Physiological and genetic characterization of sorghum exposed to early season chilling and terminal heat and drought stress

by

Anuj Chiluwal

B.S., Tribhuvan University, 2007
M.S., Fort Valley State University, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Abstract

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the hardiest crop to abiotic stresses compared with other grain crops. However early stage chilling, terminal heat and drought stress are three most damaging abiotic stresses that have limited sorghum productivity in the US Great plains and other locations having similar environmental conditions. Three studies were conducted with an overall goal aimed at increasing grain sorghum's resilience to harsh climatic conditions. In the first study, four promising chilling stress tolerant sorghum advanced breeding lines, a known early stage chilling tolerant Chinese landrace (Shan Qui Red - SQR) and a susceptible US elite cultivar (RTx430) as checks were assessed for chilling tolerance during emergence and early growth under field and controlled environments. Aerial phenotyping using unmanned aircraft systems (UAS) fitted with multispectral camera was used to capture reflectance-based vegetation indices (NDVI and NDRE) in field experiments. Some advanced breeding lines with superior agronomic background also recorded significantly better emergence, seedling growth and vigor compared to SQR under chilling conditions. Aerial phenotyping indices from images taken between 30 and 60 days after emergence were consistently correlated with destructive measurements under early plantings, indicating their effectiveness in differentiating chilling responses. Second study was conducted to understand physiological mechanisms inducing heat stress resilience in sorghum during flowering. A diverse set of sorghum inbreds and selected hybrids were tested under greenhouse, growth chamber facilities and field conditions. A highly conserved early-morning-flowering mechanism was observed across all the inbreds and hybrids, with the peak anthesis wherein >90% of florets completed flowering within 30 min after dawn. The conserved response was consistent even under drought stress and heat stress exposure imposed at different times of the day. Our findings report a novel heat escaping early-morning-flowering

mechanism effectively employed by sorghum to minimize heat stress impact at anthesis. Another experiment with sequential increase in daytime temperature treatments suggest heat stress induced loss in pollen viability to be a key factor resulting in reduced seed-set and grain yield. The findings suggest heat stress could have a greater impact on post-pollen germination processes such as fertilization, embryo formation and development. We identified a heat tolerant genotype "Macia" which appears to be a promising donor for developing improved heat tolerant sorghum hybrids. In the third study, a bi-parental recombinant inbred lines (RILs) mapping population developed from elite post flowering drought susceptible cultivar (RTx430) and a known drought tolerant cultivar (SC35) were evaluated under wide spectrum of environments and moisture conditions. Several novel and major QTL for grain yield, panicle neck diameter, effective quantum yield of photosystem II and chlorophyll content were identified. The genomic regions and the candidate genes within these regions can potentially help in improving source and sink dynamics in sorghum under diverse environments. The findings from these studies will complement ongoing efforts in developing future sorghum with enhanced resilience to different abiotic stresses that continue to limit sorghum productivity.

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Dedication

This thesis is dedicated to my beloved wife Srijana Timilsina Chiluwal, my lovely daughter Anushree Chiluwal and my respected parents Rishi Ram Chiluwal and Bimala Devi Chiluwal for their unconditional love, sacrifice and support.

Chapter 1 - Introduction and literature review

1.1 Sorghum

Sorghum (*Sorghum bicolor* (L.) Moench) is an annual warm-season crop primarily grown in arid and semi-arid region of the world. Sorghum is originated from the tropical and subtropical regions of Africa, hence is well adapted to arid, semi-arid, sub humid and humid environments (Smith and Frederiksen, 2000). Sorghum is genetically a very diverse crop and grown in many countries around the world (Mutava et al., 2011). It is the fifth most important cereal crop in the world after maize, rice, wheat and barley. United States is the highest sorghum producing country in the world (Popescu and Condei, 2014). Sorghum is widely grown as food crop in developing countries, as feed and fodder for livestock and ethanol production in developed countries. It has high production potential even under limited inputs (Popescu and Condei, 2014). Sorghum is the major staple food in many developing countries particularly in drought prone areas of Africa. It is the major source of subsistence for millions of people in semi-arid tropics of Africa, thereby directly influencing their livelihood, nutritional and economic status. Sorghum is a staple diet supporting more than 300 million people around the world, mostly in developing countries. Sorghum is a gluten free cereal and contains high antioxidant levels and hence its consumption is beneficial for human health. Sorghum along with millets are the main food sources for more than one billion people in the semi-arid tropics (Proietti et al., 2015). Sorghum is also used as animal feed, fodder and for making hay and silage (Meeske et al., 1993). In developed countries such as United States, sorghum is mostly grown for animal feed and biofuel production. It is widely used in beef, dairy, swine and poultry feed industries. Sorghum feed improves pork fat quality compared to corn because of its favorable amino acid and fatty acid profile, hence it is more preferred in swine industry (Benz et al., 2011). More than 30% of sorghum in United States was used for ethanol production in 2009 (Yan et al., 2012). In comparison, corn is the major source of bioethanol production in the US with sorghum contributing only around 4%. However, studies have showed that ethanol productivity from sorghum is comparable to corn, suggesting that it could be a potential crop for bioethanol production specially in marginal and drought prone areas where corn has very low productivity (Wu et al., 2007; Wang et al., 2008). Further, sorghum dried distillers grains with solubles (DDGS) which is the product after ethanol extraction from grain, is a good source of phosphorus and can be used as livestock feed (Jenkin et al., 2007).

1.2 Abiotic stresses - major challenge in agriculture under changing climate

Global temperature is increasing consistently due to climate change. Global mean temperature is expected to increase by up to 3.7°C by the end of the 21st Century (IPCC, 2013). Temperature rise causes rapid surface drying which would also increase the duration and severity of drought. Heat and drought stresses are considered the most damaging abiotic stresses for crop production. They are likely to occur together, and the co-occurrence of heat and drought stress is expected to become more frequent under anticipated future hotter environments. Further, to add to the complexity, unpredictable and erratic rainfall pattern is forecasted for the future. More precipitation in wet regions and less precipitation in dry regions is predicted because of climatic variability that will widen the gap in precipitation pattern between wet and dry region as well as between wet and dry season (IPCC, 2013; Trenberth, 2011). In addition, global climate is predicted to be more unpredictable in future with increased intensity and frequency of short episodes of extreme climatic events such as erratic rainfall and flooding, heat spikes, drought and chilling episodes. Abiotic stresses cause significant yield reduction in most field crops. Abiotic stresses like heat, drought and chilling stress are the major constraints for crop production in the world. Eighty percent of the cultivated land is dependent on dryland farming, contributing 60% to the world food production (Kay, 2011). Crops in dryland farming are likely to face more stress under anticipated future harsher environment. Global food production need to be doubled by 2050 (Ray et al., 2013) to meet the increasing food requirements of growing populations which is expected to reach 9 billion by then (Alexandratos and Bruinsma, 2012). Hence, it is increasingly important to enhance abiotic stress tolerance in field crops to combat the impending threat on global food security posed by climate change.

Drought stress could result in significantly higher yield reduction than any other abiotic stress and hence considered as the most damaging abiotic stress on crops (Boyer 1982; Araus et al., 2002; Farooq et al., 2009). Drought is a major constraint for food production in the world, even under current conditions (Barnabás et al., 2008). Drought stress has a profound effect on plant development as it affects all growth stages. It causes floral abnormalities, increases spikelet sterility, decreases photosynthesis and ultimately leading to reduced grain yield and quality (Kadam et al., 2014). Plant tolerance to drought has been commonly classified into 3 strategies: escape, avoidance and tolerance. Escaping mostly relies on completing plant life cycle before the onset of drought. Completion of reproduction before drought, short life cycle and pre-drought

assimilate storage as food reserves for grain formation are common plant responses for escaping drought. Avoidance to drought stress depends on either maintaining high water status or increased water uptake during drought stress. Limited transpiration through stomata closure, reduced canopy size and leaf area, leaf rolling, reduced tillering, dense trichomes, higher surface wax are some of the aboveground related traits associated with avoidance. On the other hand, deep and increased root growth can maximize water uptake. Similarly, drought tolerance refers to maintaining close to normal physiological activity even under drought stress. Tolerance strategies for drought stress involves maintaining turgor, osmotic adjustment, cell membrane stability and partial dormancy (Chaves et al., 2003; Barnabás et al., 2008; Prasad et al., 2018).

Temperature stress on both extremes have a negative impact on the growth and development of sorghum. Heat stress is another damaging abiotic stress in crops. Oxidative stress due to accumulation of reactive oxygen species (ROS) is the major consequence of heat stress damage in plants. ROS damages several biomolecules in plants like sugars and lipids and membranes (Apel et al., 2004). ROS accumulation can lead to thylakoid membrane damage and inhibit photosynthetic enzymes resulting in reduced chlorophyll content and photosynthetic activity (Prasad and Djanaguiraman, 2011). Plant combats negative effect of ROS by producing higher antioxidant enzymes like SOD (superoxide dismutase), catalase (CAT), peroxidase (POS), glutathione S-transferase (GST), glutathione reductase (GR) (Sudhakar et al., 2001; Gosavi et al., 2014; Djanaguiraman et al., 2018). Plants in response to heat stress also accumulate several heat shock proteins (Gosavi et al., 2014). Higher temperature during reproductive stages can result in loss of pollen and pistil viability, resulting in unsuccessful fertilization, lower seed-set and grain yield (Djanaguiraman et al., 2018). On the other side of the spectrum, chilling stress retards plant growth and development resulting in poor plant vigor and delayed flowering and maturity. Lower temperature also reduced photosynthetic rate, chlorophyll content and membrane stability (Sanghera et al., 2011). Chilling stress during early season inhibits germination and seedling emergence resulting in less plant stand and consequently reduced grain yield in many crops including sorghum (Yu and Tuinstra, 2001; Kapanigowda et al., 2013; Maulana and Tesso, 2013).

