversely affects the capacity of a plant to assimilate carbon, resulting in limiting growth and development (Taiz et al., 2015). Lower stomatal conductance often suggests reduced photosynthetic activity and yield. Nonetheless, genotypes that exhibit traits of higher photosynthetic activity to produce higher yield with lower stomatal conductance are desirable in water-limited areas (Mutava et al., 2011). Simulation studies on sorghum have demonstrated a possible increment in sorghum yields at high air vapor pressure deficit (VPD) when there is a restricted maximum transpiration rate under drought conditions (Sinclair et al., 2005). When VDP is high, there is reduction in stomatal conductance, and water loss remains within a restricted level (it does not exceed a certain maximum limit irrespective of the increasing VDP). This allows a higher accumulation of biomass per unit water transpired, i.e., a higher transpiration efficiency (Sinclair et al., 2005).

The VPD depended limited transpiration trait is genetically regulated in sorghum, and the trait facilitates water conservation and improves WUE. Gholipoor et al. (2010) observed that 17 out of 26 sorghum genotypes expressed limited transpiration under high VPD, which ranged from 1.6 to 2.7 kPa. The VPD depended limited transpiration trait holds great prospects for a future with elevated VPD as predicted by future climate models (Lobell et al., 2015). In this respect, a potential increase in sorghum grain yield by 9 to 13 % is anticipated when the limited transpiration trait is

employed under water-deficit conditions with high VPD (Xin et al., 2009). Simulation results by a crop modelling framework, APSIM – (The Agricultural Production System Simulator) have indicated that hot and dry regions of the world are best suited for the VPD depended limited transpiration trait, where a slow but more efficient transpiration rate increases biomass yield (Truong et al., 2017).

1.4.5 Carbon isotope discrimination

The method of carbon isotope discrimination cannot be easily applied to C₄ plants (Farquhar, 1983) unlike C₃ plants where it has been used effectively to detect C₃ plants with high TE (Xin et al., 2009). Henderson et al. (1998), for instance, detected a weak but positive and significant correlation between TE and $\Delta^{13}\text{C}$ for 30 sorghum lines. In C₄ species such as sorghum and maize, the $^{13}\text{C}/^{12}\text{C}$ isotope ratio is not a fixed value because the value is dependent on the separation of the C₄ PEP carboxylase from the C₃ Rubisco, on how developed the leaf Kranz anatomy is, and the degree of leakiness of the bundle sheath cells for CO₂ (Xin et al., 2009). Thus, instead of carbon isotope discrimination, lysimetric studies and gas exchange analyses have been employed in determining TE variation among C₄ species (Vadez, 2014).

1.4.6 Hydraulic conductivity

Limiting hydraulic conductivity in sorghum can potentially conserve water and increase yield (Sinclair et al., 2005), and this may be seen in roots of some sorghum genotypes where water is conserved for grain filling by increased hydraulic resistance of the roots. Changes in root hydraulic conductivity often corresponds with changes in stomatal conductance and transpiration (Li et al., 2011).

The leaf is one organ with significant control on hydraulic conductance (Choudhary et al., 2013b). Leaf hydraulic conductance (K_{leaf}) can be categorized as either resistance within the xylem (r_x) or resistance outside the xylem (r_{ox}), i.e., movement of water from the vascular bundle to other cells in the leaf (Choudhary et al., 2013b). In monocots such as sorghum, transport of water along the leaves occurs within the large longitudinal veins as well as in the small longitudinal and transverse veins (Ocheltree et al., 2014a). Further examination of the leaf hydraulic conductance (K_{leaf}) can enhance productivity under limiting water conditions.

To determine the functioning of the two leaf resistances (r_x) and (r_{ox}) in regulating stomatal responses of six sorghum genotypes under water-deficit conditions, Ocheltree et al. (2014a) observed a negative relationship between stomatal conductance and r_{ox} . This implied that there is low stomatal conductance in genotypes with high r_{ox} (e.g., 'SC15') when water is abundant so that there is conservation of water that is used later when water becomes limiting. The low stomatal conductance of 'SC15' correlated with a decrease in transpiration at a VPD of more than 2.1 kPa (Choudhary et al., 2013b). Overall, Ocheltree et al. (2014b) noted that both the root and leaf hydraulic conductance are crucial in regulating sorghum's responses to evaporative demand. This may be more critical under water-deficit conditions.

1.4.7 Nitrogen balance

Nitrogen is a major nutrient influencing plant growth and yield (Mahama et al., 2014). The nitrogen balance index (NBI) is determined by dividing the chlorophyll concentration by the concentration of flavonoids using an instrument called the Dualex (Maarschalkerweerd and Husted, 2015). The Dualex is an optical sensor for the assessment of flavonoids, anthocyanin, and chlorophyll contents

in leaves. It can detect the nitrogen status of plants by measuring the NBI which is purported to ensure a better correlation to the nitrogen concentration than chlorophyll measurement (Maarschalkerweerd and Husted, 2015).

While the highest concentration of nitrate is found in the petiole of the model plant *Arabidopsis thaliana*, it is not clear where and how nitrate is stored and utilized in most cereal crops (Worland et al., 2017). Nevertheless, three times the nitrate concentration in leaves were found in the leaf sheath of sorghum (Worland et al., 2017). Accumulation of more nitrate early in a plant's development has been linked with higher yields, and this is thought to be due to a remobilization and assimilation during later growth stages (Worland et al., 2017). Ciampitti and Prasad (2016) detected a strong, positive correlation between remobilized nitrogen and the whole plant nitrogen content at the vegetative stage. Some plants take up and store nitrate when plant available N is abundant, although it ultimately costs more energy to assimilate nitrate (being converted from nitrate to nitrite, then from nitrite to ammonium) compared with ammonium (Worland et al., 2017).

It has been proposed that by an interaction with the plant hormones cytokinins and abscisic acid, the carbon / nitrogen balance regulates leaf senescence resulting from water-deficit stress (Chen et al., 2015). During water-deficit stress, nitrogen in the stem and sugar in the stem, leaves, and roots were found to significantly increase, while leaf nitrogen content decreased; stem accumulation of nitrogen and sugar increases in the stem are denoted to be of adaptive significance (Dina and Klikoff, 1973). Senescence of mature leaves (which tend to have higher C:N ratios), as a result of water-deficit stress, ensures remobilization of nutrients to younger leaves or sink organs (Chen et al., 2015).

Chen et al. (2015) observed that changes in the C:N ratio were observed before there was chlorophyll loss, while both changes were noticed well ahead of variations in the Fv/Fm ratio, which suggested that Fv/Fm ratio is less sensitive to C:N ratio. This may be explained partly by the nitrogen remobilization from the C/N imbalance, which leads to chlorophyll loss before further chlorophyll loss from photo-oxidation (Chen et al., 2015).

1.4.8 Biomass accumulation and partitioning

As documented by Munamava and Riddoch (2001), differences in how genotypes produce and partition biomass can serve as useful indicators of relative drought tolerance. Comas et al. (2013) states in a review that plants alter allocation among absorptive tissues to acquire resources that most limit growth.

Water-deficit stress (one month dry-down until an average soil water deficit of - 8.85 bars) was observed to have significantly reduced stem biomass accumulation by an average of 42 %, an observation attributed to a reduction in the length of the internodes of stems rather than a reduction in the diameter of the stems (Perrier et al., 2017). In their sorghum experiments, Borrell et al. (2014b) found a significant, negative correlation between biomass accumulation during grain-filling and green leaf area at anthesis. Sorghum hybrids exposed to water-deficit stress recorded reductions in shoot dry weights by 37 % in the first year and 18 % in the following year (Perrier et al., 2017). Both Nxele et al. (2017) and Perrier et al. (2017) observed a reduction in leaf dry weight of sorghum plants under water-deficit stress. Radiation limits how much biomass is accumulated even under well-watered conditions, and dry matter accumulation can be terminated by water-deficit stress before physiological maturity (Borrell et al., 2014b). It has been noted that

partial drying of root systems under water deficit has the potential to decrease allocation of assimilates to vegetative shoots (Comas et al., 2013).

1.4.9 Cellular level

Water is vital for life and serves as a key solvent for cellular biochemical reactions. Any form of dehydration reduces turgidity of cells and affects metabolic processes. Growth of a cell is one of the physiological process that is most sensitive to water-deficit stress (Taiz et al., 2015).

Long periods of water-deficit stress cause accumulation of osmolytes, such as free amino acids and soluble sugars (glucose and fructose), which help protect against functional and structural damage resulting from dehydration (Yadav et al., 2005; Chen et al., 2015). However, sugar accumulation in leaves beyond acceptable limits deactivates photosynthetic activity and, in association with nitrogen deficiency, triggers leaf senescence (Chen et al., 2015).

Loss of cell turgor and accumulation of reactive oxygen species (ROS) as a result of stress can be detrimental, wherein a significant increase in hydrogen peroxide resulted in augmented levels of lipid peroxidation, which lead to cell death (Nxele et al., 2017). To sustain cellular functions during stress, plants secrete proteins such as proline and glycine-betaine which are shown to be involved in osmotic rebalancing while aquaporins are involved with water movement across membranes (Ngara et al., 2018). The plant may also make use of other nutrients such as silicon to increase root endodermal silification and improve cell water balance (Farooq et al., 2009). Nxele et al. (2017) recorded 37 % increase in proline in water-deficit stressed plants, but the increase was not sufficient enough to curtail oxidative damage due to H₂O₂ accumulation and the subsequent increase in lipid peroxidation. During stress conditions such as drought, cellular detoxification

enzymes are produced for oxidative damage prevention, while messenger RNA-binding proteins also ensure maintenance of macromolecules and membranes (Ngara et al., 2018).

1.4.9.1 Hormonal

When plants detect water-deficit stress, transcriptional changes are activated and mechanisms that are either abscisic acid (ABA)-dependent or ABA-independent are deployed for metabolic reprogramming to allow growth to be aligned with moisture gradients (Ngara et al., 2018; Nxele et al., 2017) . Depending on soil water availability, there is a source and sink balancing role played by the plant hormone abscisic acid (Liu et al., 2005). When soil dries, more ABA concentrates in the roots to maintain root functions; this leads to increased root hydraulic conductivity, resulting in high water uptake, which culminates in delaying water shortages in the shoots (Barnabás et al., 2008). However, there is a reduction in leaf area expansion and stomatal closure when ABA is transported to the shoots. When it is transported to the reproductive structures, it influences cell division and enzyme activity, particularly acid invertase in sorghum (Barnabás et al., 2008; Liu et al., 2005). Higher levels of ABA in the reproductive machinery constrain active cell division of the endosperm and the embryo, and eventually the ovaries abort (Liu et al., 2005). ABA appears to restrain seed-set so that survival is achieved at the expense of grain production and only a few grains are produced (Liu et al., 2005).

1.5 Water-deficit stress during the pre-anthesis and anthesis stages

During the pre-flowering reproductive growth stage of sorghum, the demand for water is reported greatest compared with the post-flowering reproductive growth stage (Devnarain et al., 2016). A month after emergence, a sorghum plant has established photosynthetic machinery, sufficient leaf

number and area while it transitions to reproductive development (Roozeboom and Prasad, 2016). Thus, physiological adjustment to water-deficit stress during the vegetative stage tends to influence reproductive processes and the eventual yield. This suggests that any effect from water-deficit stress poses enormous potential to disrupt growth and development of both the pre and post anthesis stages. The growing reproductive tissues at the early development phase are enclosed by vegetative organs so that impact from water-deficit stress is minimized by the surrounding vegetative organs (Barnabás et al., 2008).

Crop growth and development may be delayed or accelerated depending on the stage of the crop and the magnitude of the water-deficit stress. The beginning and duration of developmental stages are often disrupted by water-deficit stress; typically, the length of time from floral initiation (panicle initiation) to anthesis is decreased by moderate water-deficit stress but extended when stress gets severe (Prasad et al., 2008). Munamava and Riddoch (2001) observed a decrease in development by an average of five days when different sorghum genotypes were exposed to ten days of water-deficit stress at the vegetative stage where watering was withheld until visible signs of wilting. Irrespective of the tolerance level of a sorghum variety to soil moisture stress, Munamava and Riddoch (2001) advocated that moisture supplementation should be offered at booting and flowering stage. This was due to their observation that the vegetative stage was the least sensitive, and the booting stage was the most sensitive, to water-deficit stress. Drought tolerance traits that may be linked with pre-flowering include greater leaf photosynthetic rates, higher canopy temperature depression, improved panicle exertion, and increased pollen viability (Mutava et al., 2011; Prasad et al., 2008). A sorghum plant may remain vegetative until stress is relieved before it can transition to the reproductive phase (Prasad et al., 2008).

1.5.1 Shoots

As a result of water-deficit stress, leaf length of newly formed leaves is reduced either due to a shorter growth period or a slower growth rate (Stout et al., 1977). Sorghum plants exposed to water-deficit stress had reduced leaf dry weights and shoot length by 57 % and 32 %, respectively (Nxele et al., 2017). Specific leaf area (SLA- ratio of leaf area to leaf weight) is an indicator of the amount of carbon needed to build a unit area of leaf and a lower value implies thicker leaves and eventually lower growth rates (Munamava and Riddoch, 2001). Genotypes with high SLA are proposed to be water efficient and are useful in breeding programs. Munamava and Riddoch (2001) reported a decrease in SLA with water-deficit stress for various sorghum genotypes; loss of leaf area during stress, which affects SLA, contributes to a significant reduction of grain yield.

Ability of sorghum tillers to emerge from lower nodes, as well as their eventual growth and grain development, is dependent on photosynthate availability in the main stem. Tiller emergence from the upper nodes is dependent on how competition from neighboring plants affects light quality (Roozeboom and Prasad, 2016). The total number of tillers that a sorghum plant can produce is reliant on temperature, genotype, and nutrient resources (Ciampitti and Prasad, 2016). How many fertile tillers are produced by a sorghum plant is influenced by environmental stress, such as heat and water-deficit stress (Roozeboom and Prasad, 2016). Restricted tillering has been shown to promote deeper root growth during vegetative growth when water becomes limiting (Blum, 2011).

Devnarain et al. (2016) reported a strong, positive correlation between sorghum height and grain yield. The relationship resulted from the increased biomass of taller plants, which contributed to greater yields. Taller sorghum plants are beneficial in areas where machinery is not employed for harvesting and where the plants are harvested for building materials and fuel after grain harvest.

Genotypic variation in sorghum heights after subjecting to water-deficit stress has been noted (Devnarain et al., 2016; Stout et al., 1977). Water-deficit stress can restrain panicle exertion, and the effect is dependent upon the hybrids capacity to maintain peduncle elongation during stress periods. Slow or incomplete panicle exertion eventually affects pollination, seed set, and grain size (Roozeboom and Prasad, 2016).

1.5.2 Roots

The size of the root system and its distribution ultimately governs access to water and nutrients and eventually regulate how the shoot transports water through the plant (Comas et al., 2013). Roots are a considerable sink for assimilates. They serve as the source for the transpiration stream. In addition signals that affect stomatal conductance such as ABA originate in the roots (Xin et al., 2009). Reportedly, the root fraction of total biomass differs greatly among sorghum genotypes, such that a variation in TE was noted depending on whether it is determined using only the shoot biomass or the total biomass (Xin et al., 2009).

In their investigations, Singh and Singh (1995) found sorghum to extract more water from the sub-soil (45-135 cm) while maize extracted more from the top soil (0-45 cm). Sorghum's ability to be more drought tolerant compared with maize has been attributed to its deeper roots, which are able to access water at greater soil depths (Hasan et al., 2017). In addition, sorghum possess twice as many secondary roots per unit of primary root as maize, making it efficient at managing soil water resources (Sanchez-Diaz and Kramer, 1971). Sorghum, similar to the fellow members of the Poaceae family, develops a crown or nodal root system (post-embryonic shoot-borne root system), in which roots are initiated from belowground basal nodes of the shoot (Sebastian et al., 2016; Blum and Arkin, 1984). Development of these roots are induced when water is perceived and so

their initiation and growth may be suppressed by water-deficit stress (Sebastian et al., 2016). The nodal root system starts to take over as the primary source of support, nutrients, and water at the fifth-leaf stage (Roozeboom and Prasad, 2016).

1.5.2.1 Root morphological traits

Root traits that improve productivity under water-deficit stress have become essential for breeders and geneticists, and this requires an understanding of the root's functional traits and their relation to the operations of the shoot component. Root traits with associated quantitative trait loci (QTL) have been identified by Mace et al. (2012) for sorghum (nodal root angle and root dry weight). As reported by Comas et al. (2013), root traits with associated QTL have also been identified in wheat (increased total root biomass, seminal root number and angle, root length), rice (root biomass, increased root length, root number) and in maize (increased branching, decreased root diameter, root angle, and axial root elongation rate).

Root traits that improve the ability of plants to explore the soil and increase the soil-to-plant contact are crucial for enhanced water and nutrient uptake, which result in increased productivity. Root traits that are important for enhancing water uptake generally include rooting depth, total root length, total root surface area, root length density, fine root length, and fine root surface area, as have been reported for wheat (Narayanan and Prasad, 2014). Root traits identified with maintaining plant productivity under water-deficit stress include small or fine root diameter, long specific root length, deeper rooting, and considerable root length density at depths having accessible soil water (Comas et al., 2013). One trait that has been suggested to contribute to increased plant water acquisition during water-deficit stress is decreased root diameter (Wasson et al., 2012). In addition, a large root tip diameter is necessary for penetration and access to water in

hard, drying soils (Paez-garcia et al., 2015). This trait is crucial in selecting for root penetration in water-deficit stressed environments where water is available at depths but soil is hard and root penetration is greatly hindered (Blum, 2011). Identification of genotypes with deep roots is essential so that water stored in deeper soil layers can be accessed. Water in deeper soil layers is more predictable and is converted into grain more efficiently than with in-season rainfall (Wasson et al., 2012).

1.5.2.2 Root to shoot ratio

Consideration of the root system's function in acquiring soil resources for the plants is complete only when its size is viewed in connection to the remainder of the plant, either relative to leaf area, shoot biomass, or whole plant size (Comas et al., 2013). The root to shoot ratio is an allometric measurement of dry mass of plants that helps to quantify the growth metrics of shoots in relation to roots (Comas et al., 2013). Depending on the growth and development of a plant, the root to shoot ratio changes as limited resources are allocated for various parts of the plant. As documented by Munamava and Riddoch (2001), there are genotypic variations in root to shoot ratio for sorghum.

According to the “balanced growth” hypothesis, some plants respond to water-deficit stress by sustaining or stimulating root growth while decreasing shoot growth, so that by the growth of new root tips and the increased root to leaf surface area, the plant's hydraulics is improved to ensure productivity under stress (Comas et al., 2013). High root to shoot ratio has been noted as indispensable mechanism for coping under stress as it reflects a high water absorption capacity (Narayanan and Prasad, 2014). Munamava and Riddoch (2001) found a general increase of root to shoot ratio, especially at the vegetative stage, for all sorghum varieties exposed to water-deficit

stress. As reviewed by Comas et al. (2013), it has been argued that, although root to shoot ratio provides some understanding on the functioning and performance of a plant, it does not account for tissue plasticity, thereby concealing changes in root morphology or architecture, suggesting they are constant, even though features such as the root length or surface area change dynamically.

1.6 Effects of water-deficit stress on grain-filling stage

Water-deficit stress occurring at both the pre and post-flowering stages impacts grain development and yield (Devnarain et al., 2016). Reportedly, critical to grain development and filling is the rate of photosynthesis and the redistribution of photoassimilates stored in different plant tissues during vegetative growth (Souza et al., 2015). As such, environmental stress including water-deficit stress happening at the reproductive stage can cause poor grain filling and yield losses. Higher grain yield has been linked to water availability during grain filling (Vadez, 2014).

Drought-tolerant traits that are important during post-flowering include longer seed-filling duration, increased seed-filling rate, and improved rooting depth (Mutava et al., 2011; Prasad et al., 2008). Vadez (2014) argued that what underpins the availability of more water during the grain-filling stage is not a greater overall water extraction or water extraction by deep root, but rather a combination of shoot and root traits that function synergistically in making more water available for extraction during the grain-filling phase.

1.6.1 Yield and yield components

Yield differences in agronomic crops are related with grain number and grain weight. Sorghum has its potential grain number set during the reproductive stage that covers panicle initiation to flowering. The grain weight is determined, within genetic limits, during the period of flowering

to physiological maturity (Roozeboom and Prasad, 2016). The yield component most often associated with grain yield differences due to pre-flowering water-deficit stress is panicles per square meter or panicles per plant; that of mid-season water-deficit stress is grain number per panicle and grain weight for terminal water-deficit stress (Maman et al., 2004; Manjarrez-Sandoval et al., 1989). Production factors that directly affect yield components include water availability, weed competition, row spacing, nitrogen application, plant population, and non-uniform stands (Ciampitti and Prasad, 2016). Yields of grain sorghum are more sensitive to water-deficit stress at the reproductive stage (panicle initiation to flowering) compared with grain-filling stage, which spans flowering to physiological maturity (Maman et al., 2004).

In stressful production environments, values of harvest index are normally less than 50 % and can be higher if conditions are optimal (Roozeboom and Prasad, 2016). Post-anthesis water-deficit stress has been reported to cause up to 30 % decrease in yield (Souza et al., 2015). Under limited water conditions, adjustment in the number of panicles per square meter and grains per panicle may occur as yield compensation, earlier in the life cycle. Compensation late in the life cycle may include variation in grain weight (Maman et al., 2004). In compensation for limited tillering or low plant populations, there may be increases in grain weight and number of grains per panicle (Maman et al., 2004). Water may also be conserved for grain filling by increasing the hydraulic resistance of roots. In an investigation where sorghum genotypes were characterized for traits related to drought tolerance, Mutava et al. (2011) reported negative effects of water deficit on harvest index and grain numbers and a positive correlation of grain yield to grain weight, grain number and harvest index. Thus, a drought-tolerant sorghum would be expected to show little or no effect of the stress on its harvest index and its various yield components.

1.6.1.1 Grain number

Environmental stress, such as heat and water deficit occurring from the vegetative period to flowering stage, can reduce grain number irreversibly such that even sufficient supply of water during the effective grain-filling phase will do little to repair the yield loss incurred other than partial increase in grain weight (Maman et al., 2004). Gambin et al. (2008) reported a linear relationship between grain number and sorghum's growth rate around the time of flowering, which implied a constant early assimilate availability per grain within each genotype across growing conditions. Nevertheless, plant growth rate around the period of grain set differs among genotypes, and there are differences in grain setting efficiency as well (Gambin et al., 2008). In an experiment on drought adaptation of stay-green sorghum genotypes, Borrell et al. (2014b) identified a significant correlation between grain number and yield. They also proposed that differences in grain number were due to grain number per panicle rather than panicle number per meter square. There was also a positive correlation between grain number and biomass accumulation.

1.6.1.2 Grain weight

Any condition that diminishes availability of assimilates during the grain-filling period indirectly reduces the final grain weight (Blum et al., 1997). Almost all the water extracted during the post-anthesis time contributes to grain growth (Vadez, 2014). Results have indicated a high association of sorghum grain weight (other than number of grains per panicle) with total yield (Maman et al., 2004). Stout et al. (1977) found the 1000-kernel weight of sorghum was lower for non-irrigated plants of two cultivars of sorghum than for irrigated plants. Borrell et al. (2014b) observed slight genotypic differences in the individual grain weight of stay-green genotypes subjected to water-deficit stress. Effects of water-deficit stress on the grain yield and panicle development of sorghum

resulted in 50 % reduction of the individual grain weight (Manjarrez-Sandoval et al., 1989). In relation to impacts of high temperature stress, Prasad et al. (2015) found significant decreases in individual grain weight of a sorghum hybrid exposed to temperature stress during the grain-filling stage under both controlled environment and field conditions.

1.6.1.3 Total grain yield

Reportedly, there is mobilization of nitrogen, carbon, and other storage compounds from other organs of the plant to the grain during the grain-filling period. In sorghum, the efficiency of this storage mobilization is about 40 % under optimal conditions, and it rises to about 50 % under water-deficit stress (Souza et al., 2015). For a mid-season water stress imposed at 50 % flowering for ten consecutive days, there were yield reductions of 37 % and 77 % for sorghum and millet, respectively; the stress also strongly reduced the harvest index of both crops (Emendack et al., 2011).

Total grain yields of sorghum were found to be lower when irrigation was limited while higher grain yields, associated with an increase in grain weight, were observed under well irrigated conditions (Maman et al., 2004). There was also an increase in all the yield components with a single irrigation at the mid grain-filling phase, but only panicles per square meter increased for a single irrigation or multiple irrigations at the boot stage. The number of grain sorghum panicles per square meter was negatively correlated with grain weight in the less stressful environment but positively correlated in the more stressful environment; more so, grain weight of sorghum was positively correlated with grain number per panicle in the more stressful conditions (Maman et al., 2004).

Grain yield of sorghum tillers is dependent upon tiller emergence frequency, tiller fertility frequency, grain numbers per tiller, and on plant density, because only a few tillers survive to produce grains when plant density increases (Roozeboom and Prasad, 2016). The third-node tiller usually produces the most grain after the main stem while subsequent grain production from the other tillers follows the pattern of tiller emergence in a descending manner (Roozeboom and Prasad, 2016).

1.6.2 Grain-filling duration

Grain-filling duration is an important determinant of grain yield. Depending on limiting environmental factors such as heat and drought, a longer duration for the grain-filling period ensures greater grain yield (Prasad et al., 2008). During the soft dough phase of the grain-filling stage, the water content, volume, and dry weight of the grain increases quickly; when this phase gets extended, such that a grain obtains a larger volume to contain more assimilates, the overall duration of the grain-filling period becomes longer, and heavier grains tend to be produced (Roozeboom and Prasad, 2016).

1.7 Conclusion

The review above covers aspects of sorghum production, detailing on the physiological and agronomic effects of water-deficit stress on sorghum's pre and post anthesis development stages. This review has shown that there are several factors controlling the response of sorghum under water-deficit stress in each of these developmental stages. Thus, three experiments were conducted and reported as part of this thesis, seeking to identify and provide a thorough understanding on the traits and mechanisms contributing to the drought resistance in sorghum.

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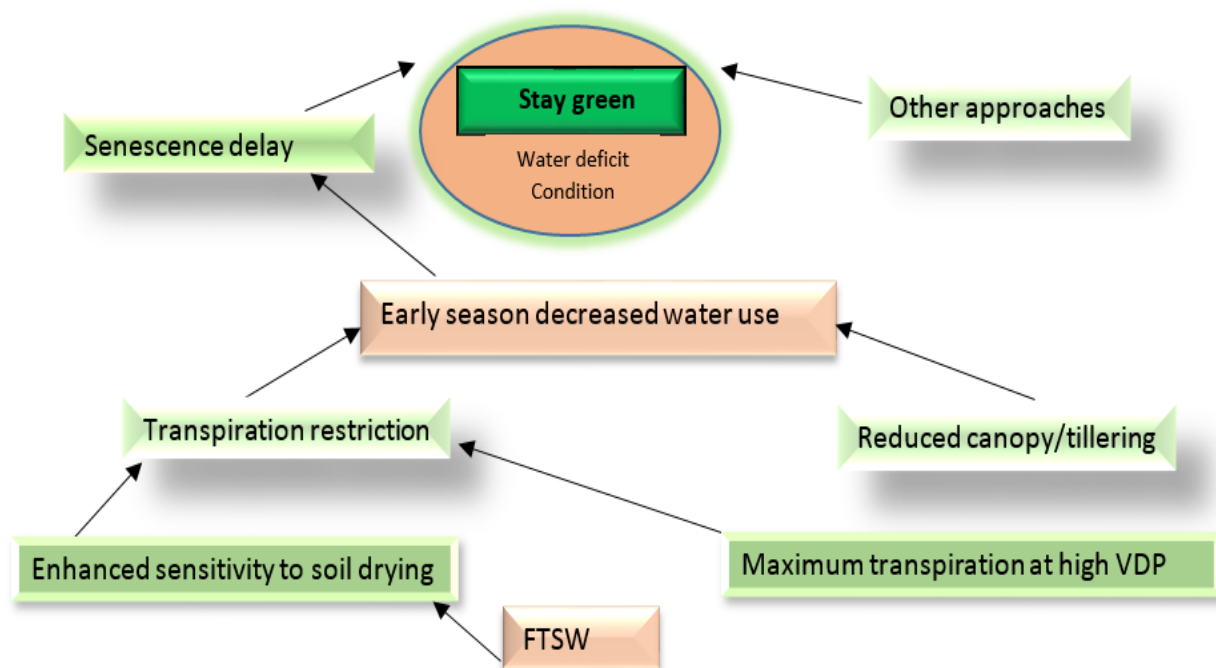


Figure 1.1 Mechanisms contributing for the expression of stay-green in sorghum. FTSW – Fraction of Soil Transpirable Water; VPD – Vapor Pressure Deficit
(Figure created by Regina Enniful, 2015)

Chapter 2 – Physiological Characterization of Parents of Sorghum

Mapping Populations Exposed to Water-Deficit Stress during

Vegetative stage

2.1 Abstract

Changing climate presents new challenges to agricultural production, and global climate models project increased intensity and magnitude in water-deficit stress conditions in the future. This is especially challenging for the arid and semi-arid regions of the world, where sorghum forms an important component of the cropping system. The research objective was to characterize eleven genetically and geographically diverse sorghum Nested Association Mapping parental lines (Ajabsido, SC1103, SC265, P898012, SC35, Macia, SC283, SC1345, Segalane, SC971 and RTx430 - the common parent) for shoot and root related physiological parameters. A lysimeter based experiment was set up in the greenhouse and water-deficit stress (55 % to 60 % field capacity) was imposed, lasting for 15-days, starting from 33-days after emergence. During this period, gravimetric pot weighing was followed on a daily basis to impose uniform level of stress and also to determine the cumulative water transpired. The experiment was repeated using four contrasting genotypes based on results from the first experiment. Most traits varied significantly between the genotypes irrespective of treatment differences in both experiments while significant reductions were seen with the water-deficit treatment. However, transpiration efficiency (TE) increased for water-deficit stressed plants which corresponded with an increase in $\delta^{13}\text{C}$ under water-deficit conditions. A positive significant correlation was thus detected between TE and $\delta^{13}\text{C}$. The root/shoot ratio was significantly greater for water-deficit plants and the biomass partitioning to the roots were also significantly higher in water-deficit stressed plants. SC35 depicted

significant percentage difference between the well-watered and water-deficit treatments for the fewest traits in both experiments suggesting a higher level of stress tolerance. On the contrary, SC971 recorded more traits with significant and higher percentage difference between the well-watered and water-deficit treatments than any other genotype in both experiments, implying its susceptibility to water-deficit stress. Outputs from this research is helpful for providing pathways to map genomic regions responsible for increased resilience to water-deficit stress.

2.2 Introduction

Although the Earth appears to be water-rich as its surface area is covered by about 70 % of water, actual available water for life renders the earth a water-scarce planet (Perlman, 2016). This situation is compounded by the changing climate. The global climate models project increased intensity and magnitude in water-deficit conditions (IPCC, 2014) which is especially challenging for the arid and semi-arid regions of the world. Even in regions of high agricultural productivity, water-deficit conditions occur within the growing seasons, as well as unexpected years of severe water-deficit stress (Callo-Concha et al., 2013; Barnabas et al., 2008). Water-deficit stress, irrespective of duration of occurrence has the potential to significantly limit productivity of crops, especially when the stress coincides with sensitive growth stages (Prasad et al., 2008).

With the changing climate presenting abiotic stress challenges to global agriculture, there is a need for water-deficit tolerant crops such as sorghum which account for a major portion of the world's food source. Sorghum is the fifth most economically important cereal and serves as a staple food for some Asian and many African regions of the world, used as feed for animals and employed for industrial purposes such as biofuels and beverages (Macauley, 2015). In the arid and semi-arid regions where there is either lack of crop production on a steady basis or some crops cannot grow

because of the harsh environmental conditions, crops such as sorghum survive by adoption of various physiological, morphological and molecular mechanisms (Callo-Concha et al., 2013; Felch et al., 2006). Water-deficit stressed plants generally transpire less, decreases both stomatal conductance and photosynthetic rates, and have reduced leaf water potential when compared with non-stressed plants (Singh, 2013). Though lower stomatal conductance may imply reduced photosynthetic activity and yield, genotypes which exhibit traits of higher photosynthetic assimilation leading to increased yield under lower stomatal conductance are desirable for water-limiting areas (Mutava et al., 2011). Thus, understanding traits that augment resilience to water-deficit conditions are indispensable under water-deficit stress scenarios.

