THESIS


CHARACTERIZATION OF *SORGHUM BICOLOR* GENOTYPES UNDER VARYING WATER REGIMES


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ABSTRACT

CHARACTERIZATION OF *SORGHUM BICOLOR* GENOTYPES UNDER VARYING WATER REGIMES

Agriculture is the largest consumer of fresh water, accounting for 70% of use globally. The availability of water is expected to decrease in future climate models. It is imperative to develop crop varieties with improved performance under targeted limited water environments. One approach to address this aim is capitalizing on inherent variability for drought tolerance traits in crops. Depending on the nature of water stress and other environmental factors, specific root morphology, shoot morphology, and the harvest index are selectable traits that can distinguish performance of different genotypes in crops. These aforementioned parameters discriminate relative water-stressed performance in many studied crop species. Among these is sorghum (*Sorghum bicolor*), a grain crop adapted to many different environments, particularly where limited rainfall is an agricultural constraint. The specific objectives of the following thesis were to 1) characterize the morphology of a sorghum recombinant inbred line (RIL) population (n = 430) of *Sorghum bicolor* for response to varying moisture regimes and 2) evaluate 10 selected genotypes for below-ground morphology in the greenhouse and field for response to varying moisture regimes. This population expresses a wide range of phenotypes in response to varying moisture regimes in panicle weight, shoot weight, harvest index, plant height, stem diameter, tillering and days to flowering. Ten selected RILs, including the two population parents, BTx623 and IS3620C, represent the population distribution and respond uniquely to different moisture environments. When subjected to drought stress and subsequent recovery

when re-watered in the greenhouse, root system response varied at both time points. Root system and stem size are indicators of this response and vascular tissue variation may play a role in these varied responses. In the field, the root system of these ten RILs, as quantified by crown root angle, had a tendency to grow more vertically than horizontally under dryland conditions while high moisture environments showed no association with root system orientation. Taken together, this study provides foundational knowledge on above and below-ground responses of RIL population BTx623 X IS3620C to varying moisture environments.

ACKNOWLEDGEMENTS

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INTRODUCTION

***Challenges in Agriculture***

Agriculture will face many challenges in the 21$^{st}$ century, mostly resulting from a

growing population expected to reach 9.8 billion people by 2050 ("World Population Prospects"

2017). Climate change is expected to raise the global average temperature by 4°C, increase the

rate of temperature flux in temperate zones, reduce rainfall in arid and semi-arid regions

further inland, and increase storm system activity and variability of rainfall quantity and

frequency (Turral, Burke, and Faurès 2011). This uncertainty in climate is predicted to decrease

food security, the reliable access to sufficient and nutritious food. Competition for land and

water concomitant with a need to generate more agricultural products to supply the growing

needs of the human population is a multi-faceted challenge.

Agriculture is the single largest use of the terrestrial surface on Earth and consumes

about 70% of fresh water withdrawals (Turral, Burke, and Faurès 2011). Advances in agricultural

irrigation practices have enabled increased cropping densities resulting in higher yield.

However, these improvements are placing a heavy burden on the freshwater supply system,

with some predictions estimating a 64% increase in global freshwater irrigation demand by

2030 (Foley et al. 2011). Furthermore, as freshwater sources become less available, water will

be preferentially diverted to urban areas for human consumption (Simon 2001). Simon (2001)

estimates that diverting as little as 5% of agricultural water to municipal use could solve urban

water needs for the next 25 years. Small improvements in agricultural water efficiency have the potential to satisfy increased water demand in all sectors.

In the past, substantial increases in crop productivity were achieved through traditional plant breeding, such as screening for natural genetic variants with shorter stems that reallocate carbon partitioning towards grains instead of stems and leaves. Other areas of agricultural improvement has resulted from leaving less land fallow, reduced tillage, use of chemical fertilizers and pesticides, large monocultures for efficient mechanical harvest, equipment advancements, and increased cropland area (Foley et al. 2011; Evenson and Gollin 2003; Altieri and Koohafkan 2008). Although historically effective, stagnated agricultural productivity is an increasing global concern (Turral, Burke, and Faurès 2011; Foley et al. 2011). Grassini et al. (2013) argue that the top three grain crops, maize, rice, and wheat, which comprise nearly one third of global crop production have reached yield stagnation. Additionally, other crops are expected to reach this productivity threshold; therefore, shifting crop consumption or cultivation will not meet growing food demands.  These yield stagnations may be due in part to plant breeding for favorable target environments but this provides small improvements in marginal lands.   Further improvements in crop production will require breakthroughs to increase yield potential under unpredictable and variable environments.

Abiotic stress is a major yield-limiting factor which restricts the choice of crops produced over large areas, and in extreme cases leads to total crop failure (Vadez et al. 2013). Of the various abiotic stress factors, drought stress is increasing in incidence in the arid and semi-arid regions of the globe and severity world-wide (Cisneros et al. 2014). Drought can be characterized in several ways and varies over spatial and in temporal scales (Wilhite 2009).

Decades of low rainfall generate major climatic change and the resulting arid environment leads to food insecurity and severe social disruption (Passioura 2007). Within a season, drought is considered a temporary aberration in normal rainfall where water demands are not met and results in a reduction in crop yield quantity and quality (Wilhite 2009). Furthermore, the frequency and intensity of rainfall reductions can differentially influence pre-planting soil moisture, germination rates, vegetative growth, flowering time and length, grain/pod fill or fruit growth and maturation (Blum 2011b). Additionally, the ability of plants to adequately respond to drought within a developmental stage may greatly reduce yield losses and decrease risk of total crop failure. Therefore, the development of plants with improved survival and yield stability under limited water is a major objective in many crop breeding programs.

### Plant response to water stress

The identification of key traits and understanding their underlying genetic mechanisms is important in breeding programs to develop drought resistant crops. Historically, the primary consideration for plant performance under drought conditions is yield, which is typically the amount of biomass or the fruit, seed, grain or fiber produced (Blum 2011c). For example, selection of high yield potential in maize has led to consistent yield increases in unfavorable environments (Castleberry et al. 1984). Yield is complex, integrating both constitutive and adapted plant responses (Blum 2011c). The relationship between yield and these responses are variable depending on the duration and intensity of drought stress and plant developmental stage. In general, drought sensitivity increases in order through the following stages: seedling, vegetative, pre-flowering, grain fill/fruit growth, and flowering. A practical approach to classify

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drought response in plants is to parse them into two larger groups, pre-flowering and post-flowering. Pre-flowering drought stress occurs earlier in the season and post-flowering drought stress coincides with terminal, end of season water limitations (Wang et al. 2014). These two categories are used because plants employ either pre-flowering or post-flowering drought-tolerant strategies; seldom are both seen in a single variety (Sanchez et al. 2002).

The evolutionary history and breeding objectives of a particular crop determine the genetic mechanisms underlying optimum plant performance in a target environment. Understanding limitations during early- and late-season growth behavior can help direct research efforts towards identifying traits best suited for a variable drought conditions (Blum 2011b). For example, plants that flower early in response to limited water may escape terminal drought and ensure yield stability, while plants that delay flowering in response to drought may time anthesis and grain fill with the return of moisture. In both cases, plasticity in flowering traits minimizes exposure to harsh growing conditions during vulnerable developmental stages. Therefore, generalizing drought susceptible or tolerant response is difficult. Reported drought-susceptible plant responses during the pre-flowering stage include the following: poor stand establishment, reduced plant height and leaf area, delayed or hastened flowering time, low root to shoot ratio, increased leaf temperature and oxidative load, and reduced stomatal aperture, transpiration rates, $CO_2$ assimilation rates, and synthesis of photosynthetic pigments (Farooq et al. 2012). Reported drought susceptible plant response during the post-flowering stage include: weak panicle exsertion, short maturity interval, increased ovule abortion, increased pollen sterility, reduced photosynthetic pigments, and early senescence (Blum 2011c; Farooq et al. 2012; Wang, Burow, and Burke 2014).

One approach to improve plant performance in limited water environments is to evaluate and characterize the drought response of crops historically and currently cultivated in arid and semi-arid regions. These crops display a high degree of adaptability to a wide range of climate scenarios and geographic locations. Commonly cultivated crops with these attributes include millet (*Eleusine*, *Pennisetum, Panicum* species), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), chickpea (*Cicer arietinum*), certain varieties of upland rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*). Sorghum is a globally important crop and serves as a drought-adapted model system.

### Sorghum as a drought-adapted model crop

*Sorghum bicolor (L.)* Moench, is the fifth most cultivated cereal crop in the world and is adapted to hot, dry, and highly variable environmental conditions (Rao et al., 2014). Sorghum originated from Eastern Africa and was disseminated via human transport throughout the continent and later to Asia (Kimber 2000). After each migration event, sorghum was locally adapted, which resulted in large genotypic and phenotypic diversity allowing for acclimation to a wide range of environments (Kimber 2000; *Lost Crops of Africa: Volume I: Grains* 1996). Additionally, cultivated and wildtype sub-groups can readily outcross, enabling the exchange of diverse genetic content uncharacteristic of many cultivated crops (*Lost Crops of Africa: Volume I: Grains* 1996). Consequently, sorghums can survive and thrive in temperate and tropical latitudes, rainy or semi-arid climates, altitudes from sea level to 3000 m, dense clayey or light sandy soils, soil pH from 5.0-8.5, and seeds germinate well at temperatures between 10-35°C (Kimber 2000).

Sorghum's genotypic and phenotypic diversity allow for cultivation on all six continents for use as food, livestock and poultry feed, fiber, building material, and a biofuel feedstock (Rao et al. 2014; Kimber 2000). This crop is grown by both subsistence and commercial growers. In more agriculturally advanced countries, sorghum is typically used for livestock and poultry feed, while less agriculturally advanced countries tend to rely on sorghum as a food product (Rao et al. 2014). The United States (US) is the leading producer and exporter of sorghum, generating approximately 16% of world supply, comprising roughly 71% of global sorghum trade. Other major producers include Mexico, Sudan, Argentina and China. The lowest rate of productivity lies in West Africa and South Asia, with more than 80% of the total production area concentrated in these regions (Rao et al. 2014). One contributing factor for low crop productivity in these regions is that sorghum is rarely grown in rows or monocultures, but rather scattered randomly among other crops such as cowpea and pigeon pea (*Lost Crops of Africa: Volume I: Grains* 1996). However, sorghum grain remains a caloric staple in the arid and semi-arid regions of the world and sorghum as a food product constitutes 55% of global production (Patil 2015; *Lost Crops of Africa: Volume I: Grains* 1996). Increasingly, sorghum is cultivated for use as a biofuel feedstock (Rao et al. 2014). Grain, juice or biomass can render ethanol, making sorghum the only crop that produces all three bioethanol precursors: sugar, starch and lignocellulose (Rao et al. 2014).  Despite its diversity in use and widespread cultivation across varying climates, sorghum production is still constrained by limited water. Of all the biotic and abiotic stress factors, drought is the greatest cause of sorghum crop loss worldwide (Assefa et al. 2010). Drought conditions can result in 60-90% yield loss and up to 50% total crop failure in sorghum (Assefa et al. 2010; Wang et al. 2014). Improving sorghum

yield quality and quantity under water limitations can have global consequences by reducing

food insecurity and benefiting the livelihoods of large scale producers and small-holder farmers

alike.

Many drought responsive traits can prove beneficial and plant breeders must select for

those best suited for the target environment. This target environment is important because a

trait in one environment may no longer be useful in another. For example, leaf rolling may help

reduce transpiration and reduce water loss, however, this reduces photosynthetic activity

(Wang et al. 2014; Corlett et al. 1994). Therefore, this response is beneficial under severe

drought of short duration but is a susceptible response in scenarios with mild water stress. In

sorghum, several traits have been implicated in pre-flowering and post-flowering drought

resistance, however this is contingent on the target environment. Traits adapted to mitigate

pre-flowering drought stress include early flowering, glaucousness, and floret retention (Wang

et al. 2014; Assefa et al. 2011; Rosenow et al. 1983). Additionally, the prevention of several

traits is indicative of pre-flowering tolerance to limited water including leaf rolling,

uncharacteristic leaf erectness, and leaf tip and margin burn. Post-flowering drought adapted

traits are less common in cultivated sorghum because water stress during reproductive and

grain fill stages results in the largest reduction in yield, sometimes with total crop failure

(Younesi and Moradi 2009; Saini and Westgate 2000). Some distinct phenotypic traits that

contribute to terminal water stress tolerance in sorghum include a reduction in canopy size,

decreased tillering, decreased number of leaves per culm, delayed senescence, maintenance of

leaf chlorophyll (stay-green), and glaucousness (Monk et al. 2014; Ogbaga et al. 2014; Sanchez

et al. 2002; Assefa et al. 2010; Blum 2011d). Tolerant varieties tend to maintain developmental

milestones and normal seed head development (Rosenow et al. 1983). Additionally, many

below-ground traits that confer sorghum post-flowering drought tolerance include an extensive

root system, high root to shoot ratio, and deeper rooting (Monk et al. 2014; Blum 2011d;

Sanchez et al. 2012).

Many above-ground non-destructive morphological and physiological traits are used to

evaluate plant responses to low moisture. Maintenance of leaf turgidity and photosynthetic

pigments allows photosynthetic metabolism to take place despite the environmental

conditions, reducing yield losses during water stressed conditions (Blum 2011b). Glaucousness

reduces water loss from shoot tissue allowing more water to be used for plant function rather

than lost to the atmosphere (Blum 2011b). In a terminal drought condition, a smaller canopy

enables growth to maturity without crop failure (Blum 2011c). In the canopy, a smaller leaf area

results in less photosynthetic capacity but this also reduces water loss through transpiration

during photosynthetic metabolism (Blum 2011d). In addition to maintaining active shoot

metabolism, sorghum root activity and appropriate root architecture is required for continued

growth under drought environments (Rouphael et al. 2012).

The study of below-ground traits is less thoroughly conducted than above-ground

morphology and physiology, largely due to the destructive and labor-intensive nature of root

evaluation. However, root studies have found that access to reservoirs of soil moisture typically

increases with a more prolific root system (Blum 2011d). A shallow root system extending more

horizontally than vertically is generally better suited for environments with short periodic

rainfall and limited stored soil moisture (Blum 2011d). Conversely, sorghum root systems can

grow deeper and more extensively than other crops (up to 2.5 meters), allowing roots to reach

otherwise inaccessible water reserves (Monk et al. 2014; Assefa et al. 2010). Typically, this type of root growth is beneficial for a terminal drought condition where water additions are not expected later in the season, but preseason heavy rains allowed for storage of water deeper in the soil profile. Together, root and shoot morphology and physiology enables maintenance of plant metabolism resulting in viable plant reproduction and continued grain fill for the duration of the season.