1.3 Drought stress tolerance in sorghum

Sorghum is a highly tolerant grain crop to drought stress, compared to other cereals. Majority of the sorghum in the world is grown in dryland conditions where it is hard to produce other grain crops (Proietti et al., 2015). Sorghum needs relatively less amount of moisture (332 kg of water) compared to other crops like maize (368 kg of water), barley (434 kg of water) and wheat (514 kg of water) for every kg dry matter production (House, 1985). Sorghum can extract water from deeper soil profile and has superior yield potential under limited water conditions compared to corn and other grains (Farré and Faci, 2006). Sorghum, like many other crops, also employs escaping, avoidance, tolerance mechanism and their combination for its resilience to drought stress depending on the growth stage and the nature and severity of drought stress.

Stay green, limited transpiration, deep root system, lower leaf senescence, higher leaf water retention, higher photosynthesis rate and canopy temperature depression are some of the key physiological traits helping sorghum lower drought stress damage. Stay-green is the most important integrated drought adaptation trait that is systematically investigated in sorghum (Vadez et al., 2013; Borrell et al., 2014a). Stay-green refers to leaf-greenness or chlorophyll retention and the ability to maintain photosynthesis during grain-filling under limited water condition. It has been one of the most prioritized area in sorghum drought tolerance research for more than 30 years (Borrell et al., 2014b). Stay-green improves grain yield in sorghum under water limited conditions (Borrell et al., 2000; Jordan et al., 2012; Borrell et al., 2014b). A recent study unraveled physiological mechanisms behind stay-green induced drought stress tolerance in sorghum (Borrell et al., 2014a). They showed that stay-green is more a result of developmental regulation which decreases tillers and size of upper leaves and reduces canopy size and transpiring leaf area before anthesis. Lower leaf area before anthesis results in lower water demand, hence conserving soil water before anthesis leading to increased water availability during grain-filling stage. Four major stay-green QTL - *Stg1*, *Stg2*, *Stg3*, and *Stg4* have been consistently identified across studies in different environments and genetic backgrounds (Tuinstra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu et al., 2000; Sanchez et al., 2002). These stay-green QTL together contributes more than 50% of the phenotypic variation. Several significant molecular markers including some user-friendly molecular markers associated with stay-green have been developed and validated (Borrell et al., 2000). These markers have facilitated marker assisted selection and breeding, helping many researchers around the world to successfully introgress stay-green trait into their adapted elite

cultivars for enhancing drought stress tolerance (Vadez et al., 2013). Ongoing fine-mapping studies (Harris et al., 2007) is expected to unravel genes responsible for the trait in near future that would facilitate molecular breeding and enable precise gene-based markers as against a QTL based makers that are current used.

Limited transpiration is another important physiological response of sorghum, that helps conserve soil moisture for minimizing drought stress damage (Vadez et al., 2011; Choudhary et al., 2013; Kholová et al., 2014). Limited transpiration allows sorghum to conserve soil water during the vegetative phase thereby increasing water availability during grain filling. Hence it is considered as an important post-flowering drought tolerance trait. High vapor pressure deficit and low soil moisture are two major factors that drive limited transpiration phenomenon in sorghum (Choudhary et al., 2013). Genotypes with higher fraction of transpirable soil water (FTSW) threshold to initiate reduced transpiration, employs soil water conservation earlier than genotypes with lower FTSW threshold. Drought tolerant genotypes are expected to have higher FTSW threshold compared with susceptible genotypes. Previous studies using 12 or 16 sorghum genotypes reported FTSW threshold for reduced transpiration ranging from 0.32 to 0.48 (Gholipoor et al., 2012; Choudhary et al., 2013). Sorghum also reduces transpiration rate in response to high vapor pressure deficit to conserve soil water. Genotypes having lower vapor pressure deficit breakpoint to initiate reduced transpiration employs water conservation earlier than genotypes with high vapor pressure deficit breakpoint. Drought tolerant genotypes are expected to have lower vapor pressure deficit breakpoint compared with susceptible entries. A study using 12 sorghum genotypes reported vapor pressure deficit breakpoint for limited transpiration ranging between 1.2 to 2.9 kPa (Choudhary et al., 2013).

Wax deposition on leaves and stem, deep root system and leaf angle adjustment are some of the physiological traits for drought stress tolerance in sorghum. Leaf rolling during limited water conditions and canopy temperature depression traits also help to minimize drought stress damage in sorghum (Chaudhur et al., 1986; Assefa et al., 2010). Maintenance of improved photosynthesis rates, panicle exertion, pollen viability, increased seed filling rate and duration and higher individual seed weight are others drought stress tolerance traits in sorghum (Mutava et al., 2011).

Osmotic adjustment through the accumulation of organic and inorganic solutes, higher antioxidant production, higher membrane and organelle stabilizing protein and amino acids under drought stress are some of the biochemical traits inducing drought stress tolerance in sorghum.

Osmotic adjustment during limited water conditions is an important drought adaption mechanism in sorghum. Accumulation of several osmolytes prevents water loss under drought stress which leads to cell turgor maintenance and improved water uptake and leaf water potential (Anjum et al., 2011; Arivalagan and Somasundaram, 2016). Sorghum, in response to drought stress, accumulates higher amount of sugars (sucrose, glucose), amino acids (proline, glycine), sugar alcohol, glycine betaine to employ osmotic adjustment (Getnet et al., 2015; Arivalagan and Somasundaram, 2016). Sorghum also induces dehydrin protein production which helps in maintaining ion balance and cell membrane integrity under drought stress (Fracasso et al., 2016). Sorghum also produces several heat shock proteins (HSPs), gluconic acid and ascorbic acid in response to drought stress. Ascorbic acid is an antioxidant and scavenger of ROS and protects lipid and protein damage. Gluconic acid plays protective role under drought stress. Although the exact role of heat shock protein is still unknown, evidence suggest its role in protein stability and degraded protein repair under drought stress (Ogbaga et al., 2016).

Response to drought in sorghum has been investigated as pre-flowering and post-flowering impact (Rosenow et al., 1983). A study by Ananda et al. (2011) investigated drought stress impact on sorghum yield during five growth stages such as pre-flowering (boot leaf emergence) to flowering, flowering to seed-set, seed-set to early seed-fill, early seed-fill to mid seed-fill, and mid seed-fill to late seed-fill stages. Although sorghum is relatively resistant to drought compared to other grain crops, drought stress during both pre and post-flowering growth stages could still cause significant grain yield reduction. Drought stress resulting in grain yield reduction in sorghum has been reported in several previous studies. Assefa and Staggenborg (2010) after analyzing yields from hybrid grain sorghum performance trials from 1957 to 2008 in Kansas reported 7.7 Mg ha^{-1} grain yield in irrigated field and only 4.8 Mg ha^{-1} at dryland sites. Grain yield reduction in sorghum largely depends on the variety, stage and severity of drought stress. A study using 6 sorghum genotypes reported 10 to 37% reduction in grain weight under same level of pre-anthesis drought stress imposed by avoiding rainfall between 31 to 63 days after sowing using rain-out shelters (Santamaria et al., 1990). Kharrazi and Rad (2011) using 7 sorghum genotypes found 40 to 76% grain yield reduction under same drought stress level with no additional irrigation after panicle emergence. A wide variation in grain yield reduction (30 to 78% in Dekalb DK54 - Ottman et al., 2001; 13 to 93% in Pioneer 8501 - Farré and Faci 2006; 22 to 58% in KMF9 - Asgharipour and Heidari, 2011) in response to different drought stress has been documented. Drought stress during

reproductive stage in sorghum is considered more detrimental for grain yield compared to vegetative stages. Drought stress during vegetative stress largely reduces grain number while drought stress during reproductive stages decreases both grain number and grain size (Ludlow et al., 1990; Santamaria et al., 1990; Asgharipour and Heidari, 2011). Haussmann et al. (1998) using 12 sorghum hybrids and their 24 parental lines found 49%, 64% and 92% grain yield reduction across all genotypes under pre-flowering (moderate drought stress), terminal (moderate drought stress) and both stages (severe drought stress), respectively. Assefa et al. (2010) reported drought stress during vegetative and reproductive stage in sorghum causing more than 36% and 55% grain yield reduction, respectively. Hence there is still ample opportunity to improve sorghum yield potential especially in dryland environment through enhancing both pre- and post-flowering drought stress tolerance.