Water-deficit stress affects various developmental stages of a plant with its effects seen at the molecular, cellular and the organismal level (Barnabas et al., 2008; Prasad et al., 2008). As a result, there are various physiological, morphological, anatomical, and biochemical features that enables a plant to tolerate these stresses (Ji et al., 2012, Nelson et al., 2007). Response to the stress is dependent on the severity and length of the stress, age and phase of development of the plant, as well as the particular organs, cell type and sub-cellular compartment (Barnabas et al., 2008; Prasad et al., 2008). Thus, understanding impacts of water-deficit conditions at various developmental stages of sorghum is prudent. Studies at the vegetative stage offers the benefit to understand rooting behavior as the dynamics of root growth and development past this stage is limited. Root growth and development of most annual agricultural crops are characterized by dominance of root growth at the early half of the planting season but root death takes over during the latter half of the season (Taiz et al., 2015; Mutava et al., 2011; Cheng et al., 1990). Importance of roots for accessing available water cannot be over-emphasized yet, not much investigations are being done on roots

compared with shoots, partly because roots are ‘buried, hidden and usually forgotten’ (Geldner and Salts, 2014).

Although sorghum is regarded as a water-deficit tolerant crop, the question remains as to whether water-deficit tolerance traits can be quantified in different sorghum genotypes to support ongoing crop improvement programs. This involves understanding the physiological basis of the stress tolerance and the variation that exists so that traits that enhance the balance between survival and productivity are capitalized (Jones, 2007). Identifying and understanding plant traits that augment resistance to water-deficit conditions are indispensable for ensuring food production and adapting to a water-scarce world, accompanied by other variable climatic factors.

Sorghum populations have been developed using the nested association mapping (NAM) technique, where ten founder lines (Ajabsido, SC1103, SC265, P898012, SC35, Macia, SC283, SC1345, Segalane, SC971) with RTx430 as the common parent were used. Understanding the effects of water-deficit stress on key traits and mechanisms of the sorghum NAM founder lines under water-deficit stress can help improve sorghum yields. It is in this light that this research seeks to characterize these founder lines for shoot related physiological parameters and rooting dynamics under water-deficit conditions. Specifically, the research aims to quantify the effects of water-deficit stress during the vegetative stage on the shoot’s physiological and growth responses as well as the root morphological dynamics in sorghum. We test the hypothesis that water-deficit stress during the vegetative stage will induce different water-deficit tolerance mechanisms and the resultant changes in the physiological and agronomic traits contrast among the tested sorghum genotypes.

2.3 Materials and Methods

The study characterized parents of sorghum mapping populations exposed to water-deficit stress during the vegetative stage. Experiments were conducted at the greenhouse of the Department of Agronomy, Kansas State University, Manhattan, KS, USA. The first experiment was conducted in 2015 using eleven parental sorghum genotypes (Ajabsido, SC1103, SC265, P898012, SC35, Macia, SC283, SC1345, Segalane, SC971, RTx430 common parent) of the Nested Association Mapping population (Table 2.1). The experiment was repeated using four contrasting genotypes (P898012, SC35, SC971 and RTx430) based on results from the first experiment.

2.3.1 Plant material and growth conditions

The eleven parental sorghum genotypes were grown in polyvinyl chloride (PVC) columns with inner diameter of 20 cm and height of 1 m. The PVC columns were sealed at the bottom with plastic caps that had drilled holes of size 0.5 cm. The growth medium used was surface® (MVP®, Profile Products LLC, Buffalo Grove, IL), which is a calcined montmorillonite clay with high cation exchange capacity, and a better drainage, porosity, and water-holding capacity when compared with other growth media such as cymad mix and sand; its properties helps in easy separation of substrate from the root system (Calonje et al., 2010). The rooting medium was fertilized with Osmocote® Classic (The Scotts Company LLC, Marysville, Ohio), a controlled-release fertilizer with 14–14–14 ratio of N–P₂O₅–K₂O; at a rate of 190 g per column (5 g per liter) as well as Scotts Micromax Micronutrients (Hummert International, Topeka, Kansas) at a rate of 22 g per column. The fertilizer was mixed with the growth medium before sowing. A systemic insecticide, Marathon 1 % Granular (OHP, Inc., Mainland, PA) was mixed with growth medium at 12 g per column before sowing. The experiment was set up in a Randomized Complete Block

Design with three blocks (replicates). Three seeds were sown at a depth of about 2 cm in each pot and thinned to one plant per pot during the three-leaf stage.

Plants were maintained under greenhouse conditions with 15 hours of photoperiod and a daytime maximum / night-time minimum temperatures of 32 and 25 °C respectively. Plants were irrigated daily using an automated drip irrigation system until 35 days after emergence, when the stress treatment was initiated. Irrigation (1.5 ± 0.1 liters) was provided for four minutes, four times per day at 0600, 1000, 1400, and 1800 h. At 35 days after emergence, gravimetric pot weighing was followed on a daily basis to impose uniform level of stress and also to determine cumulative water transpired for a 15-day period. By the gravimetric pot weighing, control pots were kept at 100 % field capacity (FC) while water-deficit pots were progressively exposed to 55 % - 60 % FC, before which all the pots were maintained at 100 % FC (Figure 2.1). Water lost by transpiration and reaching levels below the target stress level were replenished by adding back a calculated amount of water to maintain the stress levels of 55 % to 60 % FC. Pots surface was covered with a circular polythene sheet to control for evaporative water loss. A slit opening in the sheet was created to prevent heat buildup. To account for water loss through this slit opening, a set of filled PVC pots were maintained without plants (Kadam et al., 2015).

2.3.1.1 Observations

Daily pot weights for the 15 days period of stress were used to determine the daily evapotranspiration. Plant weight accrued during the weighing period was negligible (Blum and Arkin, 1984). The weights of filled pots without plants were used for evaporative loss adjustments after which the daily transpiration was calculated and summed to attain the cumulative water transpired.

2.3.2 Shoot related physiological and agronomic parameters

On days 5, 10 and 15 of the stress treatment, physiological parameters (gas exchange, chlorophyll index, and chlorophyll fluorescence a) were measured on the youngest fully expanded leaf between 10:00 and 13:00 hours. The leaf level gas exchange (photosynthetic assimilation and stomatal conductance) was measured with LICOR 6400 (Licor Inc., Lincoln, Nebraska, USA); the chlorophyll fluorescence a was measured with a fluorometer (OS30p; OptiSciences, Hudson, NH, USA) and the chlorophyll index with a chlorophyll meter (Soil Plant Analyzer Development – SPAD, Model 502; Spectrum Technologies, Plainfield, IL, USA). Both gas exchange and chlorophyll fluorescence were measured on the middle portion of the leaf blade, excluding the midrib. Instantaneous water use efficiency was obtained from the gas exchange measurements through a ratio between photosynthetic assimilation and leaf transpiration (E). After 60 minutes of dark adaptation of leaves, measurement with the chlorophyll fluorometer of the dark-adapted leaves captures the minimal fluorescence (F_o), maximum fluorescence (F_m), and photochemical efficiency of PSII (F_v/F_m) which is a ratio of variable fluorescence (F_v) to maximum fluorescence (F_m). A ratio of the F_o to F_m gives indication of the thylakoid membrane damage (Narayanan et al., 2015; Sunoj et al., 2017). For the measurement of chlorophyll index, readings were taken at top, middle, and base of the leaf blade and averaged.

At the end of the stress period, tiller and leaf numbers were counted and the stem height was recorded and stem thickness was measured using digital Vernier calipers (electronic digital caliper, VWR® Digital Calipers, USA). The second new fully expanded leaf of each plant was harvested and its leaf area determined using leaf area meter (LI-3100C Area Meter, LI-COR Inc., Lincoln, Nebraska, USA). The leaf was dried to constant weight at 60 °C and weighed. Specific leaf area (SLA) was then calculated as the ratio of the leaf area to the leaf dry weight. The other vegetative

shoot samples (leaves and stems) were oven dried separately to constant a weight at 60 °C and weighed to determine shoot biomass.

2.3.3 Leaf $\delta^{13}\text{C}$

One new fully expanded leaf from each plant was harvested and oven dried to constant weight at 60 °C. The dried leaves were ground to a fine powder using a ball mill and analyzed for carbon isotope composition in a Stable Isotope Mass Spectrometry Laboratory at the Division of Biology, Kansas State University. Stable carbon isotope ratios ($\delta^{13}\text{C}$) are expressed relative to the Pee Dee Belemite standard (‰).

2.3.4 Root Morphology

After harvesting the shoot biomass, roots were carefully removed from the surface[®] and laid horizontally for root length measurement as an estimate of rooting depth. Afterwards, roots were washed meticulously using a 1-mm sieve to minimize the loss of small roots and root hairs. Washed roots were stored in 30 % (v/v) alcohol in Ziploc bags and stored in cold room maintained at 4 °C. Stored roots were carefully cut into smaller lengths and spread to fit a water filled scanner glass trays. Grayscale images were generated by using a photo scanner (Epson perfection V800 photo scanner, Epson America Inc., Long Beach, CA, USA). An average of 20 images were obtained per plant. After scanning, root samples were oven dried to constant weight at 60 °C and weighed to determine root biomass. Total biomass was obtained by a summation of all leaves, stem and roots dry weights.

Root images from the scanning were analyzed with WinRHIZO Pro image analysis system (WinRHIZO Pro., Regent Instruments Inc., Quebec City, Quebec, Canada) to estimate

morphological features which includes total root length, total root surface area, root volume, average root diameter. Similarly, the total root length, root surface area, and root volume of the fine root system (roots with diameter < 0.50 mm) were also estimated.

2.3.5 Derived Shoot and Root Growth Parameters

Transpiration efficiency (TE) was obtained by a ratio of the shoot biomass to the cumulative water transpired. Root to shoot ratio was calculated as the ratio of root dry weight to shoot dry weight. Root weight ratio (RWR) was calculated as the ratio of the root dry weight to the total biomass. Similarly, shoot weight ratio (SWR) and leaf weight ratio (LWR) were calculated as the ratios of the stem and leaf dry weights to total biomass respectively.

2.3.6 Statistical analysis

The experimental design was a two way factorial treatment structure run in a Randomized Complete Block Design with three blocks of size 22. All data were statistically analyzed using the General linear model (GLM) procedure in SAS (SAS 9.4, SAS Institute Inc. Cary, NC, USA), with the sorghum parental genotypes and the watering regime (at two levels namely well-watered and water deficit) as factors. Pairwise comparisons using the Fisher's protected least significant difference (LSD) were done to separate means for significant treatments and interactions at probability level of 0.05. Correlation analysis were also conducted between the shoots, physiological, and root traits.

2.4 Results

In testing for statistical significance, analysis of variance showed that the genotype by watering treatment interaction (G x T) was significant ($P < 0.05$) for various agronomic, physiological, and derived traits (Tables 2.2, 2.3, and 2.4). The G x T interaction indicated stem height not only under genetic control but was as well subject to the complex effects of the watering regimes in both experiments (Table 2.2). But considering only experiment one, the following traits – stem height, leaf and tiller numbers, total root length, total root surface area, leaf weight ratio, chlorophyll fluorescence, thylakoid membrane damage, photosynthetic assimilation and stomatal conductance - exhibited G x T interaction significance. However, the following traits in experiment two showed significance for the G x T interaction: stem height, shoot biomass, cumulative water transpired, total root volume, root/shoot ratio, chlorophyll index, and instantaneous WUE. It is interesting to note that with the exception of traits that did not record a G x T significance in both experiments, traits that recorded significance for G x T effect in experiment one did not record the same in experiment two, and *vice versa* except for stem height (Tables 2.2, 2.3, and 2.4).

All traits (Tables 2.2, 2.3, and 2.4) varied significantly between water-deficit stress and well-watered treatments for both experiments regardless of genotypic variances except the rooting depth in both experiments, the stem weight ratio in experiment one, and the following traits in experiment two: number of tillers, specific leaf area, total root length, root dry weight, and the fine root's length, surface area and volume. Significant reductions ($P < 0.05$) were seen with the water-deficit treatments for most traits; however transpiration efficiency and carbon isotope discrimination in both experiments increased significantly ($P < 0.01$) under water-deficit conditions (Table 2.2).

Similarly, most traits varied significantly ($P < 0.05$) between the genotypes irrespective of treatment differences (Table 2.2, 2.3, and 2.4) except rooting depth and the average root diameter in both experiments, the cumulative water transpired and transpiration efficiency in experiment one, and for experiment two, the exception included leaf area, specific leaf area, chlorophyll fluorescence and thylakoid membrane damage.

2.4.1 Shoot and physiological traits

For shoot and physiological traits with significant T x G interactive effects, genotypes RTx430, SC265, and SC35 recorded significant ($P < 0.05$) percentage difference between the well-watered and water-deficit treatments for only 2-3 traits in experiment one (Table 2.5) unlike other genotypes which differed for 4-9 traits. SC35 had the fewest traits with significant ($P < 0.05$) percentage difference in experiment two while RTx430 behaved differently in experiment two compared with experiment one (Table 2.6). On the contrary, genotype SC971 recorded more significant ($P < 0.05$) percentage differences between the well-watered and water-deficit treatments than any other genotypes in both experiments (Tables 2.5 and 2.6). The highest significant reductions in tiller (84.4 %) and leaf (68 %) numbers was recorded in SC1103 while that of stem height (54.1 %) occurred with SC283 (Figure 2.2; Table 2.5). In experiment two, SC971 had the most significant reduction in cumulative water transpired (75.3 %), stem height (54.9 %), and shoot biomass (51 %) (Figure 2.3; Table 2.6).

All genotypes recorded significant reductions ($P < 0.05$) in photosynthetic assimilation and stomatal conductance under water-deficit conditions in experiment one (Table 2.5) and the cumulative water transpired in experiment two (Table 2.6). In experiment one, Macia had the least reduction in photosynthetic assimilation (10.9 %) and stomatal conductance (24.4 %) while SC283

had the highest level of percentage difference for both traits, recording 55.2 % and 69.3 % respectively (Table 2.5). In both experiments and under both watering regimes, there was a significant ($P < 0.05$) positive correlation between photosynthetic assimilation and stomatal conductance (Figure 2.7 and 2.9) so that the effect of the watering regimes on these traits followed similar trends across both experiments (Figure 2.5c, 2.5d; Figure 2.6c, 2.6d); more so, the correlation was significantly stronger ($P < 0.01$) under water-deficit stress than well-watered conditions.

Under water-deficit stress, segaolane had the most thylakoid membrane damage (18.7 %) relative to its well-watered condition in experiment one which also corresponded with the largest decrease in chlorophyll fluorescence (F_v/F_m), depicted by a significant percentage difference of 4.8 % (Table 2.5). In both experiments, there was a very strong significant ($P < 0.01$) negative correlation between thylakoid membrane damage and chlorophyll fluorescence (F_v/F_m) under both watering regimes (Figure 2.7 and 2.9). Although RTx430 was the only genotype that did not differ significantly between well-watered and the water-deficit stress for chlorophyll index in experiment two, it was the only genotype to have differed significantly for instantaneous WUE by an increase of 24.8 % under stress (Table 2.6).

Effects of watering regimes over time were significant ($P < 0.05$) for instantaneous WUE, chlorophyll index, stomatal conductance, and photosynthetic assimilation in both experiments plus chlorophyll fluorescence and thylakoid membrane damage in experiment two (Table 2.4; Figure 2.5 and 2.6). Instantaneous WUE for both watering regimes declined sharply between day 5 and 10 in experiment two while it declined sharply between day 10 and 15 in experiment one (Figure 2.5a and 2.6a). Although the chlorophyll index for the well-watered was greater than the water-

deficit stress treatment, both watering regimes remained relatively steady from day 5 through to day 15 in both experiments (Figure 2.5b and 2.6b). In experiment one, stomatal conductance of well-watered plants showed increasing trends between days 5 and 15 while photosynthetic assimilation remained steady; plants under water-deficit stress generally declined by day 15 of stress treatment (Figure 2.5c and 2.5d). In experiment two, stomatal conductance and photosynthetic assimilation in well-watered plants showed increasing trends between days 5 and 10 and declined by day 15; for the water-deficit plants, there was a decline between days 5 and 10 and a further decline at day 15 of stress treatment (Figure 2.6c & 2.6d).

2.4.2 Roots and derived parameters

Water-deficit stress decreased significantly ($P < 0.05$) for some root and derived traits in both experiments (Table 2.3). The most significant reductions in total root length (54.2 %) and total root surface area (56.6 %) occurred with SC1345 in experiment one (Tables 2.5; Figure 2.4) while that of total root volume (50.2 %) occurred with SC971 in experiment two (Figure 2.3d; Table 2.6). P898012 had a 29.6 % increase of total root length and a 6 % increase of total root surface area under water-deficit conditions in experiment one (Figure 2.4) but these responses were not statistically significant ($P > 0.05$) (Table 2.5). Under well-watered condition in experiment two, SC971 and RTx430 recorded the highest (80.9 cm³) and the least (38.3 cm³) root volume respectively while under water-deficit stress, P898012 and SC35 recorded the highest (54.5 cm³) and the least (31.4 cm³) root volume respectively (Figure 2.12).

In general, a greater fraction of biomass was partitioned to leaves and stems than to roots (Table 2.3). Even though total biomass accumulation in well-watered plants was significantly ($P < 0.01$) higher than water-deficit stressed plants, biomass partitioning to the roots (RWR) was significantly

higher in water-deficit stressed plants compared with the well-watered plants (Table 2.3). In this respect, the mean values for root/shoot ratio in water-deficit stress treatments were greater than well-watered treatments (Table 2.3). Thus, what accounted for the greater biomass accumulation in well-watered plants was the significantly greater biomass partitioning to the leaves (leaf weight ratio - LWR) and stems (stem weight ratio - SWR) than roots (RWR). The LWR, SWR, and RWR varied significantly ($P < 0.05$) amongst all genotypes for both experiments (Table 2.3). In experiment one, SC35 recorded significantly lowest decrease of leaf weight ratio (36.9 %) followed by P898012 (20.7 %) under water-deficit stress (Table 2.5). In experiment two, RTx430 recorded significantly the highest decrease of root/shoot ratio (66.7 %) under water-deficit stress (Table 2.6).

2.5 Discussion

2.5.1 Aboveground: Shoot's physiological and growth responses

The chlorophyll index for the well-watered sorghum plants was greater than that of the water-deficit stress. This result agrees with the findings of Mutava et al. (2011) where chlorophyll index of sorghum plants grown in moisture limited soil conditions was lower compared with well irrigated counterparts. Subjected to a moisture deficit stress, sorghum plants were also found to have decreased chlorophyll content and dry matter, attributed to altered plant water relations (Takele, 2010). In addition, chlorophyll index for both watering regimes remained relatively steady over time in both experiments in the current study. Subjected to pre-flowering dehydration, the rate of decline in chlorophyll contents of sorghum plants was detected to be slower compared to maize, with chlorophyll contents retained between 98 and 96 % when the relative water contents was between 95-51 % (Takele, 2010). Retaining chlorophyll content have been proposed as a

dehydration coping mechanism in sorghum (Thomas and Howarth, 2000). Genotype SC35 had the smallest decrease of chlorophyll index under water-deficit stress (Table 2.6) which attest to its stay green status. As an adaptive feature, the loss of chlorophyll is helpful for reducing the quantity of radiation intercepted by leaves without which a higher light load can potentially damage the photosynthetic machinery.

Chlorophyll fluorescence (F_v/F_m) significantly decreased under water-deficit stress which depicts decreased photochemical efficiency of PSII by the stress condition. Although Reactive Oxygen Species (ROS) were not investigated in this experiment, a more likely increase in ROS production (plus reduced antioxidants and ROS detoxification enzyme activities) due to the water-deficit stress would have resulted in damaging the PSII reaction center and electron flow (Taiz et al., 2015). This would increase the F_o/F_m ratio, giving an indication of increased thylakoid membrane damage by the water-deficit stress (Djanaguiraman et al., 2010). An increase in F_o/F_m ratio leads to a decrease in F_v/F_m ratio (Chlorophyll fluorescence) and a concomitant downregulation of PSII photochemistry, as evidenced by a very strong negative correlation between thylakoid membrane damage (F_o/F_m) and chlorophyll fluorescence (F_v/F_m) in this study. Relatedly, all genotypes recorded significant reductions under water-deficit conditions for photosynthetic assimilation and stomatal conductance. Both traits were positively correlated with the relation being stronger under water-deficit stress. Water-deficit stress stimulates the accumulation of abscisic acid which triggers stomatal closure in an attempt to conserve water loss through transpiration, which inversely regulates gas exchange and inhibits photosynthesis (Saradadevi et al., 2017; Yan et al., 2017, Daszkowska-Golec and Szarejko, 2013 Franks and Farquhar, 2001).

There was increased transpiration efficiency (TE) for water-deficit stressed plants (plant level WUE) which agrees to the well documented increased WUE under water-deficit conditions (Blum, 2009; Vadez, 2014). While under water-deficit condition, reduced stomatal conductance leading to reduced transpiration would increase TE, assuming biomass accumulation is minimally affected. Narayanan et al. (2013) in their evaluation of eight sorghum genotypes found a strong relation of increased WUE to increased biomass production rather than to decrease water use; thus they deduced the potential to improve WUE without compromising biomass production and yield potential.

The increased TE corresponded with an increase in $\delta^{13}\text{C}$ under water-deficit conditions. In this regard, a significant positive correlation was detected in connection with these two traits for both well-watered and water-deficit treatment of experiment one and the well-watered treatment of experiment two (Figure 2.7, 2.9, 2.11). This outcome is comparable to results of Henderson et al. (1998) where a weak but positive significant correlation between TE and $\delta^{13}\text{C}$ was found for 30 sorghum lines. Unlike C_3 plants which tend to have a negative relationship between TE and $\delta^{13}\text{C}$ such that a lower $\delta^{13}\text{C}$ would imply a higher WUE (Impa et al., 2015; Kadam et al., 2015), C_4 plants appears to exhibit the contrary. This may be that under water-deficit conditions, stomatal closure restrains the little discrimination of carbon isotopes in C_4 plants. It could also be that there is an increase in the C_i/C_a (the internal to atmospheric CO_2 ratio) due to the reduction in photosynthetic capacity (Monneveux et al., 2007). Reduced specific leaf area, a water conservation strategy, has also been linked with $\delta^{13}\text{C}$ (Kadam et al., 2015; Vadez, 2014). A negative significant correlation was identified between $\delta^{13}\text{C}$ and specific leaf area for water-deficit treatment of experiment one and the well-watered treatment of experiment two (Figure 2.7 and 2.9).

There is experimental evidence of genetic variation of $\delta^{13}\text{C}$ in sorghum (Hubick et al., 1990) and other C_4 plants which includes *Zea mays* (Monneveux et al., 2007), *Panicum coloratum* (Ohsugi et al., 1988) and *Saccharum* spp. (Meinzer et al., 1994). In addition, Monneveux et al. (2007) obtained higher $\delta^{13}\text{C}$ for water-deficit tolerant *Zea mays* hybrids and inbred lines comparatively to susceptible ones. Similarly, significant genetic variation was also recorded in both experiments in this study with SC35 posing to be the most water-deficit tolerant by having the highest $\delta^{13}\text{C}$ mean values (supplementary Tables one and two) for both experiment one (5.03 ‰) and experiment two (5.5 ‰). It's been proposed that either genetic differences in the ratio of assimilation rate to stomatal conductance or genetic differences in leakiness of the bundle sheath cells may be responsible for the variation of $\delta^{13}\text{C}$ in C_4 species (Hubick et al., 1990). Monneveux et al. (2007) reports on increase of leakiness of the bundle sheath cells as $\delta^{13}\text{C}$ increases with water-deficit stress in *Zea mays*. The NADP-ME subtype nature of sorghum would suggest a small variation in the leakiness as the cause on the variation, owing to the small differences in $\delta^{13}\text{C}$ between the water deficit and well-watered conditions in this study.

Water-deficit stress resulted in significant reductions in tiller and leaf numbers in this study. This can be understood on the basis that water-deficit stress may have adversely affected the initiation of new leaves as well as accelerated leaf senescence, especially old and matured leaves. The effects on the number of tillers is important for indicating the reproductive potential as tillers serves as source of assimilate during the grain filling stage. Restricted tillering is been observed to be adopted by plants to promote deeper root growth during vegetative growth when water becomes limiting (Blum, 2011). Nevertheless, no consistent correlation was detected between tiller number and rooting depth in this experiment (Figure 2.8 and 2.10) partly because there was no significant variation of rooting depth in both experiments. Lower tillering and leaf numbers are also noted as

water saving dynamics occurring during the vegetative stages as well as a strategy to reducing leaf area which in turn helps to control transpiration (Borell et al., 2014). Relatedly, Blum and Arkin (1984) submitted that when soil available water is limiting, leaf area of sorghum plant was reduced so as to control transpiration. Reports have indicated that water-deficit tolerant plants such as sorghum limits water loss by reducing leaf area or restricting stomatal opening, or both while at the same time having little effect on the biomass production (Farooq et al., 2009). Leaf area reduction as a means to controlling water use under stress has been documented (Vadez, 2014).

2.5.2 Belowground: Root morphological dynamics

In considering whether the effect of watering regimes on root traits differ by genotypes, the total root length, total root surface area, and total root volume emerged as significantly important traits. The other root traits generally were also significantly important except that their effects were not dependent on genotypic differences. Root traits that improves the ability of plants to explore the soil and increases the soil-to-plant contact would be crucial for enhanced water uptake for increased productivity. Regardless of which watering regime, substantial genetic variability were found for all root traits of sorghum in this study except the rooting depth and the average root diameter. However, the average root diameter was significantly smaller for water-deficit plants compared with the well-watered. In a related development, Comas et al. (2013) reports on decreased diameter in maize, another C₄ cereal, as one of the examples of QTL linked to traits associated with increasing foraging capacity of roots system and indicted fine root diameter to be vital for maintaining plant productivity under water-deficit condition. Although the increased total root length and total root surface area of P898012 under water-deficit stress was not statistically

significant, it can be considered potentially as a robust genotype for sorghum root breeding purposes.

The fine roots (diameter < 0.50 mm) generally constituted the greatest portion of the entire root system for all genotypes in this study. It comprised ~ 90 % of the total root length irrespective of the watering regime (Table 2.3). Since total root length influences the quantity of water absorbed by the root distribution in the soil profile (Manschadi et al., 2006), the massive composition of fine roots to the total root lengths suggests that sorghum's ability to thrive irrespective of moisture levels, by developing fine roots. It is also indicative that the fine roots constituted a greater proportion of sorghum's lateral roots. Development of lateral roots as a result of root branching is very critical for water uptake for most crops as it is regarded as the most active portion of the root system for water uptake and represent the majority of the length and surface area of root systems (Paez-Garcia et al., 2015). Compared with the total root surface area and total root volume, the fine roots contributed more under the water-deficit regime than under well-watered. Fine roots are known to increase the roots' surface area so as to increase the capacity for nutrients and water absorption (Narayanan and Prasad, 2014). Thus the roots' surface area was increased under water-deficit stress in this study by producing more fine roots.

A greater fraction of biomass was partitioned to leaves and stems in this study, and similar reports are available on rice and wheat (Kadam et al., 2015). In this study, the root/shoot ratio was significantly higher for water-deficit plants than for the well-watered. This could have been due to significant greater reduction in shoot biomass production under stress (Table 2.2) and higher biomass partitioning to the roots in water-deficit stressed plants compared with the well-watered plants. The root/shoot ratio is a phenotypic plasticity feature of plants, establishing the functional

balance between photosynthesis by the shoot and the root's water uptake (Taiz et al., 2015). Normally, the shoots of a well-watered plants grow until water uptake by the roots becomes limiting, while the roots grow as much as there is enough supply of photosynthates from the shoots. Under water-deficit stress, this functional balance between photosynthesis and water uptake is altered by the activity of the plant hormone abscisic acid – ABA. Depending on the soil water availability, there is a source and sink balancing role played by the ABA hormone (Liu et al., 2011). As carbon and energy consumption is curtailed through the reduction in leaf expansion, more of the plant's photosynthates are allocated for more growth of the root's system so as to explore larger soil surface area and extract water in the soil profile (Taiz et al., 2015).

2.6 Conclusion

The study was conducted to physiologically characterize sorghum NAM founder lines under water-deficit stress. For both experiments that were conducted, the genotype by watering treatment interaction (G x T) was significant for various agronomic, physiological, and derived traits. Significant reductions were seen with the water-deficit treatments for most traits though TE, iWUE, $\delta^{13}\text{C}$, R:S ratio and the RWR were significantly increased under water-deficit condition. Most shoot and root traits of the sorghum parental genotypes varied significantly between stressed and well-watered treatments. Sorghum genotypes, such as SC35 and RTx430 appear to demonstrate some level of higher tolerance to the stress based on least percentage difference exhibited between treatments and by depicting significant percentage difference for the fewest number of traits. On the other hand, genotype SC971 recorded higher significant percentage difference in more traits between the well-watered and water-deficit treatments than any other genotype in both experiments, implying its susceptibility to water-deficit stress. Root traits of

genotypes SC283, SC971 and SC1345 were significantly impaired under water-deficit stress while that of RTx430 and Segalane appears less differential under the different watering regimes. In view of the current research output, the means to identify appropriate mapping population to map genomic regions responsible for increased water-deficit resilience in sorghum is explicated. This will help complement efforts currently ongoing to enhance resilience to water-deficit stress in sorghum.

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TABLES AND FIGURES

Table 2.1: The sorghum Nested Association Mapping populations (NAM) parent lines: RTx430 as the common genotype crossed with 10 other genotypes (Bouchet et al., 2017).

Parent	Origin	Race	Characteristics
RTx430	Texas A&M	-	Pollinator parent
Ajabsido	Sudan	Caudatum	Pre-flowering drought tolerance
Macia	ICRISAT	Caudatum	Elite improved African line
P898012	Purdue	-	Pre & Post flowering drought tolerance
SC1103	Nigeria	Guinea	Grassy architecture
SC1345	Mali	Caudatum	Underutilized germplasm
SC265	Burkina Faso	Guinea	Potentially high yielding
SC283	Tanzania	Guinea	Aluminum tolerance
SC35	Ethiopia	Durra	Stay green
SC971	Puerto Rico	Durra-Kafir	Limited transpiration/Slow wilting
Segaolane	Botswana	Kafir	Potentially high yielding

Table 2.2: Probability values of effects of water-deficit stress treatment (T), genotype (G) and T x G interaction on some agronomic and physiological traits for experiments 1 and 2.

Traits	Exp. 1			Exp. 2						
	T	G	G x T	Treatment (mean \pm standard error*)		T	G	G x T	Treatment (mean \pm standard error *)	
				Well-watered	Water deficit				Well-watered	Water deficit
Stem height (cm)	<.0001	<.0001	0.0352	72.833 \pm 2.9	46.06 \pm 2.9	<.0001	0.0002	0.0007	57.08 \pm 2.40	33.44 \pm 2.40
Stem thickness (mm)	<.0001	<.0001	0.4438	14.85 \pm 0.32	12.67 \pm 0.32	0.0002	<.0001	0.0743	20.25 \pm 0.50	17.20 \pm 0.50
Tiller number	<.0001	<.0001	<.0001	3.12 \pm 0.31	1.42 \pm 0.31	0.5807	<.0001	0.3236	4 \pm 0.32	3.75 \pm 0.32
Leaf number	<.0001	<.0001	0.0017	26.0 \pm 1.43	15.151 \pm 1.43	0.0002	0.0002	0.1377	33.17 \pm 2.15	19.42 \pm 2.15
Leaf area (cm ²)	<.0001	<.0001	0.297	604 \pm 22.51	406 \pm 22.51	0.0049	0.051	0.1851	458 \pm 21.65	362 \pm 21.65
Specific leaf area (cm ² g ⁻¹)	<.0001	0.0007	0.1742	209.37 \pm 3.66	178.08 \pm 3.66	0.2634	0.087	0.3393	200.46 \pm 6.36	190.18 \pm 6.36
Shoot biomass (g plant ⁻¹)	<.0001	0.0052	0.1253	41.68 \pm 2.12	26.56 \pm 2.12	<.0001	0.0007	0.0414	50.53 \pm 2.20	29.54 \pm 2.20
Cumulative water transpired (l)	<.0001	0.23	0.3382	7.87 \pm 0.69	1.77 \pm 0.69	<.0001	0.0003	0.0041	5.27 \pm 0.25	1.55 \pm 0.25
Transpiration efficiency (g/l)	<.0001	0.0718	0.3306	7.51 \pm 0.87	15.62 \pm 0.87	<.0001	0.0444	0.8184	9.72 \pm 0.58	19.37 \pm 0.58
Carbon Isotope discrimination	<.0001	0.0002	0.5041	4.25 \pm 0.043	5.25 \pm 0.043	<.0001	0.0018	0.3351	4.86 \pm 0.05	5.76 \pm 0.05

Table 2.3: Probability values of effects of water-deficit stress treatment (T), genotype (G) and T x G interaction on roots and derived traits for experiments 1 and 2.