### *Conclusion*

Agriculture will face many challenges in coming years due to uncertainty in climate with added demand from a growing population. Thus, increasing efficiency in crop production is critically important in improving the livelihoods of large and small-scale producers while reducing food insecurity. This is of particular importance in limited water environments characteristic of arid and semi-arid regions of the globe.

By evaluating crops historically and currently cultivated in arid and semi-arid regions, the mechanisms used to tolerate water limitations can be identified. Subsequently, these traits can be applied to breeding programs to further improve crops adapted for dry environments as well as improve plant water use of crops without this adaptation. One such crop, *Sorghum bicolor,* is well-suited for drought-prone environments and contains a reservoir of genetically and phenotypically diverse traits. Although not immune to water stress, extreme diversity has enabled sorghum to develop traits valuable for growth and yield output in marginal environments.

Sorghum employs a variety of plant morphological traits and physiological behaviors in response to water stress. These attributes can be divided into two primary groups: expression during pre-flowering drought stress and expression during post-flowering drought stress. This expression involves different components of plant anatomy, developmental stages, metabolism and genetic control that remain to be fully understood. Further complicating these responses is the type of water stress imposed on the crop. The intensity, frequency, and timing of water stress is critical to identify traits suitable for plant growth and production in a given target environment. Taken together, knowledge gained from evaluating drought response in sorghum can improve the development of elite lines with remarkable ability to adapt to water stress and serve as reference model crop for non-adapted crop species.

In this project, we evaluated above ground morphological traits of a diverse sorghum population under varying moisture regimes in the field for two growing seasons. Furthermore, a subset of 10 genotypes from this population was evaluated for above- and below-ground morphology in a field and greenhouse setting. We identified several characteristics unique to this population and the selected lines. Plant height, stem diameter, tiller number and flowering are consistently heritable under varying moisture regimes in the field setting. Plasticity in different components of shoot growth affect biomass partitioning inter- and independently. Additionally, horizontal root growth in the field is valuable in moderate moisture environments, but less important under high water environments. Pre-flowering root growth is restricted under drought stress but recovers quickly following re-watering.  This study provides foundational data on the BTx623 X IS3620C RIL population response to varying moisture regimes.

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CHAPTER II. **Above- and below-ground morphological traits of *Sorghum bicolor* RIL population BTx623 X IS3620C in response to varying water regimes**

SUMMARY

With increased demands on freshwater, the development of crop varieties with improved performance under reduced water conditions is important for food and fuel security. Sorghum (*Sorghum bicolor*) is an internationally important food and fuel crop with large genetic variation in agronomically important traits such as tolerance to drought. While many traits contribute to drought adaptability, plant survivability and yield production under water-limited conditions is not fully understood. To identify associations between above- and below-ground morphological traits and different moisture conditions, we utilize a phenotypically diverse RIL population derived from an elite hybrid, BTx623, and a guinea line, IS3620C. A subset of 250 and 124 lines from the full population were grown in 2016 and 2017, respectively, in Rocky Ford, Colorado. The following traits were evaluated: panicle weight, shoot weight, harvest index (panicle weight/ dry shoot weight + panicle weight), plant height, stem diameter, stand count, tillering and days to flowering. In response to varying levels of soil moisture, the panicle weight, shoot weight, harvest index and tillering were most variable. Furthermore, shoot dry weight was highly correlated with plant height and panicle weight for dryland and irrigated moisture regime. In the dryland condition however, positive correlations were significant among all shoot traits, including tiller number, shoot dry weight, plant height and panicle weight only under water deficit. A subset of this RIL population was used to assess root biomass to determine whether particular root traits are favorable under drought stress and recovery following the addition of water to the system. Seedling evaluation in the greenhouse suggests

that plasticity in stem and root size in response to drought-stressed and irrigated conditions is a beneficial phenotype resulting from variability in environmental moisture. In the field, root system orientation is not associated with above ground performance under high moisture environments, but becomes more relevant as soil moisture decreases. Steeper, more vertically-oriented roots (approximately <38° from soil surface) are associated with higher yields as soil moisture is reduced. This population represents a wealth of genetic and phenotypic diversity in which response to different moisture environments has been characterized.

INTRODUCTION

Agriculture will face many challenges in the future and a major concern is the availability of freshwater. Agriculture consumes 70% of freshwater globally and water demand is expected to increase with the expansion of cultivated acreage. Although water available for agriculture is expected to decrease, primarily resulting from climate change and competition from a growing population, global weather patterns and precipitations events are expected to be more be variable (Turral et al. 2011; Foley et al. 2011). One approach to address reduced water availability is to develop crop varieties with improved performance under low-water environments. As drought tolerant crop varieties gain importance agriculture, they should yield competitively in water limited conditions as well as express little to no yield penalty in non-stressed environments in years where water is plentiful. Therefore, exploiting traits inherent in drought tolerant crops can reduce agricultural water demands while maintaining or improving yield to satisfy population needs.

Plant response to a particular target environment will vary depending on a number of factors and the primary strategy of drought resistance. Environmental factors include water availability, temperature, pest and pathogen pressure, weed pressure, and mechanical damage (e.g., hail) (Tardieu 2012). The three primary strategies of drought resistance are dehydration avoidance, dehydration resistance and drought escape (Blum 2011b). Dehydration avoidance is the most common form identified in plants, defined by maintenance of plant hydration. Dehydration resistance is the least common, defined by continuing plant function despite a dehydrated water status. Drought escape is the ability of plant to avoid the most severe drought conditions during anthesis or fruit/grain-fill by altering phenology. This last strategy is commonly used by plant breeders to improve drought resistance because phenology is typically genetically-driven (Blum 2011b).

Taken together, the abiotic influences and drought tolerance strategy are critical to fully understand tolerant or susceptible responses to unique environmental conditions (Tardieu 2012). For example, plant growth and development can be altered depending on the intensity, duration and timing of water limitation. Traits responsive to differences in soil moisture include root morphology, shoot morphology, root exudation, yield, photosynthetic activity and phenology (Blum 2011a; Tardieu 2012). An environment of relatively frequent, low intensity rainfall throughout the season (e.g., western Nebraska, USA) favors shallow, horizontally extensive root growth. However, in a region where terminal drought is frequent, plants rely entirely on stored soil moisture from previous season (e.g., Andra Pradesh, India) and vertically extensive roots allow exposure to soil moisture otherwise inaccessible to a shallow root system (Blum 2011e). Additionally, fitness penalties can be present with terminal drought escape,

where an adaptive characteristic to shorten the crop cycle minimizes drought exposure. When rainfall does occur later in the season, plants that maintained normal growth will benefit and yield higher than those that matured early (Tardieu 2012). Thus, it is important to identify functional traits in which plasticity may play a decisive role in plant response to a variable climate. The understanding of phenotypic plasticity will be crucial to identify a wide range of useful phenotypes and increase crop productivity in a target environment.

Grasses, including important food and bioenergy crop species, have a unique root system development. Roots are initiated from the below-ground basal nodes of the shoot, termed the crown. For majority of plants, roots are the main channels of water and nutrient uptake and differences in root systems result from genetic and environmental factors. For example, substantial phenotypic variation in crown root number, growth rate, and angle has been observed in different inbred lines of maize (York and Lynch 2015). The physiological impact of these roots has been explored and indicates that fast growing roots with steep crown root angles promote access to water resources in deep soil profiles (Lynch 1995; Lynch 2013). Additionally, crown root development has been shown to be a major target for drought stress signaling in grass species, where limited water triggers crown root arrest and is thought to be an important mechanism to conserve water under drought (Sebastian et al. 2016). Root branching is also a major determinate of plant vigor that is responsive to environmental cues, but the effect of water availability on the spatial scale of root architecture is poorly understood. Recent work indicates several plant species can respond to soil wetting and that this impacts root architecture patterning (e.g., root hair formation) (Bao et al. 2014). Understanding how

water deficit affects root development and architecture may be a useful target in breeding efforts to improve yield stability over varying moisture conditions.

Among the genetically diverse and drought-adapted crops cultivated globally, grain sorghum (*Sorghum bicolor*) is the most ubiquitous ("FAOSTAT" 2015). Cultivated sorghum and wild sorghum relatives are established worldwide, largely a result of its adaptive ability to a wide range of environments and stresses. Sorghum is also a multipurpose crop and commonly used as a livestock feed, biofuel feedstock, food and fiber crop. The high adaptability of sorghum has allowed this crop to outcompete other higher value crops where the environment restricts plant growth, particularly where limited rainfall may be an agricultural constraint (Tari and Laskay 2013).

To evaluate the phenotypic plasticity of sorghum in response to different moisture environments, a recombinant inbred line (RIL) population was used. This population was selected for its unique parental origins, high phenotypic diversity, and extensive use in research in a wide range of locations and years (McCormick et al. 2017). Our goal was to identify associated root and shoot traits found in individual lines that contribute to yield stability across environments. The objectives of this study were to 1) evaluate above-ground morphological traits for response to high and low water regimes in the field to determine trait stability, 2) determine if seedling response to pre-flowering drought is predictive of root responses in the field, and 3) identify lines exhibiting a yield penalty under high moisture environments. This RIL population expresses a wide range of above ground traits in response to different moisture environments over two field seasons.

MATERIALS AND METHODS

***Plant Materials and Plot Design***

A subset of 252 and 124 *Sorghum bicolor* $F_9$ and $F_{11}$ recombinant inbred lines (RILs), derived from a cross between BTx623 and IS3620C, was evaluated for above ground and below-ground traits in response to different water regimes (Burow et al. 2011). BTx623 (PI 659985) is an elite inbred line developed from a cross between a kafir (BTx3197) and a zera zera (SC170-6) line, with characteristics including no tillers, short stature, large seed and high yield (Hart et al. 2001; Brown et al. 2006). The region between Tanzania and South Africa is the center of origin for the kafir lines, and zera zera sorghum varieties predominate near the Ethopian-Sudanese border in Eastern Africa (*Lost Crops of Africa: Volume I: Grains* 1996). IS3620C (PI 659986) is a guinea line derived from a tropical, west African, photoperiod sensitive line, that was converted to a temperate photoperiod insensitive by crossing to BTx406 and selecting from multiple backcrossing events (Burow et al. 2011). IS3620C has been observed to produce basal tillers, loose panicles, medium to tall stature, small seed and low yield.

Germplasm was obtained from the USDA-ARS Plant Genetic Resources Conservation Unit, Griffin, Georgia. All 430 RILs and the two parental lines were self-pollinated and bulked for seed increase in the greenhouse in Fort Collins, Colorado from September 2015 to May 2016. All subsequent studies were conducted using this generated seed. A subset of randomly selected lines with good plant growth and seed set were selected for evaluation. A total of 252 and 124 RILs and parental lines were evaluated in 2016 and 2017, respectively. In each year, the RILs and parental lines were grown in a split plot design in which main plots were moisture treatments and subplots were genotypes, from June-October in 3 m (2016) and 4 m (2017)

single-row furrow plots. Field design consisted of three subplots each containing a random

arrangement of genotypes planted in a single row. Three subplots comprised each main plot

moisture treatment, either dryland or irrigated. The dryland treatment received only rainfall

and the irrigated treatment received furrow irrigation as needed throughout the season in

addition to natural rainfall.

The plants were grown in a Rocky Ford silty clay loam ("Official Soil Series Descriptions"

2010) at the Arkansas Valley Research Center, Rocky Ford, Colorado. Seeds were mechanically

planted at a rate of 0.17 seeds/inch in 10-foot single row sections at 0.76 m spacing between

furrows. Weeds were controlled by tractor cultivation until four weeks of growth, followed by

manual weeding throughout the season.

*Trait Screening*

The population was evaluated for six above ground morphological traits under varying

soil moisture for two growing seasons (Table 2.2). At plant maturity, a 0.5 m section of plant

material was harvested at the soil surface from the center of each plot. In 2016, each cut

section was then bundled in clear plastic and transported to an air-conditioned processing

facility. Samples were processed within two weeks of harvest and were evaluated for the

following traits: plant height, stem diameter, panicle weight, dry shoot weight, and harvest

index (panicle weight/ dry shoot weight + panicle weight), and total stem count. For each cut

section, biomass was separated into panicles and stems.  Plants were weighed after eight days

of drying in ovens at 35 °C. In 2017, height, stem diameter, and total stem count were

measured in the field. Samples were separated in the field and weighed after drying. In 2016

stand count, the number of total stems, was recorded while in 2017 the distinction between plant count and tiller count specific to each plant was recorded. Plant height and stem diameter were measured on three random plants within the 0.5 m section. Height was measured from the soil surface to the top of the panicle and stem diameter was measured between the first and second nodes using a digital caliper and rounded to the nearest 0.5 mm value. In 2016, flowering time was estimated based on previous studies and the drought condition (Brown et al. 2006). In 2017, flowering time was recorded as days after planting when 50% or more of the plot started shedding pollen, with an accuracy ±2 days. In 2017, field data was recorded using the smartphone application *Fieldbook* (Rife and Poland 2014).

***'Shovelomics' field evaluation of 10 selected genotypes***

Based on 2016 harvest index values, eight lines were selected from the BTx623 X IS3630C RIL population for either an increase (RIL 203, 268, 399, 54) or decrease (RIL 179, 198, 304, 356) in harvest index when comparing the dryland treatment to the irrigated treatment (Figure2.2). The remaining two genotypes were the population parents (RIL 1, IS3620C and RIL 2, BTx623). In the 2017 field trial using the same design as the 2016 field trial, three plants each of 10 selected genotypes in each moisture regime were evaluated (Table 2.1) with the shovelomics technique (Trachsel et al. 2011). To characterize a fully-developed root structure using shovelomics, plants were extracted from a 20 cm depth using a shovel. Plants were carefully washed with a hose to remove soil from the root system. The crown root angle, root system diameter, and root count in addition to shoot morphological traits including plant height, tiller count, panicle weight, dry shoot weight and harvest index, were observed for three plants

genotype$^{-1}$ treatment$^{-1}$ for comparison under different moisture environments. Crown root

angle and diameter were determined using a maize shovelomics scoreboard (Lynch 2018). The

crown root angle was determined as the degrees from the soil surface to the outer most crown

roots. Root diameter was measured as the width of the root system at a 10 cm depth. Plant

height was measured from the soil surface to the tip of the panicle. All shoot biomass was

harvested and placed in drying ovens (35 °C) for at least 8 days to achieve a stable weight and

weighed.