1.4 Heat stress tolerance in sorghum

The minimum temperature for vegetative and reproductive growth in sorghum is 8°C (Hatfield et al., 2008). The optimum temperature for vegetative and reproductive stage in sorghum is 34°C and 31°C, respectively (Hatfield et al., 2008). Sorghum is considered to be one of the more heat tolerant grain crop in the world. However, Tack et al. (2017) using 30 years data from more than 400 commercial sorghum hybrids across different locations at Kansas, USA reported temperature above 33°C could cause significant yield reduction in sorghum. They found around 7% and 44% yield reductions with 2°C (moderate heat stress) and 4°C (severe heat stress) increase in temperature, respectively. Sorghum, like many other crops, is also sensitive to higher temperature during its reproductive stage compared to vegetative stage. Temperature above 32°C starts affecting the reproductive processes in sorghum (Prasad et al., 2006; Jain et al., 2007). Season long 40/30°C, maximum day temperature/minimum night temperature or even short episodes (5 day) of 42/32°C (mean daily temperature 37°C) have been documented as ceiling temperature for grain yield in sorghum (Jain et al., 2007; Prasad et al., 2015). Jain et al. (2007) recorded 27% grain yield reduction under season long 36/26°C. Prasad et al. (2008) reported more than 90% reduction in grain yield under heat stress (40/30°C) exposure during the reproductive stage. Djanaguiraman et al. (2010) found heat stress (40/30°C) during reproductive stage decreased individual grain weight and total grain weight by 51.2% and 53.1%, respectively. Response to heat stress in sorghum varies widely among the genotypes. Djanaguiraman et al. (2014) using 4

commercial sorghum hybrids under heat stress (38/28°C) reported 37 to 67% reduction in seed-set percent. Nguyen et al. (2013) using 15 inbred lines and 3 hybrids reported 7 to 65% reduction in seed-set percentage under heat stress exposure of 38/21°C. Singh et al. (2015) using 20 genotypes (17 inbred lines, 3 hybrids) with 4 temperature treatments reported seed-set percentage of 80% with 31.9/21°C, 69% with 32.8/21°C, 59% with 63.1/21°C and 31% with 38.0/21°C. Sunoj et al. (2017) using 24 genetically and geographically diverse lines using field based tents reported grain yield reduction ranging from 16 to 73% among the genotypes exposed to post-flowering heat stress.

1.4.1 Sensitive stages in sorghum to heat stress

Sorghum like many other crops is more sensitive to high temperature stress during its reproductive stage compared to vegetative stage. Even short heat episodes of 5-10 days when coincided with critical reproductive stage causes significant reduction in sorghum yield (Prasad et al., 2008, 2015). Among the reproductive stage, flowering and 5-10 days before flowering is considered as the most sensitive stages to heat stress in sorghum (Prasad et al., 2008, 2015). Heat stress during these stages increases floret sterility leading to reduced grain number and consequently grain yield. Prasad et al. (2008) reported that heat stress (40/30°C) when started 10 days before flowering and at flowering resulted in maximum reduction in grain yield compared to other periods within the reproductive phase. They found around 74% (heat stress starting at flowering) and 73% (heat stress starting 10 d before flowering) grain yield reduction at these stages, while during other times it was around 8 to 47% lower than control. Prasad et al. (2015) imposing 5 days of heat stress (36/26°C) documented maximum reduction in floret fertility when started at flowering (57%), 10 days before flowering (55%) and 5 days before flowering (42%). Although previous studies showed heat stress during flowering reduced seed-set and grain number, number of reproductive sites was found unaffected suggesting floret sterility being the reason for grain number reduction and not floret abortion (Prasad et al., 2008, 2015). Floret sterility under higher temperature could be due to its impact on either microsporogenesis (pollen development) or megasporogenesis (ovule development), pollen germination or fertilization process. Meiosis and tetrad formation during gametogenesis occur between 10 and 5 days before anthesis. Similarly, fertilization, embryo formation and its early development occurs from anthesis till 5 days after anthesis. Hence, heat stress during these stages negatively affects pollen and ovule viability, pollen

tube growth, and/or fertilization processes resulting in lower seed-set and grain number (Prasad et al., 2008, 2015). No improvement in individual grain weight was observed even with reduced grain number per panicle under heat stress in both field and growth chamber conditions (Singh et al., 2015). Sunoj et al. (2017) reported reduction in total grain weight despite increased grain size under heat stress suggesting yield reduction due to reduced grain number was not compensated by increased grain size. However, no effect on floret sterility or seed-set were observed when heat stress was applied during grain-filling (Prasad et al., 2008, 2015). Prasad et al. (2015) found no reduction in floret sterility even under very high level of heat stress (daily mean temperature 40°C) during grain-filling.

Grain filling is another sensitive stage in sorghum to heat stress. Heat stress during grain-filling decreases grain yield primarily because of reduced grain size (individual grain weight). Prasad et al. (2015) observed even 5 days of heat stress (36/26°C) starting from 10 to 30 days after flowering reducing individual grain weight by 15 to 30%. Heat stress decreases individual grain weight in sorghum primarily by decreasing grain-filling duration and then by decreasing grain-filling rate (Prasad et al., 2006, 2008). Prasad et al. (2006) reported grain-filling duration decreased by 2 days under 36/26°C and further decreased by 6 days under 40/30°C, compared with control (32/22°C). Singh et al. (2015) also found grain-filling duration to decrease by 4-5 days under higher temperature conditions in field. Prasad et al. (2006) observed that an increase in temperature from 32/22°C to 40/30°C decreased grain-filling rate from 0.65 to 0.69 mg day^{-1}. However, studies showed that individual grain weight in sorghum was not affected when heat stress was imposed before grain-filling. Prasad et al. (2015) reported no effect on individual grain weight even under very high level of heat stress (daily mean temperature 40°C) prior to grain-filling stage. In summary, flowering (few days before flowering to flowering) and then grain-filling are two most sensitive stages in sorghum to heat stress. Heat stress during flowering results in increased floret sterility leading to lower seed-set, grain number and consequently grain yield but doesn't affect individual grain weight. Heat stress during grain-filling decreases grain-filling duration and rate resulting in reduced individual grain weight and lower grain weight per plant but doesn't affect seed-set and grain number.

1.4.2 Effect of heat stress on gametogenesis

Both pollen and pistil are sensitive to higher temperature in sorghum. Heat stress increases oxidative damage in both pollen and pistil, induces morphological and anatomical changes, alters phospholipid unsaturation level and consequently decreases their viability leading to decreased seed-set. Djanaguiraman et al. (2018) after analyzing ROS accumulation, antioxidant enzyme activity and seed-set percentage reported pollen to be more sensitive in sorghum compared with pistil under heat stress. They found reactive oxygen species including H_2O_2 and O_2^- to be generated in larger amounts in the pollen, antioxidant enzymes activity like SOD, CAT and POX were lower in pollen compared with pistil under heat stress. They also observed higher seed-set reduction when heat stressed pollen were pollinated on control pistil compared with unstressed pollen pollinated on heat stressed pistil. There are no other studies related to heat stress impact on sorghum pistil. However, several studies have been conducted to assess the heat stress impact on sorghum pollen. Nguyen et al. (2013) reported pollen germination and seed-set percentage to be highly correlated ($R^2 = 0.93$) across both control (32/21°C) and heat stress (38/21°C) conditions suggesting pollen germination to be highly associated with grain yield. Another study (Singh et al., 2015) with 20 genotypes and four different temperature treatments also found *in vitro* pollen germination and seed-set to be highly correlated ($R^2 = 0.91$) across all genotypes and temperature treatments. However, Sunoj et al. (2017) found no significant relationship between grain yield and *in vitro* pollen germination under heat stress in field conditions using 24 genetically and geographically diverse genotypes. At least 10% pollen germination is required for seed-set in sorghum under heat stress (Nguyen et al., 2013; Singh et al., 2015). Prasad et al. (2011) using a sorghum hybrid reported Tmin (minimum temperature requirement for pollen germination), Topt (optimum temperature for maximum pollen germination) and Tmax (maximum temperature beyond which pollen does not germinate) as 17.2, 29.4, and 41.7°C, respectively. Another study (Djanaguiraman et al., 2014) using 8 sorghum genotypes reported Tmin, Topt and Tmax averaged across all genotypes as 15.3, 29.4 and 42.7°C, respectively. They reported significant variation in Topt and Tmax among the genotypes but not in Tmin. They found genotypes with higher Tmax also had improved seed-set under heat stress. Pollen germination in sorghum starts decreasing exponentially 1 hour after anthesis and loose its germination ability after 5 hours (Prasad et al., 2011). Sunoj et al. (2017) observed significant reduction in pollen germination when incubated at 40°C compared with 30°C under both control and heat stress conditions. However, Tuinstra and

Wedel (2000) reported no significant differences in pollen germination when incubated between 20°C to 40°C.

Several previous studies have reported reduced pollen viability and germination under higher temperature resulting in decreased seed-set, grain number and consequently grain yield. Prasad et al. (2006) reported that by increasing growing temperature from 32/22°C to 36/26°C decreased pollen germination by 26%. Jain et al. (2007) also observed high temperature stress of 36/26°C reduced pollen viability and pollen germination. Another study by Prasad et al. (2011) found pollen germination reduced by 28% under heat stress (36/26°C) compared with control (32/22°C). Singh et al. (2015) using 20 genotypes (17 inbred lines, 3 hybrids) and 4 temperature treatments showed *in vitro* pollen germination to gradually decrease with increase in heat stress intensity i.e., 80% in 31.9/21°C, 74% in 32.8/21°C, 64% in 36.1/21°C and 37% in 38.0/21°C. Djanaguiraman et al. (2018) reported pollen viability and pollen germination were reduced by 61% and 52% under 36/26°C, which was further reduced by 20% and 12% under 39/29°C, respectively.