Traits	Exp. 1			Exp. 2						
	T	G	G x T	Treatment (mean \pm standard error*)		T	G	G x T	Treatment (mean \pm standard error *)	
				Well-watered	Water deficit				Well-watered	Water deficit
Total root length (m)	<.0001	<.0001	0.0081	987 \pm 46.9	714 \pm 46.9	0.1576	0.0007	0.3087	985 \pm 68.26	844 \pm 68.26
Total root surface area (cm²)	<.0001	<.0001	0.0162	8432 \pm 360	5265 \pm 360	0.05	0.0008	0.1349	7938 \pm 540	6446 \pm 540
Total root volume (cm³)	<.0001	0.0003	0.14	60 \pm 2.66	32 \pm 2.66	0.009	0.0026	0.0408	54.65 \pm 4.00	40.45 \pm 4.00
Rooting depth (cm plant⁻¹)	0.18	0.29	0.66	116 \pm 3.31	110 \pm 3.31	0.6446	0.573	0.1285	145 \pm 4.42	148 \pm 4.42
Root dry weight (g plant⁻¹)	0.0001	0.0003	0.09	10 \pm 0.51	7 \pm 0.51	0.0717	0.0215	0.305	9.99 \pm 0.65	8.24 \pm 0.65
Average root diameter (mm)	<.0001	0.959	0.7407	0.3 \pm 0.0074	0.25 \pm 0.0074	0.0143	0.1733	0.2322	0.3 \pm 0.01	0.26 \pm 0.01
Fine root length (m)	0.0003	0.0005	0.139	883 \pm 46.9	635 \pm 46.9	0.1749	0.0007	0.3383	888.67 \pm 62.1	765.56 \pm 62.1
Fine root surface area (cm²)	<.0001	0.0003	0.1354	3979 \pm 195	2663 \pm 195	0.134	0.0005	0.225	3704 \pm 249	3172 \pm 249
Fine root volume (cm³)	<.0001	0.0003	0.1308	23.10 \pm 1.1	14.73 \pm 1.1	0.0993	0.0005	0.1332	20.94 \pm 1.41	17.74 \pm 1.41
Total biomass (g plant⁻¹)	<.0001	0.0037	0.1111	51.61 \pm 2.58	33.62 \pm 2.58	<.0001	0.0015	0.0693	60.52 \pm 2.75	37.78 \pm 2.75
Leaf weight ratio	0.0048	<.0001	0.0082	0.39 \pm 0.0083	0.36 \pm 0.0083	0.03	0.0077	0.0684	0.5 \pm 0.01	0.48 \pm 0.01
Stem weight ratio	0.5645	<.0001	0.0561	0.42 \pm 0.009	0.43 \pm 0.009	0.0123	<.0001	0.5994	0.33 \pm 0.006	0.31 \pm 0.01
Root weight ratio	0.0224	0.0023	0.8538	0.19 \pm 0.005	0.21 \pm 0.005	<.0001	0.0198	0.0644	0.16 \pm 0.006	0.22 \pm 0.01
Root/shoot ratio	0.0055	0.0016	0.4264	0.24 \pm 0.009	0.28 \pm 0.009	<.0001	0.0134	0.0331	0.20 \pm 0.01	0.28 \pm 0.01

Table 2.4: Probability values of effects of water-deficit stress treatment (T), genotype (G), T x G interaction, day of stress (D) and the respective T/G x D interactive effects on physiological traits for experiments 1 and 2.

Traits	Exp. 1							Treatment (mean \pm standard error *)	
	T	G	G x T	D	T x D	G x D	T x G x D	Well-watered	Water deficit
Chlorophyll index (SPAD Units)	<.0001	<.0001	0.6848	0.0006	0.0129	0.8411	0.1296	53.00 \pm 0.36	45.56 \pm 0.36
Chlorophyll Fluorescence ((Fv/Fm))	<.0001	0.0016	0.0005	0.1415	0.6119	0.4535	0.1385	0.79 \pm 0.0015	0.772 \pm 0.0015
Thylakoid membrane damage (Fo/Fm)	<.0001	0.0111	0.0027	0.0428	0.3465	0.4282	0.454	0.209 \pm 0.0015	0.229 \pm 0.0015
Photosynthetic assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	26.22 \pm 0.64	18.74 \pm 0.94
Instantaneous WUE (A/T, $\mu\text{mol mmol}^{-1}$)	<.0001	0.0005	0.314	<.0001	0.0171	0.2081	0.0006	9.47 \pm 0.12	10.69 \pm 0.12
Stomatal conductance	<.0001	<.0001	<.0001	0.8514	<.0001	0.0002	<.0001	0.19 \pm 0.0038	0.11 \pm 0.0038
Exp. 2									
Chlorophyll index (SPAD Units)	<.0001	<.0001	0.0107	<.0001	0.0431	0.1401	0.3078	54.22 \pm 0.42	48.16 \pm 0.42
Chlorophyll Fluorescence ((Fv/Fm))	<.0001	0.1595	0.7924	<.0001	0.0254	0.7846	0.4859	0.785 \pm 0.002	0.77 \pm 0.002
Thylakoid membrane damage (Fo/Fm)	<.0001	0.1504	0.7382	<.0001	0.0335	0.8293	0.4357	0.214 \pm 0.002	0.229 \pm 0.002
Photosynthetic assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	<.0001	0.0031	0.1484	<.0001	<.0001	0.1523	0.4279	17.01 \pm 0.51	9.73 \pm 0.51
Instantaneous WUE (A/T, $\mu\text{mol mmol}^{-1}$)	0.0323	0.0006	0.0107	<.0001	0.0003	0.4481	0.0425	7.18 \pm 0.18	7.61 \pm 0.18
Stomatal conductance	<.0001	<.0001	0.3202	0.0004	<.0001	0.1688	0.3543	0.14 \pm 0.01	0.07 \pm 0.01

Table 2.5: Percent difference between water deficit and well-watered treatments for traits with significant T x G interactive effects – Experiment 1

Traits/Genotypes	Ajabido	Macia	P898012	RTx430	SC1103	SC1345	SC265	SC283	SC35	SC971	Segaolane
Stem height	-53.4	-32.0	-31.8	-31.9	-17.5	-48.0	-34.9	-54.1	-2.7	-43.2	-28.6
Tiller number	-66.7	0	-27.3	0	-84.4	-50	100	-40	-40	-76.9	-50
Leaf number	-48.9	-24	-28.8	-5	-68	-42.3	24.4	-42.9	-35.6	-64.4	-37.3
Total root length	-18.6	-28.1	29.6	-12.2	-35	-54.2	-13.6	-38.2	-24.3	-52	-5.2
Total surface area	-36.7	-39.1	6	-13.8	-41.6	-56.6	-37.7	-46.9	-37.2	-54.3	-22.4
Leaf weight ratio	0.39	-9.25	-20.68	-2.28	-7.60	13.42	-11.85	4.14	-36.89	-2.86	3.02
Chlorophyll fluorescence	-1.84	-2.99	-3.76	-0.57	-1.8	-1.46	-0.29	-1.74	-1.09	-4.58	-4.77
Thylakoid membrane damage	7.48	11.77	14.27	2.46	6.55	9.44	0.83	6.56	4.10	17.64	18.66
Photosynthetic assimilation	-11.4	-10.9	-43.8	-15.4	-22.4	-26.0	-36.7	-55.2	-52.1	-26.9	-15.1
Stomatal conductance	-34.2	-24.4	-55.8	-41.3	-38.2	-24.7	-47.7	-69.3	-62.8	-52.8	-29.7

*Statistically significant percentages are in bold.

Table 2.6: Percent difference between water deficit and well-watered treatments for traits with significant T x G interactive effects – Experiment 2

Traits/Genotypes	P898012	RTx430	SC35	SC971
Stem height	-42.3	-28.8	-31.3	-54.9
Shoot biomass	-42.7	-39.2	-27.3	-51.0
Cumulative water transpired	-69.9	-70.5	-62.4	-75.3
Total root volume	-8.7	-6.8	-21.2	-50.2
Root/Shoot ratio	58.33	66.67	18.97	20.69
Chlorophyll concentration	-14.8	-5.5	-10.7	-14.2
Instantaneous WUE	6.5	24.8	-5.6	2.1

*Statistically significant percentages are in bold.

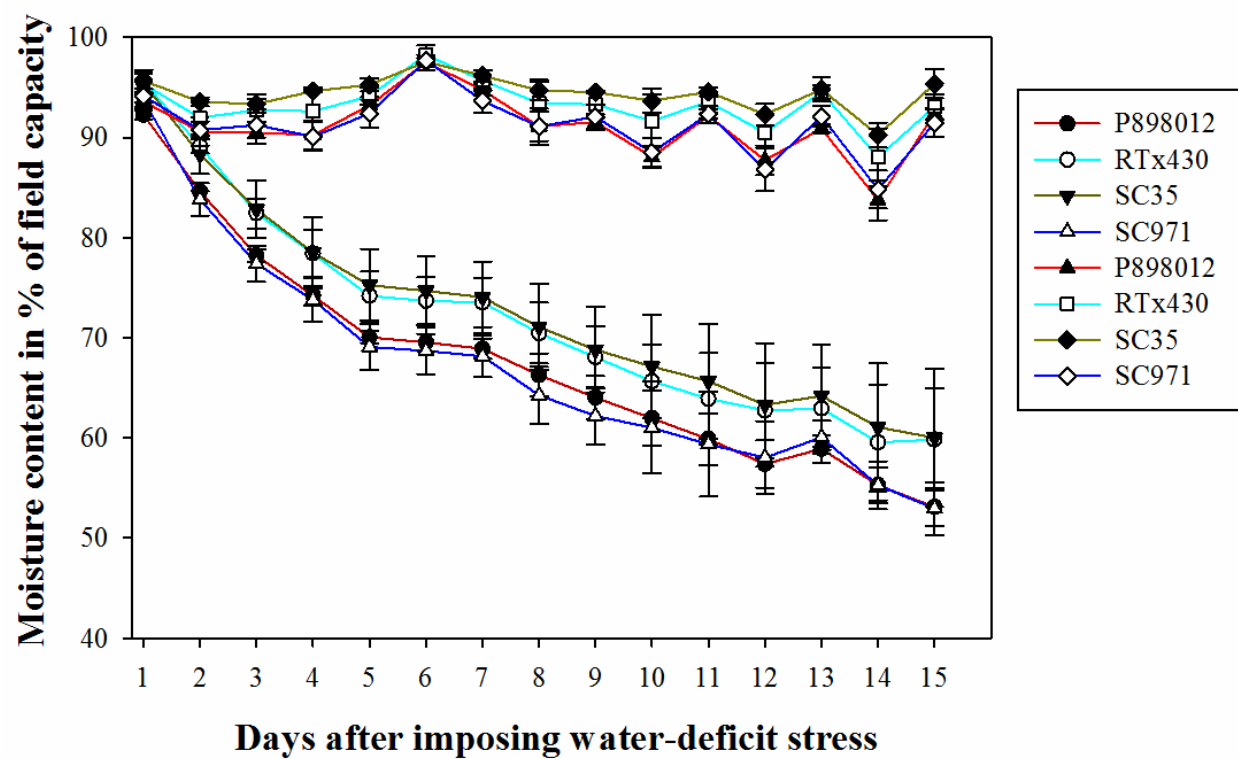


Figure 2.1: Rate of water depletion calculated based on gravimetric pot weighing and expressed as moisture content in % field capacity for sorghum parental genotypes selected for validation.

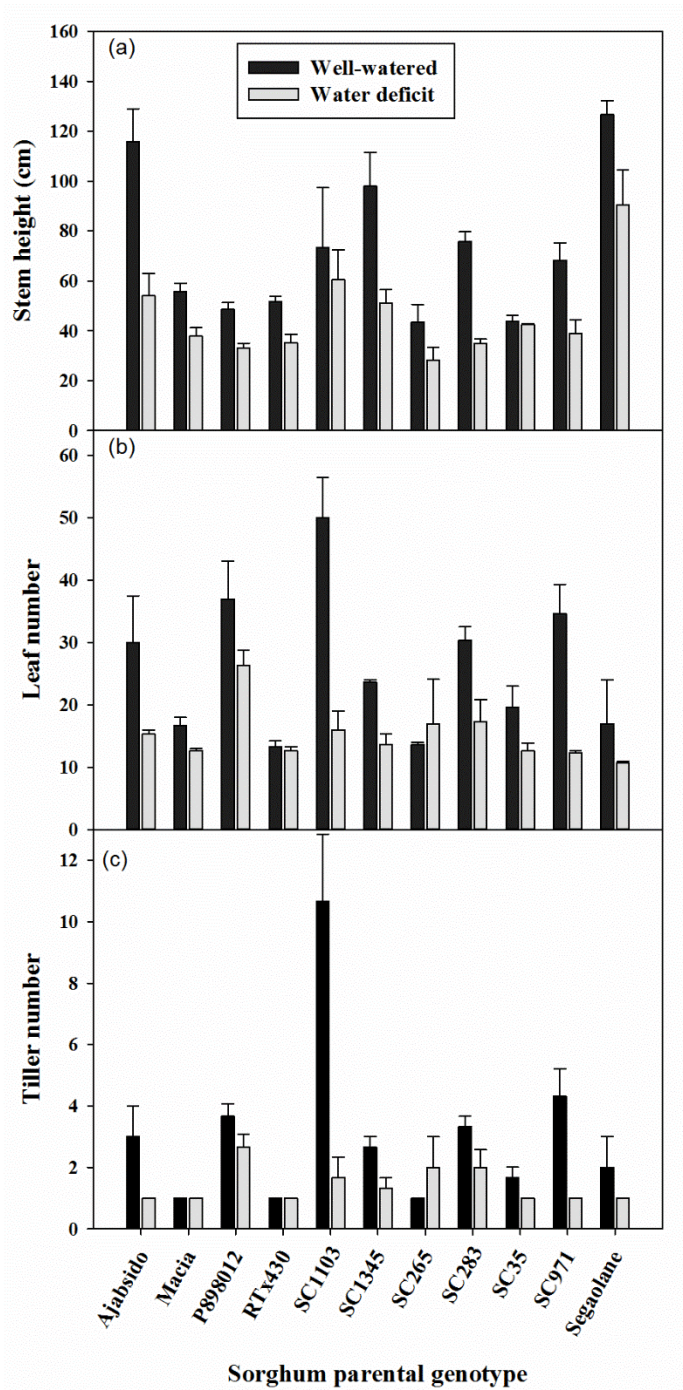


Figure 2.2: Effects of water-deficit stress on (a) stem height (b) leaf number and (c) tiller number in experiment one. Black columns indicate well-watered and grey columns indicate water-deficit stress. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$).

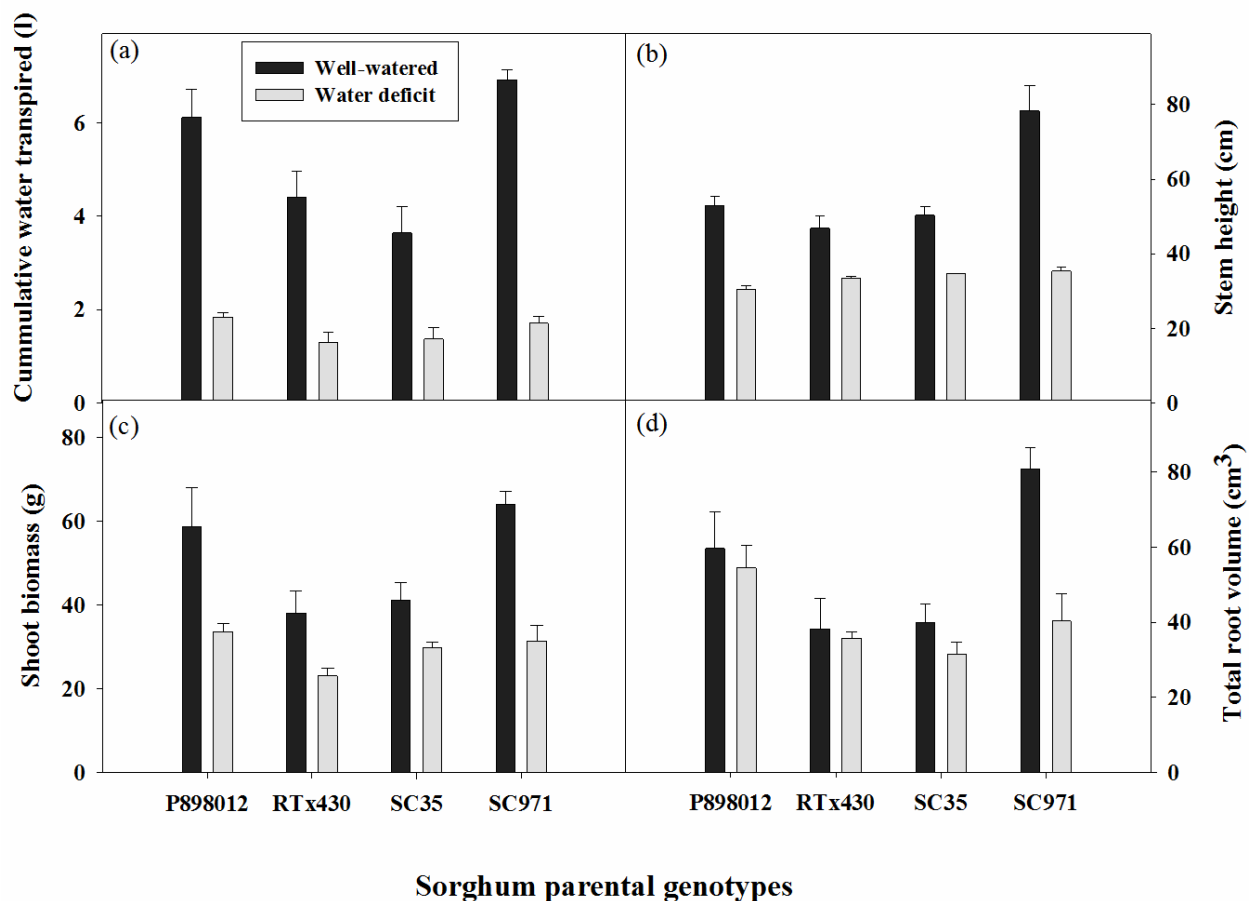


Figure 2.3: Effects of water-deficit stress on (a) cumulative water transpired (b) stem height (c) shoot biomass and (d) total root volume for validation experiment on selected contrasting genotypes from experiment one. Black columns indicate well-watered and grey columns indicate water-deficit stress. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$).

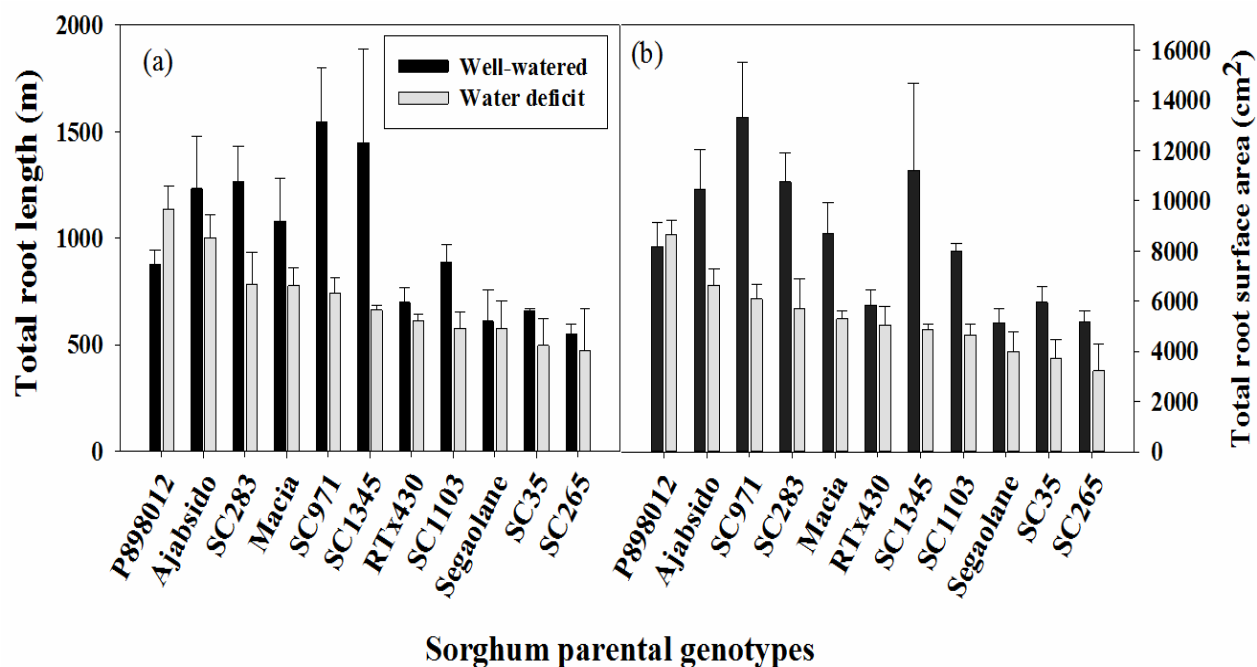


Figure 2.4: Effects of water-deficit stress on (a) total root length and (b) total root surface area in experiment one. Black columns indicate well-watered and grey columns indicate water-deficit stress. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$).

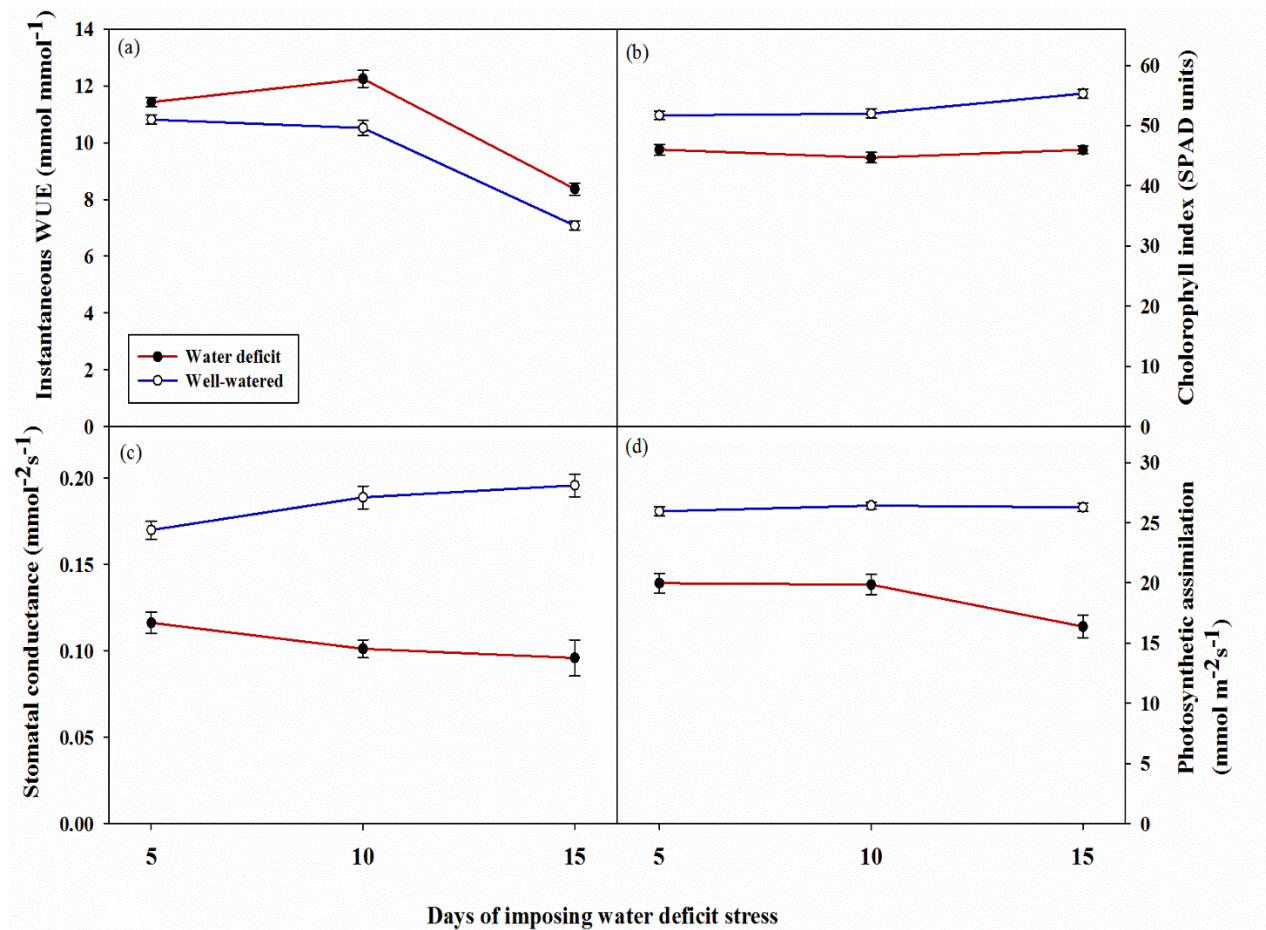


Figure 2.5: Experiment one time series data on (a) instantaneous WUE (b) chlorophyll concentration (c) stomatal conductance and (d) photosynthetic assimilation measured on days 5, ten and fifteen days of imposing stress. Blue lines indicate well-watered condition and red lines indicate water-deficit stress. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$).

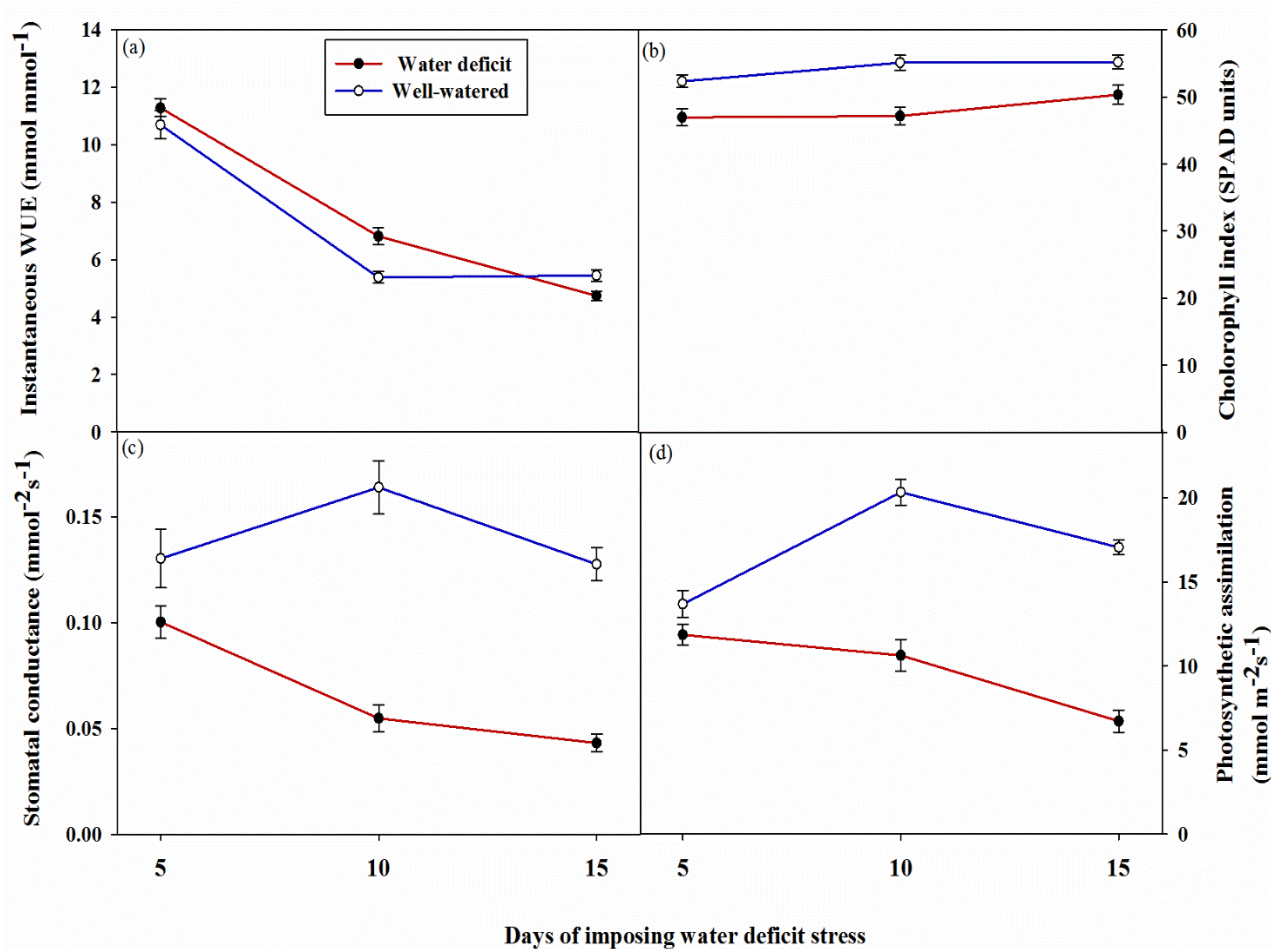


Figure 2.6: Experiment two time series data on (a) instantaneous WUE (b) chlorophyll concentration (c) stomatal conductance and (d) photosynthetic assimilation measured at days 5, ten and fifteen of imposing stress for validation experiment on selected contrasting genotypes. Blue lines indicate well-watered condition and red lines indicate water-deficit stress. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$).

(A)

	SH	ST	TN	LN	LA	SLA	SB	CWT	TE	$\delta^{13}\text{C}$	PA	SC	IWUE	CI	CF	TMD
SH	-															
ST	-0.14	-														
TN	0.04	-0.41	-													
LN	0.01	-0.30	0.90	-												
LA	0.02	0.48	-0.35	-0.27	-											
SLA	-0.02	-0.19	0.23	0.22	-0.21	-										
SB	0.38	0.32	-0.06	0.14	0.60	-0.43	-									
CWT	0.32	0.18	-0.11	0.07	0.34	-0.08	0.47	-								
TE	-0.16	-0.04	0.01	-0.06	-0.12	0.03	-0.08	-0.59	-							
$\delta^{13}\text{C}$	0.08	-0.17	0.18	0.08	-0.14	-0.14	0.13	-0.24	0.30	-						
PA	0.40	0.00	-0.39	-0.24	0.19	-0.11	0.28	0.38	-0.19	-0.37	-					
SC	0.21	-0.17	-0.08	-0.08	0.20	0.08	0.04	0.13	-0.11	-0.11	0.56	-				
IWUE	-0.06	-0.01	-0.26	-0.25	-0.15	-0.14	-0.26	0.08	-0.29	-0.04	0.10	-0.27	-			
CI	0.46	-0.04	-0.19	-0.24	0.00	-0.55	0.28	-0.04	0.07	-0.15	0.26	0.05	-0.02	-		
CF	0.44	0.29	0.10	0.15	0.33	0.16	0.32	0.09	-0.10	-0.17	0.26	0.15	-0.29	0.16	-	
TMD	-0.45	-0.28	-0.11	-0.15	-0.33	-0.16	-0.32	-0.09	0.10	0.17	-0.26	-0.15	0.29	-0.16	-1.00	-
	1	0.8	0.6	0.4	0.2	0	-0.2	-0.4	-0.6	-0.8	-1					

Shoot traits: Stem height (SH), Stem thickness (ST), Tiller number (TN), Leaf number (LN), Leaf area (LA), Specific Leaf Area (SLA), Shoot biomass (SB). **Physiological traits:** Cumulative water transpired (CWT), Transpiration efficiency (TE), Carbon Isotope ($\delta^{13}\text{C}$), Photosynthetic assimilation (PA), Stomatal conductance (SC), Instantaneous WUE (IWUE), Chlorophyll index (CI), Chlorophyll fluorescence (CF), Thylakoid membrane damage (TMD).