***Greenhouse evaluation of pre-flowering drought stress response***

The same selected 10 genotypes were evaluated for plant response to pre-flowering

drought in both above- and below-ground morphological traits in a controlled greenhouse

environment at the Colorado State University Plan Growth Facilities, Fort Collins, Colorado

(Table 2.1). Each of the 10 genotypes were germinated on paper towels in a petri plate using a

systemic and contact fungicide solution (Maxim XL, Syngenta, Greensboro, NC, USA).  After 5

days (3 days dark, 2 days light), the seedlings were transplanted into pots. The pots (7.6 cm

diameter) were filled with fritted clay (Field & Fairway, Profile Products LLC, Buffalo Grove, IL,

USA), saturated with water, allowed to drain for two hours and weighed to determine 100%

field capacity. Plants were grown in a completely randomized design and received 16-hours of

combined supplemental and natural lighting, daily temperature range between 20°C - 30°C and

an average relative humidity between 50-60%. All plants were maintained at 100% field

capacity using calculations based on pot weight collected every other day. After 5 weeks, four

or five replicates of each of the 10 lines were subjected to each of two treatments: (1) a water

stressed treatment where water was withheld for a 6-day drought treatment, then returned to a 100% field capacity (severe drought stress and recovery), and (2) a well-watered treatment maintained at 100% gravimetric moisture. The use of fritted clay in a dry down experiment emulates the gradual loss of water in a field setting, leading to moderate water stress (Lovell et al. 2015).

Data was collected at two time-points, the first at day six of the drought treatment. The second destructive sampling time point, recovery, occurred nine days later. During recovery, water was added to maintain 100% field capacity in both treatments. Plants were evaluated for plant height, stem diameter, dry shoot weight, and dry root weight. Plant height was measured using a ruler from the soil surface to the tip of the last fully expanded leaf. Stem diameter was measured using digital calipers (Titan 23175 Electronic Digital Calipers, Star Asia, USA) and rounded to the nearest 0.5 mm. Dry weights were collected nine days after oven drying at 35 °C. Root index was calculated as dry root weight divided by total biomass dry weight (shoot + root weight). Additionally, various below-ground traits, including root surface area and tip count, were measured using WinRhizo root scanning technology (Epson Expression 1100XL, Epson America, Inc., Long Beach, CA, USA) and software (Regent Instruments, Inc. Quebec, QC, CA). At the recovery time point, green leaf area was measured using the LICOR LI-3100C leaf area meter (LI-COR, Inc., Lincoln, NE, USA).

***Statistical analysis***

RStudio was used for statistical analysis (RStudio Team 2015). The xlsx package was used to import and export data files through RStudio. The package dplyr determined trait

means and standard deviations. The car package was used to test normality assumptions and determine analysis of variance by treatment, genotype, and treatment by genotype interaction. The Hmisc package determined Spearman rank correlation coefficients and p-values. Spearman correlations were used because the data was non-normal and transformations could not achieve normality. The GGally and Mass packages were used to produce the numerical output for correlations. Standard deviations were used to display the spread of the data. Data that did not meet normality assumptions was Tukey transformed using the rCompanion package. Log2 fold changes of means between treatments were calculated in Microsoft Excel 2013 and the ggplot2 package. The lme4 package was used to compute genetic variance components and heritability for each environment separately. In this model, genotype and replication were considered random effects for each trait. $V_G$ is variance due to genotype. $V_E$ is variance due to environment as a result of differences between replications. $V_P$ is the sum of $V_G$ and $V_E$, representing total phenotypic variance for each trait. Broad-sense heritability ($H^2$) was calculated as: $1 - Error/ (V_G * n + V_E + Error)$. The packages ggplot2, reshape2, grid, gtable and knitr were used for data visualization.

Upper and lower tails are represented by the mean of the highest and lowest 10% of genotypes for a trait in a given treatment each year. In 2016 and 2017, the tails represent 25 genotypes and 13 genotypes each, respectively. Each year and treatment combination was considered an environment, totaling four environments.

RESULTS

**The target environment experiences unpredictable rainfall**

In the 2016 and 2017 field trials, 252 and 126 genotypes were evaluated (respectively) for developmental and yield traits that contribute to differential response to water (Figure 2.1). In 2016, the dry treatment received a cumulative 112 mm of water from natural rainfall (shaded in blue) (Figure 2.1a). The irrigated treatment (shaded in light purple) received over double this amount at 340 mm of water from natural rainfall and 3 irrigation events of 76 mm. In 2017 the dry treatment (shaded in blue) received one irrigation event of 76mm and 222 mm of natural rainfall, totaling 298 mm of soil water additions (Figure 2.1b). The irrigated treatment (shaded in purple) received 450 mm of water from natural rainfall and three irrigation events of 76mm. The difference in rainfall between the two years is substantial, enabling the observation of a range of responses. However multi-year validation of individual genotype responses to one environmental condition is reduced.

**Above-ground traits are variable in BTx623 X IS3620C RIL population under different moisture regimes**

The sorghum RIL subsets showed a wide range of expression in eight above ground phenotypes including panicle weight, shoot weight, harvest index, plant height, stem diameter and stand count (Table 2.2). The response of the parental lines, BTx623 and IS3620C, was variable by year and treatment. Between moisture regimes in 2016, harvest index was similar for the parental genotypes, BTx623 and IS3620C. However, BTx623 maintained a high harvest index in both treatments in 2017, while IS3620 responded with proportionally more

partitioning to non-grain biomass under the irrigated treatment. BTx623 produces consistently larger panicles than IS3620C. This is particularly evident in the 2016 and 2017 irrigated treatments where BTx623 yielded approximately 60% more in panicle weight than IS3620C.

The largest trait ranges were seen in panicle weight and shoot weight. In 2016, shoot weight was the most variable trait for both the irrigated and dryland treatments (Table 2.2a). In 2017, panicle weight was most variable in the irrigated treatment and shoot weight was most variable in the dryland treatment (Table 2.2b). The 2017 harvest index values were relatively stable, deviating only 14-18% whereas 2016 saw nearly double the variance at 33-35%. None of the trait values for the upper and lower tails between treatments were significantly different. In 2016, the upper and lower tails in both the dryland and irrigated treatments had nearly identical trait values for panicle weight, shoot weight, harvest index, plant height and diameter and differed by 0-7% (Table 2.2a). In some traits, a similar trend was seen in 2017. Harvest index, stem diameter, stand count, tillering, and flowering time differed only 10% between corresponding upper and lower tails in each treatment (Table 2.2b).

Correlation coefficients provide insight to the relative changes among traits within and between differences in water availability. Stem diameter and plant height show a negative association in all years and treatments (Figure 2.3, Table S2.1). The correlation coefficients are more pronounced in 2017 and statistically significant (Figure 2.4, Table S2.1). In 2017, harvest index and panicle weight have a positive correlation in both the dryland (r = 0.18, p-value = 0.047) and irrigated (0.25, p-value = 0.006) treatments. Harvest index and shoot weight have a strong negative correlation in both the dryland (r = -0.67, p-value < 0.0001) and irrigated (r = -0.61, p-value < 0.0001) treatments.

***Sorghum above-ground traits are heritable in different moisture environments***

Variance components and heritability differed in each environment (Table 2.3). Plant height was the most heritable trait in all environments, ranging from 0.87 to 0.93. Stem diameter and stand count were also highly heritable, with values ranging from 0.59 to 0.89 and 0.53 to 0.79, respectively. In 2017, flowering time and tiller count was also highly heritable in both environments, with values of 0.96 and 0.63-0.93, respectively. Heritability estimates were generally lower in 2016 than 2017.

***Some RILs perform better under drought conditions***

The means and standard deviations for ten traits show a wide range of responses in ten selected genotypes under two treatments in the field in 2017 (Table 2.4). Flowering time ranged from 48 to 79 days for each genotype per treatment. Harvest index was also highly variable among genotypes and ranged from 0.31 to 0.61. Despite these differences, flowering time occurred within two days between treatments and harvest index remained low. The dryland treatment harvest index was higher than the irrigated treatment by only 10%.

In 2017 RIL 268 produced 29% more roots in the dryland treatment compared to the irrigated treatment (Table 2.4). This coincided with an increased panicle weight and 36% more grain in the dryland treatment. However, this line suffered a yield penalty under surplus moisture conditions. This is also seen in 2016 where RIL 268 reduced panicle weight by 45% under normal conditions with the highest yields seen under the water limited environments (data not shown). In 2016, RILs 198 and 1 (IS3620C), also saw a fitness penalty under normal moisture conditions compared to the water-limited treatment, with panicle weight reductions

of 13% and 58%, respectively. The reaction norms from the full population exemplify this trend with IS3620C compared to the rest of lines grouped by tails or full averages, with the driest conditions resulting in the highest yield for the guinea parent (Figure 2.5).

***Root and shoot traits respond differently to varying moisture regimes***

In the 2017 field evaluation of the 10 selected genotypes, harvest index tends to maintain a strong positive correlation between treatments ($r = 0.85$, p-value < 0.002). Crown root angle and harvest index were negatively correlated ($r = -0.64$, p-value = 0.028) in the dry treatment, however this relationship fell apart without water limitation ($r = -0.15$, p-value = 0.69). In the wet treatment, panicle weight showed a strong positive correlation with harvest index ($r = 0.76$, p-value = 0.011) while shoot weight showed a moderate negative correlation ($r = -0.57$, p-value = 0.08).

The trait means for the six traits of ten selected sorghum RILs grown under different moisture treatments in the greenhouse also varied (Table 2.5). The data was collected at two different time points, peak drought stress (day 6) and recovery, nine days following peak drought stress (day 15). RILs 1, 2, 198, 54, 203 and 268 saw an increase in root index in the dry treatment but at recovery the trend nearly flips, with only RILs 1, 2, 304 and 356 increasing root index after drought stress. Genotype 1, the parental line IS3620C, is the only genotype that maintains the same root index at peak drought stress and recovery. Root indices show a positive trend between treatments at day 6 but virtually no association between treatments at day 15, the recovery time point.

Root tip count was variable among the ten genotypes at the two time points. At peak water deficit, day 6, RILs 198 and 399 increase their root tip count compared to the irrigated control. At the recovery time point all genotypes increased root tip count and saw higher values in the irrigated treatment except RIL 1, IS3620C. The average root area of day 6 at the end of the drought treatment is not correlated with root tip count (Figure 2.4). However, at day 15 average root area is positively correlated with tip count (r = 0.64, p-value = 0.025). In 9 out of 10 RILs, stem diameter was smaller under water deficit.

The stem diameter in the irrigated treatment for RIL 268 is twice the stem diameter in the water stressed treatment. At the recovery time point, the irrigated treatment tends to maintain a higher stem diameter. RIL 2 and 178 see an increase in stem diameter in drought recovery, which matches an increase in average root area. RIL 198 also responds with an increased stem diameter upon recovery from drought, but no associated trends are seen in root growth.  Between greenhouse and field data, average crown root angle is positively correlated with average root area at six days of water stress (r =0.64, p-value = 0.048).

DISCUSSION

In this study, a subset of the RIL population BTx623 X IS3620C was evaluated for above ground traits under different moisture levels in the field over two years. Additionally, 10 selected genotypes, including the parental lines, were evaluated for root traits in response to varying moisture regimes in both the greenhouse and field setting. To our knowledge, this population has not yet been characterized for response to different moisture environments. Correlation matrices provide insight to the associations between traits and an indication of the

relative importance of root and shoot response to water availability. The results of this study

provide a foundation to guide future experiments to understand the complex response of

sorghum to variable water conditions.

Environments were separated by treatments and years due to the large differences in

precipitation between years (Figure 2.1). Although the irrigation applications were consistent

year to year, the amount of rainfall was variable, with the dry treatment in 2017 receiving

moisture from rainfall reaching 93% of water received in the 2016 irrigated treatment.

Although the type of moisture treatment is variable among years, the difference in moisture

between treatments within a year is 50%, thus plant response to relative differences in

moisture regimes is consistent year to year. Without a water limited treatment in 2017,

genotype by environment interaction was not significant for traits evaluated in the entire

population (data not shown).


***In high moisture environments, total stem count could serve as a proxy for main stem and
tiller number***

Tillering, or the growth of secondary stems, is an important trait in grasses in that it has

the potential to increase grain yields and biomass production (Belhassen 1996). Early-season

water deficit may reduce yield in the main stem, even after the availability of water later in the

season. Tillers exhibit rapid growth upon rehydration following water deficit and this recovery

could result in drought tolerance in target environments. Under water deficit however, the

additional biomass results in increased leaf area at the expense of normal growth and

development, and ultimately reduces final yield.

Although tiller number may provide insight to drought-adaptive responses in sorghum, phenotyping this time-sensitive trait requires a substantial amount of resources and time to carry out precise data collection by manually counting tiller numbers per plant (Berger, de Regt, and Tester 2009). Measuring only stems at harvest could eliminate one extra step in the phenotyping process without compromising interpretations of plant response. In 2017 the plant to tiller ratio and stand count are positively correlated (p-values <0.001) in dryland and wet treatments with r values of 0.73 and 0 .67, respectively (Figure 2.3, Table S2.1). This suggests total stem count could serve as a proxy for plant to tiller ratio and save time and resources in data collection in environments with non-stressed moisture levels. However, previous studies have shown this trait is more variable under water-limited conditions. Under water deficit, maximum tiller number is strongly reduced in wheat (Cone, Slafer, and Halloran 1995; Steinemann et al. 2015) and rice (Sarvestani et al. 2008). This may not be the case in all sorghum varieties because tillering is highly plastic phenotype (Wani, Albrizio, and Vajja 2012). Sorghum may delay tiller production under water stress, but depending on genotype tillers will still be produced.

***Plant height, stem diameter, tiller number and flowering are consistently heritable under dryland and irrigated field conditions***

High heritability estimates for plant height, stem diameter, tiller number and flowering time were seen in this experiment and have been recorded in other studies of sorghum (Murray et al. 2008; Shehzad and Okuno 2015; Brown et al. 2006), maize (Liu, Cai, and Chu 2011) and rice (Ahmad et al. 2015; Jahn et al. 2011). Panicle weight heritability estimates across the four

environments were highly variable (Table 2.3) and although yield components are commonly

calculated to have high heritabilities, inconsistencies in these estimates have been found in

other grasses (Kuchel et al. 2007; Tefera and Peat 1997). In general, heritability in the

population field trials was lower in 2016 than in 2017. This could be a result of many factors,

including the effect of high insect pressure throughout the season and moderate hail damage

occurring at the boot stage (Gerik, Bean, and Vanderlip 2000). The latter is most likely the cause

for low heritability estimates in that hail specifically damages biomass and photosynthetic

ability, both of which directly influence shoot weight, panicle weight and harvest index (Table

2.3). The error calculated on all values was proportionally high and is likely a result of

uncontrollable environmental factors as mentioned above, unaccounted variation in phenology

and plant number per replication.