Reduction in pollen germination under heat stress also varies among the genotypes. Genotypic variability on pollen viability in response to heat stress have been observed in previous studies. Djanaguiraman et al. (2014) using 4 commercial sorghum hybrids under heat stress (38/28°C) documented 30 to 68% reduction in pollen germination. Nguyen et al. (2013) using 15 inbred lines and 3 hybrids reported 17 to 63% *in vitro* pollen germination under heat stress (38/21°C). Similarly, another study (Singh et al., 2015) using 20 genotypes (17 inbred lines, 3 hybrids) observed significant variations in pollen germination among the genotypes under heat stress in controlled environment chambers and 6 genotypes under field conditions. Sunoj et al. (2017) using 24 genotypes reported pollen germination under heat stress ranging from 23 to 69% in field grown plants.

Studies have showed that heat stress altered carbohydrate metabolism and inhibited sucrose biosynthesis, consequently resulting in starch deficiency in developing pollen grains (Jain et al., 2007, 2010). Starch provides energy during pollen germination hence a shortage under heat stress would lead to reduced pollen germination and seed-set. Heat stress (32/28°C) induced higher reactive oxygen species (ROS) and altered phospholipid in pollen grains leading to lower pollen germination and viability has been documented (Prasad and Djanaguiraman, 2011). Phospholipids play an important role in pollen development, viability, germination and pollen tube elongation (Xue et al. 2009). Hence, changes in phospholipid composition under heat stress may be associated

with decreased pollen germination and seed-set in sorghum. ROS accumulation in pollen grains under heat stress have been reported in previous studies (Prasad and Djanaguiraman, 2011; Djanaguiraman et al., 2018) which would lead to membrane damage and reduce pollen germination. Abnormal exine in pollen grain has been observed under heat stress indicating tapetal cells disruption that might have obstructed nutrient supply to developing pollen grains (Djanaguiraman et al., 2014).

1.4.3 Effect of heat stress on growth and development

Moderately higher temperature accelerates growth and development in sorghum resulting in earlier flowering and maturity compared with cooler temperatures (Nguyen et al., 2013; Singh et al., 2015). However, temperatures above the optimum can significantly delay reproductive development. Jain et al. (2007) reported days to panicle initiation delayed by 20-25 days and only 10% of the plants produced panicles under season long heat stress (40/30°C) while no panicle initiation was observed under season long severe heat stress (44/34°C). Prasad et al. (2008) found high temperature stress (40/30°C) during the reproductive stage delayed panicle emergence and flowering by 28 and 20 days, respectively.

1.4.3.1 Plant biomass

Effect of heat stress on plant biomass varied widely among the previous studies, depending on stage and severity of heat stress. Jain et al. (2007) found no effect on leaf area, leaf weight and total dry weight under season long high temperature (36/26°C) exposure. Prasad et al. (2008) reported heat stress (40/30°C) during reproductive stage decreased total biomass. Another study (Prasad et al., 2008) documented even with short episodes of heat stress (40/30°C for 10 days) resulting in vegetative dry weight reduction when stress was imposed during flowering (starting from 10 days before or at flowering) but no effect when stress was imposed during grain-filling stages. Djanaguiraman et al. (2010) found heat stress (40/30°C) during reproductive stage decreased total plant dry weight. Contrastingly, Prasad et al. (2006) reported increase in vegetative dry weight under season long severe heat stress (44/34°C).

1.4.3.2 Photosynthesis

Several studies have reported no effect on photosynthesis rate in sorghum under high temperature stress. No difference in photosynthesis rate was observed under season long heat stress (36/26°C) (Jain et al., 2007) and under heat stress (40/30°C) during reproductive stage (Prasad et al., 2008). Prasad et al. (2006) found that even under a severe season long heat stress (44/34°C), photosynthetic rate was reduced by only 4%. Contrastingly, some studies have reported reduced photosynthesis rate under heat stress. Djanaguiraman et al. (2010) reported about 30% reduction in photosynthesis rate under heat stress (40/30°C) during reproductive stage. Djanaguiraman et al. (2014) found heat stress (38/28°C) during flowering decreased photosynthetic rate by 22% compared with control. Sunoj et al. (2017) using 24 diverse sorghum genotypes observed wide genotypic response to photosynthesis rate under heat stress, ranging from no change up to 48% reduction in photosynthesis rate compared with control. Djanaguiraman et al. (2010) reported heat stress (40/30°C) during reproductive stage increased stomatal conductance and transpiration rate by 19.9% and 17.4%, respectively, under controlled environment conditions. Similarly, Prasad et al. (2006) also recorded an increase in stomatal conductance and transpiration rate under severe season long heat stress (44/34°C), again under controlled conditions. However, Sunoj et al. (2017) found reduced transpiration rate (upto 60%) in all the tested 24 genotypes under heat stress in field conditions. Differences in transpiration rate under heat stress may be due to variable growing conditions and different mechanisms employed by sorghum in response to heat stress. Sorghum exposed to heat stress under field conditions may have employed limited transpiration to conserve soil moisture, while under non-water limiting conditions in growth chambers may have increased transpiration rate to reduce canopy temperature.

1.4.3.3 Chlorophyll content

Several studies in the past have reported heat stress induced decrease in chlorophyll content, increased thylakoid membrane damage resulting in reduced photochemical efficiency of photosystem II in sorghum. Prasad et al. (2008) reported a decrease in chlorophyll content and photochemical efficiency of PSII (Fv/Fm - ratio of variable fluorescence i.e. difference between maximum and minimum fluorescence to maximum fluorescence) under heat stress (40/30°C) during reproductive stage. Another study by Djanaguiraman et al. (2010) also observed similar

results as heat stress (40/30°C) during reproductive stage decreased chlorophyll content, Fv/Fm, and increased Fo/Fm (ratio of minimum fluorescence to maximum fluorescence) indicating thylakoid membrane damage. Djanaguiraman et al. (2014) also found high temperature stress (38/28°C) during flowering decreased chlorophyll content by 13%, effective quantum yield of photosystem II by 18% while increased thylakoid membrane damage (Fo/Fm) by 26% under heat stress compared with control. Sunoj et al. (2017) using 24 genotypes reported heat stress increased Fo/Fm, decreased Fv/Fm and chlorophyll index under heat stress compared with control in field conditions.

1.5 Early stage chilling tolerance in sorghum

Sorghum is a warm season crop originated from tropical and subtropical regions of Africa. It is adapted to arid, semi-arid, sub humid and humid environments (Smith and Frederiksen, 2000). Sorghum is one of the hardiest crops under abiotic stresses such as drought and heat stress, which makes it an ideal crop for hot and arid regions of the world (Doggett, 1988; Blum, 2004; Pennisi, 2009). However, sorghum with its tropical adaptation is highly sensitive to early season chilling temperature stress (Peacock, 1982; Rooney, 2004; Burow et al., 2011). Optimum air temperature for sorghum growth and development is between 21° and 35°C. Sorghum is sensitive to small temperature changes below 20°C. Minimum temperature requirement for sorghum germination and emergence are 7°C and 10°C respectively (Peacock, 1982; Rooney, 2004). It is damaged by chilling injury if temperature drops below 15°C and the seedling dies if temperature drops below 0°C (Peacock, 1982).

Sensitivity of sorghum to low temperature is the major limitation for expanding sorghum cultivation in temperate zone, including northern United States. Introducing greater chilling tolerance during early season is seen as an opportunity to escape from some of the terminal drought and heat stress damage (detailed earlier). However, sensitivity to chilling temperature majorly limits sorghum cultivation to hot summer months in the Great Plains of the US and other regions of the world (Maulana et al., 2017). Majority of the sorghum is grown in dry land conditions in the world (Assefa et al., 2010). Early stage chilling tolerance would allow early sorghum planting in many locations of the world including Kansas. Early planting in these locations provides opportunity to effectively utilize early season residual moisture present in the field due to late spring and early summer rainfall. Furthermore, sorghum sensitivity to low temperature has also

narrowed sorghum growing season in many areas of the world. For example, based on temperature requirement for its emergence, recommended sorghum planting date in United States is after May 15 (Shroyer et al., 1996). Moreover, low temperature during early fall also affects its grain maturity limiting sorghum growing season from May to September. Corn is more chilling tolerant during germination and early seedling stages than sorghum. The minimum temperature requirement for corn emergence is 10°C (Warrington and Kanemasu. 1983; Gesch and Archer, 2005), which has allowed planting of corn at least a month ahead of sorghum i.e. between April 20 and May 14, under Kansas conditions (Shroyer et al., 1996). Early planting using early stage chilling tolerant sorghum genotypes is a viable option to provide extended growing season in these locations. Early planting in these locations also provides more conducive environment (good soil moisture and warm temperature) during pre-flowering stage and good ground cover which can potentially improve sorghum productivity. Hence, early stage chilling tolerance is one of the most desired trait in sorghum as it allows sorghum production beyond the conventional growing areas and provide opportunity to benefit from the above-mentioned advantages.