(B)

	SH	ST	TN	LN	LA	SLA	SB	CWT	TE	$\delta^{13}\text{C}$	PA	SC	IWUE	CI	CF	TMD
SH	-															
ST	-0.16	-														
TN	-0.21	0.03	-													
LN	-0.21	0.15	0.95	-												
LA	0.08	0.35	-0.18	-0.19	-											
SLA	0.06	-0.25	-0.01	-0.06	0.25	-										
SB	0.17	0.77	0.28	0.43	0.26	-0.33	-									
CWT	0.30	0.42	0.17	0.24	0.27	-0.33	0.58	-								
TE	-0.02	0.36	0.04	0.12	0.02	0.04	0.43	-0.43	-							
$\delta^{13}\text{C}$	-0.22	0.43	0.12	0.16	0.12	-0.35	0.49	0.08	0.50	-						
PA	0.43	0.10	-0.35	-0.30	0.30	-0.06	0.03	0.30	-0.33	-0.17	-					
SC	0.39	0.14	-0.29	-0.29	0.34	-0.10	0.08	0.30	-0.27	0.01	0.87	-				
IWUE	0.11	-0.30	0.17	0.13	-0.04	0.00	-0.18	-0.17	-0.17	-0.32	0.19	-0.04	-			
CI	0.13	0.20	-0.26	-0.32	0.20	-0.18	0.05	0.24	-0.16	-0.01	0.23	0.20	-0.04	-		
CF	-0.16	0.03	-0.23	-0.22	0.03	0.18	-0.18	-0.09	0.02	0.22	-0.03	0.08	-0.27	0.32	-	
TMD	0.14	0.00	0.21	0.20	0.03	-0.24	0.20	0.14	-0.04	-0.16	0.05	0.02	0.21	-0.35	-0.97	-
	1	0.8	0.6	0.4	0.2	0	-0.2	-0.4	-0.6	-0.8	-1					

Figure 2.7: Correlation matrix between physiological and shoot traits under well-watered (A) and water deficit (B) conditions of experiment one

Shoot traits: Stem height (SH), Stem thickness (ST), Tiller number (TN), Leaf number (LN), Leaf area (LA), Specific Leaf Area (SLA), Shoot biomass (SB). **Physiological traits:** Cumulative water transpired (CWT), Transpiration efficiency (TE), Carbon Isotope ($\delta^{13}\text{C}$), Photosynthetic assimilation (PA), Stomatal conductance (SC), Instantaneous WUE (IWUE), Chlorophyll index (CI), Chlorophyll fluorescence (CF), Thylakoid membrane damage (TMD).

(A)

	SH	ST	TN	LN	LA	SLA	SB
TRL	0.32	0.21	0.12	0.27	0.58	-0.39	0.90
TRSA	0.30	0.19	0.14	0.30	0.58	-0.34	0.87
TRV	0.28	0.19	0.12	0.28	0.52	-0.27	0.76
RD	0.22	0.25	0.18	0.22	0.46	-0.28	0.49
RB	0.25	0.27	0.02	0.22	0.59	-0.42	0.92
ARD	0.14	0.03	-0.07	-0.05	-0.10	0.08	-0.02
FRL	0.24	0.10	0.17	0.37	0.50	-0.34	0.82
FRSA	0.25	0.09	0.21	0.40	0.48	-0.32	0.82
FRV	0.26	0.09	0.22	0.41	0.47	-0.30	0.82

(B)

	SH	ST	TN	LN	LA	SLA	SB
TRL	0.00	0.37	0.41	0.55	0.13	-0.18	0.52
TRSA	-0.05	0.30	0.37	0.52	0.18	-0.07	0.48
TRV	-0.07	0.16	0.29	0.43	0.21	0.05	0.35
RD	0.14	0.18	0.25	0.33	0.45	0.21	0.34
RB	-0.09	0.46	0.49	0.64	0.21	-0.08	0.69
ARD	-0.10	-0.35	-0.12	-0.12	0.01	0.14	-0.23
FRL	0.08	0.50	0.46	0.60	0.27	-0.20	0.65
FRSA	0.05	0.49	0.45	0.61	0.31	-0.17	0.64
FRV	0.05	0.47	0.45	0.61	0.33	-0.13	0.63

Figure 2.8: Correlation matrix between shoot and root traits under well-watered (A) and water deficit (B) conditions of experiment one

Shoot traits: Stem height (SH), Stem thickness (ST), Tiller number (TN), Leaf number (LN), Leaf area (LA), Specific Leaf Area (SLA), Shoot biomass (SB). **Root traits:** Total root length (TRL), Total root surface area (TRSA), Total root volume (TRV), Rooting depth (RD), Root biomass (RB), Average root diameter (ARD), Fine root length (FRL), Fine root surface area (FRSA), Fine root volume (FRV).

(A)

	SH	ST	TN	LN	LA	SLA	SB	CWT	TE	$\delta^{13}\text{C}$	PA	SC	IWUE	CI	CF	TMD
SH	-															
ST	-0.40	-														
TN	0.59	-0.71	-													
LN	0.41	-0.68	0.95	-												
LA	0.27	-0.38	0.63	0.66	-											
SLA	-0.05	-0.45	0.21	0.31	0.45	-										
SB	0.67	-0.24	0.79	0.77	0.63	0.04	-									
CWT	0.69	-0.47	0.89	0.87	0.67	0.13	0.93	-								
TE	-0.09	0.64	-0.41	-0.43	-0.19	-0.25	0.00	-0.36	-							
$\delta^{13}\text{C}$	0.13	0.51	-0.17	-0.25	-0.32	-0.41	0.13	-0.11	0.67	-						
PA	-0.59	0.31	-0.26	-0.24	-0.41	-0.41	-0.39	-0.46	0.21	0.40	-					
SC	-0.52	0.07	-0.42	-0.37	-0.62	-0.28	-0.61	-0.56	-0.12	0.09	0.66	-				
IWUE	0.06	0.30	0.16	0.14	0.24	0.08	0.28	0.22	0.21	0.26	0.01	-0.65	-			
CI	-0.22	0.28	-0.02	0.07	0.41	-0.18	0.15	0.11	0.11	0.23	0.12	-0.23	0.34	-		
CF	-0.17	0.19	-0.13	0.00	-0.27	-0.25	0.08	-0.11	0.39	0.07	-0.05	0.05	-0.21	0.02	-	
TMD	0.15	-0.19	0.13	0.00	0.27	0.26	-0.09	0.09	-0.38	-0.07	0.06	-0.05	0.22	-0.02	-1.00	-

Shoot traits: Stem height (SH), Stem thickness (ST), Tiller number (TN), Leaf number (LN), Leaf area (LA), Specific Leaf Area (SLA), Shoot biomass (SB). **Physiological traits:** Cumulative water transpired (CWT), Transpiration efficiency (TE), Carbon Isotope ($\delta^{13}\text{C}$), Photosynthetic assimilation (PA), Stomatal conductance (SC), Instantaneous WUE (IWUE), Chlorophyll index (CI), Chlorophyll fluorescence (CF), Thylakoid membrane damage (TMD).

(B)

	SH	ST	TN	LN	LA	SLA	SB	CWT	TE	$\delta^{13}\text{C}$	PA	SC	IWUE	CI	CF	TMD
SH	-															
ST	0.06	-														
TN	-0.30	-0.89	-													
LN	-0.54	-0.79	0.95	-												
LA	0.51	-0.27	0.13	0.04	-											
SLA	-0.33	-0.39	0.39	0.34	-0.48	-										
SB	-0.04	0.08	-0.01	-0.04	0.06	-0.22	-									
CWT	-0.25	-0.26	0.39	0.33	-0.11	0.14	0.79	-								
TE	0.36	0.58	-0.67	-0.62	0.24	-0.53	0.03	-0.59	-							
$\delta^{13}\text{C}$	0.39	0.10	-0.23	-0.42	0.22	-0.15	0.56	0.45	-0.01	-						
PA	0.13	0.11	-0.19	-0.14	-0.33	0.18	-0.78	-0.74	0.19	-0.55	-					
SC	0.29	0.27	-0.37	-0.35	-0.29	0.06	-0.65	-0.71	0.30	-0.39	0.93	-				
IWUE	-0.38	-0.32	0.29	0.33	0.16	0.20	0.13	0.33	-0.38	0.16	-0.43	-0.67	-			
CI	0.46	0.20	-0.26	-0.35	0.21	-0.40	-0.52	-0.40	0.02	0.04	0.23	0.28	-0.19	-		
CF	-0.01	0.39	-0.39	-0.30	-0.11	-0.26	-0.08	-0.41	0.61	-0.18	0.47	0.49	-0.23	-0.18	-	
TMD	0.03	-0.39	0.38	0.28	0.14	0.25	0.06	0.37	-0.58	0.18	-0.46	-0.48	0.22	0.20	-1.00	-
	1	0.8	0.6	0.4	0.2	0	-0.2	-0.4	-0.6	-0.8	-1					

Figure 2.9: Correlation matrix between physiological and shoot traits under well-watered (A) and water deficit (B) conditions of experiment two.

Shoot traits: Stem height (SH), Stem thickness (ST), Tiller number (TN), Leaf number (LN), Leaf area (LA), Specific Leaf Area (SLA), Shoot biomass (SB). **Physiological traits:** Cumulative water transpired (CWT), Transpiration efficiency (TE), Carbon Isotope ($\delta^{13}\text{C}$), Photosynthetic assimilation (PA), Stomatal conductance (SC), Instantaneous WUE (IWUE), Chlorophyll index (CI), Chlorophyll fluorescence (CF), Thylakoid membrane damage (TMD).

(A)

	SH	ST	TN	LN	LA	SLA	SB
TRL	0.62	-0.32	0.80	0.81	0.72	0.24	0.95
TRSA	0.74	-0.36	0.83	0.79	0.63	0.18	0.94
TRV	0.81	-0.37	0.84	0.73	0.48	0.05	0.88
RD	0.62	0.26	0.11	-0.03	0.12	-0.32	0.48
RB	0.60	-0.24	0.73	0.73	0.56	0.17	0.92
ARD	0.01	0.10	-0.08	-0.19	-0.62	-0.65	-0.21
FRL	0.60	-0.31	0.79	0.81	0.73	0.25	0.95
FRSA	0.70	-0.35	0.82	0.80	0.71	0.20	0.96
FRV	0.76	-0.38	0.83	0.79	0.69	0.17	0.95

(B)

	SH	ST	TN	LN	LA	SLA	SB
TRL	-0.31	-0.18	0.38	0.40	0.01	-0.08	0.83
TRSA	-0.39	-0.22	0.40	0.45	-0.06	-0.03	0.78
TRV	-0.46	-0.26	0.39	0.47	-0.15	0.04	0.66
RD	0.00	0.24	-0.39	-0.32	0.04	-0.42	-0.42
RB	-0.47	-0.15	0.26	0.33	-0.34	0.07	0.68
ARD	-0.09	-0.24	0.06	0.14	-0.22	0.28	-0.55
FRL	-0.29	-0.17	0.37	0.39	0.03	-0.09	0.84
FRSA	-0.33	-0.17	0.36	0.39	0.01	-0.08	0.85
FRV	-0.36	-0.19	0.37	0.41	-0.01	-0.05	0.83

Figure 2.10: Correlation matrix between shoot and root traits under well-watered (A) and water deficit (B) conditions of experiment 2
Shoot traits: Stem height (SH), Stem thickness (ST), Tiller number (TN), Leaf number (LN), Leaf area (LA), Specific Leaf Area (SLA), Shoot biomass (SB). **Root traits:** Total root length (TRL), Total root surface area (TRSA), Total root volume (TRV), Rooting depth (RD), Root biomass (RB), Average root diameter (ARD), Fine root length (FRL), Fine root surface area (FRSA), Fine root volume (FRV).

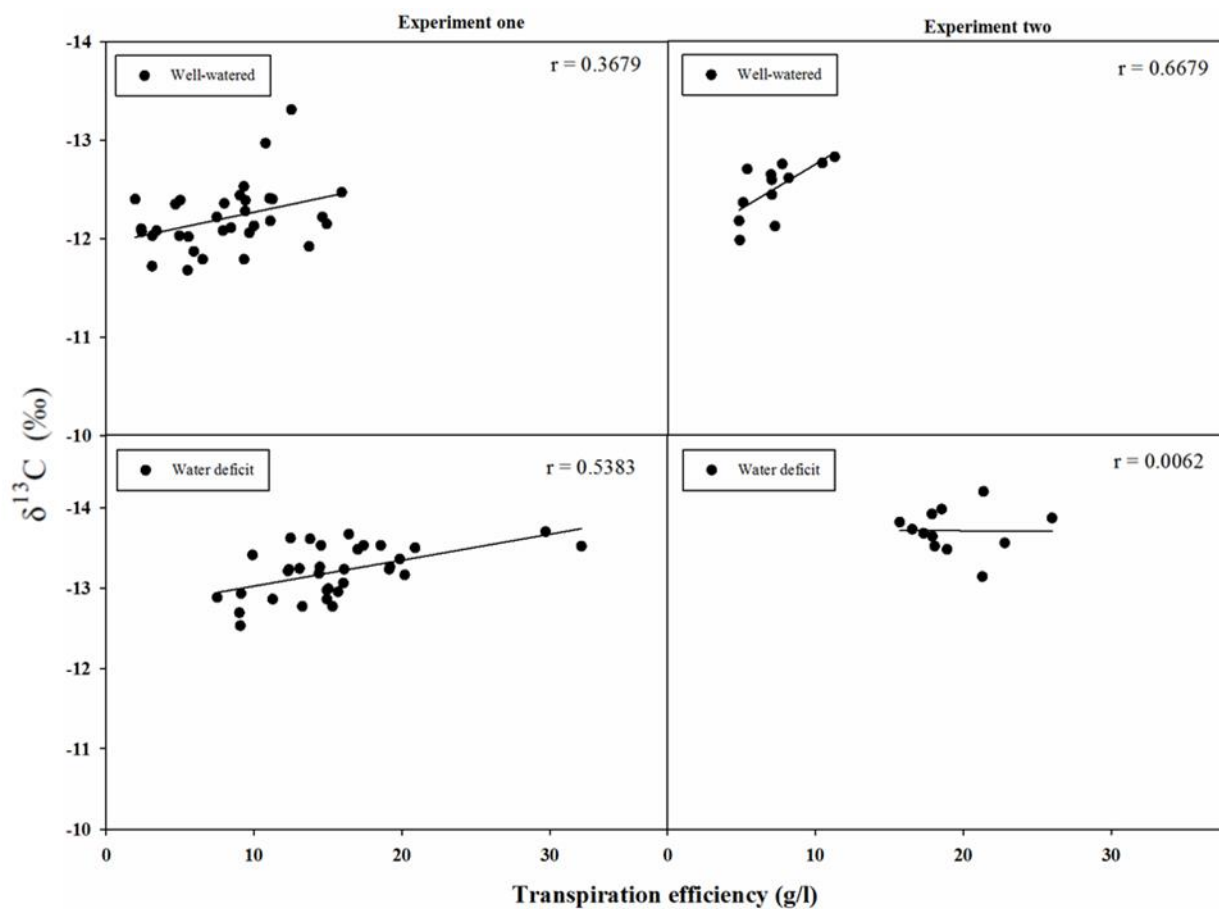


Figure 2.11: Relationship between transpiration efficiency and carbon isotope composition ($\delta^{13}\text{C}$) of sorghum parental genotypes under well-watered and water-deficit stress conditions for experiment one and experiment two (validatory). Test for significance was considered at the $P < 0.05$ level.

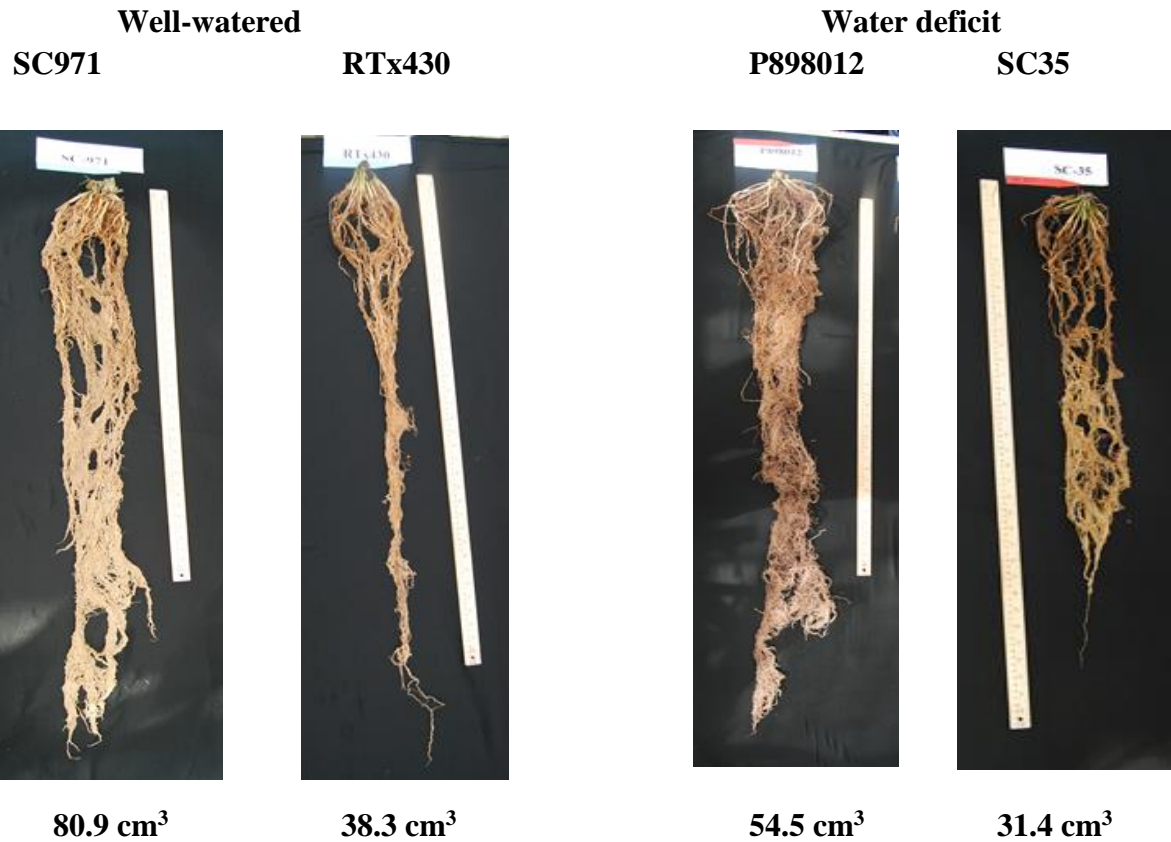


Figure 2.12: Root volume of validation experiment (experiment two) involving contrasting parental sorghum genotypes under well-watered (*left*) and water-deficit (*right*) conditions. The largest and least on the right and left respectively for each watering condition.

Chapter 3 – Characterization of Parents of Sorghum Mapping

Populations Exposed to Water-Deficit Stress during the Grain

Filling Stage

3.1 Abstract

Agricultural productivity is threatened by scarcity of water and with sorghum's ability to thrive in arid environments, it is useful to understand what constitutes its adaptive strategies, especially during the grain filling period. Although studies have been conducted on other cereals to determine the influence of panicle architecture on yield, similar studies on sorghum are limited. Using parents of the nested association mapping (NAM) sorghum populations, water-deficit stress induced through lysimeters in the greenhouse and rainout shelters in the field were conducted. Investigations were focused on determining the variability in the grain-filling pattern across differential positions within panicles in the parental sorghum genotypes (Ajabsido, SC 1103, SC 265, P898012, SC 35, Macia, SC 283, SC 1345, Segalane, SC 971, and RTx430). Due to water-deficit stress, there were variations in grain number and grain weight along different positions on the panicle among the tested genotypes. Differences in panicle positional grain number accounted for differences in panicle positional grain weight as the positional individual grain weight was not affected by the stress. Regardless of the watering treatments however, differences in grain numbers and grain weight among genotypes resulted from the diversity in panicle architecture other than grain filling dynamics. In both the greenhouse and field experiments, SC1103 did not record any significant difference between the watering treatment for all growth and yield traits measured while SC265 and SC35 recorded a similar response only with the greenhouse experiment. In comparing results of the greenhouse study to the field study, Spearman's rank correlations

indicated the ability to select for water-deficit tolerance traits in the greenhouse that would partially represent rankings on the field.

3.2 Introduction

Agricultural productivity is threatened by scarcity of water (Lopes et al., 2011), which is even more challenging for the arid and semi-arid regions of the world. Water scarcity or drought events can be episodic and can extend through most of the growing season (Comas et al., 2013). In the year 2011 and 2012 for instance, drought affected 63 % of agricultural lands in some areas of the United States of America, with an estimated loss of more than \$ 8 billion (Kadam et al., 2014). However, in arid and semi-arid regions where some crops cannot grow in the harsh environmental conditions, crops such as millet and sorghum have acclimitized to survive and sustain a moderate level of productivity. Sorghum is the fifth most economically important cereal and serves as a staple food for some Asian and many African nations (Felch et al., 2006).

With sorghum's ability to thrive in arid environments, it is useful to understand what constitutes its adaptive strategies, especially during the grain filling period. Whereas the extent to which water-deficit stress limits crop productivity depends mainly on the phase of development during which the stress is experienced (Dolferus et al., 2011), almost all growth and developmental phases of the sorghum cycle are affected by water-deficit stress (Kadam et al., 2014). At the boot phase, the potential number of grains has been determined so that yield potential after this phase is based on successful grain-set and grain filling (Roozeboom and Prasad, 2016). Water-deficit stress during anthesis results in reduced seed-set, and water-deficit-stress during the post-anthesis stage mainly results in decreased grain weight (Prasad and Staggenborg, 2009; Maman et al., 2004). Typically, water-deficit stress during the grain-filling period induces early senescence, limits

assimilate availability, shortens the grain-filling period, and ultimately reduces yield (Zhang et al., 2018; Samarah, 2005; Yang and Zhang, 2005). Yield differences in agronomic crops are related with grain number and grain weight (Prasad and Staggenborg, 2008).

In the development and growth of most cereals, almost all the water extracted during the post-anthesis phase contributes to grain growth (Vadez, 2014). From simulation studies on sorghum, it has been established that 1 mm of additional water transpired during grain filling has the potential to increase grain yield by 30 kg ha⁻¹ (Hammer, 2006). This increase in grain yield is attributable to the findings that water extracted late in the season is channeled largely for grain growth and not for building more structural crop biomass (Borrell et al., 2014). Since higher grain yield has been strongly linked to sufficient water supply during grain filling, any condition such as water-deficit stress that reduces availability of assimilate during the grain-filling period indirectly reduces the final grain weight (Blum et al., 1997).

Although studies have been conducted on other cereals to determine the influence of panicle architecture on yield and grain quality (Sathishraj et al., 2016; Zhang et al., 2013), similar studies on sorghum are limited. With regards to the basipetal flowering pattern of sorghum, and the later phases of grain development that follow same pattern (where grain development commences first at the top and continues down the panicle in circular bands - Roozeboom and Prasad, 2016), it is interesting to assess how sorghum grain yields are influenced by developmental patterns of grains along the different positions on the panicle, particularly under water deficit conditions.

Using parents of the nested association mapping (NAM) sorghum populations (Ajabsido, SC 1103, SC 265, P898012, SC 35, Macia, SC 283, SC 1345, Segalane, SC 971, and RTx430 as the common parent), greenhouse and field experiments were conducted to examine the effects of

water-deficit stress on the grain-filling stage. It is of interest to dissect how biomass partitioning for growth and yield traits is affected by water-deficit stress and the impact it has on the grain filling patterns within panicles. Investigations were focused on determining the variability in the grain-filling pattern across differential positions within panicles in the above genotypes, exposed to water-deficit stress during the grain-filling stage. The study specifically aimed to (1) compare the impact of water-deficit stress during grain filling on the yield and related traits under greenhouse and rainout shelter conditions and (2) explore the genetic diversity for within-head grain-filling dynamics under water-deficit stress induced through lysimeters in the greenhouse and rainout shelters in the field. We tested the hypothesis that water deficit stress during the grain filling stage will cause differences in yield and growth related traits among the sorghum genotypes and there will be variations in grain filling dynamics within the sorghum panicles.

3.3 Materials and Methods

Experiments were conducted at the greenhouse and under field conditions to characterize sorghum genotypes exposed to water-deficit stress during the grain-filling stage. These genotypes are the eleven parental sorghum genotypes (Ajabsido, SC1103, SC265, P898012, SC35, Macia, SC283, SC1345, Segalane, SC971, RTx430) of the Nested Association Mapping population available at KSU.

3.3.1 Greenhouse Experiment

3.3.1.1 Plant material and growth conditions

For the greenhouse experiment, lysimetric PVC pots (polyvinyl chloride columns with inner diameter dimension of 20 cm and height of 1 m) were used. The PVC columns were sealed at the

bottom with plastic caps that had drilled 5 holes of diameter 0.5 cm. The growth medium used was Turface® (MVP®, Profile Products LLC, Buffalo Grove, IL), which is a calcined montmorillonite clay with high cation exchange capacity, and with a better drainage, porosity, and water-holding capacity when compared with other growth media such as cymad mix and sand (Calonje et al., 2010). The rooting medium was fertilized with Osmocote® Classic (The Scotts Company LLC, Marysville, Ohio), a controlled-release fertilizer with 14–14–14 ratio of N–P₂O₅–K₂O and at a rate of 5 g per liter. The fertilizer was mixed with the growth medium before sowing. A systemic insecticide, Marathon 1 % Granular (OHP, Inc., Mainland, PA) was mixed with growth medium at 12 g per column before sowing.

The experiment was set up in a randomized complete block design with three blocks (replicates). Three seeds were sown at a depth of about 2 cm in each pot and thinned to one plant per pot during the three-leaf stage. Plants were maintained under greenhouse conditions of 15 hours photoperiod and daytime maximum / night-time minimum temperatures of 32 and 25 °C, respectively. Plants were irrigated (1.5 ± 1 liters) daily using an automated drip irrigation system. Irrigation was provided for four minutes, four times per day at 0600, 1000, 1400, and 1800 h.

Based on the results from a preliminary experiment conducted at the vegetative stage, water-deficit stress was imposed for 15 days starting on the day when 70 – 80 % of the panicle had flowered. The flowering of sorghum is basipetal (flowering starts at the top and continues gradually to the base, with an average of four days for complete flowering). Thus exposure to the water-deficit stress started when two-thirds of the panicle had anthers (70 - 80 %) so that plants could utilize any amount of water left in the pots to complete flowering.

Plants were treated individually depending on their phenology, mainly based on the time of initiation of flowering. Flowering date was recorded as the day on which anthers first appeared on the main stem. Dates for complete panicle flowering (when anthers/pollen are found at the base of panicle) were noted. The first main tiller was tagged upon flowering. After the 15-days of imposing stress, plants were re-watered until maturity. Physiological maturity was determined as the time of appearance of the black layer on the grain which was about 40-45 days after anthers were observed at the bottom of panicle.

3.3.1.2 Data collection

On days 5, 10 and 15 during the stress period, chlorophyll index, and chlorophyll fluorescence a were measured on the flag leaf between 10:00 and 13:00 hours. Chlorophyll fluorescence a was measured with a chlorophyll fluorometer (OS30p; OptiSciences, Hudson, NH, USA) and the chlorophyll index with a chlorophyll meter (Soil Plant Analyzer Development – SPAD, Model 502; Spectrum Technologies, Plainfield, IL, USA). Chlorophyll fluorescence a was measured on the middle portion of the flag leaf blade, after 60 minutes of dark adaptation of leaves. Measurement with the chlorophyll fluorometer on the dark-adapted leaves captures the minimal fluorescence (F_0), maximum fluorescence (F_m), and photochemical efficiency of PSII (F_v/F_m) which is a ratio of variable fluorescence (F_v) to maximum fluorescence (F_m). For chlorophyll index measurements, readings were taken at top, mid, and base of the flag leaf and averaged per plant.

At physiological maturity, based on the grains in the main panicle, the main panicle was harvested together with the main stem. The stem height and stem thickness were measured using meter rule and Vernier calipers (electronic digital caliper, VWR® Digital Calipers, USA) respectively. The

leaves, leaf sheath, and the stem were separated and oven dried separately to constant weight at 60 °C. They were weighed to determine the dry weights of main stem leaves, main stem leaf sheath, and the main stem. Using a technique similar to that of Sathishraj et al. (2016), rachises of the main panicle were divided into three (top, middle, bottom) based on panicle length: the length of panicle at the base and top portions were kept equal and the middle portion varied (by +1 or -1 cm) depending on divisibility of the measured panicle rachis/branches by three. Grains of these positional zones of the panicle were hand-threshed, oven dried to constant weight at 60 °C and then counted and weighed to determine the panicle positional grain number and the panicle positional grain weight, respectively. Each of the panicle positional grain numbers (i.e., grain number with respect to the top, middle, bottom) were added to obtain the grain number per main panicle. Likewise, the panicle positional grain weights were added to obtain the grain yield per main panicle. Individual grain weight was obtained as a ratio of the grain yield per main panicle to the grain number per main panicle.

At physiological maturity of the first tiller panicle, the number of additional panicles bearing tillers were counted and the plants were harvested. Panicles of the first tiller (which flowered following the main tiller) were dried, threshed using a stationary thresher (Model LDB, ALMACO, 99 M Avenue Nevada, US), weighed, and counted separately from the panicles of all the other tillers. The rest of the shoot biomass was separated into leaves and stems, and oven dried to constant weight at 60 °C and weighed. Harvest index was computed as the ratio of the total grain yield (summation of grain yield per main panicle, first tiller grain yield, and the grain yield of all other panicles) to the total aboveground biomass (main stem leaves dry weight + main stem leaf sheath dry weight + and the main stem dry weight + all other leaves dry weight + all other stem dry weight). All other stems and leaves referred to the stems and leaves of the tillers.

3.3.2 Field Experiment

The field research was conducted in 2016 summer growing seasons at Kansas State University Agronomy Research Farm at Manhattan-Ks (39 11'N, 96 35'W). The soil type was a Kennebec silt loam. Before planting, soil test was conducted on soil samples collected at the 0-15 cm depth. Soil test analysis indicated the soil to contain contained 2.0 % OM, 27.0 mgkg⁻¹ of Melich-P, 317 mgkg⁻¹ K, 7.2 mgkg⁻¹ of NH₄-N and 15.3 mgkg⁻¹ of NO₃-N and had a pH of 6.9. The field experiment was set up using two rainout shelters (for the water-deficit stress treatment), two control plots (for the well-watered treatment) located opposite to the rainout shelters, and ten of the parental sorghum genotypes instead of the eleven used in the greenhouse (genotype SC265 was omitted due to seed supply issues). Each plot size was 12.2 m by length and 10.5 m by width.

Before planting, a pre-emergence herbicide (i.e. glyphosate-4 plus [N-(phosphonomethyl) glycine] (Alligare LLC) at 1.67 a.i. L ha⁻¹, was applied. Planting was done using a hand pushed single row seeder (Rowseed 1R, Wintersteiger, Ried im Innkreis, Austria). To ensure uniformity of flowering among the genotypes, planting was conducted in 2 phases: the late flowering genotypes (P898012, SC35, Macia, RTx430, SC971) were planted first, before the early flowering genotypes (Ajabsido, SC1103, SC1345, Segalane, SC283) were planted, i.e., a week later. Between row spacing was 64 cm, and within rows spacing was approximately 18 cm. To handle the effects of borders on the experimental genotypes, rows of sorghum plants (DeKalb hybrids) were planted around the plots as borders. Hand weeding was done weekly to keep plots free of weeds, mostly monocotyledonous weeds. Drip tape irrigation was used for all plots to ensure adequate soil moisture before imposing stress, and the drip tapes were continually used to irrigate the control plots during the water-deficit stress treatments in the rainout shelters. Nitrogen fertilizer (urea, 46 % N) was hand broadcast at about 15 days after emergence at the rate of 90 kg ha⁻¹ N. Since the plots were irrigated using drip

tape, the rainout shelters were under operation early in the growing season to ensure minimal soil moisture during the grain-filling period.