***Changes in harvest index between treatments depends on the moisture environment and***

***plasticity in biomass and panicle growth***

In grasses, harvest index is a ratio used to quantify relative partitioning to the seed head

versus the remaining above-ground biomass and is a measure of reproductive efficiency

(Unkovich, Baldock, and Forbes 2010). This ratio is affected to varying degrees by many

environmental conditions and is particularly responsive to moisture availability. Here, change in

harvest index in the subset of the 10 selected genotypes is more likely a result of partitioning

differences to the panicle, not the shoot (Figure 2.4, Table S2.3). In the irrigated treatment,

panicle weight has a strong positive correlation with harvest index (r = 0.76, p-value = 0.011)

while shoot weight has a moderate negative correlation (r = -0.57, p-value = 0.086). Although

not significant, the same trend is seen in the dry treatment. This suggests that with sufficient moisture, biomass production fluctuates more than panicle weight. This is in contrast to high moisture conditions where plasticity in biomass lessens as more assimilates are shuttled to the panicle. This is contrary to the full RIL population evaluated in 2017, where harvest index and shoot weight are more strongly correlated than harvest index and panicle weight in both treatments (Figure 2.3, Table S2.1). This association where shoot weight is more variable under different moisture regimes is also seen between the upper and lower tails in each environment. Shoot weight has an average 3.4 fold reduction between the lines in the upper and lower tail across all environments (Table 2.2). Alternatively, panicle weight is reduced by an average 2.1 fold from upper to lower tail values in all environments. This suggests moisture regime affects assimilate partitioning to both panicles and the remaining above-ground biomass, but the changes in remaining non-grain biomass is more plastic within this population. The variability in harvest index under different moisture regimes is also seen in studies of sorghum (A. Blum et al. 1992), barley (Thomas and Fukai 1995) and wheat (Unkovich, Baldock, and Forbes 2010) with the index influenced by varying degrees of change in biomass and panicle weight.

***Sorghum trait values generally respond the same to varying levels of adequate moisture***

With surplus rainfall comes an increased panicle weight, shoot weight, plant height and flowering time as shown in virtually all group divisions and years (Table 2.2). Stem diameter was variable between treatments, but the irrigated environment almost always resulted in a larger stem diameter. Despite these differences, the coefficient of variation was 18 or 19% in all four environments.  In the tails for each environment, the values for all traits are comparable, with

differences of 0-7% among the two upper and two lower tails each in the dry treatment. In

2017, larger differences are seen in panicle weight, shoot weight and plant height, but

differences range from 0-9% in all other traits among upper and lower tails, respectively. In

2017, correlation coefficients of the six traits collected in both years, in addition to flowering

time and tiller to plant ratio collected, verify the similar response between treatments (Figure

2.3 and Table S2.1). Most traits are positively correlated with the corresponding trait between

treatments, indicating that varying high moisture environments did not significantly influence

plant behavior. This is most pronounced with flowering time, stem diameter and plant height.

Flowering time was positively correlated between both treatments with an r = 0.92 and p-value

<0.0001 (Table S2.1), suggesting that the difference in moisture availability within a high

moisture category does not affect phenology.

In contrast, water deficit strongly influences phenology, with greater effects depending

on plant ontogeny and intensity of stress. This has been observed in many crop species

including sorghum, soybean and wheat, with some responding with early reproductive and

shorter maturation stages (Desclaux and Roumet 1996; Angus and Moncur 1977; Donatelli,

Hammer, and Vaderlip 1992). Alternatively, other crop species including sorghum (Gardner et

al. 1981; A. Blum et al. 1992; Donatelli, Hammer, and Vaderlip 1992) and wheat (Berliner and

Oosterhuis 1987; Angus and Moncur 1977) can delay the onset of flowering to avoid water

stress during the vulnerable flowering stage.

The 2016 field data collected on the full population did not include precise phenological

data, but instead predicted flowering times were estimated (Figure 2.1a) based on previous

studies where flowering time was determined based on the nature of drought stress (Brown et

al. 2006; Blum 2011a). More severe stress during early vegetative growth tends to hasten the onset of flowering and maturity, resulting in an earlier estimated time of anthesis in dryland treatment compared to the irrigated treatment (Angus and Moncur 1977; Donatelli et al. 1992). Despite the lack of phenological data, plant responses were different in 2016 as compared to the 2017 population response. Between 2016 and 2017 irrigated and dryland conditions, panicle weight and harvest index are not correlated, suggesting different plant responses year to year. In 2016, shoot weight is weakly correlated between treatments (r = 0.18, p-value = 0.0498). This suggests limited water in 2016 induces a severe response in both components of harvest index, but affects final grain yield to a higher degree. Although explicit flowering data was not recorded, the terminal drought condition affecting moisture availability during anthesis and grain fill likely influenced the aforementioned response.

The exception to this trend typically seen in crop production clearly exemplified in this in this population is the behavior of IS3620C (Figure 2.5). The guinea line shows the highest yield in the most water-limited condition among all years and treatments. IS3620C has valuable adaptive qualities to limited moisture environments, but also suffers a severe fitness penalty with the addition of water. This may be the result of survival traits outcompeting high yielding traits selected for in cultivated species.


***Under non-stress moisture environments, a vertically extensive root system is associated with a high biomass and less panicle weight***

The plant root system is essential for acquisition of water and nutrients and the architecture of plant roots can change depending on the environment (Wasson et al. 2012;

Trachsel et al. 2011). The angle of crown root growth through the soil may also indicate rooting

depth. Generally a horizontally extensive root system tends to remain closer to the soil surface

and is favorable under periodic rainfall or environments with minimal stored soil water (Ehdaie,

Layne, and Waines 2012). However, a more vertically extensive root system tends to grow to

deeper soil layers and can capitalize on stored soil moisture and the low likelihood of rainfall

throughout the growing season.  Root angle is generally measured as the angle of the root from

the soil surface, with values closer to °0, representing a shallower, horizontally-oriented root

system (Trachsel et al. 2011; J. Lynch 2018). Alternatively, a higher angle approaching 90°

indicates a steeper, vertically-oriented root system. In wheat, modelling has suggested that a

steeper root system results in deeper root growth and higher yields (Manschadi et al. 2008).  In

a comparison between varieties of wheat adapted to water-limited and -unlimited

environments, plants adapted to lower water environments have steeper root systems while

wheat varieties adapted to frequent but low rainfall had a shallower, horizontally-oriented root

system. However, other studies in wheat have found no correlation between yield and root

angle in water-unlimited environments (McDonald 2010). These results indicate this trait may

be genotype dependent and strongly influenced by soil structure which can impact root growth

and distribution at depths (White and Kirkegaard 2010).

   Root growth was shown to influence shoot growth in the 2017 shovelomics evaluation

(Figure 2.4, Table 2.5). Here, we found that harvest index has a strong positive correlation ($r^2$

=0.85, p-value < 0.01) between moisture regimes in the field, indicating difference in moisture

regimes did not significantly affect relative harvest index values (Figure 2.4, Table S2.3).

Additionally, crown root angle and harvest index were negatively correlated (r = -0.69, p-value =

0.028) in the dry treatment and this relationship falls apart without water limitation (r = -0.15, p-value = 0.69). This suggests that in high water environments, more horizontally oriented root growth (smaller crown root angle) is associated with increased partitioning to the panicle relative to the remainder of the plant.

Crown root angle and panicle weight were negatively associated in the dryland treatment (r = -0.62, p-value = 0.056), with a similar trend in the irrigated treatment (r = -0.19, p-value = 0.6). This suggests sorghum exhibits plasticity in crown root angle depending on the moisture environment. Crown root angle is more important in driving increases in grain yield under water limitations, but with sufficient soil moisture, crown root angle and root system orientation becomes less important for grain yield. Similarly, other studies have determined vertical root distribution is favorable under water deficit in sorghum (Liang et al. 2016; Mace et al. 2012), as well as maize (Grieder, Trachsel, and Hund 2014) and dry bean (Lasley 2013).

Given the increase of moisture both in the dryland and irrigated treatments in 2017 when we evaluated field root architecture, a follow-up evaluation with water deficit to determine the plasticity in root angle and depth at maturity in this population could provide valuable insight into root growth response to limited water. Further characterization of this mapping population regarding crown root angle, or more vertical or horizontal root growth, could corroborate existing or identify a new genetic basis related to this trait in sorghum (Mace et al. 2012). Controlling for different durations, intensities and timing of water limitations would result identification of specific plant response and root response unique to each genotype. In this way, the best suited phenotype will outcompete less adapted lines for a given

drought scenario. The added challenge for these studies is the consistent and thorough

evaluation of each plant root system.

***Plasticity in root tip count relative to average root area may be important for recovery***

***following water stress***

Root plasticity, the adjustment of root growth in response to the environment, may be

important for drought adaptability in response to water limitations (O'Toole and Bland 1987).

Plasticity in root systems can be expressed in response to influences of the environment and

different stresses. Among the most influential stress on root plasticity is water availability and

plant response may be essential for consistent yields among different moisture environments

(Ehdaie, Layne, and Waines 2012). Responses to low water environments can include increased

root branching at depth to increase surface area (Wasson et al. 2012; Hurd 1964), slower

seedling root elongation rates (Nicotra, Babicka, and Westoby 2002), root cavitation reversal

(Stiller, Lafitte, and Sperry 2003), smaller root vascular tissue (Nicotra, Babicka, and Westoby

2002; Boughalleb et al. 2014) and thicker root diameter (Lasley 2013). Additional environmental

stimuli can further influence root plasticity, but specific root phenotypes may help screen for

favorable drought-adapted varieties (Ehdaie, Layne, and Waines 2012).

Root plasticity is particularly important for plant recovery following early vegetative

stage drought stress. In the greenhouse experiment of the ten selected genotypes, the average

root area of water-stressed plants at the end of the treatment (day 6) is not correlated with

root tip count (Figure 2.4). However, at the recovery time point (9 days after re-water),

increasing average root area is positively correlated with root tip count ($r = 0.64$, p-value <

0.05). This suggests that under water stress root branching is restricted, particularly with very fine roots, but the root system recovers following the availability of water. In these selected genotypes, root growth returns to levels as seen in the control (Table S2.3). A parallel study with the same water limitations in the field could provide valuable information regarding greenhouse screening and the predictive capacity end of season plant performance, particularly in terms of yield and harvest index. Alternatively, an intermediate step between could involve a large-scale greenhouse study where roots are harvested an evaluated at different levels of water limitations throughout the season. The primary challenge in a greenhouse setting however, is the ability to enable unrestricted root growth caused by growth in pots, especially as sorghum reaches near maximum root size at flowering.

Average root area also shows trends when grouped by field harvest index data (Figure 2.6). Low harvest index genotypes generally increase root size under drought stress and either increase or decrease root growth at recovery. High harvest index genotypes either increase or decrease root growth under limited water but limit root growth in the droughted plants following recovery. At six days of drought stress the parental lines respond differently, with IS3620C (RIL 1) increasing root area under moisture stress and BTx623 (RIL2) decreasing average root area under water stress. Three out of four low harvest index RILs decrease average root area under drought stress, but at recovery root growth responses are variable. The high harvest index RILs respond variably, with half increasing average root area and the other half decreasing average root area to different degrees compared to the well-watered control at recovery. All the high harvest index RILs, however, either remain unchanged or substantially increase average root area upon recovery. This suggests plasticity in root growth

in response to seedling water deficit is valuable for recovery, and subsequently a higher harvest

index at maturity as seen in other crops such as drought tolerant dry bean (Lasley 2013).

The variable results in root size and architecture compared to above ground biomass, as

well as root plasticity in response to drought stress, suggest that below-ground phenotypes

must be evaluated in conjunction with above-ground traits. Shoot size provides an indication of

root behavior underground but is insufficient to serve as a proxy for root behavior diverse

genotypes, especially under stressful environmental conditions.

***Together, stem diameter and root morphology may play a role in limited water environments***

Stem diameter size in sorghum (Ramesh and Mahadevaswamy 2000), sugarcane (Silva

et al. 2008), tamarind (Van den Bilcke, Simbo, and Samson 2013) and woody plants (Ortuño et

al. 2010; Ogigirigi, Kozlowski, and Sasaki 1970; Miralles-Crespo et al. 2010) is reduced under

water deficit. In *Miscanthus*, variation in stem composition may specifically affect vascular

tissue under water deficit (van der Weijde et al. 2017). In that study, all but one cellulosic

component was reduced under moisture stress in 49 *Miscanthus* genotypes. Below ground,

root length and biomass is reduced in *Albizzia*, *Erythrina*, *Eucalyptus microthec*a, *Populus*

species, *Avocado* and pearl millet, as described by Shao et al. (2008). Further, perennial plants

adapted to low rainfall environments have smaller root vascular components compared to

higher rainfall adapted species (Nicotra, Babicka, and Westoby 2002).

Under limited water in the greenhouse, stem diameter decreases or remains unchanged

in the 10 selected RILs. The greatest diameter plasticity is seen in RIL 268 with a 50% decrease

in stem diameter. RIL 268 responded strongly to the stressful environment but was able to

quickly recover when the water was added back to the system. The variation in stem diameter is less pronounced and minimally variable among the other evaluated genotypes, suggesting water-limited driven plasticity in stem diameter is generally low, but this depends genotype (Table 2.5).

Root tip count, as an indication of root growth and extent, is reduced under drought stress in eight out of ten RILs (Table 2.5). Average root area, a metric of total biomass, is both reduced and increased under drought stress and is continually variable among the 10 genotypes following re-water (Figure 2.5). Despite these inconsistencies, root tip count and stem diameter are positively correlated in the dry treatment at peak drought stress (r = 0.79, p-value = 0.065) and recovery (r = 0.78, p-value = 0.075). Average root area and stem diameter under drought stress show a similar trend (r = 0.52, p-value = 0.12) but average root area exhibits no plasticity between time points.

This suggests drought adaptability under low-water environments is in part a result of stem and root size. Inherently smaller systems or plasticity in stem or root size may be favorable for a variety of limited water conditions. As seen in the recovery time points, recovery of stem size and the root system may be just as critical for plant productivity when favorable conditions occur as when plants are continually growing under water deficit. Further evaluation of root and stem anatomy is required to identify the specific morphological changes occurring within the plant tissues (Boughalleb et al. 2014). Reduced vessel size, increased vessel thickness or other characteristics of vascular tissue may be responsible for diameter plasticity in sorghum in limited water environments.

*Concluding Remarks*

Evaluation of genotypically and phenotypically diverse sorghum lines enables the elucidation of plant responses to variable moisture conditions. Investigations of plant response to water stress is continually evolving as experimental design is changed to improve data resolution and minimize confounding elements. In this study, further evaluation of genotypes within this population would benefit from using genotypes with a similar flowering time or monitoring flowering time closely throughout the season as was done in the 2017 field trial. This would ensure that any conclusions made regarding drought response are not confounded by phenology, but in fact are the result of stress exposure during comparable developmental stages (Farooq et al. 2012). Additionally, planting 3- or 4-row plots instead of single-row plots, would minimize effects from unique neighbor associations and the center plot(s) would behave as if in a large, single variety field setting (Hurlbert 1984). Planting more seeds per plot and culling to ensure consistent plant density among replications would also reduce unintended environmental influences. Seed limitations restricted multiple row plots and increasing seeding rate for this study. Harvesting a select number of plants per plot rather than the plants contained within a pre-determined distance may improve data resolution and minimize confounding, particularly when using lines with a low seed count and an unknown germination success rate. This would increase quality of phenotypic data and downstream estimations, hasten the data collection process, and lessen error when working with a team.