Early season chilling stress in sorghum mainly affects germination, emergence, seedling growth, resulting in poor plant stand and vigor (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Kapanigowda et al., 2013; Maulana and Tesso, 2013; Maulana et al., 2017). Yu and Tuinstra (2001) using 9 sorghum inbreds and 20 hybrids in Kansas reported chilling stress under early planting (April 19 to May 8), negatively affecting seedling vigor and seedling emergence percent and index compared with regular planting (May 31 to June 11). They found 37% and 11% reduction in emergence percentage and seedling vigor rating, respectively, while nearly doubled emergence index (double the emergence duration) under early planting compared with normal. Emergence index of 7.3 days and 15.3 days were recorded under regular and early planting, respectively, in their study. Another study by Yu et al. (2004) using 30 sorghum hybrids in controlled growth chamber conditions resulted in similar effect of lower temperature on sorghum germination, emergence and early seedling growth. Kapanigowda et al. (2013) using 48 sorghum genotypes in two locations (Hays and Colby) in Kansas reported chilling stress conditions under early planting (May 2) leading to 11% and 86% reduction in emergence percentage and 30 days seedling biomass, respectively compared with regular plantings (May 31). They found emergence index increased by 6 days under early planting compared with regular planting. Another study involving 136 sorghum accessions in Manhattan, Kansas reported emergence percentage, seedling

vigor ratings and seedling dry weight to decrease by 35%, 48% and 87% under early planting i.e., during second week of April compared with regular planting in May end to early June (Maulana and Tesso, 2013; Maulana et al., 2017). Although sorghum demonstrated the ability to maintain grain yield per plant after stress recovery, decreased final plant stand due to early stage chilling stress is irreversible and perceived to be a major challenge reducing grain yield (ha^{-1}) in sorghum (Chiluwal et al., 2018). Early season chilling stress in sorghum also delays days to flowering and maturity. Kapanigowda et al. (2013) documented 12 to 15 days delay in days to flowering under early planting compared with regular planting in field. Similarly, Chiluwal et al. (2018) in Hays, Kansas using 4 planting dates intended for mild to severe chilling stress imposition under field conditions, recorded a delay of 18 days to flowering with mid-April (early) planting and further a 32 days delay under April 1 (very early) planting compared with regular plantings. Maulana and Tesso (2013) reported even short episodes of chilling stress (10 days) during early stage delayed flowering and maturity by 8 and 4 days, respectively. Chilling stress decreases chlorophyll content (Maulana and Tesso, 2013; Chiluwal et al., 2018) and photosynthesis rate (Ortiz et al., 2017; Chiluwal et al., 2018) in sorghum. A study reported photosynthesis in sorghum to be more sensitive to chilling conditions compared to other crops like maize, soybean, paspalum and ryegrass (Taylor and Rowley, 1971). Photosynthetic capacity of sorghum resulted in close to complete damage even with 10°C temperature lasting 3 days, with light intensity of 170 $w.m^{-2}$, which is equivalent to a cloudy day. Chilling stress permanently damaged photosynthesis process and sorghum plants couldn't recover under normal temperature (Taylor and Rowley, 1971). The study also showed that the intensity of light had a significant role in damaging photosynthetic machinery under chilling conditions. Sorghum couldn't recover even with 2 days of chilling stress (10°C) in combination with high light levels of 215 $w.m^{-2}$, which is equivalent to a sunny day. However, damage was relatively less and reversible under chilling stress accompanied with low light level of 50 $w.m^{-2}$, which is equivalent to a dull overcast day (Taylor and Rowley, 1971). Hence, combined chilling and high light could damage photosynthetic apparatus permanently resulting in lack of recovery even after returning to control conditions (Taylor and Craig, 1971; Taylor and Rowley, 1971). About 75 to 85% of the absorbed light in chloroplast is dissipated as heat under normal condition. Despite the small proportion of light retained, chilling stress induced damage to photosynthetic machinery can result in ultrastructure changes in chloroplast (Taylor and Craig, 1971). Swelling of stroma, separation of thylakoids, loss in granal stacking and

consequently disintegrated and swelled chloroplast and disappearance of starch grains were reported in two weeks old sorghum seedlings when exposed to chilling stress (10°C) with the light level of 170 $w.m^{-2}$, which is equivalent to a light cloudy day (Taylor and Craig, 1971).

Developing sorghum with the ability to emerge and possess superior seedling vigor is essential to minimize the damages mentioned above and to simultaneously provide opportunities for earlier planting compared to the current planting during hotter months. Hence, early stage chilling tolerance is one of the prioritized areas in sorghum research and a lot of efforts have and are undergoing in this area. However, satisfactory achievement has not been achieved yet. Chinese sorghum landraces which evolved in temperate regions (kaoliangs types) are the major sources of chilling tolerance in sorghum. Sorghum crops moved from Africa to India and later through Himalayans to the temperate region of China (Dogget, 1988). Adaptation in temperate environments in China is believed to have conferred chilling tolerance in these landraces. Chinese landraces have been extensively used in many previous sorghum chilling tolerance studies (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Franks et al., 2006; Knoll et al., 2008; Kapanigowda et al., 2013; Maulana and Tesso, 2013). These Chinese kaoliang accessions have higher germination and emergence under chilling conditions. Franks et al. (2006) using 10 Chinese Kaoliang accessions, 10 US inbred lines and 10 hybrids reported Chinese accessions having better germination, seedling emergence and seedling vigor than US inbreds and hybrids under both laboratory and field studies. However, these chilling tolerant Chinese landraces also possess undesirable agronomic and grain quality traits like tall plant architecture, low yield, and high grain tannin content. Several QTL and markers associated with chilling tolerance have been identified in Chinese background (Knoll and Ejeta, 2008; Knoll et al., 2008). However, many of them were found to overlap with QTL for tannin content which has limited their use in chilling tolerance breeding and continues to remain as a bottleneck in chilling tolerance research in sorghum (Wu et al., 2012; Maulana et al., 2017). Hence, it has become increasingly important to identify and develop other chilling tolerant sorghum genotypes with desirable agronomic traits to be integrated into ongoing sorghum breeding programs. Many sorghum chilling tolerance breeding programs have focused their effort to identify alternative donor sources for chilling tolerance with good agronomic background. Recently some encouraging progress has been achieved. Tannin free advanced breeding lines developed from Kansas State University, Agricultural Research Center, Hays, Kansas showed comparable or even better chilling tolerance than Chinese landraces which

are currently being used in chilling tolerant hybrid sorghum development (Kapanigowda et al., 2013; Chiluwal et al., 2018).

1.6 Abiotic stresses tolerance in sorghum - opportunities and challenges

Sorghum is one of the most heat and drought resilient cereal crop in the world (Doggett, 1988; Blum, 2004; Pennisi, 2009). Sorghum has a C_4 pathway for carbon fixation allowing more efficient utilization of light energy even under high temperatures and water limited conditions (Lara and Andreo, 2011). Hence, sorghum has great potential to be a leading cereal crop in future hotter environment to feed the growing population. However, early stage chilling, post flowering heat and drought stress remains three major damaging abiotic stresses or challenges in sorghum. Hence, there is a need to enhance sorghum's tolerance to these abiotic stresses to improve current and more importantly future sorghum production and productivity. This dissertation research comprises three studies which mainly focused on those three major challenges related to sorghum response to weather variability, with an overall goal aimed at increasing grain sorghum's resilience to future harsher climatic conditions. In the first study, we quantified early stage chilling in some promising advanced breeding lines with good agronomic background using both destructive and high throughput aerial phenotyping. The objective of second study was to uncover physiological mechanisms for heat stress resilience in sorghum during flowering. Third study was conducted to identify QTL associated with source and sink related traits, and panicle neck diameter under wide range of environments and to some extent including terminal drought stress.

1.7 References

Alexandratos, N., and J. Bruinsma. 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. Rome, FAO.

Ananda, N., P.V. Vadlani, and P.V.V. Prasad. 2011. Evaluation of drought and heat stressed grain sorghum (*Sorghum bicolor*) for ethanol production. Ind. Crop. Prod. 33:779-782.

Anjum, S.A., X. Xie, L. Wang, M.F. Saleem, C. Man, and W. Lei. 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6:2026-2032.

Apel, K., and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55:373-399.

Araus, J.L., G.A. Slafer, M.P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C_3 cereals: What should we breed for? Ann. Bot. 89:925-940.

Arivalagan, M., and R. Somasundaram. 2016. Induction of drought stress tolerance by propiconazole and salicylic acid in *Sorghum bicolor* is mediated by enhanced osmoregulation, compatible solutes and biochemical accumulation. J. Appl. Adv. Res.1:41-52.

Asgharipour, M.R., and M. Heidari. 2011. Effect of potassium supply on drought resistance in sorghum: Plant growth and macronutrient content. Pak. J. Agri. Sci. 48:197-204.

Assefa, Y., and S.A. Staggenborg. 2010. Grain sorghum yield with hybrid advancement and changes in agronomic practices from 1957 through 2008. Agron. J. 102:703-706.

Assefa, Y., S.A. Staggenborg, and V.P.V. Prasad. 2010. Grain sorghum water requirement and responses to drought stress: a review. Crop. Manage. 9. https://doi.org/10.1094/CM2010-1109-01-RV.

Barnabás, B., K. Jäger, and A. Fehér. 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 31:11-38.

Benz, J., M. Tokach, S. Dritz, J. Nelssen, J. DeRouchey, R. Sulabo, and R. Goodband. 2011. Effects of increasing choice white grease in corn-and sorghum-based diets on growth performance, carcass characteristics, and fat quality characteristics of finishing pigs. J. Anim. Sci. 89:773-782.

Blum, A. 2004. Sorghum physiology. In: H.T. Nguyen, and A. Blum (Eds.), Physiology and biotechnology integration for plant breeding. Marcel Dekker Inc., New Nork, pp. 141–223.

Borrell, A.K., E.J. van Oosterom, J.E. Mullet, B. George-Jaeggli, D.R. Jordan, P.E. Klein, and G.L. Hammer. 2014b. Stay-green alleles individually enhance grain yield in sorghum under drought by modifying canopy development and water uptake patterns. New Phytol. 203:817-830.