3.3.2.1 Data collection

During the stress period, three plants per each genotype per plot were tagged for weekly measurements of chlorophyll fluorescence (using a similar method described as in the greenhouse experiment) and using Dualex (Dualex 4 Scientific, Force-A, Orsay, France) for determining the chlorophyll index. At physiological maturity, main stems of the three tagged plants of each genotype per treatment were harvested. The same procedure as in the greenhouse was followed to obtain data on main stem leaves dry weight, main stem leaf sheath dry weight, main stem dry weight, panicle positional grain number, panicle positional grain weight, grain number per main panicle, grain yield per main panicle, and the individual grain weight, on the harvested main stems from the field. Thereafter, plant harvest was done for a 3 m row length per genotype for panicles and shoot biomass. The shoot biomass was separated into leaves and stems, oven dried to constant weight at 60 °C and then weighed (harvest components of the 3 m row, such as stems, leaves, and panicles were referred to as ‘all other’ in the results section). Panicles were threshed using a stationary thresher (Model LDB, ALMACO, 99 M Avenue Nevada, US) and the grains were weighed to determine grain yield of all other panicles. Other estimated yield related parameters included harvest index (procedure same as stated under the greenhouse experiment) and total grain yield (kg/ha).

3.3.2.2 Determining soil water content under field conditions

Prior to planting, aluminum access tubes (length 150 cm, diameter 5 cm, and wall thickness 0.128-cm) were installed in the field using a hydraulic probe. The tubes were driven by a slide hammer to a depth of 135 cm with 15 cm of the tube exposed above the soil surface. There were six access tubes per rainout shelter and three tubes per control plot. Hence a total of 12 tubes were used for monitoring the soil water-deficit in two independent rainout shelters, while six tubes were used for the well-watered control. Two additional tubes were also installed to calibrate the neutron probe (i.e. to find a relationship between neutron counts and volumetric water content). One of the calibration tubes was used to calibrate the neutron probe under dry conditions and the remaining access tube to calibrate the neutron probe under wet (i.e. near saturation) conditions. For the dry calibration tube, sorghum plants were planted around it to ensure dry conditions across the entire profile. To ensure high moisture conditions around the wet calibration tube, water was applied periodically for about two months and the soil surface was kept covered with a tarp to minimize evaporative losses.

A neutron probe (Model 503DR Hydroprobe Moisture Depth Gauge by Campbell Pacific Nuclear International, Inc. Martinez, CA) was used to record neutron counts during 18 seconds at five soil depths (15, 45, 75, 105, and 135 cm) at weekly intervals during the growing season. Readings were conducted for eight weeks which spanned a period before the onset of stress to the end of the stress period. Standard counts were recorded in the field prior to and after each weekly measurements. Count ratio (C_R) was calculated as

$$C_R = \frac{C}{C_s}$$

where C and C_s were the access tube and standard counts respectively.

3.3.2.3 Calibration of neutron probe

Calibration of the neutron probe was carried out using the method and principles outlined by Chanasyk and Naeth (1996) and Gardner and Kirkham (1952). At the end of the experiment, three replicate soil samples were taken around each calibration access tube to the laboratory to determine the volumetric moisture content. Undisturbed core sub-samples 5 cm in diameter were collected from four soil depths (e.g. 3 – 8, 9 – 14, 16 – 21, and 24 – 29 cm) to calibrate the neutron probe in the 0 – 30 cm depth layer. For the remaining depths (30 – 60, 60 – 90, 90 – 120, and 120 – 150 cm), two subsamples were taken within each depth.

Each soil sub-sample was weighted in the laboratory to record the field moisture conditions. The soil samples were used to determine the saturation point and the soil water retention at -10 kPa and -33 kPa respectively using the tempe cells before oven-drying the soil. Samples were saturated from the bottom by placing the soil cores in container with 5 mM solution of calcium chloride. Samples that did not reach saturation within the first five days were placed under vacuum conditions. Mass was recorded at saturation to calculate saturation point of each soil core. The tempe cell method (Dane and Hopmans, 2002) was used to determine the volumetric water content at two nominal suctions (i.e. -10 kPa and -33 kPa) representing the drained upper limit of the soil, points that are also known as “field capacity”. The tempe cells method consists of placing saturated soil samples at incrementally higher pressures and waiting until soil samples reach equilibrium. Equilibrium was assumed to have been reached when the change in tempe cell mass over two consecutive days was lower than 1 g, time at which we recorded the mass of the samples. At the end of the process, samples were oven-dried at 105°C for 48 hours. The data collected were used to calculate the volumetric water content (θ) in field conditions, saturation, -10 kPa and -33 kPa. Volumetric moisture content was calculated as

$$\theta = \left(\frac{M_{wet} - M_{dry}}{M_{dry}} \right) \frac{\rho_b}{\rho_w}$$

where M_{wet} is the mass of wet soil (e.g. mass at saturation point or field conditions), M_{dry} represents the mass of oven-dry soil, ρ_b is the bulk density of the soil, and ρ_w is the density of water at room temperature. Bulk density was calculated as:

$$\rho_b = \frac{M_{dry}}{V}$$

where V is the volume of the containing ring, which in this case was 98.2 cm³.

The soil water retention at -1500 kPa (commonly known as permanent wilting point) was determined using the WP4C soil water potentiometer (Meter Environment, Pullman, WA). About 4 g of disturbed and oven-dried soil were placed in four stainless steel cups. Different amount of water droplets was added to each cup to generate cups with slightly different matric potentials. Water potential for each was measured with the WP4C. In this study we assumed that the osmotic potential of the samples was negligible relative to the value of the matric potential. Gravimetric water content of the soil in each cup was measured by oven drying the soil at 105°C for 24 hours. Volumetric water content was approximated by using the value of gravimetric water content and the previously calculated bulk density of the soil. In addition, soil samples from each soil depth were air dried, sieved through a 2 mm sieve and analyzed for particle size distribution using the hydrometer method (Gaylark et al., 2005; Gee and Or, 2002). The soil textural class determined for all depths in the experimental plots was predominantly silty clay loam. Calibration equations were developed using linear regression of the count ratio and the volumetric moisture content in the equation below (Evelt et al., 2003).

$$\theta = a + b * C_R$$

where θ , a , b , and C_R were the volumetric moisture content, intercept of regression, slope of regression, and count ratio, respectively. Calibration equations were developed separately for the surface (0 – 30 cm) and for the subsurface (30 -150 cm) depth (Figure 3.1).

3.3.2.4 Plant available water (PAW) and the fraction of available water capacity (FAW)

In accordance with Krueger et al. (2015), the plant available water (PAW) was determined in this context as the soil water content between measured value and permanent wilting point, and was calculated as

$$PAW = (\theta - \theta_{WP}) d$$

where θ is measured volumetric water content, θ_{WP} is volumetric water content at the permanent wilting point, and d is the thickness of the layer represented by the measurement. The plant available water capacity was then determined as

$$PAWC = (\theta_{FC} - \theta_{WP}) d$$

Where θ_{FC} is the field capacity (the drained upper limit of the soil). Since the available water capacity (maximum PAW) varies immensely across soils due soil properties such as texture and porosity that dictate how much water is available for plant uptake, the fraction of available water capacity (FAW) was calculated to account for the effect of soil properties on moisture available to crops (Figure 3.2) and also to normalize the PAW across the plots (Krueger et al., 2015). FAW was calculated as

$$FAW = PAW/PAWC$$

Values of FAW are typically between 0 (no PAW) and 1 (maximum PAW) and values less than approximately 0.5 indicate conditions of vegetative moisture stress (Krueger et al., 2015; Allen et al., 1998).

3.3.2.5 Statistical analysis

For the greenhouse experiment, the experimental design was a 2-way factorial treatment structure run in an Randomized Complete Block Design with three blocks of size 22. The two factors were the eleven sorghum parental genotypes and the watering regime. For the field experiment, the experimental design was a split-plot design where the main-plot treatment was the watering regime: water-deficit (rainout shelters) and well-watered (open field plots); subplot factors were the ten parental sorghum genotypes. All data were statistically analyzed using the GLM procedure in SAS (SAS 9.4, SAS Institute Inc. Cary, NC, USA). Pairwise comparisons using Tukey-Kramer test in SAS were done to separate means for significant treatment interactions at probability level of 0.05. Correlation analysis (Rahmani et al., 2015) were also conducted as follows: Spearman's rank correlations to compare greenhouse and field results and Pearson correlation (simple linear regression) between some growth and yield traits in both experimental settings.

3.4 Results

In testing for statistical significance, analysis of variance showed that the genotype by watering treatments interaction ($G \times T$) was significant for some traits (main stem height, main stem thickness, main stem leaf sheath dry weight, all other stem dry weight, grain number per main panicle, grain yield per main panicle, all other panicles' grain yield and harvest index) in both the greenhouse and the field conditions (Table 3.1). In addition, the $G \times T$ interactive effect was significant for the following traits in the greenhouse: all other stems' dry weight, number of panicle bearing tillers, first tiller grain number, individual grain weight, and all other panicles' grain number, as well as the following traits in the field: main stem leaf dry weight, main stem dry weight, all other leaves dry weight and total grain yield (Table 3.1).

Traits that did not record G x T significance but varied significantly between water-deficit stress and well-watered treatments (irrespective of genotypic differences) include the following: main stem leaf dry weight, main stem dry weight, and first tiller grain weight – in the greenhouse experiment and the individual grain weight in the field (Table 3.1). Likewise, traits that did not record a G x T significance but had significant genotypic differences (regardless of treatment variation) include main stem leaf dry weight, main stem dry weight, all other leaves dry weight, and first tiller grain weight in the greenhouse experiment and the individual grain weight in the field (Table 3.1). There was significant genotypic variation (irrespective of treatment differences) for all traits measured in both the greenhouse and the field experiments (Tables 3.1, 3.2).

Analysis of variance also showed that the genotype by watering treatment and its interactive effects on grain position on panicle (T x G x P) was significant ($P < 0.01$) for panicle positional grain weight for both the field and greenhouse experiments plus the panicle positional grain number in the field (Table 3.2). Panicle positional grain number in the greenhouse however, had significant ($P < 0.01$) G x P interactive effects. Grain position on panicle did not have any significant effect on the individual grain weight nor did it have any significant interactive effect with the watering treatments or genotypes in both greenhouse and field experiments (Table 3.2).

Regarding physiological traits, the genotype (G) by watering treatment (T) and its interactive effects with days (D) of imposing stress (T x G x D) was significant ($P < 0.001$) for only chlorophyll fluorescence in the field (Table 3.2). The T x G and T x D were significant for chlorophyll index and chlorophyll fluorescence in both greenhouse and field (Table 3.2).

In both the greenhouse and field experiments, SC1103 did not record any significant difference between the watering treatment for all growth and yield traits measured (Table 3.3, 3.4), while SC265 and SC35 recorded a similar response only with the greenhouse experiment.

3.4.1 Growth traits

The measured growth parameters include main stem height, main stem thickness, main stem leaf sheath dry weight, main stem leaves dry weight, main stem dry weight, all other leaves dry weight, and all other stems dry weight. Generally, all genotypes had reductions in the growth traits under water-deficit stress in both greenhouse and field conditions except for a few parameters that increased under water-deficit stress (Figure 3.3, 3.4) although these increments were mostly not significant (Table 3.3, 3.4). SC35 had the most growth with the main stem's thickness (Figure 3.3c) and leaf sheath dry weight in the greenhouse (Figure 3.3e); even its growth under water-deficit stress was significantly higher than the well-watered treatments of most genotypes for both these growth traits. Dry weight of all other stems in the greenhouse (which relates with number of tiller production) was zero for Macia (Figure 3.3g) due to its lack of tiller development.

Genotypes SC1103, SC1345, SC265, SC283, SC35, SC971, and Segalane did not record any significant difference between the well-watered and water-deficit treatments for growth traits in the greenhouse (Table 3.3). Ajabsido and RTx430 recorded significantly lower main stem height and thickness, respectively, in the greenhouse experiment while P898012 had a reduction in main stem leaf sheath dry weight (Table 3.3). However in the field, Segalane and Macia had the most significant reduction in main stem height and thickness, respectively. Particularly with SC35 in the field, the most significant reduction in the main stem leaf sheath dry weight corresponded with the most significant reduction in the main stem dry weight as well as main stem leaves dry weight

(Table 3.4). SC971 had the most significant reduction in shoot biomass (all other stems and leaves' dry weight) in the field (Table 3.4).

3.4.2 Yield traits

3.4.2.1 Panicle positional characteristics

Generally, for all genotypes, panicle positional grain weight (Figure 3.5) and panicle positional grain number (Figure 3.6) were both higher at the bottom and middle sections compared with the top in both experiments. Thus, fewer grain number and hence weight were recorded at the top section of the panicle. Notably however, the mean panicle positional grain number of SC35 was essentially same across all three positions compared with all the other genotypes in the greenhouse (Figure 3.6).

For both greenhouse and field experiments, Macia appeared mostly to have the highest grain weight and numbers in all divisional sections irrespective of the watering regime (Figure 3.5, 3.6). Nevertheless, Macia recorded the most significant reduction in the panicle bottom positional grain weight in the greenhouse (Table 3.3) as well as in all three positions of the panicle grain weight in the field (Table 3.4). For the panicle positional grain number in the field, SC971 had the most significant reductions at the top and mid sections while Macia recorded the highest reduction for the bottom section (Table 3.4). In the greenhouse, there was no significant difference between well-watered and water-deficit stress treatments for the top section of the panicle positional grain weight for all genotypes (Table 3.3).

3.4.2.1 Other yield aspects

The observed peak grain weight and numbers in majority of the divisional sections irrespective of the watering regime for Macia (Figs 3.5 and 3.6) culminated mostly to the highest grain number per main panicle (Figure 3.7a,b) and highest grain yield per main panicle (Figure 3.7c,d) under both greenhouse and field conditions. In the greenhouse, there were not many genotypes differing significantly between the well-watered and water-deficit treatments for grain number per main panicle compared with grain yield per main panicle (Figure 3.7a,c; Table 3.3). Grain yield of all other panicles of all genotypes in both greenhouse and fields were significantly reduced by water-deficit stress (Figure 3.7e,f). Similarly, harvest indices of all genotypes in both greenhouse and fields were generally reduced by water-deficit stress (Figure 3.7g,h) with significant reductions ranging from 0.27 to 0.62 in the greenhouse (Table 3.3) and 0.17 to 0.61 in the field (Table 3.4).

In the greenhouse, the number of panicle bearing tillers of SC1345, SC283, and SC971 were higher under water-deficit condition (Figure 3.8a) although only the increments of SC1345 and SC971 were significant (Table 3.3). Likewise, first tiller grain number of RTx430, SC1103, SC1345, and SC265 was more under water-deficit condition (Figure 3.8b) but none of these increments were significant; however, Ajabsido and SC971 decreased significantly under water-deficit stress (Table 3.4). All other panicles grain number (Figure 3.8c) and the individual grain weight (Figure 3.8d) of all genotypes in the greenhouse were reduced by water-deficit stress, with significant reductions ranging from 8.6 g to 18.1 g for the individual grain weight (Table 3.3).

In both greenhouse and field experiments, a strong and significantly positive correlation (greenhouse well-watered: $r = 0.76$, water deficit: $r = 0.85$; field well-watered: $r = 0.73$, water deficit: $r = 0.45$) was determined between grain yield per main panicle and main stem thickness

(Figure 3.9). However, a weak positive correlation (greenhouse well-watered: $r = 0.31$, water deficit: $r = 0.33$; field well-watered: $r = 0.19$, water deficit: $r = 0.34$) was obtained for grain yield per main panicle and main stem height (Figure 3.10). Within treatments, the correlations were stronger for water-deficit conditions than for well-watered conditions, except the relationship between grain yield per main panicle and main stem thickness in the field.

3.4.3 Comparison of greenhouse and field traits

Comparison of results of the greenhouse study to the field study using the Spearman's rank correlations indicated the following traits to significantly ($P < 0.05$) correlate ($r_s = 0.72$ to 0.96) for both watering regimes: main stem height, main stem leaves dry weight, main stem leaf sheath dry weight, and main stem dry weight (Table 3.5). This indicated that for these traits, the greenhouse rankings corresponded very closely with field rankings irrespective of the watering regime (Table 3.6). Thus, on average, Macia, P898012, and SC35 ranked as top performers for these growth traits while SC1103, SC1345, and SC283 were ranked amongst the least (Table 3.6). For the following traits, a significant correlation ($r_s = 0.65$ to 0.96) was detected for only the well-watered regime: chlorophyll fluorescence, individual grain weight, and the positional (top, mid, bottom) individual grain weight (Table 3.5). Under well-watered conditions, Ajabsido and P898012 thus ranked among the top for grain weight while SC1103 and SC971 ranked among the lowest (Table 3.6). There was no significant correlation ($r_s = 0.54$ and 0.58) for the main stem thickness in both watering regimes (Table 3.5) and for each corresponding genotype, the main stems in the greenhouse were thicker than those in the field (Figure 3.3c,d).

3.5 Discussion

A general reduction in growth related traits under water-deficit stress in both greenhouse and field conditions was recorded. Similarly, significant reductions in stem biomass (42 %), shoot dry weight (37 %), and leaf dry weight of sorghum plants due to water-deficit stress have been reported (Nxele et al., 2017, Perrier et al., 2017). The reduction in growth related traits observed in the current study may have been related to the conversion and mobilization of stem reserves into soluble sugars for the grain-filling process when photosynthesis decreases due to water-deficit stress (Barnabás et al., 2008; Prasad et al., 2008). The significantly lower chlorophyll index and chlorophyll fluorescence (maximum quantum yield of PSII – Fv/Fm) recorded for water-deficit stressed plants in this study attested to the reduced photosynthetic activity. Studies have shown that sugar concentration in the leaves, as well as nitrogen and sugar content in the stem, significantly increase during water-deficit stress (Dina and Klikoff, 1973). It has also been indicated that decrease in deposition rate of carbohydrates, proteins, and other nutrients could account for loss in stem weight (Gambin et al., 2008). It has been noted that water-deficit stress terminates dry matter accumulation before physiological maturity (Borrell et al., 2014).

Possible indicators of relative tolerance to water-deficit stress are differences in how genotypes produce and partition biomass (Munamava and Riddoch, 2001). While Devnarain et al. (2016) reported a strong positive correlation between sorghum height and grain yield, a positive but weak correlation between main stem height and grain yield (per main panicle) was observed for this study. Instead, a strong correlation was detected for grain yield per main panicle with main stem thickness, which points to the vitality of this trait in grain production, even under water-deficit stress. The implication is that increased biomass due to thicker stems contributes to greater yields. In this regard, the thick stem growth of Macia and especially SC35 could well contribute to towards

their tolerance to water-deficit stress. In a related finding, Borrell et al. (2014) identified a positive correlation between grain number and biomass accumulation.

Leaf senescence usually results during grain filling, because a significant amount of nitrogen needed by the grains is obtained by remobilized nitrogen from leaves (Prasad et al., 2008). Water-deficit stress causes an imbalance in the carbon and nitrogen ratio that results in leaf senescence of especially mature leaves, which tend to have higher C:N ratios (Chen et al., 2015). In effect, there is chlorophyll loss in relation to leaf senescence which culminates in potential yield loss through reduction in photosynthetic activity. More so, almost all the water extracted during the post-anthesis time contributes to grain growth (Vadez, 2014). Hence, any condition such as water-deficit stress that diminishes availability of assimilates during the grain-filling period indirectly reduces the final grain weight (Blum et al., 1997). Hence, stress exposure during grain filling would have resulted in the significant differences between the well-watered and water deficit treatments accounting for reduction in yields observed in this study. There was also a notable panicle infestation (most likely from sorghum midge) on P898012, RTx430, and SC35 (Figure 3.11), especially on the control plots in the field. This adversely affected yield components reported for the field study such that field grain and yield related results, in connection with these genotypes may not reflect their actual potential under control conditions.

Significant differences between the well-watered and water-deficit treatments for grain number per main panicle occurred as a result of seed-set ability and its development, depending on the timing of stress. Because the potential number of grains is determined during the early development stage i.e., before flowering, it is generally well-noted that water-deficit stress experienced before or at anthesis mainly affects the grain number while water-deficit stress that

occurs after anthesis affects grain weight (Prasad and Staggenborg, 2009; Maman et al., 2004). Thus, it can be reasoned that when fertilization process coincides with the onset of stress, anther dehiscence, pollen germination, pollen tube growth and pollen-stigma interactions are adversely affected. This will result in unsuccessful fertilization (Prasad et al., 2015), leading to partial seed-set and eventual reduction in grain numbers. However, considering that sorghum fertilization occurs within 6-12 hours after pollination (Stephens, 1934), and grain formation begins immediately after flowering (Ciampitti et al., 2015), it is very unlikely that unsuccessful fertilization can be the cause in this study where sufficient moisture was maintained at the onset of stress i.e. with 50 % flowering completed (Figure 3.2). Hence, the most likely aspect that would have been affected is the embryo abortion, where fertilized ovules are not able to grow due to insufficient assimilate supply resulting from the stress. According to Ciampitti (2013), sorghum seed number can still vary weeks after flowering. Genotypes that had no significant difference between watering treatments for grain numbers but recorded a significant variation in grain weight (as observed especially in the greenhouse –Table 3.3) indicates limited allocation of available assimilates to producing many small sized grains from water-deficit stressed plants.

With reference to the panicle positional grain weight and number, higher yields (weight and numbers) were generally recorded at the bottom and middle sections compared with the top for all genotypes in both experiments, regardless of watering treatments. Later phases of grain development (soft dough and hard dough) are reported to follow a basipetal pattern with spikelets in the top portion filling first. When the soft dough stage gets extended such that a grain obtains a larger volume to contain more assimilates, heavier grains tend to be produced (Roozeboom and Prasad, 2016) and studies have shown that longer duration ensures greater grain yield (Prasad et al., 2008). It can therefore be intuitively conclusive that the topmost section fills first so that the

mid and bottom section gets to experience longer grain-filling duration. However, the fact that the positional individual grain weight did not differ among the three sectional areas (Table 3.2) implies that the positional grain weight differences resulted from differences in the positional grain numbers other than grain filling dynamics. Differences in positional grain numbers (regardless of watering treatments) will then be due to panicle architecture, wherein a narrower panicle width at the top will hold less flowers, and hence fewer grains. Determinant of panicle architecture that is strongly associated with yield include rachis length, number of branches, panicle length and panicle width (Hmon et al., 2013; 2014). Consequently, genotypic variation in grain numbers and grain weight irrespective of stress are primarily attributed to differential panicle architecture of the genotypes (Table 2.1; Figure 3.12). The parental sorghum genotypes used for the study belongs to diverse botanical races (namely Guniea, Caudatum, Durra, and Kafir) that have distinctive panicle architecture. In addition, plant growth rate around the period of grain set differs among genotypes, and there are differences in grain setting efficiency as well (Gambin et al., 2008).

With respect to watering treatments affecting panicle positional yield, differences in positional grain number would account for differences in positional grain weight. This is due to positional individual grain weight which was not affected by the stress across the three sectional areas (T x P of Table 3.2). Differences in panicle positional grain numbers with regards to watering treatments may be connected with either embryonic abortion or panicle architecture.

In connection with embryonic abortion, insufficient assimilates resulting from the stress usually leads to embryonic abortion, as explained above. Furthermore, the fact that there were generally smaller differences between watering treatments for the positional grain number at the top section compared with the middle and bottom sectional areas (as seen especially on the field - Table 3.4)

appear to follow the ‘basipetal filling pattern’ which is also similar to the concept of superior and inferior spikelets as identified in rice (Hongthong et al., 2012; Mohapatra et al., 2011; Yang et al., 2006). Hypothetically, based on the response in rice, superior spikelet which would mostly be located at the top sectional area fills preeminently and thus have access to copious amount of assimilate supply as opposed to diminishing assimilate supply during the stress condition for the inferior spikelets when they start to fill. An investigation on rice where the grain filling of inferior spikelets improved by increased grain filling rate and grain weight when the superior spikelets were removed (during flowering) indicates a restriction of the latter on the former (You et al., 2016). An experiment on effect of high temperature on rice reports on the poor development of inferior spikelets as arising from conversion efficiency of sucrose into starch (Cao et al., 2016). On the premise of dominance by earlier-filling superior spikelets over late-filling inferior spikelet due to assimilate accessibility, the differences in positional grain number in relation to the stress can be understood ideally on a more embryonic abortion at the middle and bottom sectional areas than the top.

In connection with panicle architecture affecting the differences in panicle positional grain numbers as a result of the watering treatments: it may be that the growth of some determinants of panicle architecture were affected by inadequate assimilate during the stress period. While it may be possible for water-deficit stress to influence panicle positional grain number by modifying panicle morphological characteristics such as branch length, panicle width, and rachis length, it is not clear how these characteristics are exactly affected by the post-flowering water-deficit stress.

In the greenhouse, the number of panicle bearing tillers of SC1345, SC283, and SC971 were more under water-deficit condition. In addition, the first tiller grain numbers of RTx430, SC1103,

SC1345, and SC265 were more under water-deficit condition. Number of fertile tillers produced by a sorghum plant is influenced by environmental stress, such as heat and water-deficit stress (Roozeboom and Prasad, 2016). Under stress conditions, some plants tend to be dedicated to seed production and thus accelerate maturity (Munamava and Riddoch, 2001) so that early grain formation is completed before the onset of severe stress (Barnabás et al., 2008). Differences in all-other-stem and its related parameters both in the greenhouse and on the field will be in relation to tiller emergence frequency, tiller fertility frequency, and on grain numbers per tiller; (Roozeboom and Prasad, 2016).

There were more significant Spearman's rank correlations for growth traits than yield traits in comparing results of the greenhouse study to the field study. This must have been influenced by changes in grain output for some genotypes in the field due to panicle infestation (most likely from sorghum midge) as reported above. Overall, Spearman's rank correlations indicated the ability to select for water-deficit tolerance traits in the greenhouse that would have partial representative rankings on the field. The rank correlations also demonstrated the usefulness of utilizing greenhouse evaluations in screening large numbers of genotypes (i.e. under optimal conditions) for further assessments on the field as well as the possibility for assessing performance of genotypes under water-deficit conditions.

3.6 Conclusion

A general reduction in growth and yield traits under water-deficit stress in both greenhouse and field conditions was recorded due to the conversion and mobilization of the stem reserves into soluble sugars in response to a declining photosynthesis. Differences in grain numbers and grain weight regardless of watering treatments was due to variation in the panicle architecture, other

than grain filling dynamics, where a narrower panicle width at the top resulted in fewer grains. This led to differences in positional grain numbers and positional grain weight regardless of the watering treatments. Embryonic abortion due to insufficient assimilate supply appeared more likely to account for the significant differences in grain number observed between well-watered and water-deficit stress. With regards to watering treatments affecting panicle positional yield, absence of uniformity in grain filling of spikelets at different positions on the panicle led to differences in panicle positional yield. Since positional individual grain weight was not affected by the stress across the three sectional areas, differences in panicle positional grain number accounted for differences in panicle positional grain weight. A genotype is considered “drought tolerant” when it has the ability to yield significantly better than another genotype during severe water-deficit stress (Hasan et al., 2017). In consideration with measured growth and yield traits of high yielding genotypes in both experiments, and with particular respect to individual grain weight, total grain yield, and harvest index, yield performance of SC35 under water-deficit stress outperformed other genotypes altogether.

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Table 3.1: Probability values of effects of water deficit stress (T), genotype (G) and T x G interaction on agronomic and physiological traits for greenhouse and field studies.

Traits	Greenhouse			Field						
	T	G	G x T	Treatment (mean \pm standard error*)		T	G	G x T	Treatment (mean \pm standard error *)	
				Well-watered	Water deficit				Well-watered	Water deficit
Main stem height (cm)	0.2912	<.0001	0.0152	110.03 \pm 2.00	107.38 \pm 2.00	<.0001	<.0001	0.0012	112.66 \pm 1.54	99.64 \pm 1.54
Main stem thickness (mm)	<.0001	<.0001	0.0054	17.33 \pm 0.32	15.35 \pm 0.32	<.0001	<.0001	<.0001	10.13 \pm 0.15	8.24 \pm 0.15
Main stem leaves dry weight (g)	0.0003	<.0001	0.26	24.09 \pm 0.96	18.79 \pm 0.96	<.0001	<.0001	<.0001	23.79 \pm 0.64	15.53 \pm 0.64
Main stem leaf sheath dry weight (g)	<.0001	<.0001	0.025	16.49 \pm 0.68	12.18 \pm 0.68	<.0001	<.0001	<.0001	15.31 \pm 0.35	11.44 \pm 0.35
Main stem dry weight (g)	<.0001	<.0001	0.091	38.58 \pm 1.80	27.60 \pm 1.80	<.0001	<.0001	<.0001	45.99 \pm 1.06	26.77 \pm 1.06
All other leaves dry weight (g)	0.17	<.0001	0.38	38.95 \pm 2.84	33.36 \pm 2.84	0.0134	<.0001	0.0314	515.18 \pm 6.80	488.82 \pm 6.80
All other stem dry weight (g)	0.014	<.0001	0.041	87.84 \pm 8.93	58.37 \pm 8.93	<.0001	<.0001	<.0001	1074.26 \pm 11.72	895.13 \pm 11.72
Panicle bearing tillers	0.2891	<.0001	0.0003	4.91 \pm 0.36	4.49 \pm 0.36	-	-	-	-	-
First tiller grain number	0.056	0.0032	0.0314	715.33 \pm 118.29	420.55 \pm 118.29	-	-	-	-	-
First tiller grain weight (g)	0.0096	0.0023	0.0619	20.35 \pm 3.28	8.78 \pm 3.28	-	-	-	-	-
Grain number per main panicle	0.0038	<.0001	<.0001	1676.01 \pm 68.71	1449.92 \pm 67.47	<.0001	<.0001	<.0001	1903.88 \pm 52.74	797.20 \pm 52.74
Grain yield per main panicle	<.0001	<.0001	<.0001	44.87 \pm 2.13	27.75 \pm 2.09	<.0001	<.0001	<.0001	49.59 \pm 1.22	22.76 \pm 1.22
Individual grain weight (mg)	<.0001	<.0001	0.0148	26.41 \pm 0.9	18.7 \pm 0.88	0.0137	<.0001	0.5785	26.71 \pm 0.63	28.93 \pm 0.63
All other panicles' grain number	<.0001	<.0001	<.0001	1756.55 \pm 188.07	597.57 \pm 199.96	-	-	-	-	-
All other panicles' grain yield (g)	<.0001	<.0001	<.0001	38.20 \pm 3.97	11.42 \pm 4.22	<.0001	<.0001	<.0001	711.21 \pm 20.62	338.01 \pm 20.62
Harvest index	<.0001	<.0001	0.0007	0.5 \pm 0.03	0.35 \pm 0.03	<.0001	<.0001	<.0001	0.48 \pm 0.01	0.27 \pm 0.01
Total grain yield (kg/ha)	-	-	-	-	-	<.0001	<.0001	<.0001	4737.4 \pm 108.3	2234.7 \pm 108.3

Table 3.2: Probability values of effects of water deficit stress (T), genotype (G), grain position on panicle (P), and days of imposing stress (D) and the respective T/G x P/D interactive effects on agronomic and physiological traits for greenhouse and field studies.

Traits	Greenhouse							Treatment (mean \pm standard error *)	
	T	G	T x G	P	T x P	G x P	T x G x P	Well-watered	Water deficit
Panicle positional grain weight (g)	<.0001	<.0001	<.0001	<.0001	0.0079	<.0001	0.0018	14.84 \pm 0.43	9.39 \pm 0.43
Panicle positional grain number	0.0019	<.0001	<.0001	<.0001	0.3615	<.0001	0.062	558.56 \pm 21.02	485.67 \pm 20.93
Positional individual grain weight (mg)	<.0001	<.0001	<.0001	0.5003	0.5371	0.8181	1	26.19 \pm 0.45	18.76 \pm 0.45
Field									
Panicle positional grain weight (g)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	16.58 \pm 0.33	7.63 \pm 0.33
Panicle positional grain number	<.0001	<.0001	<.0001	<.0001	<.0001	0.0014	0.0006	642.66 \pm 15.02	266.23 \pm 15.02
Positional individual grain weight (mg)	0.0004	<.0001	0.0535	0.1697	0.9187	0.7000	0.4288	26.62 \pm 0.37	28.49 \pm 0.37
Greenhouse									
	T	G	T x G	D	T x D	D x G	T x G x D	Well-watered	Water deficit
Chlorophyll index (SPAD units)	<.0001	<.0001	<.0001	<.0001	<.0001	0.5149	0.8819	59.7 \pm 0.6	50.2 \pm 0.6
Chlorophyll fluorescence (Fv/Fm)	<.0001	<.0001	<.0001	<.0001	<.0001	0.1654	0.1978	0.77 \pm 0.01	0.67 \pm 0.01
Field									
Chlorophyll index (dualex units)	0.019	<.0001	0.0085	<.0001	0.0435	0.175	0.2324	52.9 \pm 0.61	51.09 \pm 0.46
Chlorophyll fluorescence (Fv/Fm)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.76 \pm 0.001	0.74 \pm 0.003

Table 3.3: Mean differences between water deficit and well-watered treatments for traits with significant T x G interactive effects – **Greenhouse experiment.** Significance of the means differences were carried out using Tukey-Kramer test in SAS ($P < 0.05$).