Another valuable tool in terms of the moisture environment, would be the use of soil moisture probes throughout the season. This would provide a better estimate of soil water available for plant use rather than relying on water inputs as the only metric, allowing more

repeatable and interpretable experiments (Jones 2006). The use of lysimeters would also enable determinations of evapotranspiration and more detailed evaluation of crop response to varying moisture levels (Berliner and Oosterhuis 1987). Furthermore, technologies such as pivot or drip irrigation and rainout shelters would more uniformly supply irrigation water or prevent undesired rainfall, respectively, during the field study (Blum 2011c). Furthermore, precise control and tracking of soil moisture would enable the evaluation of different types of drought stress, depending on frequency, intensity and duration of soil water additions throughout the season. This level of control could assist breeders by elucidating traits uniquely suited for a specific moisture environment.

The research included in this thesis has set the stage for further investigation on this population exposed to unique moisture environments. This population has never previously been evaluated for response to variable moisture environments and shows a wide range of morphological response to different moisture regimes in both the field and greenhouse. There is large phenotypic variation for both above ground and below-ground response to moisture environments, with some genotypes expressing a large degree of variation for a particular traits than others with less plasticity. The genetic diversity between the RIL population parents enables the expression of highly variable gene combinations. This transgressive segregation not only provides unique combinations of evaluated phenotypes, but often this results in trait values exceeding those of the parents (Figure 2.2, Figure 2.5). These relatively extreme phenotypes valuable in that traits of interest, particularly for performance under a given environmental stress, can be assessed for differences in their individual genotype.

Root system architecture of 10 phenotypically diverse genotypes was characterized and provides some clues on how pre-flowering drought stress affects root behavior. The evaluation of a larger bulked sample of the population under consecutive years of drought stress may provide more clues into the adaptive capacity of sorghum to withstand limited water environments. Ultimately, traits related to high yield under unique moisture environments can be used in breeding programs to improve sorghum production or incorporated into programs improving other crops less adapted to water limited environments.

## TABLES AND FIGURES

**Table 2.1** RIL subsets evaluated for various traits at different locations, planting dates, planting structures, phenotyping collection dates, number of replications, and sampling subplot.

| Population | RILs (*n*) | Planting | | | Phenotyping | | | |
| | | Location | Date | Structure | Date(s) | Reps. | Sample subplot | Evaluated traits |
|---|---|---|---|---|---|---|---|---|
| BTx623 X IS3620C $F_8$-$F_{11}$ | 252 | Field Rocky Ford, CO | 06/22/2016 | Single-row plot | 10/20/2017-11/03/2017 | 3 | 0.5 m, center | plant height (PH) stand count (stand) stem diameter (D) panicle weight (PW) dry shoot weight (SW) |
| | 126 | Field Rocky Ford, CO | 06/01/2017 | Single-row plot | 09/15/2017, 09/30/2017-10/01/2017, 10/07/2017 | 3 | 0.5 m, center | plant height (PH) stand count (stand) stem diameter (D) panicle weight (PW) dry shoot weight (SW) flowering time (F) tiller:plant (Till:Plt) |
| | 10 | Field Rocky Ford, CO | 06/01/2017 | Single-row plot | 09/16/2017 | 3 | 1 plant, center | plant height (PH) stand count (stand) stem diameter (D) panicle weight (PW) dry shoot weight (SW) flowering time (F) tiller:plant (Till:Plt) crown root angle (CRA) root diameter (RD) root tip count (RC) |
| | 10 | Greenhouse Fort Collins, CO | 07/10/2017 | One per pot | 08/07/2017, 08/15/2017 | 5 | 1 plant | plant height (PH) stem diameter (D) dry shoot weight (SW) leaf area (LA) root dry weight (RW) root surface area (RSA) root tip count (RC) |

*Field row plots were mechanically planted in furrows at 3m intervals spaced at 0.76 m between rows.

**Table 2.2** Trait means (X̄) and standard deviations (SD) of BTx623, IS3620C, upper and lower tails (10%) of the recombinant inbred lines (RILs), the RILs and trait ranges and coefficients of variation of the RILs for each moisture regime in two years.

(a) Data for 6 shoot traits for dryland and irrigation treatments in 2016

| Traits | 2016 Irrigated Treatment | | | | | | | 2016 Dryland Treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BTx623 | IS3620C | RIL Upper Tail | RIL Lower Tail | RIL Population | | | BTx623 | IS3620C | RIL Upper Tail | RIL Lower Tail | RIL Population | | |
| | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | Range | CV (%) | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | Range | CV (%) |
| PW | 388 ± 281 | 237 ± 66 | 491 ± 48 | 213 ± 19 | 334 ± 133 | 44-934 | 39 | 356 ± 0 | 404 ± 31 | 472 ± 41 | 196 ± 31 | 325 ± 128 | 36-771 | 39 |
| SW | 526 ± 162 | 524 ± 317 | 878 ± 107 | 272 ± 36 | 528 ± 264 | 88-1574 | 50 | 907 ± 239 | 563 ± 204 | 900 ± 94 | 274 ± 34 | 551 ± 288 | 96-2056 | 52 |
| HI | 0.36 ± 0 | 0.36 ± 0.16 | 0.56 ± 0.03 | 0.26 ± 0.03 | 0.41 ± 0.13 | 0.07-0.81 | 33 | 0.40 ± 0.23 | 0.43 ± 0.07 | 0.56 ± 0.04 | 0.25 ± 0.04 | 0.39 ± 0.14 | 0.04-0.83 | 35 |
| PH | 109 ± 5 | 122 ± 7 | 199 ± 13 | 81 ± 6 | 137 ± 34 | 70-243 | 26 | 115 ± 17 | 131 ± 18 | 187 ± 12 | 77 ± 4 | 128 ± 34 | 65-219 | 26 |
| D | 23 ± 1 | 13 ± 2 | 20.5 ± 3 | 11 ± 1 | 15 ± 3 | 9-32 | 19 | 16.5 ± 4 | 14.5 ± 3 | 19.5 ± 1 | 10 ± 1 | 14.5 ± 2.5 | 8.5-22 | 18 |
| Stand | 6.3 ± 1.5 | 13.6 ± 3.5 | 15.5 ± 1.2 | 7 ± 0.9 | 11 ± 2.4 | 5-17.6 | 22 | 10 ± 2 | 18 ± 4.4 | 22.8 ± 3.5 | 7.5 ± 1.3 | 14.6 ± 2.1 | 4-34 | 14 |

(b) Data for 8 shoot traits for dryland and irrigation treatments collected in 2017

| Traits | 2017 Irrigated Treatment | | | | | | | 2017 Dryland Treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BTx623 | IS3620C | RIL Upper Tail | RIL Lower Tail | RIL Population | | | BTx623 | IS3620C | RIL Upper Tail | RIL Lower Tail | RIL Population | | |
| | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | Range | CV (%) | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | X̄ ± SD | Range | CV (%) |
| PW | 620 ± 92 | 387 ± 30 | 469 ± 150 | 237 ± 54 | 352 ± 278 | 26-996 | 79 | 407 ± 60 | 263 ± 35.1 | 355 ± 76 | 222 ± 39 | 294 ± 90 | 100-640 | 30 |
| SW | 667 ± 285 | 577 ± 25 | 701 ± 69 | 186 ± 32 | 245 ± 94 | 80-1140 | 38 | 333 ± 75.7 | 427 ± 227 | 536 ± 64 | 151 ± 22 | 294 ± 132 | 80-1190 | 45 |
| HI | 0.49 ± 0.07 | 0.42 ± 0.1 | 0.60 ± 0.02 | 0.33 ± 0.04 | 0.53 ± 0.08 | 0.06-0.71 | 14 | 0.55 ± 0.02 | 0.38 ± 0.42 | 0.63 ± 0.02 | 0.36 ± 0.03 | 0.5 ± 0.09 | 0.25-0.71 | 18 |
| PH | 140 ± 6 | 184 ± 6 | 212 ± 14 | 109 ± 10 | 160 ± 31 | 91-244 | 19 | 133 ± 0.5 | 165 ± 8 | 194 ± 11 | 95 ± 6.7 | 140 ± 29 | 85-217 | 21 |
| D | 23.5 ± 2 | 15.5 ± 1 | 22.5 ± 2 | 12 ± 0.5 | 16.5 ± 3 | 9.5-27 | 19 | 19 ± 1.5 | 16 ± 2 | 21 ± 1 | 11 ± 0.5 | 15.5 ± 3 | 10-23 | 19 |
| Stand | 10 ± 1 | 24.3 ± 5.1 | 21 ± 3.7 | 8.6 ± 1 | 14 ± 4 | 6.7-32 | 28 | 9 ± 1.7 | 17 ± 3.6 | 20 ± 3.5 | 8 ± 0.8 | 12.7 ± 3.5 | 6-28 | 27 |
| Plt:till | 1.2 ± 1 | 5.1 ± 1.3 | 5.2 ± 0.6 | 1.1 ± 0.2 | 2.9 ± 1.2 | 0.5-6.4 | 42 | 1.1 ± 0.4 | 3.6 ± 0.4 | 5.1 ± 0.8 | 1 ± 0.3 | 2.7 ± 1.1 | 0.6-6.6 | 42 |
| FT | 60 ± 1 | 77 ± 1.1 | 74. ± 6.1 | 50 ± 1.5 | 60 ± 5 | 46-87 | 9 | 61 ± 0.6 | 79 ± 5.2 | 75 ± 5.9 | 49 ± 1.5 | 59 ± 8 | 44-90 | 13 |

*PW: Panicle weight (g), SW: Shoot weight (g), HI: Harvest index (g), PH: Plant height (cm), D: Stem diameter (mm), Stand: Stand count/0.5m, Plt:till: Tillers per plant, FT: Flowering time (Days after planting).*

**Table 2.3** Genetic Variance Components and heritability for five traits in 2016 and seven traits in 2017 under varying moisture environments.

| | | | **2016** | | | | | | **2017** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Trait** | $V_G$ | $V_E$ | $V_P$ | **Error** | $H^2$ | **Trait** | $V_G$ | $V_E$ | $V_P$ | **Error** | $H^2$ |
| **Irrigated** | PH | 1075.6 | 0 | 1238.12 | 487.56 | 0.87 | PH | 823.51 | 90.73 | 975.79 | 366.11 | 0.87 |
| | SW | 7732 | 0 | 28438.67 | 62120 | 0.27 | SW | 15790 | 0 | 23456.67 | 23000 | 0.67 |
| | PW | 0 | 903.3 | 6154.37 | 17559.8 | 0.05 | PW | 8925 | 0 | 12224.33 | 9898 | 0.73 |
| | HI | 0.0001 | 0 | 0.006 | 0.02 | 0.02 | HI | 4.41E-03 | 0 | 0.006 | 0.006 | 0.69 |
| | D | 4.405 | 0 | 7.46 | 9.16 | 0.59 | D | 8.82 | 0.08 | 10.18 | 3.31 | 0.89 |
| | Stand | 2.998 | 0 | 5.61 | 7.82 | 0.53 | Stand | 10.62 | 0 | 14.56 | 11.82 | 0.73 |
| | | | | | | | FT | 50.9 | 0 | 52.86 | 5.82 | 0.96 |
| | | | | | | | Plt:Till | 0.82 | 0 | 1.36 | 1.62 | 0.60 |
| **Dryland** | PH | 1038.7 | 0 | 1120.17 | 244.4 | 0.93 | PH | 754.65 | 34.55 | 845.23 | 237.18 | 0.91 |
| | SW | 2213 | 0 | 29258.67 | 81137 | 0.08 | SW | 10320 | 512.9 | 12865.9 | 7124.8 | 0.82 |
| | PW | 0 | 1440 | 5947 | 16400 | 0.08 | PW | 3877 | 939.9 | 5598.3 | 4224 | 0.75 |
| | HI | 0.0002 | 0.0006 | 0.007 | 0.02 | 0.07 | HI | 0.005 | 0 | 0.006 | 0.003 | 0.84 |
| | D | 4.28 | 0.9902 | 6.85 | 6.71 | 0.67 | D | 7.46 | 0 | 8.76 | 3.89 | 0.85 |
| | Stand | 12.51 | 0.033 | 18.1 | 16.43 | 0.70 | Stand | 9.63 | 0 | 12.24 | 7.82 | 0.79 |
| | | | | | | | FT | 53.91 | 0.20 | 55.96 | 5.95 | 0.96 |
| | | | | | | | Plt:Till | 0.857 | 15.38 | 6.47 | 1.45 | 0.93 |

Each year x moisture regime is considered an independent environment.