Borrell, A.K., G.L. Hammer, and R.G. Henzell. 2000. Does maintaining green leaf area in sorghum improve yield under drought? II. dry matter production and yield. Crop Sci. 40:1037-1048.

Borrell, A.K., J.E. Mullet, B. George-Jaeggli, E.J. van Oosterom, G.L. Hammer, P.E. Klein, and D.R. Jordan. 2014a. Drought adaptation of stay-green sorghum is associated with canopy development, leaf anatomy, root growth, and water uptake. J. Exp. Bot. 65:6251-6263.

Boyer, J.S. 1982. Plant productivity and environment. Science 218:443-448.

Burow, G., J.J. Burke, Z. Xin, and C.D. Franks. 2011. Genetic dissection of early-season cold tolerance in sorghum (*Sorghum bicolor* (L.) Moench). Mol. Breed. 28:391–402.

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

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

Chiluwal, A., R. Bheemanahalli, R. Perumal, A.R. Asebedo, E. Bashir, A. Lamsal, D. Sebela, N.J. Shetty, and S.V.K. Jagadish. 2018. Integrated aerial and destructive phenotyping differentiates chilling stress tolerance during early seedling growth in sorghum. Field Crops Res. 227:1-10.

Choudhary, S., R.N. Mutava, A. Shekoofa, T.R. Sinclair, and P.V.V. Prasad. 2013. Is the stay-green trait in sorghum a result of transpiration sensitivity to either soil drying or vapor pressure deficit? Crop Sci. 53:2129-2134.

Cisse, N., and G. Ejeta. 2003. Genetic variation and relationships among seedling vigor traits in sorghum. Crop Sci. 43:824–828.

Crasta, O.R., W.W. Xu, D.T. Rosenow, J. Mullet, and H.T. Nguyen. 1999. Mapping of post-flowering drought resistance traits in grain sorghum: Association between QTLs influencing premature senescence and maturity. Mol. Gen. Genet. 262:579-588.

Djanaguiraman, M., P.V.V. Prasad, M. Murugan, R. Perumal, and U.K. Reddy. 2014. Physiological differences among sorghum (*Sorghum bicolor* L. Moench) genotypes under high temperature stress. Environ. Exp. Bot. 100:43-54.

Djanaguiraman, M., P.V.V. Prasad, and M. Seppanen. 2010. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol. Bioc. 48:999-1007.

Djanaguiraman, M., R. Perumal, S.V.K. Jagadish, I.A. Ciampitti, R. Welti, and P.V.V Prasad. 2018. Sensitivity of sorghum pollen and pistil to high-temperature stress. Plant Cell Environ. 41:1065-1082.

Doggett, H. 1988. Sorghum, second ed. Wiley, New York, NY.

Farooq M., A. Wahid, N. Kobayashi, D. Fujita, and S.M.A. Basra. 2009. Plant drought stress: effects, mechanisms and management. In: Lichtfouse E., M. Navarrete, P. Debaeke, S. Véronique, and C. Alberola (eds) Sustainable Agriculture. Springer, Dordrecht.

Farré, I., and J.M. Faci. 2006. Comparative response of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) to deficit irrigation in a Mediterranean environment. Agric. Water Manage. 83:135-143.

Fracasso, A., L. Trindade, and S. Amaducci. 2016. Drought tolerance strategies highlighted by two *Sorghum bicolor* races in a dry-down experiment. J. Plant Physiol. 190:1-14.

Franks, C.D., G.B. Burow, and J.J. Burke. 2006. A comparison of US and Chinese sorghum germplasm for early season cold tolerance. Crop Sci. 46:1371–1376.

Gesch, R.W., and D.W. Archer. 2005. Influence of sowing date on emergence characteristics of maize seed coated with a temperature-activated polymer. Agron. J. 97:1543-1550.

Getnet, Z., A. Husen, M. Fetene, and G. Yemata. 2015. Growth, water status, physiological, biochemical and yield response of stay green sorghum (*Sorghum bicolor* (L.) Moench) varieties - a field trial under drought-prone area in Amhara Regional State, Ethiopia. J Agron. 14:188-202.

Gholipoor, M., T.R. Sinclair, and P.V.V. Prasad. 2012. Genotypic variation within sorghum for transpiration response to drying soil. Plant Soil 357:35-40.

Gosavi, G.U., A.S. Jadhav, A.A. Kale, S.R. Gadakh, B.D. Pawar, and V.P. Chimote. 2014. Effect of heat stress on proline, chlorophyll content, heat shock proteins and antioxidant enzyme activity in sorghum (*Sorghum bicolor*) at seedlings stage. Indian J. Biotechnol. 13:356-363.

Harris, K., P.K. Subudhi, A. Borrell, D. Jordan, D. Rosenow, H. Nguyen, P. Klein, R. Klein, and J. Mullet. 2007. Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. J. Exp. Bot. 58:327-338.

Hatfield, J.L., K.J. Boote, P. Fay, L. Hahn, C. Izaurralde, B.A. Kimball, T. Mader, J. Morgan, D. Ort, W. Polley, A. Thomson, and D. Wolfe, 2008. Agriculture. In: The effects of climate change on agriculture, land resources, water resources, and biodiversity in the United States. A Report by the U.S. Climate Change Science Program and the Subcommittee on Global Change Research. Washington, DC., USA, p. 21–74.

Haussmann, B.I.G., A.B. Obilana, A. Blum, P.O. Ayiecho, W. Schipprack, and H.H. Geiger. 1998. Hybrid performance of sorghum and its relationship to morphological and physiological traits under variable drought stress in Kenya. Plant Breeding 117:223-229.

House, L.R. 1985. A Guide to Sorghum Breeding Second Edition. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Patancheru, India.

IPCC. 2013. Summary for policymakers. In: T.F. Stocker et al., editors, Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, Cambridge, UK, and New York. p. 1–27.

Jain, M., P.S. Chourey, K.J. Boote, and L.H. Allen Jr. 2010. Short-term high temperature growth conditions during vegetative-to-reproductive phase transition irreversibly compromise cell wall invertase-mediated sucrose catalysis and microspore meiosis in grain sorghum (*Sorghum bicolor*). J. Plant Physiol. 167:578-582.

Jain, M., P.V.V. Prasad, K.J. Boote, A.L. Hartwell Jr., and P.S. Chourey. 2007. Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolor* L. Moench). Planta 227:67-79.

Jenkin, S., S. Carter, J. Bundy, M. Lachmann, J. Hancock, and N. Cole. 2007. Determination of P-bioavailability in corn and sorghum distillers dried grains with solubles for growing pigs. J. Anim. Sci. 85:113.

Jordan, D.R., C.H. Hunt, A.W. Cruickshank, A.K. Borrell, and R.G. Henzell. 2012. The relationship between the stay-green trait and grain yield in elite sorghum hybrids grown in a range of environments. Crop Sci. 52:1153-1161.

Kadam N.N., G. Xiao, R.J. Melgar, R.N. Bahuguna, C. Quinones, A. Tamilselvan, P.V.V. Prasad, and K.S.V. Jagadish. 2014. Agronomic and physiological responses to high temperature, drought and elevated CO_2 interaction in cereals. Adv. Agron. 127:111–156.

Kapanigowda, M.H., R. Perumal, R.M. Aiken, T.J. Herald, S.R. Bean, and C.R. Little. 2013. Analyses of sorghum (*Sorghum bicolor* (L.) Moench) lines and hybrids in response to early-season planting and cool conditions. Can. J. Plant Sci. 93:773–784.

Kay, M. 2011. Water for food: innovative water management technologies for food security and poverty alleviation. UNCTAD/DTL/STICT/2011/2. United Nations.

Kharrazi, M.A.S., and M.R.N. Rad. 2011. Evaluation of sorghum genotypes under drought stress conditions using some stress tolerance indices. Afr. J. Biotechnol. 10:13086-13089.

Kholová, J., T. Murugesan, S. Kaliamoorthy, S. Malayee, R. Baddam, G.L. Hammer, G. McLean, S. Deshpande, C.T. Hash, P.Q. Craufurd, and V. Vadez. 2014. Modelling the effect of plant water use traits on yield and stay-green expression in sorghum. Funct. Plant. Biol. 41:1019-1034.

Knoll, J., and G. Ejeta. 2008. Marker-assisted selection for early-season cold tolerance in sorghum: QTL validation across populations and environments. Theor. Appl. Genet. 116:541-553.

Knoll, J., N. Gunaratna, and G. Ejeta. 2008. QTL analysis of early-season cold tolerance in sorghum. Theor. Appl. Genet. 116:577–587.

Lara, M.V., and C.S. Andreo, 2011. C4 plants adaptation to high levels of CO2 and to drought environments. In: Shanker, A. (Ed.), Abiotic Stress in Plants-Mechanisms and Adaptations. InTech, pp. 415–428.

Ludlow, M.M., J.M. Santamaria, and S. Fukai. 1990. Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) Moench under water-limited conditions. II. water stress after anthesis. Aust. J. Agric. Res. 41:67-78.

Maulana, F., D. Weerasooriya, and T. Tesso. 2017. Sorghum landrace collections from cooler regions of the world exhibit magnificent genetic differentiation and early season cold tolerance. Front. Plant Sci. 8:756

Maulana, F., and T.T. Tesso. 2013. Cold temperature episode at seedling and flowering stages reduces growth and yield components in sorghum. Crop Sci. 53:564–574.