Genotypes	Ajabsido	Macia	P898012	RTx430	SC1103	SC1345	SC265	SC283	SC35	SC971	Segaolane
Traits											
Main stem height (cm)	-37.5	4.8	-10.0	3.2	-0.2	-10.7	0.0	5.3	8.7	8.5	-1.3
Main stem thickness (mm)	-3.4	-3.1	-3.6	-7.0	-0.7	-0.1	0.5	-2.2	-1.4	-0.5	-0.4
Main stem leaf sheath dry weight (g)	-1.7	-10.2	-12.5	-9.5	-0.7	-0.8	0.1	-1.9	-4.8	-3.6	-1.8
All other stems' dry weight (g)	-131.7	0.0	-144.7	-3.3	-25.0	-8.9	-3.0	12.9	-30.5	18.0	-7.9
Panicle bearing tillers	-6.0	0.0	-2.3	-1.0	-0.3	4.0	-0.7	2.0	-1.7	3.0	-1.7
First tiller grain number	-1786.3	0.0	-203.0	283.3	201.0	354.3	59.3	-146.0	-175.0	-1740.7	-89.7
Grain number per main panicle	-55.7	-287.3	0.0	116.3	-155.7	167.5	-263.0	-440.7	244.3	-1669.0	-27.5
Grain yield per main panicle	-18.7	-47.7	-13.9	-34.4	-3.1	-0.8	-2.9	-19.5	2.9	-38.4	-5.8
All other panicles' grain yield (g)	-61.6	0.0	-58.9	-17.3	-11.2	-33.4	-0.1	-9.4	-4.9	-101.8	-2.9
All other panicles' grain number	-2279.5	0.0	-1609.3	-554.0	-705.8	-880.3	-10.0	-349.7	-313.7	-5864.7	-223.0
Harvest index	-0.27	-0.18	0.09	-0.31	-0.06	-0.21	-0.03	-0.22	0.06	-0.62	0.02
Individual grain weight (mg)	-16.1	-12.0	-8.6	-18.1	-1.4	-7.0	-1.3	-7.7	-2.7	-5.8	-3.4
Panicle positional grain weight											
Top	-3.6	-4.1	-3.2	-5.1	-0.1	-0.7	-2.0	-4.7	0.0	-8.9	-1.6
Middle	-8.0	-9.6	-5.8	-12.8	-1.9	0.6	-0.7	-8.0	-1.4	-12.5	-3.5
Bottom	-7.0	-34.0	-4.9	-16.6	-1.1	-0.7	-0.3	-6.8	4.3	-17.2	-0.7

*Statistically significant differences are in bold.

Table 3.4: Mean difference between water deficit and well-watered treatments for traits with significant T x G interactive effects –

Field experiment. Significance of the means difference was carried out using Tukey-Kramer test in SAS ($P < 0.05$).

Traits	Genotypes	Ajabsido	Macia	P898012	RTx430	SC1103	SC1345	SC283	SC35	SC971	Segaolane
Main stem height (cm)		-1.5	-13.8	-21.7	-9.8	0.8	-2.6	-30.0	-22.3	1.2	-31.1
Main stem thickness (mm)		-2.4	-4.6	-1.2	0.4	-0.6	-2.0	-1.7	-2.0	-2.9	-1.9
Main stem leaf sheath dry weight (g)		0.7	-5.4	0.2	-3.8	0.2	-4.3	-4.7	-13.5	-3.6	-4.4
Main stem leaves dry weight (g)		-7.7	-14.4	1.5	-5.9	0.2	-10.0	-6.1	-16.0	-13.6	-11.0
Main stem dry weight (g)		-26.2	-37.2	-12.3	-8.9	-2.1	-10.1	-10.5	-43.7	-17.6	-23.7
All other leaves dry weight (g)		-5.5	-62.7	-1.9	31.6	0.7	-36.6	-24.7	-102.3	-104.3	42.0
All other stem dry weight (g)		-471.9	-299.5	-192.4	-61.6	6.2	-14.5	-118.1	-122.1	-506.7	-10.9
Grain number per main panicle		-710.9	-2958.4	-343.5	-619.9	-54.3	-1380.1	-269.5	-363.0	-2956.6	-1410.6
Grain yield per main panicle		-30.8	-75.1	-13.4	-18.8	-0.1	-35.8	-6.2	-10.0	-46.9	-31.1
All other panicles' grain yield (g)		-496.4	-761.9	26.0	-283.6	-15.5	-552.3	-434.8	-119.2	-678.3	-416.1
Harvest index		-0.17	-0.36	0.00	-0.19	-0.01	-0.61	-0.28	0.06	-0.32	-0.29
Total grain yield (kg/ha)		-3226.7	-5533.8	-144.8	-1868.5	-83.3	-3621.5	-2394.1	-829.5	-4509.7	-2816.0
Panicle positional grain weight											
Top		-1.0	-12.9	-3.6	-0.9	-0.3	-8.4	-0.7	-7.9	-12.6	-5.6
Middle		-9.1	-26.0	-4.9	-6.3	0.6	-14.3	-1.7	-2.8	-19.5	-13.9
Bottom		-20.7	-36.3	-4.9	-10.2	-0.7	-13.0	-3.8	-0.6	-14.8	-11.7
Panicle positional grain number											
Top		-74.4	-492.5	-71.9	-64.4	-40.4	-311.1	-19.4	-248.7	-856.0	-297.5
Middle		-251.4	-995.6	-142.4	-214.8	15.6	-559.8	-28.0	-85.6	-1180.3	-598.3
Bottom		-536.5	-1470.3	-129.3	-340.8	-51.0	-500.2	-222.1	-55.8	-879.8	-514.9

*Statistically significant differences are in bold.

Table 3.5: Spearman rank correlation coefficients (r_s) for agronomic and physiological traits of ten sorghum parental genotypes subjected to water deficit stress at the greenhouse and on field.

Traits	Well-watered	Water deficit
Main stem height (cm)	0.94***	0.77**
Main stem thickness (mm)	0.54 ^{ns}	0.58 ^{ns}
Main stem leaves dry weight (g)	0.96***	0.88***
Main stem leaf sheath dry weight (g)	0.9***	0.77**
Main stem dry weight (g)	0.92***	0.72*
Chlorophyll index	0.56 ^{ns}	0.44 ^{ns}
Chlorophyll fluorescence	0.65*	0.6 ^{ns}
Grain number per main panicle	0.30 ^{ns}	0.31 ^{ns}
Grain yield per main panicle	0.31 ^{ns}	0.09 ^{ns}
Individual grain weight (mg)	0.94***	0.61 ^{ns}
Harvest index	0.41 ^{ns}	0.22 ^{ns}
Panicle positional grain number		
Top	0.42 ^{ns}	0.16 ^{ns}
Mid	0.53 ^{ns}	0.58 ^{ns}
Bottom	0.43 ^{ns}	0.44 ^{ns}
Panicle positional grain weight (g)		
Top	0.38 ^{ns}	0.006 ^{ns}
Mid	0.44 ^{ns}	0.21 ^{ns}
Bottom	0.37 ^{ns}	0.2 ^{ns}
Positional individual grain weight (mg)		
Top	0.96***	0.54 ^{ns}
Mid	0.89***	0.53 ^{ns}
Bottom	0.82**	0.49 ^{ns}

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3.6: Spearman rankings of traits measured under well-watered and water-deficit condition at the greenhouse and field.

	MS height					MS leaves dry wt					MS dry wt					MS leaf sheath dry wt					Fluorescence			
	WW		WD			WW		WD			WW		WD			WW		WD			WW		WD	
	G	F	G	F		G	F	G	F		G	F	G	F		G	F	G	F		G	F		
Ajabsido	1	2	4	1		6	7	6	5		4	2	6	2		9	9	9	6		9	10	9	5
Macia	5	6	3	6		2	1	2	2		2	3	2	4		2	2	2	3		6	5	3	4
P898012	3	3	5	5		3	3	3	1		3	4	3	1		3	3	3	1		4	8	5	9
RTx430	9	9	9	8		4	4	5	4		6	7	7	8		4	4	4	4		5	7	7	3
SC1103	6	4	6	3		10	10	10	10		8	9	8	7		10	10	10	10		10	9	4	8
SC1345	10	10	10	10		9	8	8	8		9	8	10	9		8	6	8	7		3	4	6	6
SC283	8	7	8	7		8	9	9	9		10	10	9	10		6	8	6	9		7	2	8	7
SC35	7	8	7	9		1	2	1	3		1	1	1	5		1	1	1	2		1	3	2	2
SC971	4	5	2	4		5	5	4	7		5	6	5	6		5	7	5	8		2	1	10	10
Segaolane	2	1	1	2		7	6	7	6		7	5	4	3		7	5	7	5		8	6	1	1

† Ranks are in descending order with 10 as the least value. MS: Main stem Wt: Weight WW: Well-watered WD: Water deficit

G: Greenhouse F: Field

	Positional individual grain wt																		
	Individual grain wt					Top			Mid					Bottom					
	WW		WD			WW			WD		WW			WD		WW		WD	
	G	F				G	F				G	F				G	F		G
Ajabsido	1	1	3	1		1	1	4	1		1	1	3	1		1	1	4	1
Macia	6	5	6	6		6	5	6	5		6	5	6	6		3	6	6	6
P898012	2	3	2	5		2	2	2	6		2	3	2	3		2	2	1	3
RTx430	4	2	8	2		4	3	5	2		5	2	8	2		5	3	9	2
SC1103	10	10	10	10		10	10	10	10		10	10	10	10		10	10	10	10
SC1345	5	6	4	7		5	6	3	7		4	6	4	4		6	4	3	7
SC283	7	8	7	8		7	8	8	8		7	8	7	8		8	8	7	8
SC35	3	4	1	3		3	4	1	4		3	4	1	7		4	7	2	4
SC971	9	9	9	9		9	9	9	9		9	9	9	9		9	9	8	9
Segaolane	8	7	5	4		8	7	7	3		8	7	5	5		7	5	5	5

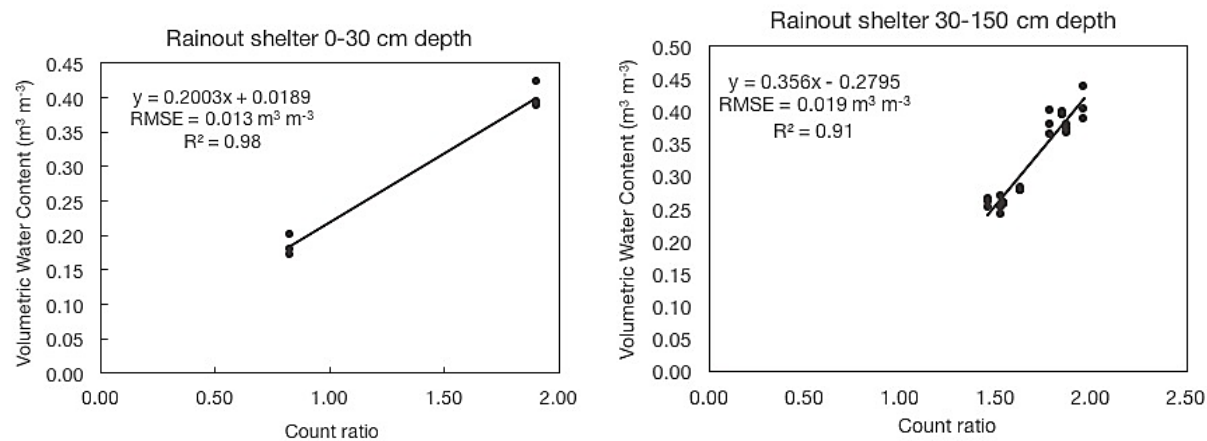


Figure 3.1: Linear regression derived for calibrating neutron probe count readings

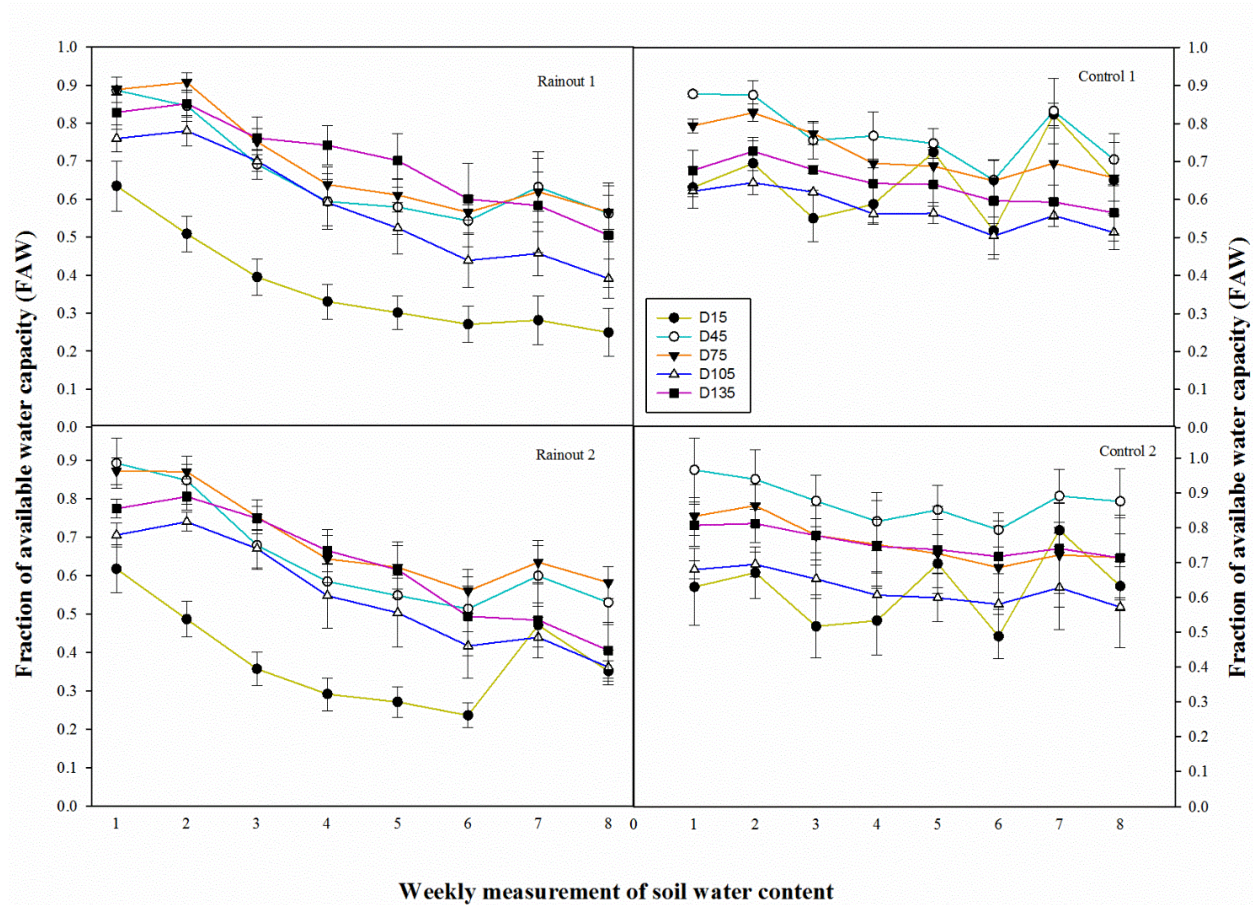


Figure 3.2. Fraction of available water capacity (FAW) derived to describe soil water content during the experimental period in the rainout shelters and control plots. D in legend refers to depths in cm.

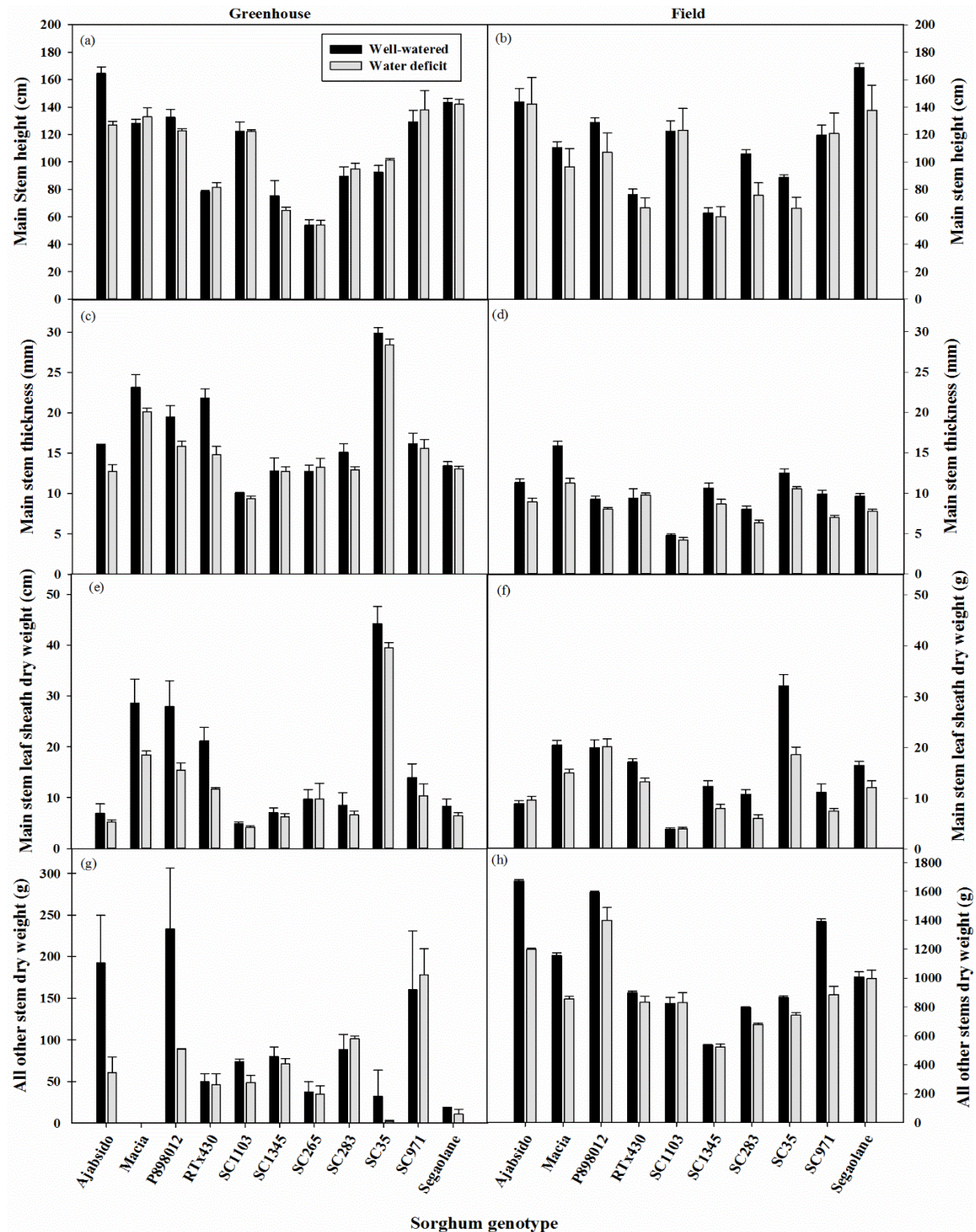


Figure 3.3: Effects of water-deficit stress on main stem height (a,b) main stem thickness (c,d) main stem leaf sheath dry weight (e,f) and all other stems' dry weight (g,h) in the greenhouse (left) and field (right). Each data point indicates mean value with \pm standard errors of means.

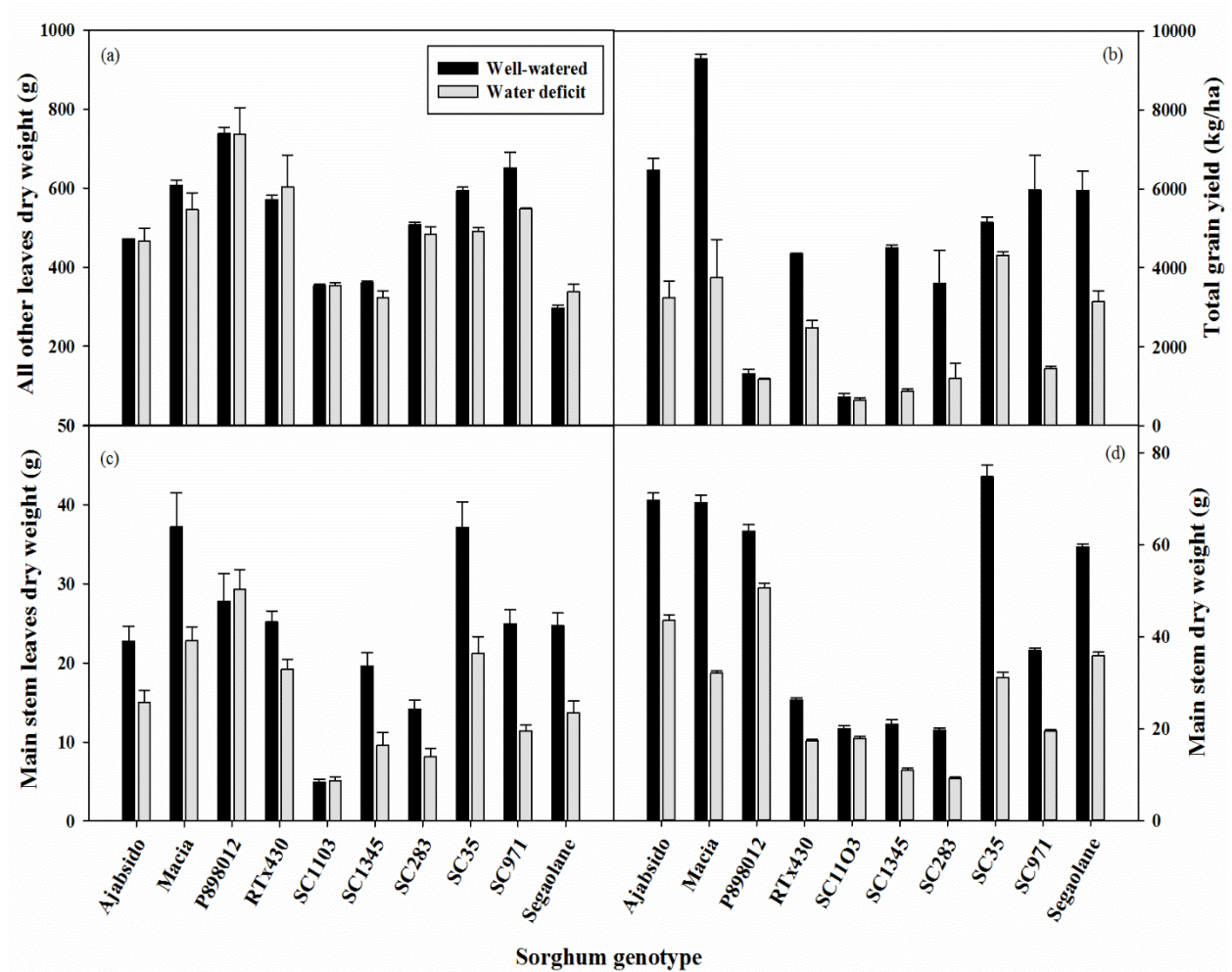


Figure 3.4: Effects of water-deficit stress on (a) all other leaves dry weight (b) total grain yield (c) main stem leaves dry weight and (d) main stem dry weight in the field. Each data point indicates mean value with \pm standard errors of means.

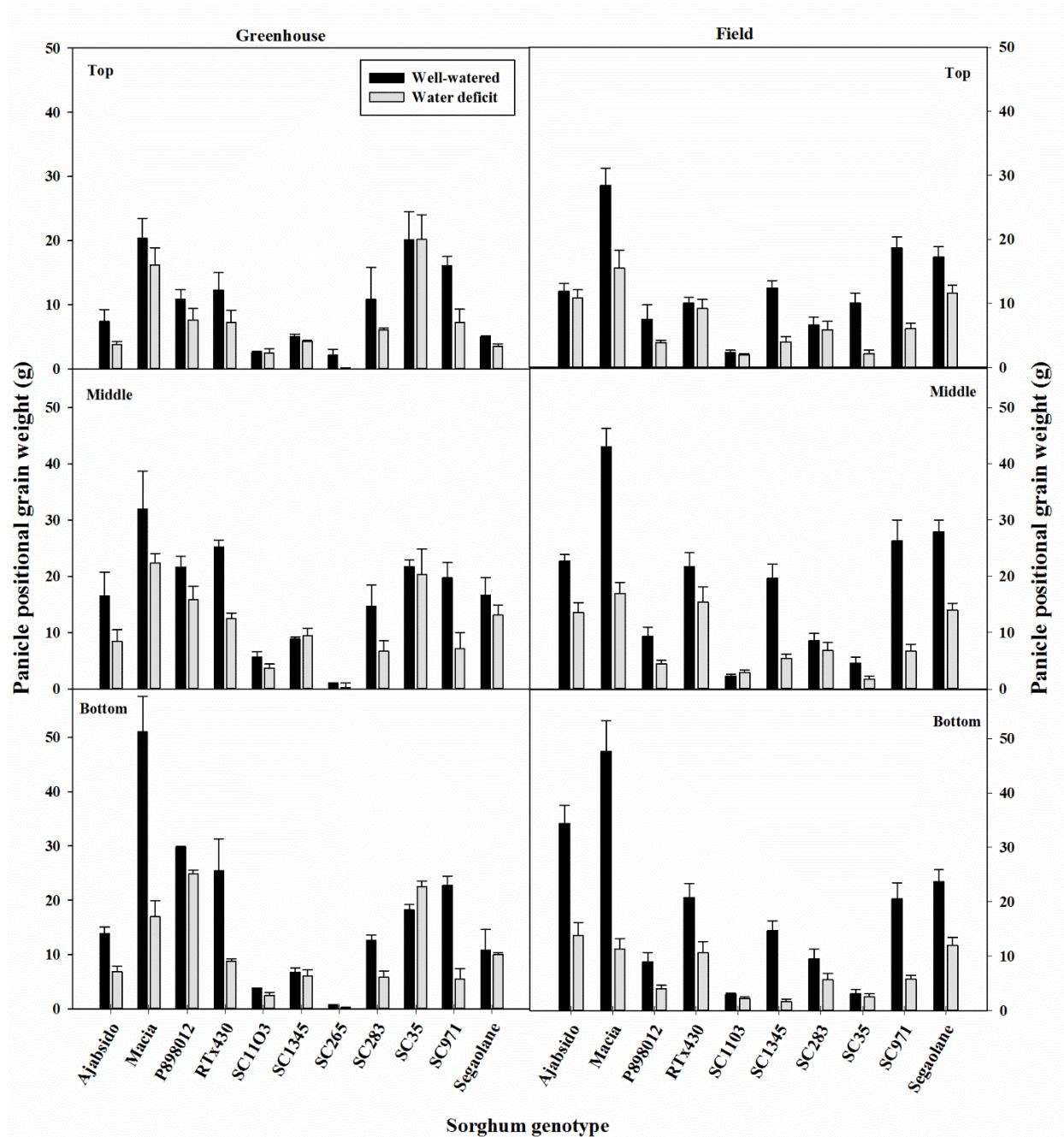


Figure 3.5: Effects of water-deficit stress on panicle positional (top, middle, bottom) grain yield in the greenhouse (left) and field (right). Each data point indicates mean value with \pm standard errors of means.

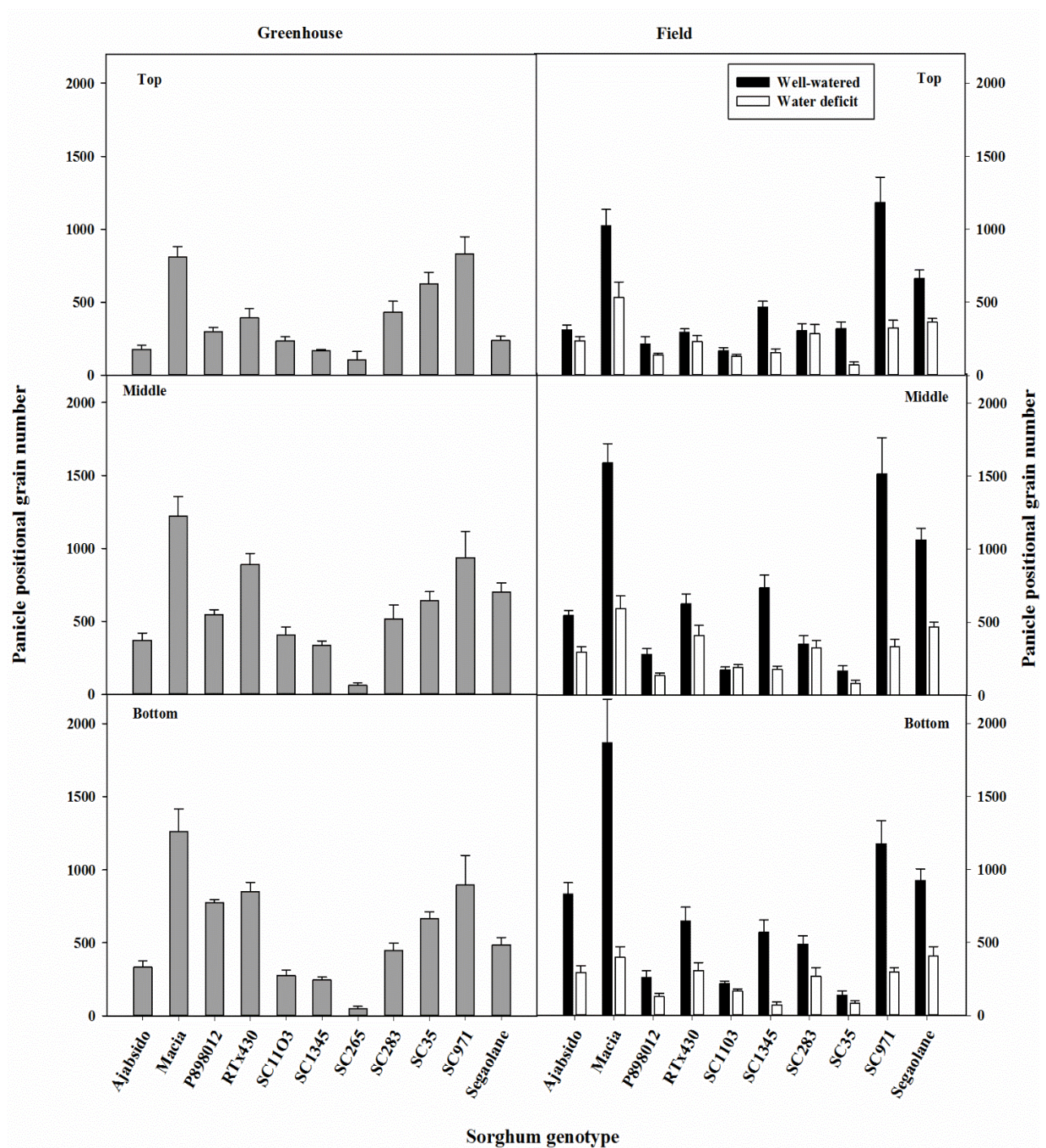


Figure 3.6: Effects of water-deficit stress on panicle positional (top, middle, bottom) grain number in the greenhouse (left) and field (right). Each data point indicates mean value with \pm standard errors of means.