*$V_G$: Genetic variance, $V_E$ = Environmental variance, $V_P$ = Phenotypic variance, $H^2$ = Broad-sense heritability, PH: Plant height (cm), SW: Shoot weight (g), PW: Panicle weight (g), HI: Harvest index, D: Stem diameter (mm), Stand: Stand count/0.5m, F: Flowering time (days after planting), Plt:Till: Tiller count per plant.*

**Table 2.4** Trait means and standard deviations of traits from 10 selected sorghum RILs grown in the field in Rocky Ford, CO under two moisture regimes.

| RIL | Treatment | PH | D | CA | RD | RC | RC:Till | SW | PW | HI | FT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Dryland | 143 ± 11 | 16.4 ± 3.3 | 38 ± 8 | 29 ± 3 | 76 ± 22 | 36 ± 28 | 86 ± 31 | 47 ± 6 | 0.36 ± 0.06 | 79 ± 1 |
| 1 | Irrigated | 129 ± 22 | 14.7 ±3.8 | 42 ± 6 | 28.3 ± 4.5 | 190 ± 87 | 23 ± 3 | 253 ± 75 | 160 ± 62 | 0.38 ± 0.03 | 77 ± 5 |
| 2 | Dryland | 139 ± 20 | 19 ± 2.7 | 35 ± 6 | 24 ± 4.4 | 44 ± 6 | 44 ± 6 | 103 ± 6 | 133 ± 15 | 0.56 ± 0.03 | 61 ± 1 |
| 2 | Irrigated | 113 ± 5 | 24.4 ± 1.6 | 38 ± 8 | 26 ± 3.6 | 89 ± 26 | 30 ± 5 | 193 ± 57 | 257 ± 75 | 0.57 ± 0.02 | 60 ± 1 |
| 54 | Dryland | 120 ± 6 | 10.4 ± 2.7 | 37 ± 9 | 24 ± 1.5 | 83 ± 63 | 11 ± 3 | 70 ± 66 | 77 ± 67 | 0.56 ± 0.09 | 48 ± 1 |
| 54 | Irrigated | 121 ± 5 | 10.8 ± 0.6 | 40 ± 4 | 22 ± 3.1 | 102 ± 12 | 10 ± 1 | 116 ± 21 | 90 ± 26 | 0.43 ± 0.05 | 49 ± 1 |
| 178 | Dryland | 230 ± 7 | 15.5 ± 3.1 | 39 ± 1 | 26 ± 2.3 | 76 ± 40 | 38 ± 15 | 160 ± 79 | 73 ± 42 | 0.32 ± 0.07 | 62 ± 2 |
| 178 | Irrigated | 229 ± 15 | 16.5 ± 0.3 | 31 ± 8 | 30 ± 5 | 101 ± 98 | 25 ± 9 | 243 ± 78 | 110 ± 26 | 0.31 ± 0.02 | 65 ± 2 |
| 198 | Dryland | 129 ± 8 | 15.4 ± 1.4 | 44 ± 3 | 22 ± 2.5 | 76 ± 20 | 16 ± 9 | 130 ± 36 | 110 ± 20 | 0.46 ± 0.03 | 62 ± 1 |
| 198 | Irrigated | 154 ± 40 | 14.8 ± 1.4 | 43 ± 8 | 25 ± 1.5 | 99 ± 28 | 31 ± 31 | 187 ± 113 | 140 ± 98 | 0.40 ± 0.09 | 64 ± 1 |
| 203 | Dryland | 163 ± 7 | 14.6 ± 1.1 | 36 ± 5 | 29 ± 11.9 | 101 ± 50 | 20 ± 6 | 170 ± 53 | 177 ± 90 | 0.50 ± 0.05 | 61 ± 1 |
| 203 | Irrigated | 158 ± 12 | 14.8 ± 0.7 | 36 ± 6 | 24 ± 1 | 119 ± 22 | 25 ± 4 | 193 ± 35 | 197 ± 15 | 0.51 ± 0.03 | 62 ± 2 |
| 268 | Dryland | 170 ± 8 | 16.4 ± 1.5 | 37 ± 5 | 28 ± 3 | 129 ± 22 | 31 ± 8 | 240 ± 82 | 167 ± 47 | 0.41 ± 0.02 | 63 ± 3 |
| 268 | Irrigated | 203 ± 6 | 15.8 ± 2.5 | 36 ± 6 | 26 ± 5.2 | 92 ± 21 | 24 ± 4 | 190 ± 53 | 107 ± 15 | 0.37 ± 0.09 | 65 ± 3 |
| 304 | Dryland | 100 ± 17 | 17.7 ± 5.1 | 33 ± 9 | 23 ± 3.6 | 86 ± 39 | 26 ± 8 | 127 ± 57 | 217 ± 146 | 0.61 ± 0.06 | 58 ± 2 |
| 304 | Irrigated | 91 ± 13 | 21.5 ± 2 | 31 ± 6 | 23 ± 5.1 | 95 ± 26 | 33 ± 8 | 133 ± 40 | 213 ± 108 | 0.59 ± 0.08 | 57 ± 3 |
| 356 | Dryland | 150 ± 25 | 20.1 ± 2.2 | 50 ± 0 | 27 ± 3.1 | 90 ± 19 | 39 ± 6 | 140 ± 56 | 77 ± 40 | 0.34 ± 0.03 | 79 ± 2 |
| 356 | Irrigated | 168 ± 53 | 17.3 ± 3.5 | 40 ± 5 | 27 ± 5.1 | 121 ± 48 | 27 ± 12 | 237 ± 49 | 167 ± 64 | 0.41 ± 0.06 | 78 ± 1 |
| 399 | Dryland | 154 ± 25 | 12.1 ± 1.6 | 41 ± 1 | 22 ± 3.5 | 77 ± 34 | 27 ± 3 | 90 ± 44 | 107 ± 76 | 0.51 ± 0.1 | 62 ± 3 |
| 399 | Irrigated | 154 ± 7 | 13.7 ± 2.6 | 38 ± 7 | 24 ± 1.5 | 112 ± 47 | 35 ± 13 | 153 ± 65 | 200 ± 66 | 0.57 ± 0.03 | 64 ± 1 |

*RIL: Recombinant isogenic line, PH: Plant height (cm), D: Stem diameter (mm), CA: Crown root angle (degrees), RD: Root diameter (cm), RC: Root count, RC:till: Root count to tiller ratio, SW: Shoot weight (g), PW: Panicle weight (g), HI: Harvest index, F: Flowering time (days after planting).*

**Table 2.5a** Trait means and standard deviations of traits from 10 selected sorghum RILs grown in the greenhouse under two moisture regimes at two time points.

| RIL | Treatment | Day | ARA | | SD | | PH | | TC | | RI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | X̄ | SD | X̄ | SD | X̄ | SD | X̄ | SD | X̄ | SD |
| 1 | Dryland | 6 | 197 ± 177 | | 1.86 ± 0.75 | | 20.3 ± 9 | | 1159 ± 828 | | 0.47 ± 0.07 | |
| 1 | Irrigated | 6 | 249 ± 84 | | 2.36 ± 0.15 | | 23.4 ± 1.9 | | 2587 ± 735 | | 0.44 ± 0.09 | |
| 2 | Dryland | 6 | 245 ± 48 | | 2.78 ± 0.58 | | 30.5 ± 6.9 | | 2600 ± 409 | | 0.38 ± 0.08 | |
| 2 | Irrigated | 6 | 229 ± 65 | | 3.14 ± 0.73 | | 31.8 ± 3.3 | | 2836 ± 643 | | 0.31 ± 0.06 | |
| 54 | Dryland | 6 | 160 ± 80 | | 1.55 ± 0.29 | | 17.7 ± 6.1 | | 1087 ± 275 | | 0.41 ± 0.11 | |
| 54 | Irrigated | 6 | 147 ± 48 | | 1.87 ± 0.76 | | 18.5 ± 6.7 | | 1703 ± 961 | | 0.38 ± 0.11 | |
| 178 | Dryland | 6 | 140 ± 70 | | 1.55 ± 0.72 | | 19.5 ± 7.5 | | 1290 ± 727 | | 0.41 ± 0.1 | |
| 178 | Irrigated | 6 | 149 ± 64 | | 1.96 ± 0.23 | | 22.7 ± 2.4 | | 1749 ± 412 | | 0.42 ± 0.06 | |
| 198 | Dryland | 6 | 204 ± 85 | | 1.97 ± 0.29 | | 19.6 ± 2.4 | | 2250 ± 1795 | | 0.44 ± 0.07 | |
| 198 | Irrigated | 6 | 175 ± 115 | | 2.70 ± 0.54 | | 22 ± 2.9 | | 1974 ± 678 | | 0.37 ± 0.1 | |
| 203 | Dryland | 6 | 175 ± 108 | | 2.29 ± 1.09 | | 26 ± 9.4 | | 1721 ± 1076 | | 0.36 ± 0.06 | |
| 203 | Irrigated | 6 | 192 ± 45 | | 2.21 ± 0.7 | | 24.8 ± 5.6 | | 2259 ± 1119 | | 0.35 ± 0.09 | |
| 268 | Dryland | 6 | 180 ± 103 | | 1.70 ± 0.53 | | 19.1 ± 9 | | 1429 ± 790 | | 0.46 ± 0.06 | |
| 268 | Irrigated | 6 | 229 ± 46 | | 3.12 ± 0.82 | | 29.5 ± 6.8 | | 2870 ± 1258 | | 0.36 ± 0.03 | |
| 304 | Dryland | 6 | 150 ± 61 | | 1.83 ± 0.41 | | 20.2 ± 5.3 | | 1286 ± 217 | | 0.37 ± 0.08 | |
| 304 | Irrigated | 6 | 135 ± 35 | | 1.90 ± 0.16 | | 25.3 ± 2.6 | | 1686 ± 432 | | 0.38 ± 0.1 | |
| 356 | Dryland | 6 | 164 ± 100 | | 2.28 ± 0.28 | | 24.6 ± 2.8 | | 1504 ± 426 | | 0.35 ± 0.06 | |
| 356 | Irrigated | 6 | 157 ± 34 | | 2.41 ± 0.48 | | 23.4 ± 2.9 | | 1604 ± 484 | | 0.37 ± 0.1 | |
| 399 | Dryland | 6 | 155 ± 123 | | 1.99 ± 0.59 | | 21.7 ± 7.3 | | 1625 ± 1282 | | 0.39 ± 0.07 | |
| 399 | Irrigated | 6 | 146 ± 91 | | 2.14 ± 0.73 | | 19.5 ± 6.9 | | 1469 ± 585 | | 0.42 ± 0.07 | |

Time points represent the day of sampling after the onset of the moisture treatment. Time point 6 represents peak drought stress and time point 15 represents recovery, nine days of normal water following drought stress. *Average root area: ARA (cm², Stem diameter: SD (mm), Plant height: PH (cm), Root tip count: TC, LA: Leaf area (cm²), RI: Root index (root weight/shoot + root weight).*

**Table 2.5b** Trait means and standard deviations of traits from 10 selected sorghum RILs grown in the greenhouse under two moisture regimes at two time points.

| RIL | Treatment | Day | ARA | | SD | | PH | | TC | | LA | | RI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | X̄ | SD | X̄ | SD | X̄ | SD | X̄ | SD | X̄ | SD | X̄ | SD |
| 1 | Dryland | 15 | 329 ± 46 | | 2.82 ± 0.45 | | 26.6 ± 3.2 | | 4298 ± 1226 | | 37.74 ± 7.31 | | 0.40 ± 0.03 | |
| 1 | Irrigated | 15 | 295 ± 49 | | 3.11 ± 0.14 | | 27.3 ± 5.7 | | 3546 ± 994 | | 43.48 ± 18.3 | | 0.38 ± 0.03 | |
| 2 | Dryland | 15 | 336 ± 57 | | 3.35 ± 0.47 | | 29 ± 6.6 | | 4583 ± 1672 | | 50.06 ± 20.53 | | 0.39 ± 0.05 | |
| 2 | Irrigated | 15 | 330 ± 61 | | 3.19 ± 0.5 | | 28.9 ± 4.5 | | 4947 ± 1029 | | 48.64 ± 24.46 | | 0.39 ± 0.04 | |
| 54 | Dryland | 15 | 216 ± 83 | | 2.39 ± 0.48 | | 25.4 ± 7 | | 2667 ± 894 | | 28.84 ± 12.26 | | 0.33 ± 0.04 | |
| 54 | Irrigated | 15 | 253 ± 58 | | 2.45 ± 0.71 | | 22.9 ± 7.4 | | 2927 ± 1859 | | 25.58 ± 14.28 | | 0.35 ± 0.07 | |
| 178 | Dryland | 15 | 284 ± 103 | | 2.64 ± 0.61 | | 26.8 ± 8.4 | | 2708 ± 1119 | | 33.68 ± 19.3 | | 0.35 ± 0.05 | |
| 178 | Irrigated | 15 | 237 ± 115 | | 2.19 ± 0.84 | | 22.5 ± 12.5 | | 3146 ± 2512 | | 27.99 ± 20.45 | | 0.37 ± 0.04 | |
| 198 | Dryland | 15 | 237 ± 121 | | 3.43 ± 1.2 | | 22.7 ± 7.9 | | 2784 ± 2250 | | 44.13 ± 28.82 | | 0.27 ± 0.11 | |
| 198 | Irrigated | 15 | 277 ± 78 | | 2.95 ± 0.94 | | 22.3 ± 7.8 | | 3578 ± 2132 | | 40.75 ± 23.58 | | 0.32 ± 0.1 | |
| 203 | Dryland | 15 | 227 ± 60 | | 3.06 ± 0.69 | | 30.5 ± 7.1 | | 3074 ± 1304 | | 43.07 ± 20.38 | | 0.29 ± 0.03 | |
| 203 | Irrigated | 15 | 340 ± 105 | | 3.59 ± 0.52 | | 37 ± 4.6 | | 5225 ± 2881 | | 66.87 ± 18.64 | | 0.31 ± 0.03 | |
| 268 | Dryland | 15 | 311 ± 101 | | 3.14 ± 0.61 | | 27.6 ± 6.7 | | 3668 ± 2059 | | 45.14 ± 24.02 | | 0.36 ± 0.08 | |
| 268 | Irrigated | 15 | 348 ± 82 | | 3.20 ± 0.37 | | 32.8 ± 3.5 | | 5637 ± 1174 | | 53.77 ± 14.88 | | 0.39 ± 0.06 | |
| 304 | Dryland | 15 | 170 ± 42 | | 2.14 ± 0.43 | | 19.7 ± 6.3 | | 1568 ± 653 | | 19.58 ± 16.36 | | 0.40 ± 0.08 | |
| 304 | Irrigated | 15 | 148 ± 79 | | 2.38 ± 0.84 | | 19 ± 10.5 | | 1580 ± 1377 | | 20.67 ± 19.9 | | 0.30 ± 0.09 | |
| 356 | Dryland | 15 | 238 ± 61 | | 2.60 ± 0.60 | | 23.9 ± 7.9 | | 2302 ± 857 | | 31.40 ± 22.21 | | 0.36 ± 0.06 | |
| 356 | Irrigated | 15 | 270 ± 60 | | 2.99 ± 0.20 | | 24.1 ± 5.3 | | 2527 ± 909 | | 23.65 ± 16.95 | | 0.28 ± 0.08 | |
| 399 | Dryland | 15 | 315 ± 44 | | 2.66 ± 1.05 | | 31.3 ± 4.4 | | 2579 ± 529 | | 40.92 ± 2.8 | | 0.33 ± 0.06 | |
| 399 | Irrigated | 15 | 307 ± 86 | | 3.10 ± 0.6 | | 29.6 ± 8.3 | | 3520 ± 1107 | | 41.50 ± 32.25 | | 0.40 ± 0.09 | |