Meeske, R., G. Ashbell, Z.G. Weinberg, and T. Kipnis. 1993. Ensiling forage sorghum at two stages of maturity with the addition of lactic acid bacterial inoculants. Anim. Feed Sci. Technol. 43:165-175.

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

Nguyen, C.T., V. Singh, E.J. van Oosterom, S.C. Chapman, D.R. Jordan, and G.L. Hammer. 2013. Genetic variability in high temperature effects on seed-set in sorghum. Funct. Plant. Biol. 40:439-448.

Ogbaga, C.C., P. Stepien, B.C. Dyson, N.J. Rattray, D.I. Ellis, R. Goodacre, and G.N. Johnson. 2016. Biochemical analyses of sorghum varieties reveal differential responses to drought. PloS One 11:e0154423.

Ortiz, D., J. Hu, and M.G. Salas Fernandez. 2017. Genetic architecture of photosynthesis in *Sorghum bicolor* under non-stress and cold stress conditions. J. Exp. Bot. 68:4545–4557.

Ottman, M.J., B.A. Kimball, P.J. Pinter, G.W. Wall, R.L. Vanderlip, S.W. Leavitt, R.L. LaMorte, A.D. Matthias, and T.J. Brooks. 2001. Elevated CO_2 increases sorghum biomass under drought conditions. New Phytol. 150:261-273.

Peacock, J.M. 1982. Response and tolerance of sorghum to temperature stress. In: Sorghum in the Eighties: Proceedings of the International Symposium on Sorghum. Patancheru, A.P, India. pp. 143–159.

Pennisi, E. 2009. How sorghum withstands heat and drought. Science 323:573.

Popescu, A., and R. Condei. 2014. Some considerations on the prospects of sorghum crop. Sci. Pap. Ser. Manag. Econ. Eng. Agric. Rural Dev. 14: 295-304.

Prasad, P.V.V., K.J. Boote, and L.H. Allen Jr. 2006. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [*Sorghum bicolor* (L.)

Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. Agric. for. Meteorol. 139:237-251.

Prasad, P.V.V, K.J. Boote, and L.H. Allen Jr. 2011. Longevity and temperature response of pollen as affected by elevated growth temperature and carbon dioxide in peanut and grain sorghum. Environ. Exp. Bot. 70:51-57.

Prasad, P.V.V., and M. Djanaguiraman. 2011. High night temperature decreases leaf photosynthesis and pollen function in grain sorghum. Funct. Plant. Biol. 38:993-1003.

Prasad, P.V.V., M. Djanaguiraman, R. Perumal, and I.A. Ciampitti. 2015. Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Front. Plant Sci. 6:820.

Prasad, P.V.V., M. Djanaguiraman, S.V.K. Jagadish, and I.A. Ciampitti 2018. Drought and high temperature stress and traits associated with tolerance. In: I. Ciampitti, V. Prasad, editors, Sorghum: State of the art and future perspectives, Agron. Monogr. 58. ASA and CSSA, Madison, WI.

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

Proietti, I., C. Frazzoli, and A. Mantovani. 2015. Exploiting nutritional value of staple foods in the world's semi-arid areas: risks, benefits, challenges and opportunities of sorghum. Healthcare 3:172–193.

Ray, D.K., N.D. Mueller, P.C. West, and J.A. Foley. 2013. Yield trends are insufficient to double global crop production by 2050. PloS One 8(6):e66428.

Rooney, W.L. 2004. Sorghum improvement-integrating traditional and new technology to produce improved genotypes. Adv. Agron. 83:37–109.

Rosenow, D.T., J. Quisenberry, C. Wendt, and L.E. Clark. 1983. Drought tolerant sorghum and cotton germplasm. Agric. Water Manage. 7:207-222.

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

Sanghera, G.S., S.H. Wani, W. Hussain, and N.B. Singh. 2011. Engineering cold stress tolerance in crop plants. Curr. Genomics 12:30-43.

Santamaria, J.M., M.M. Ludlow, and S. Fukai. 1990. Contribution of osmotic adjustment to grain yield in *Sorghum bicolor* (L.) Moench under water-limited conditions. I. water stress before anthesis. Aust. J. Agric. Res. 41:51-65.

Shroyer, J.P., C. Thompson, R. Brown, P. Ohlenbach, D. Fjell, S. Staggenborg, S. Duncan, and G. Kilgore. 1996. Kansas crop planting guide. L-818. Manhattan, Kansas: Kansas State University Cooperative Extension Service.

Singh, V., C.T. Nguyen, E.J. van Oosterom, S.C. Chapman, D.R. Jordan, and G.L. Hammer. 2015. Sorghum genotypes differ in high temperature responses for seed set. Field Crops Res. 171:32-40.

Smith, C.W., and R.A. Frederiksen. 2000. History of cultivar development in the United States: From "memoirs of A.B. Maunder - sorghum breeder'. In: Smith, C.W., and R.A. Frederiksen (Eds.), Sorghum: Origin, history, technology, and production. John Wiley & Sons, New York, NY, pp. 191–223.

Subudhi, P.K., D.T. Rosenow, and H.T. Nguyen. 2000. Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. Theor. Appl. Genet. 101:733-741.

Sudhakar, C., A. Lakshmi, and S. Giridarakumar. 2001. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci. 161:613-619.

Sunoj, V.S.J., I.M. Somayanda, A. Chiluwal, R. Perumal, P.V.V. Prasad, and S.V.K. Jagadish. 2017. Resilience of pollen and post-flowering response in diverse sorghum genotypes exposed to heat stress under field conditions. Crop Sci. 57:1658-1669.

Tack, J., J. Lingenfelser, and S.V.K. Jagadish. 2017. Disaggregating sorghum yield reductions under warming scenarios exposes narrow genetic diversity in US breeding programs. Proc. Natl. Acad. Sci. USA. 114:9296-9301.

Taylor, A.O., and A.S. Craig. 1971. Plants under climatic stress: II. low temperature, high light effects on chloroplast ultrastructure. Plant Physiol. 47:719-725.

Taylor, A.O., and J.A. Rowley. 1971. Plants under climatic stress: I. low temperature, high light effects on photosynthesis. Plant Physiol. 47:713-718.

Trenberth, K.E. 2011. Changes in precipitation with climate change. Clim. Res. 47:123-138.

Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta. 1997. Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol. Breed. 3:439-448.

Tuinstra, M.R., and J. Wedel. 2000. Estimation of pollen viability in grain sorghum. Crop Sci. 40:968-970.

Vadez, V., S.P. Deshpande, J. Kholova, G.L. Hammer, A.K. Borrell, H.S. Talwar, and C.T. Hash. 2011. Stay-green quantitative trait loci's effects on water extraction, transpiration efficiency and seed yield depend on recipient parent background. Funct. Plant. Biol. 38:553-566.

Vadez, V., S. Deshpande, J. Kholova, P. Ramu, and C.T. Hash. 2013. Molecular breeding for stay-green: Progress and challenges in sorghum. Genomic Applications to Crop Breeding 2:125-141.

Wang, D., S. Bean, J. McLaren, P. Seib, R. Madl, M. Tuinstra, Y. Shi, M. Lenz, X. Wu, and R. Zhao. 2008. Grain sorghum is a viable feedstock for ethanol production. J. Ind. Microbiol. Biotechnol. 35:313-320.

Wang, N., Y. Wang, J. Yu, Y. Zhou, Q. Wu, Y. Gao, W. Xu, and R. Huang. 2015. Prioritization of feasible physiological parameters in drought tolerance evaluation in sorghum: A grey relational analysis. Žemdirbystė (Agriculture) 102:457-464.

Warrington, I.J., and E.T. Kanemasu. 1983. Corn growth response to temperature and photoperiod I. seedling emergence, tassel initiation, and anthesis. Agron. J. 75:749-754.

Wu, X., R. Zhao, S.R. Bean, P.A. Seib, J.S. McLaren, R.L. Madl, M. Tuinstra, M.C. Lenz, and D. Wang. 2007. Factors impacting ethanol production from grain sorghum in the dry-grind process. Cereal Chem. 84:130-136.

Wu, Y., X. Li, W. Xiang, C. Zhu, Z. Lin, Y. Wu, J. Li, S. Pandravada, D.D. Ridder, G. Bai, M.L. Wang, H.N. Trick, S.R. Bean, M.R. Tuinstra, T.T. Tesso, and J. Yu. 2012. Presence of tannins in sorghum grains is conditioned by different natural alleles of *Tannin1*. Proc. Natl. Acad. Sci. USA. 109:10281-10286.

Xue, H.W., X. Chen, and Y. Mei. 2009. Function and regulation of phospholipid signalling in plants. Biochem. J. 421:145-156.

Xu, W., P.K. Subudhi, O.R. Crasta, D.T. Rosenow, J.E. Mullet, and H.T. Nguyen. 2000. Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43:461-469.

Yan, S., X. Wu, J. Faubion, S.R. Bean, L. Cai, Y.C. Shi, X.S. Sun, and D. Wang. 2012. Ethanol-production performance of ozone treated tannin grain sorghum flour. Cereal Chem. 89:30–37.

Yu, J., and M.R. Tuinstra. 2001. Genetic analysis of seedling growth under cold temperature stress in grain sorghum. Crop Sci. 41:1438–1443.

Yu, J., M.R. Tuinstra, M.M. Claassen, W.B. Gordon, and M.D. Witt. 2004. Analysis of cold tolerance in sorghum under controlled environment conditions. Field Crops Res. 85:21-30.