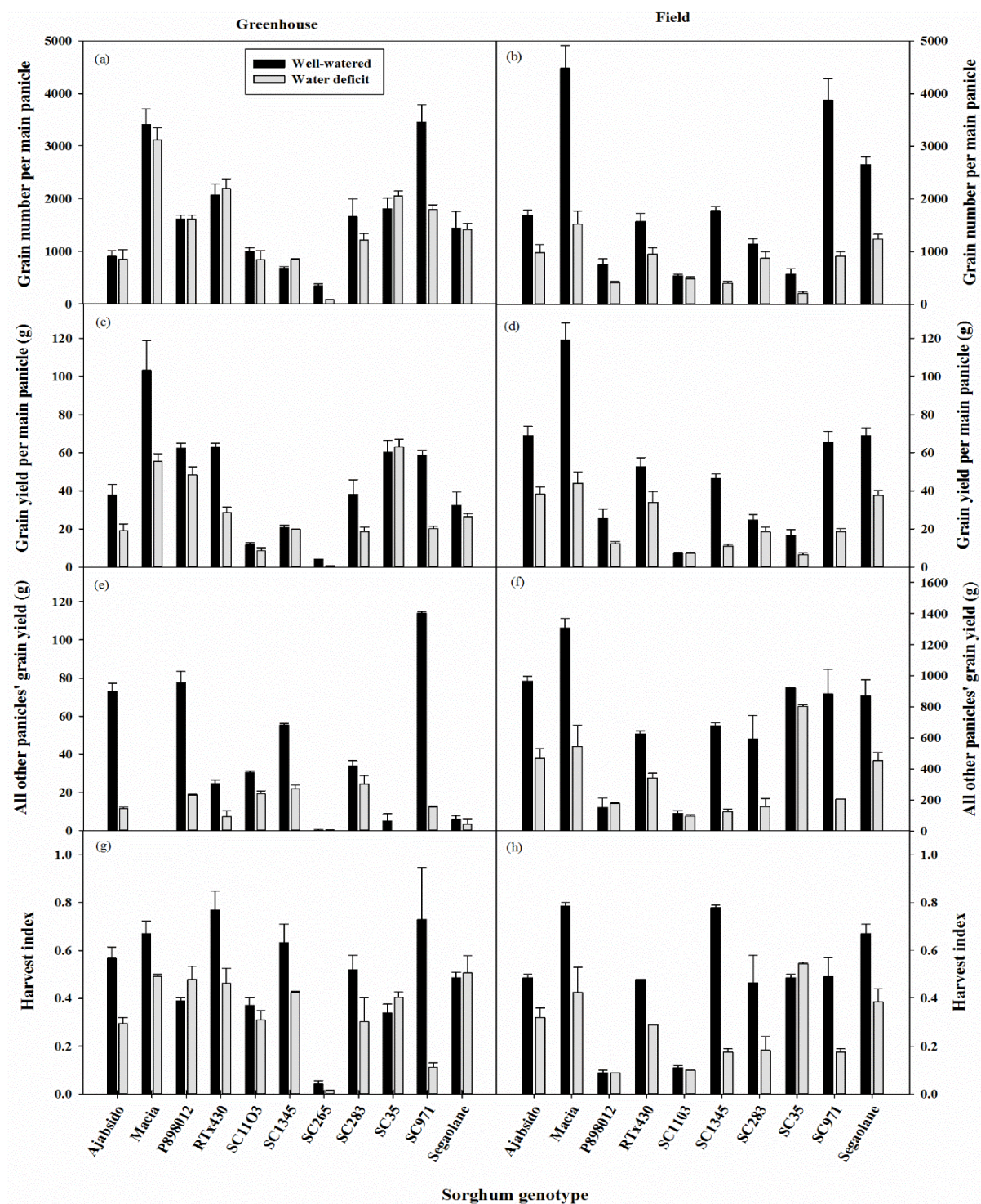


Figure 3.7: Effects of water-deficit stress on grain number per main panicle (a,b) grain yield per main panicle (c,d) all other panicles' grain yield (e,f) and harvest index (g,h) in the greenhouse (left) and field (right). Each data point indicates mean value with \pm standard errors of means.

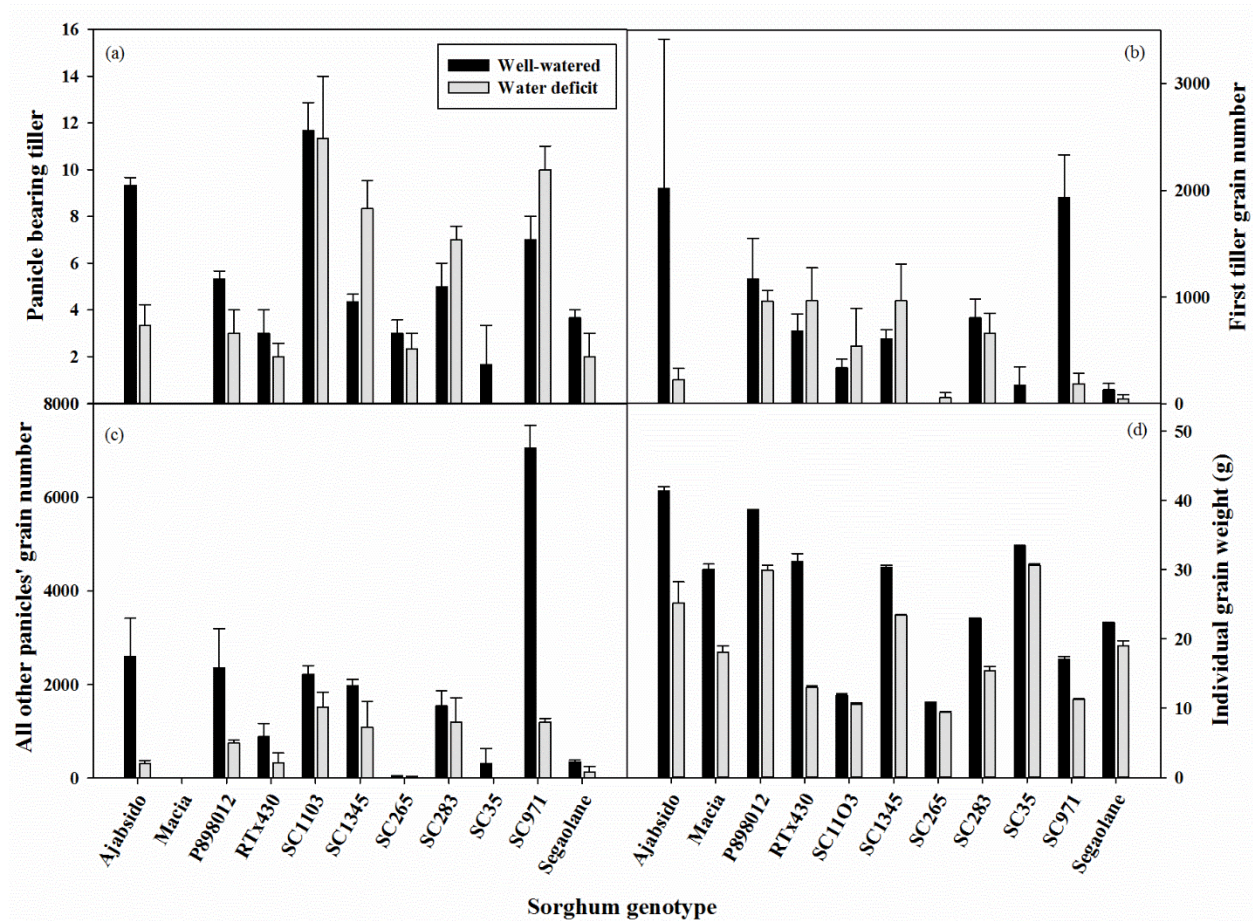


Figure 3.8: Effects of water-deficit stress on (a) panicle bearing tillers (b) first tiller grain number (c) all other panicles' grain number and (d) individual grain weight in the greenhouse. Each data point indicates mean value with \pm standard errors of means.

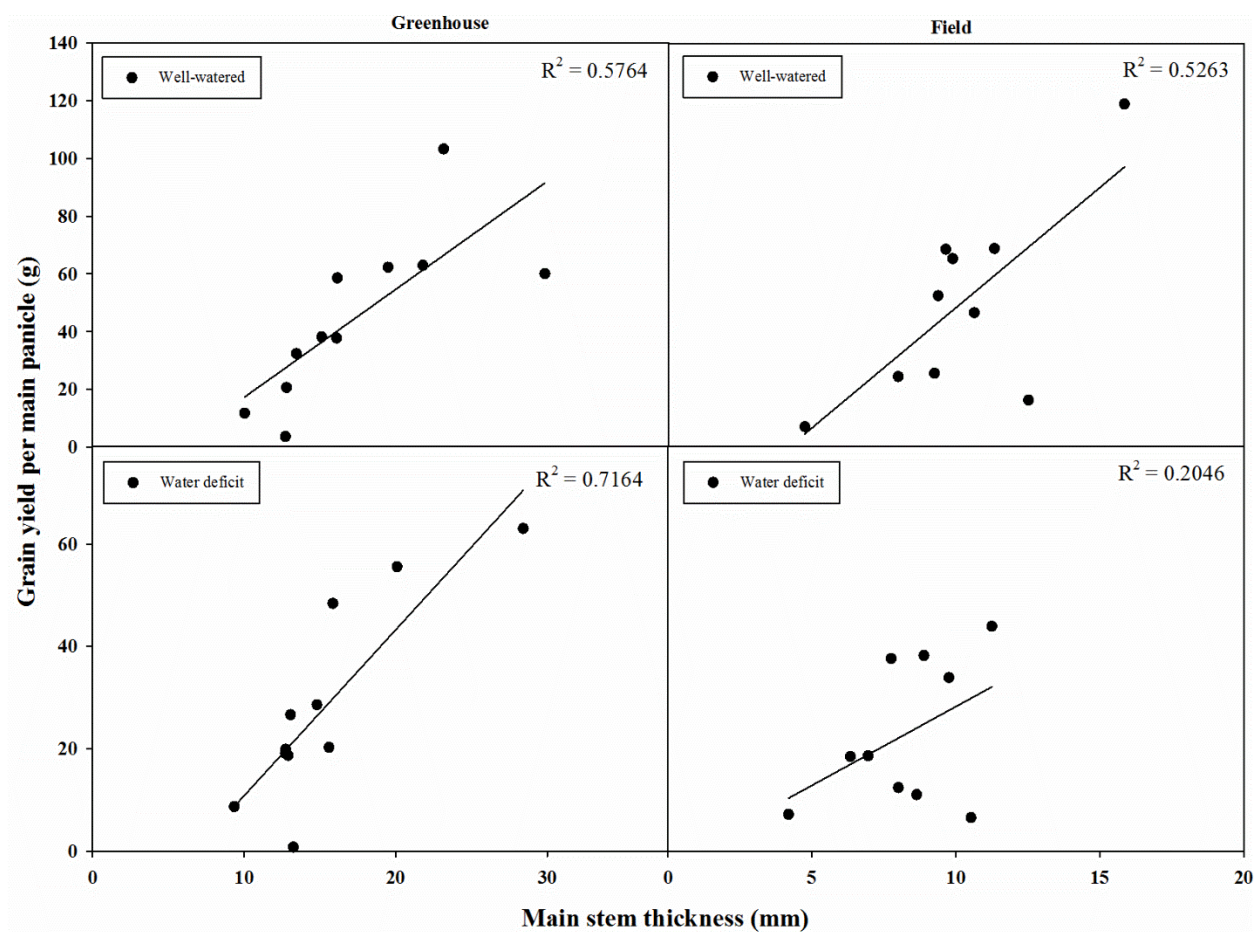


Figure 3.9: Pearson correlation (r) between grain yield per main panicle and main stem thickness of sorghum parental genotypes under well-watered and water-deficit stress conditions in the greenhouse and field. Test for significance was considered at the $P < 0.05$ level.

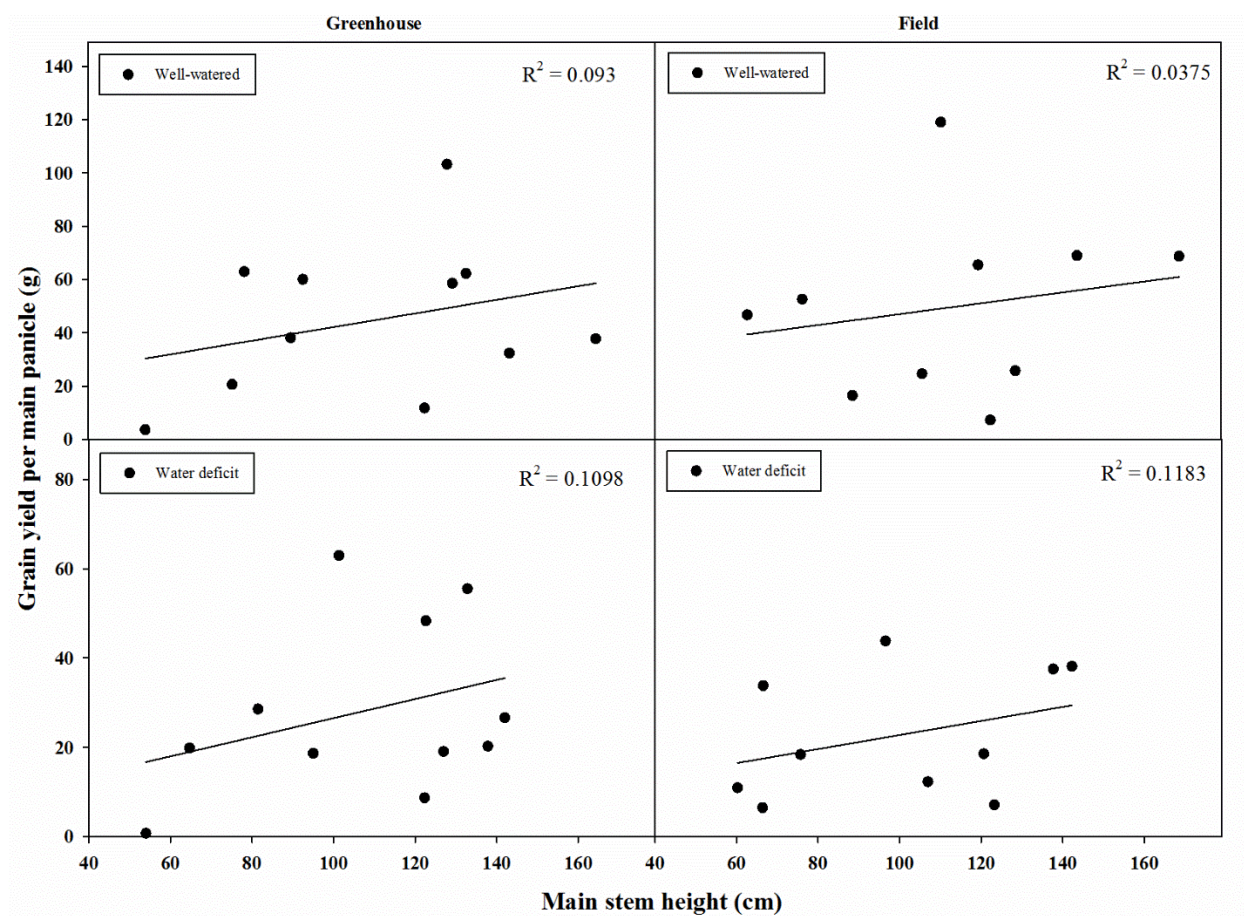


Figure 3.10: Pearson correlation (r) between grain yield per main panicle and main stem height of sorghum parental genotypes under well-watered and water-deficit stress conditions in the greenhouse and field. Test for significance was considered at the $P < 0.05$ level.



Figure 3.11: Sorghum panicles of (A) P898012 (B) SC35 and (C) RTx430 with unfilled and damaged seed capsules which is likely due to sorghum midge infestations especially on control plots (well-watered) on the field.



Figure 3.12: The parental sorghum genotypes from diverse families with distinctive panicle architecture. Panicles with blue tags are from well-watered plants and those with red tags are from water-deficit stressed plants in the greenhouse.

Chapter 4 – Physiological Characterization of Sorghum Genotypes Exposed to Moderate and Severe Water-Deficit Stress during the Vegetative stage

4.1 Abstract

The demand for food is increasing in relation with increasing global population. Though food insecurity is present in every society, it will be more prevalent and challenging in Africa where more than half of the projected increase in global population is expected. Agriculture in developing countries is dependent mainly on the climate and therefore, the threat to food security is exacerbated by the anthropogenic-accelerated changing climate. Nevertheless, as the climate gets drier and harsher making it difficult for some crops to grow, crops such as sorghum have acclimatized in being productive under these dry conditions. Yet, this achievement comes with a yield penalty. Impact of water-deficit stress on the yield of sorghum is dependent on sorghum's sensitivity to the intensity and duration of the stress in relation to a particular growth stage. Using Ghanaian soil as a growth medium, the research objective was to evaluate physiological characteristics of four sorghum genotypes exposed to water-deficit stress during the vegetative stage. Using a standardized gravimetric approach of pot weighing, water-deficit pots were progressively exposed to two different levels of water-deficit stress, before which all the pots were maintained at 100 % field capacity (FC). Severe water-deficit stressed pots were exposed to 50 % to 55 % FC, starting at 28 days after emergence and lasting for 21 days while moderate water-deficit stressed pots were exposed to 60 % to 65 % FC, starting at 35 days after emergence, and lasting for 15 days. Analysis of variance showed that the genotype by watering treatment interaction was significant for the following traits: stem height, stem thickness, number of leaves,

cumulative water transpired, stem dry weight, leaf dry weight, shoot biomass, total biomass, and stem weight ratio. Expectedly, there was a general greater decrease for shoot and root traits under the severe water-deficit stress than the moderate water-deficit conditions. Root dry weight for instance, decreased significantly by 38 % for moderate water-deficit stressed plants and by 52 % for severe water-deficit stressed plants. The study demonstrated that varying intensities of water-deficit stress during the vegetative stage induced different physiological and agronomic responses which accounts for genotypic variations relating to water-deficit stress tolerance in sorghum.

4.2 Introduction

Agriculture is a key sector in many developing countries, serving as a major source of livelihood. In Ghana, agriculture (which includes fisheries and forestry) generates the most employment (44.7 %) when compared with the services (40.9 %) and industrial (14.4 %) sectors of the economy, with agriculture contributing 18.9 % to the Ghanaian GDP (SRID-MOFA, 2017). However, the demand for food is increasing in relation with increasing global population and Ghana is experiencing a similar increase. In cognizance of the current world's population which is ~7.6 billion, the United Nations has predicted a world's population of 9.8 billion by 2050 with more than half of this projected increase expected to occur in Africa (UN, 2017). Although food insecurity is present in every society, the above change will make it more prevalent and challenging in developing countries.

Global food production has been increasing over the last several decades yet, there are limits to elements that contribute to sustainable productivity such as water resources, which are key to the progress achieved. Environmental factors are shifting, driven mainly by effects of anthropogenic-accelerated climate change. In the year 2011 and 2012 for instance, heat and drought affected 63

% of agricultural lands in some areas of the United States, with an estimated loss of more than \$ 8 billion (Kadam et al., 2014). Agriculture in developing countries is dependent mainly on the climate and therefore, the threat to food security is exacerbated by the changing climate. Simulations generated through modelling approaches report possible climatic scenarios in West Africa resulting in crop yield loss of about 41 % for a temperature rise of 6 °C and 20 % reduction in rainfall (IPCC, 2014; Sylla et al., 2016). Small-holder farmers in West Africa entirely depend on rainfall and any shift in timing of onset, distribution, duration, and amount of rainfall received in a season has significant impact and critically disrupts productivity (Callo-Concha et al., 2013).

Water is an indispensable component of life and its scarcity, in the form of water-deficit stress has detrimental consequences. Water is an essential constituent of plant cells, critical for cell division, cell enlargement, and cell differentiation which are fundamental to plant growth (Taiz et al., 2015). Water-deficit stress reduces the rate of cell expansion and it interrupts the flow of water from xylem vessels to the surrounding elongating cells such that cell elongation is hindered (Farooq et al., 2009). This ultimately reduces stem height, decreases the rate of leaf appearance, as well as the overall leaf area (Blum and Arkin, 1984). Water-deficit stress induces nutrient deficiency by decreasing rate of nutrient uptake by roots from the soil (Alam, 1999). Water-deficit stress has been closely linked with plant's diseases and pests due to the weakening defense system caused by the stress (Assefa et al., 2010).

Nevertheless, as the climate gets drier and harsher making it difficult for some crops to grow, crops such as sorghum have remained productive under dry conditions. About 80 % of sorghum grown worldwide is on drylands (Assefa et al., 2010) and sorghum is a reliable crop in the harsh environments of West Africa, (Callo-concha et al., 2013). In Ghana, sorghum (commonly referred

to as guinea corn) is crucial for food security especially for the three northern regions namely the Northern, Upper East, and Upper West regions (Lampitey et al., 2014; Angelucci, 2013). The different water-deficit tolerance and avoidance mechanisms identified in grain sorghum are summarized as leaf rolling, better adjustment in leaf angle, stomatal closure when stress increases in severity, antioxidant production, cuticle and epicuticular wax on leaves and stems, deep root system, and higher root density (Assefa et al., 2010).

Although grain sorghum tolerates and avoids water-deficit stress compared to many other cereal crops, this achievement comes with a yield penalty. Water-deficit stress at the vegetative and reproductive stages can lead to more than 36 % and 55 % reduction in yield, respectively (Assefa et al., 2010). Impact of water-deficit stress on the yield of sorghum is dependent on sorghum's sensitivity to the intensity and duration of the stress in relation to a particular growth stage. Sorghum is capable of tolerating less severe water-deficit stress (at an average leaf water potential of -18.1 bars) for shorter duration (~ 13 to 15 days) than a more severe stress (-21.7 bars) for longer duration (> 27 to 28 days) during the booting, heading and early grain filling stages (Eck and Musick, 1979). Water-deficit stress, lasting 16 and 28 days during the vegetative stage of sorghum resulted in 16 % and 36 % reduction in yield, respectively (Inuyama et al., 1976).

By virtue of the complexity associated with water-deficit tolerance of sorghum as highlighted above, and the fact that water-deficit stress can occur in any varying combination, different approaches have been used to quantify the impact. These have included variation relating to stress duration, stress intensity, growth stage, and a combination of these factors. In this experiment, two levels of water-deficit stress were imposed on selected sorghum genotypes (based on findings from chapter two) to elucidate water-deficit stress tolerance mechanisms by understanding the

physiological basis using Ghanaian soil as a growth medium. The specific objectives were to determine the effect of two levels of water-deficit stress on agronomic traits (number of leaves, number of tillers, stem height, stem thickness, shoot biomass), physiological traits, and root and root derived characteristics of four sorghum genotypes during the vegetative stage. We tested the hypothesis that varying intensities of water-deficit stress during the vegetative stage will induce different physiological and agronomic responses which will account for genotypic variations relating to water-deficit stress tolerance in sorghum.

4.3 Materials and Methods

The study characterized selected sorghum genotypes exposed to two different levels of water-deficit stress during the vegetative stage. The sorghum genotypes selected were - P898012, SC35, and SC971 which are parents of the sorghum Nested Association Mapping populations. The fourth genotype used is Kapaala which is a local high yielding sorghum obtained from SARI (Savannah Agricultural Research Institute) in Ghana. The experiment was conducted at the greenhouse of the Department of Horticulture, College of Agriculture and Natural Resources, KNUST, Kumasi in the Ashanti Region of Ghana.

4.3.1 Plant material and growth conditions

The four sorghum genotypes were grown in polyvinyl chloride (PVC) columns with inner diameter of 20 cm and height of 1 m. The PVC columns were sealed at the bottom with PVC plastic caps that had five drilled holes of size 0.5 cm. The growth medium used was a loamy sandy soil obtained from the departmental farm of Theoretical and Applied Biology, KNUST. The soil was passed through a netlike metallic filter to remove unwanted materials. The soil was then spread on

polythene sheets and air dried for about 15 days in greenhouse with an average temperature of 39°C. Each pot was filled with 50 kg of dried soil. Fertilizers that were mixed with the soil before filling up the pots, included Amiran Smart Fertilizer (Dizengoff Ghana Limited, Kumasi) a controlled-release fertilizer with 15–7–25 ratio of N–P₂O₅–K₂O; at a rate of 5 g per liter and YaraVita micronutrients (Yara UK limited, Pocklington, York, UK). The experiment was set up in a Randomized Complete Block Design with three blocks. Four seeds were sown at a depth of about 2 cm in each pot and thinned to one plant per pot during the three-leaf stage. Plants were maintained under greenhouse conditions with day-time maximum / night-time minimum temperatures of 38 °C and 22 °C, respectively, and 60 % to 80 % day/night relative humidity.

All pots were kept well-watered until the start of imposing stress. A standardized gravimetric approach of pot weighing (Kadam et al., 2015) was followed daily during stress periods to impose uniform level of stress across all the genotypes. The same approach was used with experiments conducted at KSU (chapter two) and used to determine cumulative water transpired by the plant during the stress period. By this gravimetric pot weighing, control pots were kept at 100 % field capacity (FC) while water-deficit pots were progressively exposed to two different levels of water-deficit stress, before which all the pots were maintained at 100 % FC (Figure 4.1). Severe water-deficit stress pots were progressively exposed to 50 % to 55 % FC (starting at 28 days after emergence, lasting 21 days) and moderate water-deficit stress pots were progressively exposed to 60 % - 65 % FC (starting at 35 days after emergence, lasting 15 days). Water lost by transpiration and reaching levels below the target stress level were replenished by adding back a calculated amount of water to maintain the stress levels. Pots surface was covered with a circular polythene sheet to control for evaporative water loss. A slit opening in the sheet was created to prevent heat

buildup. To account for water loss through this slit opening, a set of filled PVC pots were maintained without plants.

4.3.1.1 Observations

Daily pot weights for the 21 and 15 days stress period were used to determine the daily evapotranspiration. Plant weight accrued during the weighing period was negligible (Blum and Arkin, 1984). The weights of filled pots without plants were used for evaporative loss adjustments after which the daily transpiration was calculated and summed to attain the cumulative water transpired.

4.3.2 Physiological and agronomic parameters

At the end of the stress period, chlorophyll index was measured with a chlorophyll meter (Soil Plant Analyzer Development – SPAD, Model 502; Spectrum Technologies, Plainfield, IL, USA). Readings were taken at top, middle, and base of the leaf blade and averaged. Tiller and leaf numbers were counted and the stem height was recorded. Stem thickness was measured using digital Vernier calipers (electronic digital caliper, VWR® Digital Calipers, USA). The vegetative shoot samples (leaves and stems) were oven dried separately to constant weight at 60°C. They were weighed to determine leaf and stem dry weights and summed to determine the shoot biomass.

After harvesting the shoot biomass, roots were carefully removed from the soil and washed meticulously to minimize the loss of small roots. Root samples were oven dried to constant weight at 60°C and weighed to determine root dry weight. Total biomass was obtained by a summation of shoot biomass and roots biomass.

4.3.3 Derived Shoot and Root Growth Parameters

Transpiration efficiency (TE) was obtained by a ratio of the shoot biomass to the cumulative water transpired. Root to shoot ratio was calculated as the ratio of root dry weight to shoot dry weight. Root weight ratio (RWR) was calculated as the ratio of the root dry weight to the total biomass. Similarly, shoot weight ratio (SWR) and leaf weight ratio (LWR) were computed as the ratios of the stem and leaf dry weights to total biomass respectively.

4.3.4 Statistical analysis

The experimental design was a two way factorial treatment structure run in a Randomized Complete Block Design with three blocks of size 12. All data were statistically analyzed using the GLM procedure in SAS (SAS 9.4, SAS Institute Inc. Cary, NC, USA), with the sorghum genotypes and the watering regime (at three levels namely well-watered, moderate water-deficit stress and severe water-deficit stress) as factors.

4.4 Results

In testing for statistical significance, analysis of variance showed that the genotype by watering treatment interaction (G x T) was significant for the following traits: stem height, stem thickness, number of leaves, cumulative water transpired, stem dry weight, leaf dry weight, shoot biomass, total biomass, and stem weight ratio (Tables 4.1).

Traits that did not record G x T significance ($P > 0.05$) but varied significantly between water-deficit stress and well-watered treatments (irrespective of genotypic differences) include number of tillers, transpiration efficiency, root dry weight, and leaf weight ratio (Tables 4.1). Likewise, traits that did not record G x T significance but had significant genotypic differences (regardless

of treatment variation) include chlorophyll index, number of tillers, transpiration efficiency, root dry weight and leaf weight ratio (Tables 4.1). Root weight ratio and root/shoot ratio did not record any significant difference for genotype, treatment or G x T effects (Tables 4.1). Generally, there was a greater decrease for shoot and root traits under the severe water-deficit stress than the moderate water-deficit condition.

4.4.1 Shoot and physiological traits

Chlorophyll index significantly decreased in both moderate water-deficit stressed plants (9 %) and severe water-deficit stressed plants (27 %) (Tables 4.1). However, transpiration efficiency was significantly high (124 %) for severe water-deficit stressed plants and least for well-watered plants (Tables 4.1). Regardless of treatment effects, Kapaala recorded the highest value for transpiration efficiency, significantly differing from P898012 and SC971 (Figure 4.2a). SC35 had the next highest value for TE, significantly differing from SC971 which had the least value (Figure 4.2a). The number of tillers significantly decreased in both moderate water-deficit stressed plants (70 %) and severe water-deficit stressed plants (83 %) (Tables 4.1). SC971 produced the most tillers irrespective of watering treatments while Kapaala and SC35 had none (Figure 4.2b).

Effect of the different watering regimes on the cumulative water transpired by all four genotypes is shown in Figure 4.3a. All genotypes recorded significantly higher cumulative water transpired under well-watered condition compared with both water-deficit stressed conditions. Under moderate water-deficit stressed condition, P898012 had the most cumulative water transpired while SC35 had the least. There was significant reduction in the cumulative water transpired by all genotypes under severe water-deficit stressed condition.

Effect of the different watering regimes on the number of leaves of all four genotypes is shown in Figure 4.3b. SC35 under well-watered condition had significantly the highest number of leaves while number of leaves significantly differed between moderate and severe water-deficit stress for SC35 and SC971. The number of leaves for SC971 with moderate water-deficit stress was 17 % significantly more than those under well-watered as well as 22 % significantly more than those under severe water-deficit stress conditions. The least number of leaves was recorded with both SC35 and SC971 under severe water-deficit stressed conditions as well as the well-watered SC971 plants.

Effect of the different watering regimes on stem height of all four genotypes is shown in Figure 4.3c. There were no significant variations in stem height for Kapaala and SC35 under all three watering regimes. However, stem height differed within watering treatments for P898012 and SC971, with the stem height of well-watered P898012 being significantly taller (47 %) than severe water-deficit stressed P898012. However, SC971 under moderate water-deficit stress was significantly taller (~ 47 %) than those under both severe water-deficit stressed and well-watered conditions, following a similar pattern as number of leaves. The stem height of SC971 under moderate water-deficit stress was also significantly higher (~ 76 %) than SC35 under both well-watered and moderate water-deficit stress conditions as well as severe water-deficit stressed P898012 (49 %). However, there was no significant difference for stem thickness under various treatment levels for all genotypes except SC35. Stem thickness of well-watered SC35 was significantly the thickest (ranging from 65 % to 43 %) compared with watering treatments within and across other genotypes (Figure 4.3d).

Effect of the different watering regimes on the leaf dry weight of all four genotypes is shown in Figure 4.4a. Regardless of the watering regime, leaf dry weight of Kapaala was significantly greater than P898012 and SC971. Leaf dry weight of well-watered SC35 was significantly higher than water-deficit treatments of SC35 (~ 70 %) as well as all watering treatments of other genotypes. Similarly, well-watered SC35 recorded significantly highest stem dry weight and shoot biomass followed by the well-watered Kapaala (Figure 4.4b, c). SC35 had the least stem dry weight and shoot biomass under moderate water-deficit stress, significantly differing from Kapaala and P898012 under same watering treatments (Figure 4.4b, c). Kapaala had the most shoot biomass under severe water-deficit condition, significantly differing from those of P898012 and SC971 (Figure 4.4c). Generally, water-deficit stress induced significant reduction in the leaf dry weight, stem dry weight, and shoot biomass of SC35 but there were no significant variation in P898012 and SC971, among the two levels of water-deficit stress treatments. (Figure 4.4a, b, c). In addition, both water-deficit stress treatments had significant reductions in the stem dry weight and shoot biomass of Kapaala compared with well-watered treatment (Figure 4.4b, c).