Time points represent the day of sampling after the onset of the moisture treatment. Time point 6 represents peak drought stress and time point 15 represents recovery, nine days of normal water following drought stress. *Average root area: ARA (cm²), Stem diameter: SD (mm), Plant height: PH (cm), Root tip count: TC, LA: Leaf area (cm²), RI: Root index (root weight/shoot + root weight).*
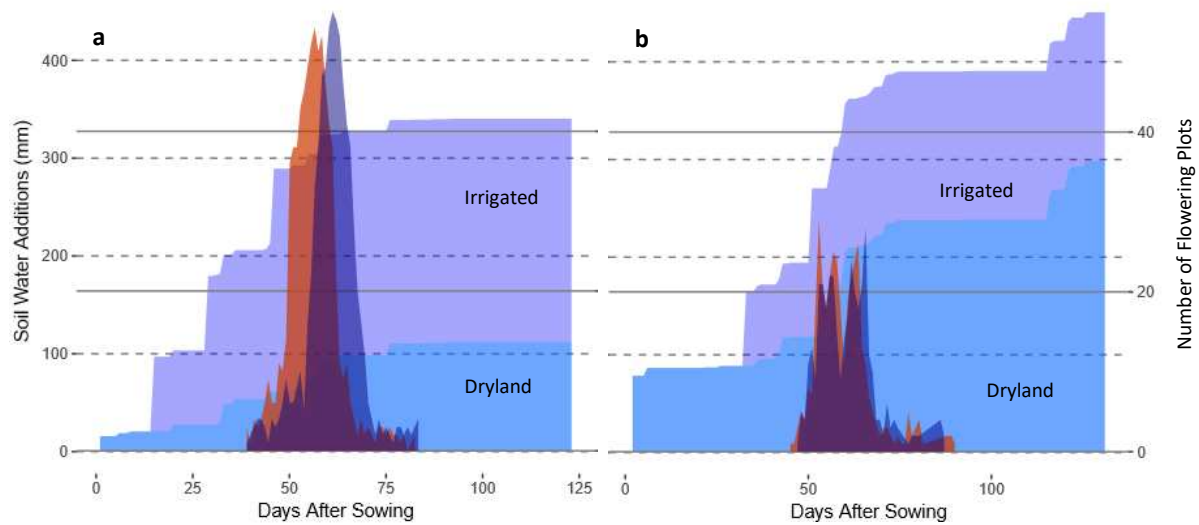
**Figure 2.1** 2016 and 2017 Soil Water Additions and Flowering Time for two moisture treatments over two years. The x-axis represents the number of days after sowing. The left y-axis represents the amount of cumulative precipitation (dryland treatment) or precipitation + irrigation (irrigated treatment). The right y-axis represents the number of plots which initiated flowering and is distinguished by dryland treatment (orange) or irrigated treatment (purple). **(a)** 2016 sorghum field trial (plots = 1487). Flowering time is estimated based on observations in Brown et al. 2006. **(b)** 2017 sorghum field trial (plots = 786). Flowering data was collected throughout the season for accuracy +/- 2 days.
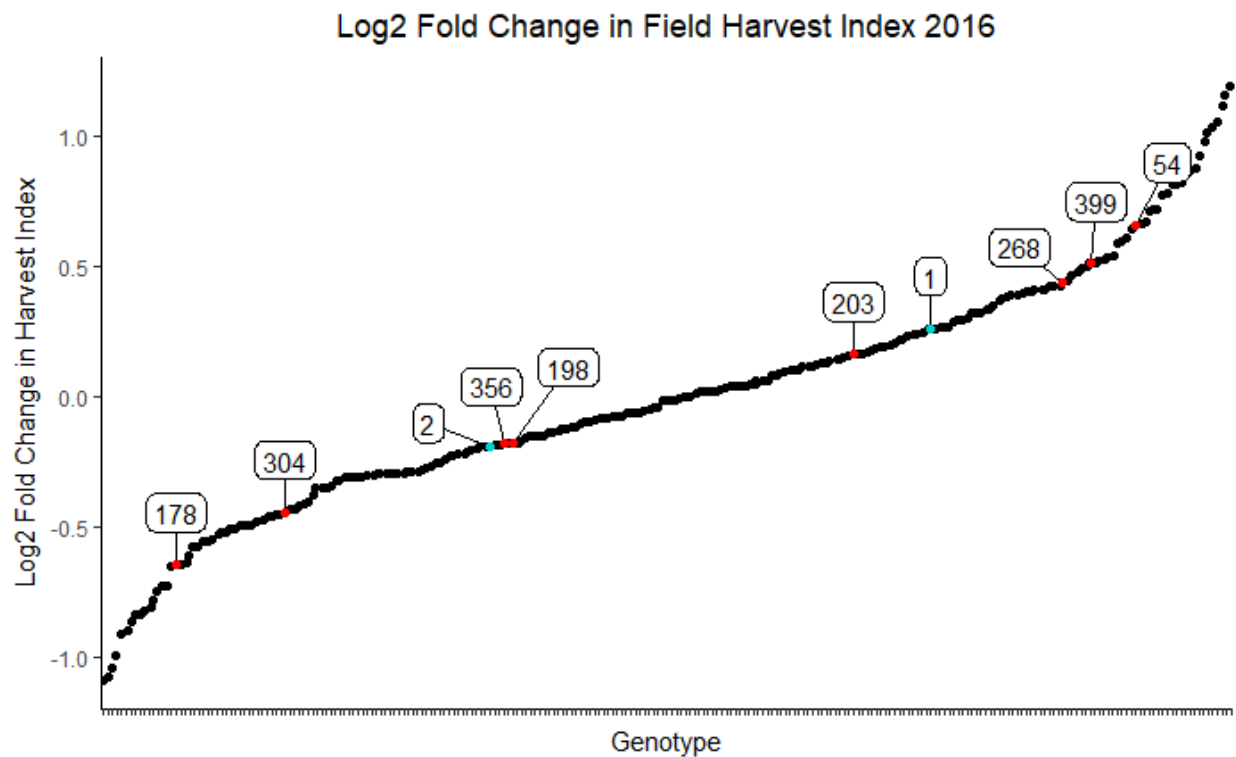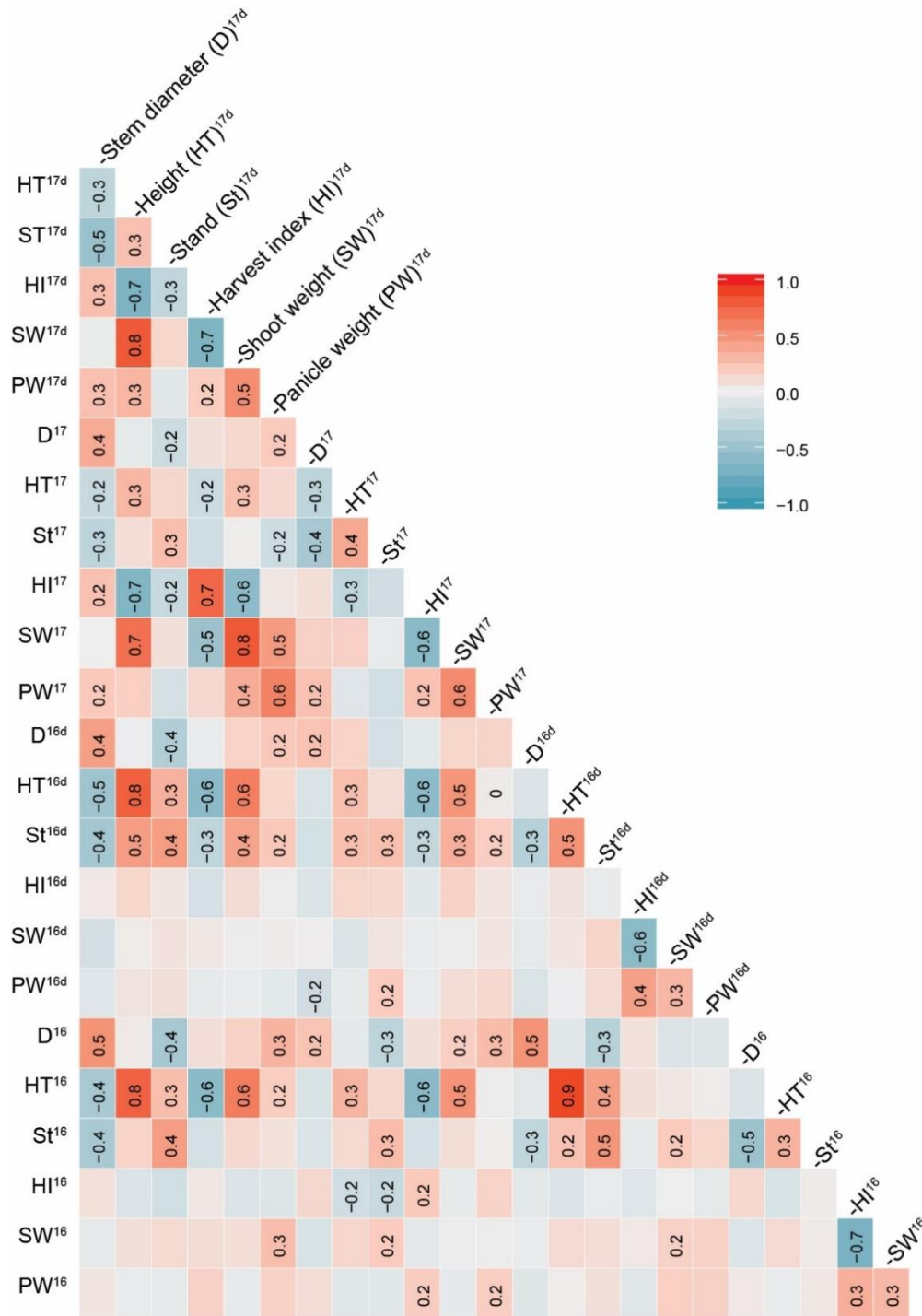
**Figure 2.2** Scatter plot of log$_2$ fold change values in harvest index between moisture treatments in 252 RILs. The 10 selected genotypes for root analysis are labeled and highlighted in red (8 filial RILs) or blue (2 parents of RILs). The x-axis represents genotype and is ordered by values on the y-axis, representing the log2 fold change in harvest index (log$_2$(dryland HI/irrigated HI)). Values > 0 represent a higher harvest index in the dryland treatment and values < 0 represent a higher harvest index in the irrigated treatment.
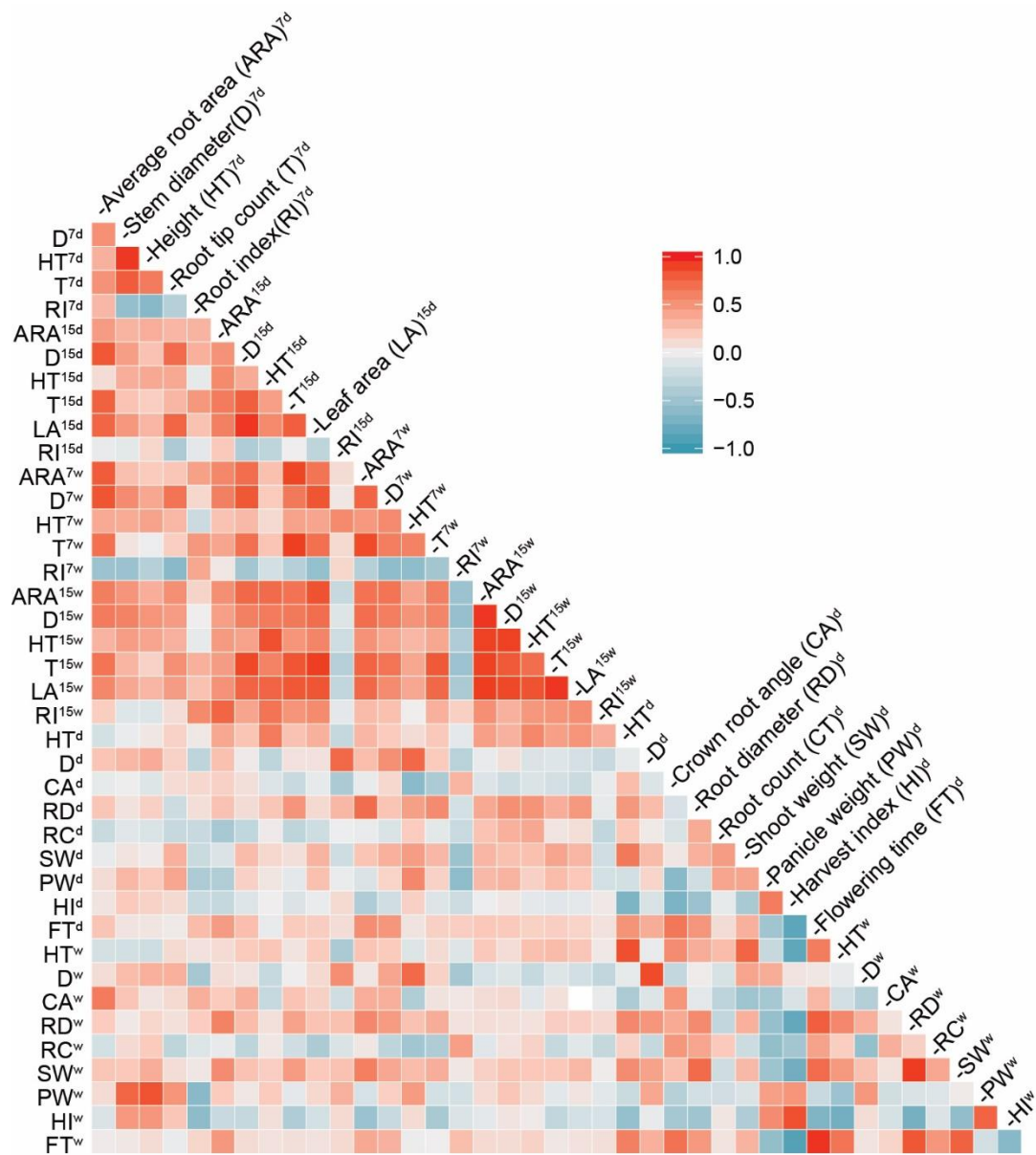
**Figure 2.3** Heat map showing Spearman's correlation coefficients for 5 above-ground traits in sorghum under two moisture regimes in 2016 and 2017. Significant correlations (-0.18 ≤ r ≥ 0.18) are colored either in red (positive) or blue (negative) hues, while non-significant correlations are white. Numerical values are shown in Supplemental Table S1. Superscript indicates harvest year, 16: 2016 and 17: 2017; n = 3 plots.

**Figure 2.4** Heat map showing Spearman's correlation coefficients for 13 total above- and below-ground traits in 10 sorghum RILs under two moisture regimes in the greenhouse and field in 2017. Significant correlations (-0.64 ≤ r ≥ 0.64) are colored in red (positive) or blue (negative) hues, while non-significant correlations are white. Numerical values are shown in Table S2.3. Superscript indicates the treatment and for the greenhouse plants, day harvested during treatment; n = 3-5 plants.
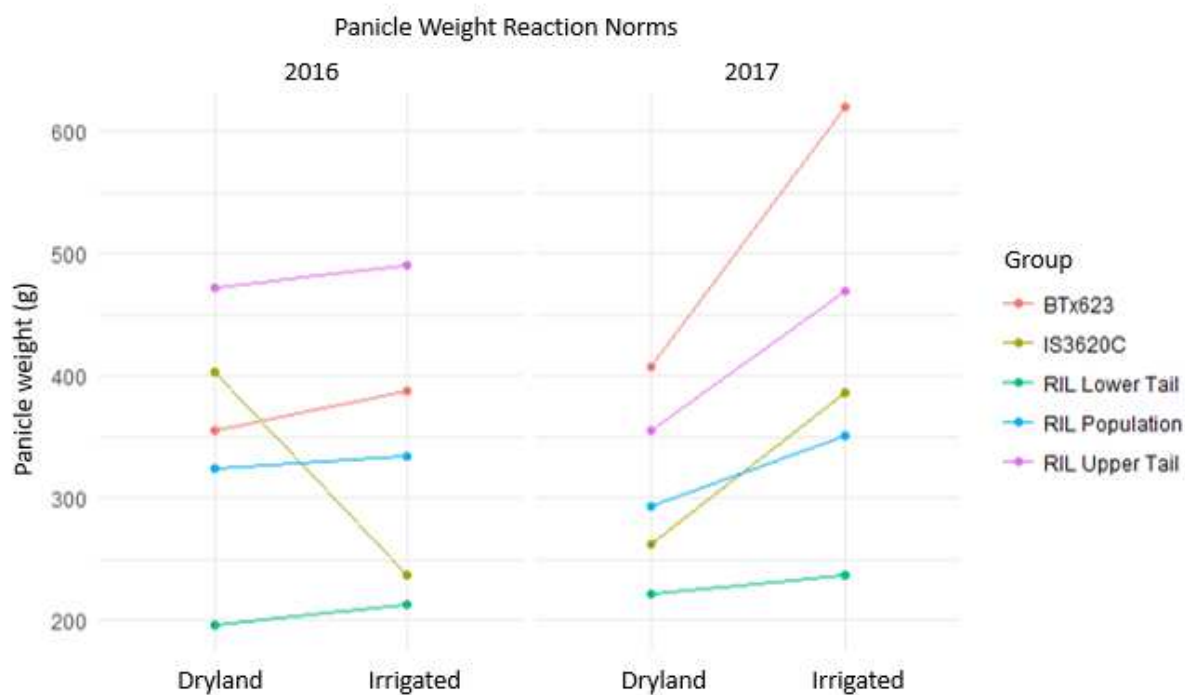
**Figure 2.5** Reaction norms for mean panicle weight (g) of the RIL population parents, BTx623 (red), IS3620C (yellow), the upper tail (pink), lower tail (green) of the RIL population and the entire population (blue). The left graph depicts 2016 and 2017 on the left and right panels, respectively, separated by dryland or irrigated treatment.
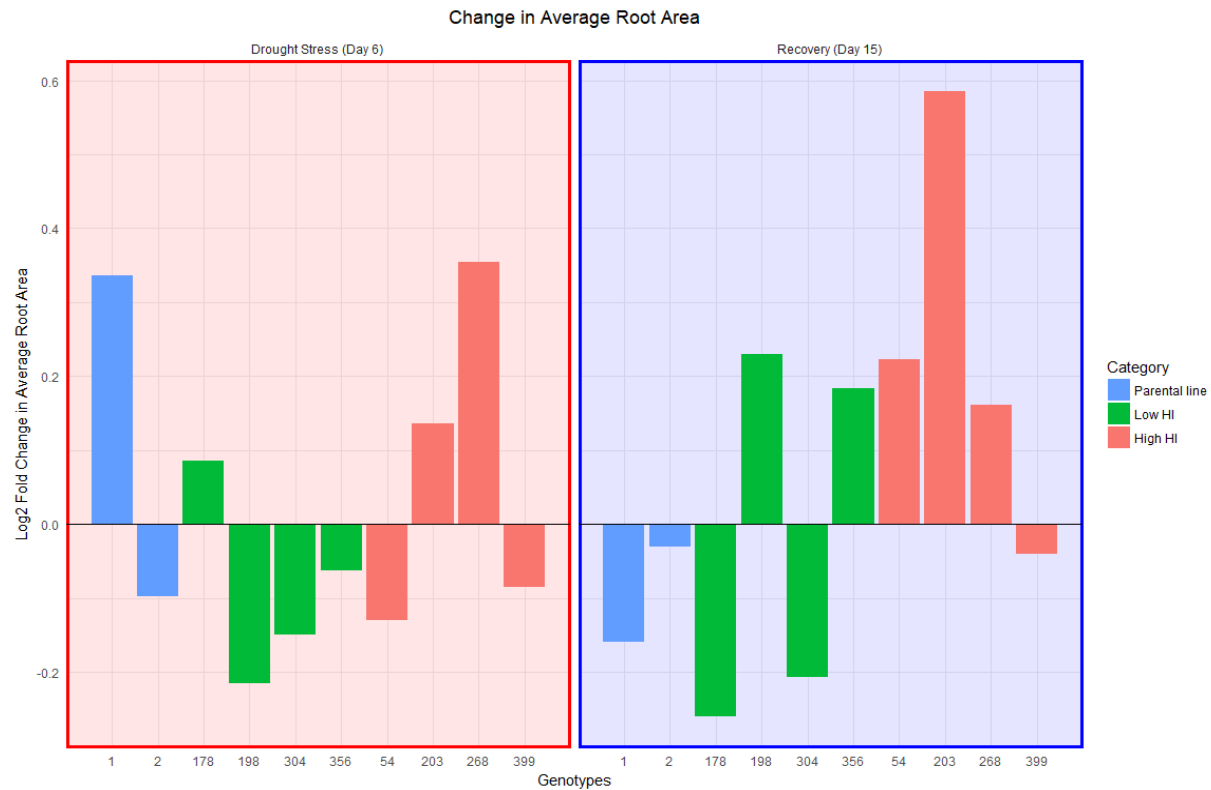
**Figure 2.6** Log$_2$ fold change in average root area (ARA) for 10 selected sorghum RILs. The two panels represent the harvest time points at 6 days of drought stress (red background) or at day 15 of recovery, after 9 days of re-water following the drought treatment (blue). The y-axis represents log2 fold change in ARA between treatments (Log$_2$(dryland ARA/irrigated ARA)), with values > 0 represent a higher ARA in the irrigated treatment and values < 0 represent a higher ARA in the dryland treatment. The x-axis lists the 10 genotypes in order by parental lines (blue), low harvest index from the 2016 field trial (green), and high harvest index from the 2016 field trial (red).

**Supplementary Table S2.1** Spearman correlation coefficients for five above ground phenotypic traits are included for two moisture regimes over 2 field seasons in Rocky Ford, Colorado. *, ** and *** denote significances at p-levels of 0.05, 0.01 and 0.001. See Taylor Person Supplementary Tables.xlsx

**Supplementary Table S2.2** Spearman correlation coefficients for seven above ground phenotypic traits are included for two moisture regimes in 2017 in Rocky Ford, Colorado. *, ** and *** denote significances at p-levels of 0.05, 0.01 and 0.001. See Taylor Person Supplementary Tables.xlsx

**Supplementary Table S2.3** Spearman correlation coefficients for various above- and below-ground traits of sorghum grown in the greenhouse and field. The greenhouse experiment involve two destructive data time points for sorghum at ~6 weeks growth. Field traits data was collected from sorghum at maturity from two moisture regimes in 2017 in Rocky Ford, Colorado. *, ** and *** denote significances at p-levels of 0.05, 0.01 and 0.001. See Taylor Person Supplementary Tables.xlsx

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Appendix I. **National Needs Fellowship international experience**

Internship in Crop Physiology at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India

      In the spring of 2017, I was awarded the opportunity to intern at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Patancheru, India with funding from the USDA - NIFA National Needs Graduate Fellowship Program (Award no. 2014-38420-21801 to TRP). I worked under the mentorship of Dr. Vincent Vadez and Dr. Jana Kholova in the crop physiology lab. The Vadez lab housed researchers from around the world (Figure A.1) studying a wide range of crops, biological principles and the complex relationship between agriculture and society. This internship provided hands-on working experience with many different lab techniques, novel high throughput technology, various cropping systems, and expanded my global network.

      During the three-month internship, I was able to work with over ten crops, most of which I had never seen before. I involved myself with nearly every project in the lab and gained a broad understanding of physiology research from seed preparation to final analysis. I spent the majority of my time evaluating shoot architecture of both legumes and cereals using a high throughput phenotyping technology called Phenospex (Figure A.2). The main objective of this evaluation was to compare and validate the measurements collected by Phenospex, which uses a 3D laser scanner to estimate leaf area, with manual leaf area meters (Figure A.3). For these evaluations, the plants were conventionally grown without a moisture treatment. I was heavily

involved in the entire process from seed preparation to harvest and was able to participate in two full experiments evaluating chickpea, soybean, mungbean, cowpea, pigeon pea, maize, sorghum, finger millet and pearl millet.

In other projects I learned a wide range of skills otherwise untenable in a traditional graduate degree program. I learned how to extract various pigments (Chlorophyll a, chlorophyll b, carotenoids) from chickpea leaves in a study assessing the relevance of stay-green phenotype under drought stress. Another experiment sought to determine the root characteristics of a back-cross nested association mapping population of sorghum. The preliminary study involved observations of the 32 lines from the population. For this aim, I gained experience in shovelomics, the excavation and evaluation of root architecture, and determining shoot biomass partitioning in grain sorghum in the field (Figure A.4, A.5). In another project I spent considerable time evaluating shoot branch architecture to compare growth patterns in wild and cultivated chickpea genotypes (Figure A.6). The goal of this study, as part of a larger project (Von Wettberg et al. 2018), was to classify the shoot architecture of wild and cultivated chickpea to ultimately identify genetic bottle-necks from domestication and understand the genetic history of wild chickpea.

In addition to technical research, I learned how to generate a barcode labeling system for simple, accurate and efficient data collection throughout the season and at harvest that I can easily apply to my research. I also used a convenient data collection application called *Fieldbook* that only requires a smartphone and an excel file. I used this technology throughout my entire 2017 sorghum field trial in Colorado and taught others in my department how to use the application. Further, I was able to gain valuable insight and new knowledge through regular

presentations and discussions. As fate would have it, the fifth Interdrought Conference was held in Hyderabad, India one month after the onset of my internship. I presented a poster of my 2016 field and greenhouse research from Colorado State University and attended several days of talks from scientists across the world. Nearly every week during my practicum, I attended the Vadez lab physiology seminars and gave a talk on my research carried out at Colorado State University. Another intern and I started a journal club where we rotated topics and discussion leaders weekly. During my internship, I was given the opportunity to meet with the legume breeders at the World Vegetable Center. I also toured the Food Science Research and Development Center at the Agricultural Innovation Partnership. This organization is focused on developing popular food products produced from semi-arid crops such as sorghum, millet and mungbean to support the value-chain system in desperate need of a stable value chain. In association with a middle school teacher at the International School of Hyderabad, we planned a field trip to visit the high throughput phenotyping platform and the lysimeter research facility to show them which plants we studied, how we conducted our research and the rationale for studying drought adaptability (Figures A.7 and A.8). Collectively, this practicum was an accelerated educational experience in an environment that encourages learning from a diverse workplace, both in terms of social community and research interests.

Although the exposure to different facets of research and discussion are valuable on their own, none of this would be possible without the hard work of the scientists and technicians carrying out the research and guiding one another. I was able to learn from the leadership of supervisors who effectively managed large groups of people of different ages, nationalities, educational levels, spoken languages and skillsets. My internship was very

rewarding and I have incorporated many of the new skills and efficient practices used in the

field and lab into my research program at Colorado State University.

**Figure A.1** Group photo ICRISAT staff and visiting scientists in the Crop Physiology Lab in February 2017.

**Figure A.2** Research scientists programming Phenospex for high throughput data collection of cereal and legume crops

**Figure A.3** ICRISAT staff harvesting legume plants for biomass and leaf area measurements at the LeasyScan platform
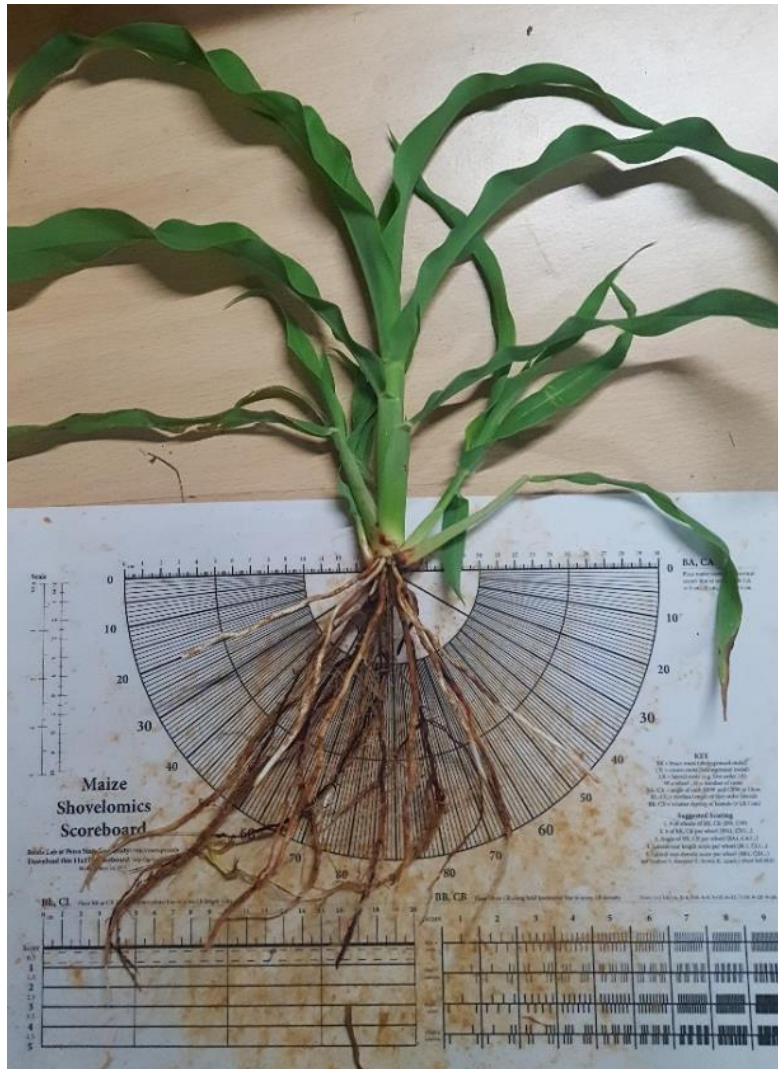
**Figure A.4** Shovelomics evaluation of sorghum at the 5-leaf stage.

**A.5** Plot of 6-week old sorghum plants for shovelomics evaluation.

**A.6** Recording of branch architecture in wild and cultivated chickpea lines.

**Figure A.7** Outreach field trip with a class of 6<sup>th</sup> grade students at the International School of Hyderabad to explain discuss the research of the Vadez lab.

**Figure A.8** Lysimeter experiment in the rainout shelter to measure water use in selected chickpea lines.