4.4.2 Root and root related traits

Values of root dry weight were significantly decreased in both moderate water-deficit stressed plants (38 %) and severe water-deficit stressed plants (52 %) (Tables 4.1). On the contrary, leaf weight ratio was significantly highest for severe water-deficit stressed plants and least for well-watered plants (Tables 4.1). Irrespective of treatment effects, there were significant reductions in leaf weight ratio of Kapaala (28 %), P898012 (32 %) and SC971 (41 %) compared with SC35 (Figure 4.2c). Stem weight ratio of SC971 reduced significantly compared with SC35, P898012, and Kapaala regardless of treatment effects (Figure 4.2d). Total biomass significantly decreased

with moderate water-deficit stressed plants (37 %) and severe water-deficit stressed plants (47 %) (Tables 4.1). Water deficit led to significant reductions in the total biomass of Kapaala and SC35 but it had no significant variations in SC971 (Figure 4.4d). Kapaala accrued the most total biomass under both conditions of severe and moderate water-deficit stress (Figure 4.4d).

4.5 Discussions

Chlorophyll index was significantly higher for well-watered plants and least for severe water-deficit stressed plants. Abiotic stresses such as water-deficit stress generate higher amounts of reactive oxygen species (ROS) such as O_2 and H_2O_2 , which can lead to changes in chlorophyll content (Jajic et al., 2015). Water-deficit stress leads to reduction in photosynthetic pigmentation and this varies with genotypes depending on tolerance level (Nxele et al., 2017; Devnarain et al., 2016). There was however no significant genotypic variation for chlorophyll index in this experiment. Chlorophyll influences a plant's ability to absorb light for photosynthesis, hence a reduction in chlorophyll index has growth and yield consequences. Thus, a likely decrease in photosynthesis (not measured in this study) must have contributed largely to the large decrease in shoot and roots characteristics under water-deficit conditions. A general reduction in leaf dry weight, stem dry weight, and shoot biomass was observed, significantly differing between the watering regimes in SC35 and Kapaala (except leaf dry weight). Comparable reports on sorghum genotypes exposed to water-deficit stress recorded reduction in leaf dry weight (Nxele et al., 2017) and shoot dry weight by 37 % (Perrier et al., 2017). Reduced biomass in response to water-deficit stress has been documented in wheat and rice (total biomass – Kadam et al., 2015; leaf dry weight – Praba et al., 2009), and barley and corn (shoot biomass - Teulat et al., 1997). However, the few instances in this study where growth traits under water-deficit stress was significantly greater than

the well-watered counterpart must have resulted from experimental challenges related with the well-watered plants being under some other form of stress.

Well-watered plants had the most tillers and number of leaves while severe water-deficit stressed plants had the least. Ability of sorghum tillers to emerge from lower nodes, as well as their eventual survival and growth is dependent on photosynthate availability in the main stem (Roozeboom and Prasad, 2016). Water-deficit stress may reduce tillering either by lowering CO₂ assimilation due to stomatal regulation or by preferential allocation of available photosynthates for root growth to ensure maximum water absorption. Similarly, inadequate assimilates during the water-deficit condition would reduce leaf number (as seen with SC35) by a shortfall in carbohydrate supply to the shoot apex (Taiz et al., 2015).

Significant variations in stem height were recorded for P898012 and SC971 under the three watering regimes although stem thickness did not differ significantly for all genotypes except SC35. A decrease in stem elongation has been noted as a consequence of water-deficit stress due to reduced rate of cell expansion, cell size, loss of turgor pressure, and eventual decline in growth rate (Assefa et al., 2010). Water-deficit stress (at soil water deficit of - 8.85 bars for 30 days) significantly reduced stem biomass accumulation by 42 % which was attributed to a reduction in the length of the internodes of stems rather than a reduction in the stem diameter (Perrier et al., 2017).

Transpiration efficiency was significantly highest for severe water-deficit stressed plants which agrees with the well-known increased WUE under water-deficit conditions (Kadam et al, 2015; Vadez, 2014; Blum, 2009). A high TE could mean either an increase in biomass production for the same amount of water transpired, same biomass resulting from reduced transpiration, or a mix

of these two scenarios (Xin et al., 2009). Similarly to reports on genotypic variation in TE among sorghum genotypes by Emendack et al. (2011) and Xin et al. (2009), genotypes varied in TE in this study with Kapaala recording the highest followed by SC35.

While in most cases root/shoot ratio has been reported to increase under water-deficit stress for sorghum (Younis et al., 2000; Salih et al., 1999), the root weight ratio and root/shoot ratio did not record any significant genotypic or watering treatment differences in this study. An increase in root/shoot ratio under water-deficit condition usually results from decrease in shoot growth other than outright growth of root (Assefa et al., 2010). In the current context, the non-significance of the root/shoot ratio denotes lesser biomass partitioning for the roots (root weight ratio) of water-deficit stressed plants.

4.6 Conclusions

The lysimeter based experiment sought to evaluate physiological characteristics of four sorghum genotypes exposed to water-deficit stress during the vegetative stage. Gravimetric pot weighing was followed on a daily basis to impose uniform level of stress and also to determine the cumulative water transpired. Severe water-deficit stressed pots were progressively exposed to 50 % - 55 % FC, starting at 28 days after emergence and lasting for 21 days while moderate water-deficit stressed pots were progressively exposed to 60 % - 65 % FC, starting at 35 days after emergence, and lasting for 15 days. In testing for statistical significance, analysis of variance showed that the genotype by watering treatment interaction was significant for the following traits: stem height, stem thickness, number of leaves, cumulative water transpired, stem dry weight, leaf dry weight, shoot biomass, total biomass, and stem weight ratio. Transpiration efficiency was significantly higher for severe water-deficit stressed plants and lower for well-watered plants.

Water-deficit stress led to significant reductions in the total biomass of moderate water-deficit stressed plants (37 %) and severe water-deficit stressed plants (47 %). The root/shoot ratio did not record any significant genotypic or watering treatment differences in this study which was attributable to lesser biomass partitioning for the roots (root weight ratio) of water-deficit stressed plants.

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Table 4.1: Probability values of effects of water-deficit stress treatment (T), genotype (G) and T x G interaction on agronomic and physiological traits for experiments 1 and 2.

Traits	T	G	G x T	Treatment effect (mean \pm standard error*)		
				Well-watered	Moderate Water deficit	Severe Water deficit
Chlorophyll index (SPAD Units)	0.012	0.8116	0.8015	52.11 \pm 3.32	47.39 \pm 2.84	37.85 \pm 2.99
Stem height (cm)	0.0084	0.003	0.0218	73 \pm 6.45	64.19 \pm 5.52	44.93 \pm 5.81
Stem thickness (mm)	0.0011	<.0001	<.0001	12.3 \pm 0.40	10.03 \pm 0.34	10.54 \pm 0.36
Tiller number	0.0319	0.0016	0.1614	1.35 \pm 0.30	0.41 \pm 0.26	0.20 \pm 0.28
Leaf number	0.0001	<.0001	0.0002	6.5 \pm 0.20	6.17 \pm 0.17	5.13 \pm 0.18
Cumulative water transpired (l)	<.0001	<.0001	0.0017	10.7 \pm 0.16	6.88 \pm 0.14	2.32 \pm 0.14
Transpiration efficiency (g/l)	<.0001	0.0012	0.2356	1.29 \pm 0.21	1.17 \pm 0.19	2.9 \pm 0.19
Stem dry weight (g plant ⁻¹)	<.0001	<.0001	<.0001	7.49 \pm 0.48	4.03 \pm 0.41	3.2 \pm 0.44
Leaf dry weight (g plant ⁻¹)	0.0003	<.0001	<.0001	4.09 \pm 0.19	3.08 \pm 0.16	2.97 \pm 0.17
Shoot biomass (g plant ⁻¹)	<.0001	<.0001	0.0001	13.35 \pm 0.85	8.33 \pm 0.73	6.71 \pm 0.77
Root dry weight (g plant ⁻¹)	0.0195	0.221	0.0619	1.84 \pm 0.25	1.14 \pm 0.23	0.89 \pm 0.28
Total biomass (g plant ⁻¹)	0.0008	0.0021	0.0029	15.71 \pm 10	9.9 \pm 0.92	8.27 \pm 1.2
Leaf weight ratio	0.0044	0.0206	0.2805	0.27 \pm 0.03	0.36 \pm 0.03	0.39 \pm 0.04
Stem weight ratio	0.1413	0.0038	0.0037	0.45 \pm 0.02	0.43 \pm 0.02	0.41 \pm 0.02
Root weight ratio	0.4863	0.4278	0.858	0.12 \pm 0.02	0.11 \pm 0.01	0.10 \pm 0.02
Root/shoot ratio	0.5393	0.4573	0.8736	0.14 \pm 0.02	0.12 \pm 0.02	0.11 \pm 0.02

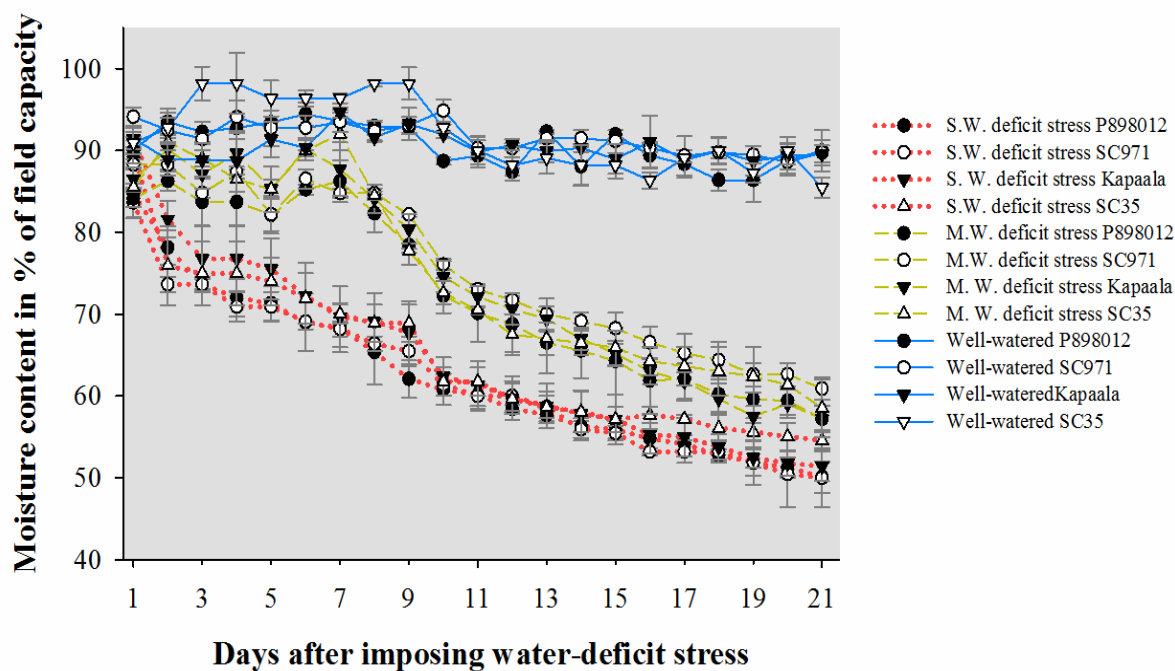


Figure 4.1: Rate of water depletion calculated based on gravimetric pot weighing and expressed as moisture content in % field capacity for four sorghum genotypes. SW – Severe water-deficit; MW – Moderate water-deficit

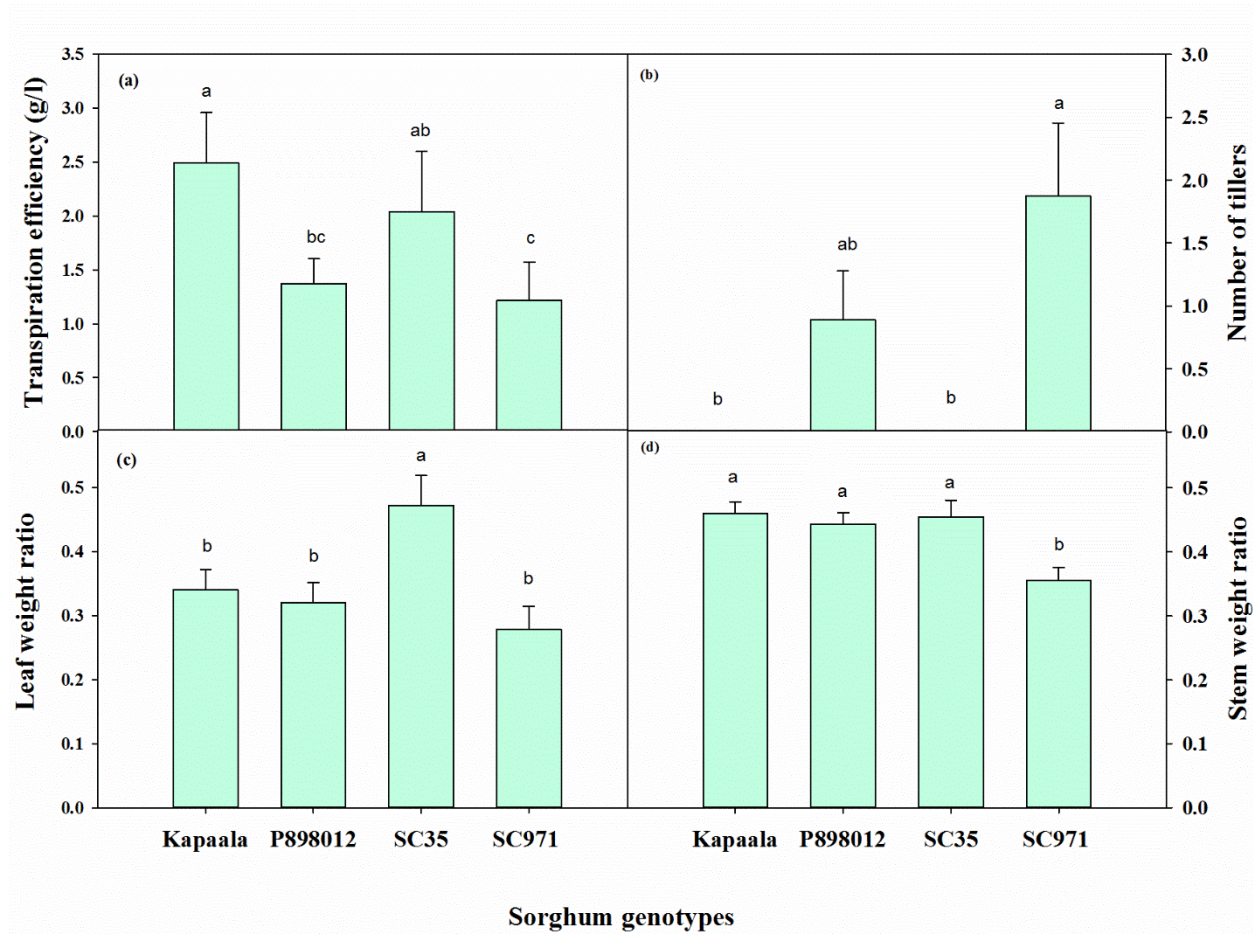


Figure 4.2: Genotypic variation (irrespective of watering treatments) for (a) transpiration efficiency (b) number of tillers (c) leaf weight ratio and (d) stem weight ratio. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$).

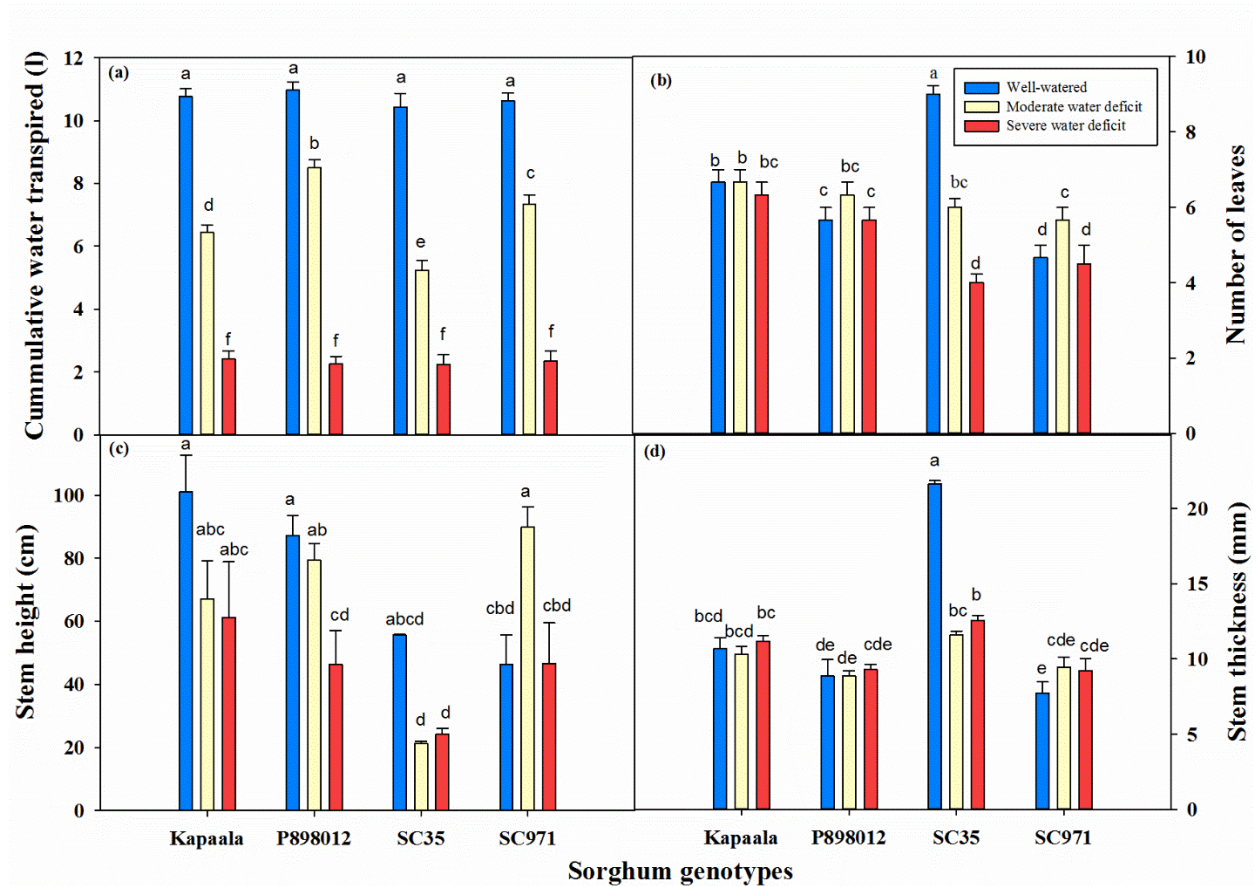


Figure 4.3: Effects of water-deficit stress on (a) cumulative water transpired (b) Number of leaves (c) stem height and (d) stem thickness for four sorghum genotypes. Red columns indicate severe water-deficit stress, the yellow columns indicate moderate water-deficit stress while the blue columns indicate well-watered. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$) and different letters on bars indicate statistically different means ($P < 0.05$).

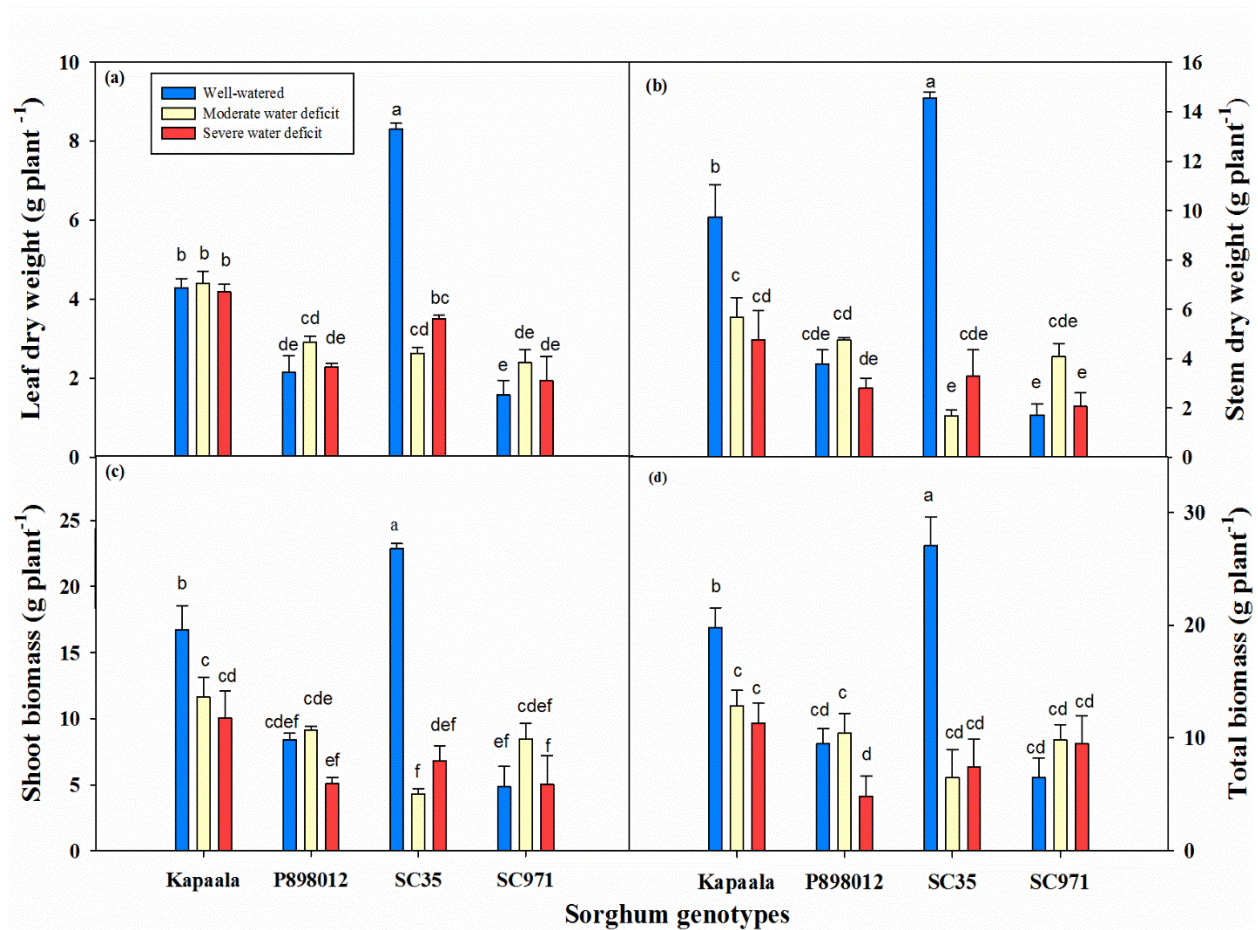


Figure 4.4: Effects of water-deficit stress on (a) cumulative water transpired (b) Number of leaves (c) stem height and (d) stem thickness for four sorghum genotypes. Red columns indicate severe water-deficit stress, the yellow columns indicate moderate water-deficit stress while the blue columns indicate well-watered. Each data point indicates mean value with \pm standard errors of means. Means and standard errors were estimated using the general linear model (GLM) procedure in SAS. Separation of means was carried out using the least significant difference (LSD) test ($P < 0.05$) and different letters on bars indicate statistically different means ($P < 0.05$).

Chapter 5 – General Summary

5.1 General Discussion

Water-deficit stress at the vegetative stages led to a decrease in chlorophyll index so that photosynthetic assimilation was constrained. This development was further supported by the findings of significant decrease of chlorophyll fluorescence (F_v/F_m) under water-deficit stress which depicted a decreased photochemical efficiency of PSII, signaled by an increased thylakoid membrane damage. In a culminated effect, there were reductions in tillering and leaf numbers and an overall reduction of growth and shoot biomass accumulation. Similar reductions in growth related traits were observed at the grain filling stages which were related to the conversion and mobilization of stem reserves into soluble sugars for the grain filling process as photosynthesis decreased due to the water-deficit stress.

Differences in how genotypes produce and partition biomass serve as possible indicators of tolerance to water-deficit stress. The main traits for studies on water-deficit stress at the vegetative and grain filling stages are biomass accumulation and grain yield respectively so that how biomass is partitioned in these stages is key in determining an overall performance. With a strong correlation determined for grain yield per main panicle with main stem thickness in the present study, a more biomass partitioning for increased stem thickness especially during the vegetative stage is crucial for grain production, even under water-deficit stress. Thus increased biomass due to thicker stems contributes to greater yields. In this regard, the thick stem growth of genotypes Macia and especially SC35 could well contribute to towards their tolerance to water-deficit stress.

At the root level, a significantly higher biomass partitioning to the roots was detected for the water-deficit stressed plants compared with well-watered plants in the US greenhouse vegetative stage experiments. However, the root/shoot ratio of another greenhouse vegetative stage experiment conducted in Ghana did not record any significant genotypic or watering treatment differences which was attributable possibly to lesser biomass partitioning for the roots (root weight ratio) of water-deficit stressed plants. The differential results in these two experimental settings are indicative of some variant role played by the different root media used, turface® (MVP®, Profile Products LLC, Buffalo Grove, IL) being the rooting media used for the US experiment and a Ghanaian soil for the Ghana experiment. There were no root studies for the grain filling experiments as the dynamics of root growth and development past the vegetative stage is limited.

In both greenhouse and rainout shelter studies on how water-deficit stress affects the genetic diversity for within-head grain-filling dynamics, it emerged that panicle positional (top, middle, and bottom) individual grain weight did not differ so that grain number served to influence differences in grain yield. It is however not clear what is responsible for the differences in panicle positional grain number under the stress although embryo abortion and panicle architecture have been proposed. It is also not clear how grain filling duration at each respective panicle position affects the grain yield of the respective positions in any way.

In consideration with growth and yield traits measured in all experiments, performance of genotypes such as SC35 portrayed a higher level of tolerance to water-deficit stress whereas other genotypes such as SC971 depicted significantly higher level of susceptibility. This was generally observed when SC35 (and other well performing genotypes) recorded significant percentage difference between the well-watered and water-deficit treatments for the fewest traits while SC971

(and other poor performing genotypes) recorded more significant percentage differences between the well-watered and water-deficit treatments. Additionally, SC35 posed to be the most water-deficit tolerant by having the highest $\delta^{13}\text{C}$ mean values for both US greenhouse vegetative stage experiments. In another development, SC1103 is posing to be an interesting genotype as it did not record any significant difference between the watering treatments for all measured growth and yield traits for the grain filling stage experiments at both the greenhouse and field.

5.2 General Conclusions

The most expected outcome of the impact of water-deficit stress, will be growth and development impairments. Water-deficit stress mostly decreased a number of shoot, root, and physiological characteristics measured in this study. In many of these traits, reductions and declines may generally be undesirable consequence of the water-deficit stress, but alternatively they could serve as adaptive mechanisms for coping with stress.

In the vegetative stage experiment involving all the eleven sorghum parental lines exposed to water-deficit stress level of 55 % to 60 % field capacity, significant reductions were seen with the water-deficit treatments for most traits though TE, iWUE, $\delta^{13}\text{C}$, R:S ratio and the RWR were significantly increased under water-deficit condition. Root traits of genotypes SC283, SC971 and SC1345 were significantly impaired under water-deficit stress while the total root length and total root surface area of P898012 increased under water-deficit stress though this increase was statistically not significant.

In Ghana, another experiment focusing on the vegetative stage with two levels of water-deficit stress imposed on selected set of sorghum genotypes, recorded a greater decrease for shoot and

root related traits under the more severe stress (50 % to 55 % FC; lasting for 21 days) than the moderate water-deficit condition (60 % to 65 % FC; lasting for 15 days). However, the root/shoot ratio did not record any significant genotypic or watering treatment differences in this study which was attributable to lesser biomass partitioning for the roots (root weight ratio) of water-deficit stressed plants.

In the grain filling experiments conducted in both the greenhouse and on the field, differences in grain numbers and grain weight among genotypes, irrespective of the watering treatments, resulted from the diversity in panicle architecture other than grain filling dynamics. This led to differences in positional grain numbers and positional grain weight regardless of the watering treatments. With respect to watering treatments affecting panicle positional yield, differences in panicle positional grain number accounted for differences in panicle positional grain weight as the positional individual grain weight was not affected by the stress. Therefore, significant differences between the well-watered and water-deficit treatments for grain number per main panicle may most likely be associated with embryo abortion, where fertilized ovules are not able to grow due to insufficient assimilate supply resulting from the stress.

Furthermore, the fact that there were generally smaller differences between watering treatments for the positional grain number at the top section compared with the middle and bottom sectional areas suggests compliance to the ‘basipetal filling pattern’ which is also similar to the concept of superior and inferior spikelets as identified in rice. On the premise of dominance by earlier-filling superior spikelets (located predominantly at the top) over late-filling inferior spikelet (located predominantly at the bottom) due to assimilate accessibility, the differences in positional grain number in relation to the stress can be understood ideally on a more embryonic abortion at the

middle and bottom sectional areas than the top. There were more significant Spearman's rank correlations for growth traits than yield traits in comparing results of the greenhouse study to the field study. Thus, Spearman's rank correlations indicated the ability to select for water-deficit tolerance traits in the greenhouse that would have partial representative rankings on the field.

Overall, findings from the various studies concur with the presumption (at the start of the study) that water deficit stress during the vegetative and grain filling stages will induce different water-deficit tolerance traits and the resultant changes in the physiological and agronomical traits varies among sorghum genotypes. Sorghum parental genotypes, such as SC35 appears to demonstrate some level of higher tolerance to the stress based on least percentage difference exhibited between treatments and by depicting significant percentage difference for the fewest number of traits. On the other hand, genotype SC971 recorded higher significant percentage difference in more traits between the well-watered and water-deficit treatments than any other genotype, implying its susceptibility to water-deficit stress.

5.3 Future Direction

- Root studies during the vegetative stages in the current research focused on agromorphological traits. Further research to investigate the root anatomical complexity along different positions of the root length would add to exposition on the below ground characteristics of the sorghum parental genotypes under water-deficit stress.
- The current study researched on roots morphological characteristics under controlled conditions. Further research is needed to quantify the effects of water-deficit stress under field conditions which may be achieved using the minirhizotron method.

- In the present research, significant differences between the well-watered and water-deficit treatments for yield was due to differences in grain number. Because the potential number of grains is determined before flowering, embryo abortion is presumed to have affected the grain numbers where fertilized ovules are not able to grow due to insufficient assimilate supply resulting from the stress. Further experiment in this direction would help to elucidate how embryo abortion affects grain yield of the sorghum parental lines during post-flowering water-deficit stress. The experiment would also help to ascertain how embryonic abortion relates with differences in the panicle positional grain number under the stress where it's been assumed in the present study that a more embryonic abortion occurred at the middle and bottom sectional areas than at the top.
- In the current study, panicle differences in positional grain number accounted for differences in positional grain weight under water-deficit stress. However, it is not clear as to what is contributing to this differences in panicle positional grain numbers. Proposedly, panicle architecture is one of the factors that may be responsible. Since determinants of panicle architecture such as rachis length, number of branches, panicle length and panicle width have been strongly linked with yield, further research is needed to investigate how panicle architecture of the sorghum parental genotypes affects grain number (and thus panicle positional grain numbers), even so under water-deficit stress.
- The approach adopted in the present study involved separates experiments conducted at the vegetative and grain filling stages. In addition, variation of water-deficit stress levels in relation to duration and intensity was experimented only at the vegetative stage. Thus further research is required to provide understanding of the effects of varying levels of the

stress exposed at the vegetative stage and re-watered for the grain filling stage until physiological maturity. In this respect, how the different levels of stress at vegetative stage affects flowering and the grain filling stages can be assessed.

- Water-deficit conditions usually occur alongside high temperatures and an integrated understanding of the parental sorghum's response to both types of stresses is key. As it is difficult to isolate influence of individual abiotic stress on sorghum, cross-synergistic and cross-adaptation researches will be a better expository. In this regard, water-deficit stressed pots can be subjected to heat stress in controlled chambers while on the field, rain out shelters and heat tents may be used to impose combined heat and water-deficit stresses.
- The sorghum Nested Association Mapping parental lines used in this study have been used to develop a genetic mapping population that is instrumental for dissecting the genetic basis of complex traits. In this regard, identified traits linked with water-deficit tolerance in the current research will be useful for further work on the general population.