Chapter 2 - Integrated aerial and destructive phenotyping differentiates chilling stress tolerance during early seedling growth in sorghum

Abstract

Sorghum with greater chilling tolerance during early season has the potential to minimize terminal drought and heat stress damage and efficiently utilize early season residual moisture. Four promising chilling stress tolerant sorghum advanced breeding lines, a known early stage chilling tolerant Chinese landrace (Shan Qui Red - SQR) and a susceptible US elite cultivar (RTx430) as checks were assessed for chilling tolerance during emergence and early growth under field and controlled environments. Plants were exposed to a maximum day-time/minimum night-time temperature of 6/6°, 8/8°, 10/10°, 15/15°, 20/10°, 20/15° and 32/22°C in two independent controlled environment growth chamber experiments. Field studies were conducted with two early (April 01 and 15) and regular (May 13 and June 02) plantings in 2016 and with one early (April 21) and regular (May 25) planting in 2017. Emergence rate and from seedlings harvested one month after emergence, leaf area, shoot weight, root weight, and other root morphological traits were recorded from all experiments. Aerial phenotyping using UAS fitted with multispectral camera was used to capture reflectance-based vegetation indices (NDVI and NDRE). Some advanced breeding lines recorded significantly better emergence, seedling growth and vigor, root biomass compared to SQR under chilling conditions. Aerial phenotyping indices from images taken between 30 and 60 days after emergence were consistently correlated with destructive measurements under early plantings, indicating their effectiveness in differentiating chilling responses. Aerial phenotyping indices have the potential to be utilized effectively for phenotyping genetically diverse populations for improving early season chilling tolerance in sorghum

2.1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) an annual warm season crop originating from tropical and subtropical regions of Africa, is adapted to arid, semi-arid, sub humid and humid environments (Smith and Frederiksen, 2000). Sorghum is one of the hardiest crops under abiotic stresses such as drought and heat stress, which makes it an ideal crop for hot and arid regions of the world (Doggett, 1988; Blum, 2004; Pennisi, 2009). In most sorghum growing regions including the United States, post-flowering drought and heat stresses are documented to induce significant yield losses (Assefa et al., 2010; Prasad et al., 2015). Introducing greater chilling tolerance during early season is seen as an opportunity to escape some of the terminal drought and heat stress damage and efficiently utilize early season residual moisture. However, sorghum with its tropical adaptation is highly sensitive to chilling temperature stress (Peacock, 1982; Rooney, 2004; Burow et al., 2011), which has restricted sorghum cultivation to hot summer months in the Great Plains of the US and other regions of the world.

Early season chilling stress is known to negatively affect sorghum germination, emergence, seedling growth, resulting in poor plant stand and vigor (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Kapanigowda et al., 2013; Maulana and Tesso, 2013; Fernandez et al., 2015). Hence, developing sorghum with the ability to emerge and possess superior seedling vigor is essential to minimize the damages mentioned above and simultaneously provide opportunities for earlier planting compared to the current planting during hotter months. Major challenges faced when trying to induce chilling tolerance in sorghum is their ability to germinate under chilling temperature and the potential to emerge by protecting the tender developing coleoptile. Another dimension to the challenge, is the ability of the emerged seedling to maintain sufficient vigor to allow photosynthetic activity and grow normally, without undergoing physiological arrest or death of the emerged seedling which has restricted sorghum cultivation to hot summer months in the Great Plains of the US and other regions of the world. Hence, seedling emergence associated with plant stand and above and belowground seedling growth as a measure of growth and development which define early season chilling tolerance in sorghum (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Kapanigowda et al., 2013; Maulana and Tesso, 2013; Fernandez et al., 2015) were investigated in our studies.

Chinese sorghum landraces which originate from temperate regions (kaoliang types) have been used in many previous sorghum chilling tolerance studies (Franks et al., 2006; Kapanigowda

et al., 2013). These Chinese kaoliang accessions have higher germination and emergence under chilling conditions than other sorghum adapted lines and hybrids (Franks et al., 2006). Of these, Shan Qui Red (SQR) has been extensively used in physiological and genetic analysis and is known to possess better seedling emergence and seedling vigor under chilling conditions (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Knoll et al., 2008; Kapanigowda et al., 2013; Maulana and Tesso, 2013). However, most of these chilling tolerant accessions including SQR possess undesirable agronomic and grain quality traits like tall plant architecture, low yield, and high grain tannin content. Thus, it is increasingly important to identify or develop other chilling tolerant sorghum genotypes with desirable agronomic traits, to be integrated into ongoing sorghum breeding programs.

Aerial High Throughput Phenotyping (HTP) indices such as NDVI (Normalized Difference Vegetation Index) and NDRE (Normalized Difference Rededge index) are extensively used to capture some of the agronomic traits such as plant height, biomass and physiological parameters including chlorophyll content and overall plant health (Daughtry et al., 2000; Bendig et al., 2015; Dash et al., 2017; Gracia-Romero et al., 2017). With classical destructive phenotyping, extracting and recording seedling biomass and growth is restricted to one or two sampling time points, while aerial HTP provides the opportunity to capture the response under chilling conditions at high temporal frequency. Additionally, multiple years of breeding efforts at Agriculture Research Center, Hays (ARCH) has developed promising chilling tolerant advanced breeding lines in desired agronomic background. These promising inbreds provide alternative sources of chilling tolerance compared to the Chinese sources. Hence, the overall goal was to assess the suitability of these promising lines in comparison to tolerant (SQR) and susceptible checks (RTx430), by integrating destructive and aerial HTP. We hypothesized that (i) ARCH lines along with good agronomic background also have comparable early stage chilling tolerance as SQR and (ii) aerial phenotyping can effectively differentiate chilling stress resilience in sorghum. Specific objectives were to (i) quantify the degree of chilling tolerance in the agronomically superior ARCH lines with checks under field and growth chamber conditions; and (ii) Correlate aerial and destructive phenotyping to test the possibility of using aerial phenotyping to differentiate chilling tolerance under field conditions.

2.2 Materials and Methods

2.2.1 Plant material

Adapted lines SC35B and 803B were crossed to PI 574599 and PI 574560, respectively; PI 574562 was crossed to DLO357B followed by crossing the F_1 with the genetic male-sterile (GMS) KP 8B. A total of about 600 F_6 progenies were obtained from the three crosses mentioned above. All these progenies were tested for chilling tolerance in growth chamber conditions using sand as germinating medium. Based on the criteria of >70% emergence under 15/12°C day/night temperature with a 12-h photoperiod, 24 lines were selected. These lines were tested under field conditions in Colby, KS, along with tolerant Chinese donors and susceptible checks. Preliminary investigation in Colby, KS, resulted in identifying few promising lines with chilling stress tolerance and superior agronomic performance. Hence six inbreds, i.e., four promising chilling stress tolerant advanced sorghum breeding lines (ARCH10747-1, ARCH10747-2, ARCH12012 and ARCH12045) identified from the preliminary investigation, a well-known donor for early stage chilling tolerance (SQR) and a US elite susceptible cultivar (RTx430) were selected for detailed phenotyping to study their responses to chilling conditions in the growth chambers and field conditions. Seeds of all six genotypes were collected from the same season nursery grown at Agriculture Agricultural Research Center, Hays, Kansas. Germination percentage of the seeds was tested prior to the experiment in the laboratory using Petri plates where all genotypes recorded more than 90% germination percentage.

2.2.2 Crop husbandry

2.2.2.1 Growth chamber experiments

Two independent experiments were conducted in walk-in controlled environment growth chamber facility at Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas, USA. These growth chambers are equipped with automated control of temperature, relative humidity and light levels making it possible to impose conditions following a diurnal pattern. Experiments were laid out in a Split Plot Design. Growth chamber temperature was considered as whole plot and genotypes as sub-plots. The first experiment was conducted with four different temperature treatments (6/6° 8/8°, 10/10°, and 15/15°C day/ night temperatures).

The second experiment included three different chilling temperatures (15/15°, 20/10° and 20/15°C) and 32/22°C as control. The set of six genotypes mentioned above were sown in 1.6 L pots (24 cm length and 10 cm width, MT49 Mini-Treepot) filled with 2:1 proportion of sand and farm-soil, respectively. The pots were filled with soil mixed with Osmocote, a controlled-release fertilizer at 3 g per pot (14:14:14% N: P: K, respectively; Hummert International, Topeka, KS, USA). The plants were grown in temperature conditions mentioned above for 30 days after 50% emergence. Each pot had a single plant with each treatment having six replicate plants. A 12 h photoperiod (06:00 to 18:00 h) was provided in control and chilling stress chambers with a light intensity of ~850 μmol m^{-2} s^{-1} at the plant canopy. Microclimatic conditions were monitored in all the growth chambers at 15 min interval throughout the experiment using a HOBO data logger (Onset Computer Corporation, Bourne, MA, USA). The soil temperature data loggers (Onset Computer Corporation, Bourne, MA, USA) were used to record soil temperature at 5 cm depth at 15 min intervals. Details related to planting, emergence, soil and air temperature conditions measured in the growth chambers are given in Table 2.1.

2.2.2.2 Field experiments

Two field experiments were conducted at Kansas State University, Agricultural Research Center, Hays, Kansas in 2016 and 2017. Based on growth chamber results and previous long-term minimum soil temperature recorded across Kansas, natural chilling conditions in the field were opted to match the base temperatures for sorghum growth and development (Brar et al., 1992; Fiedler et al., 2012; Bekele et al., 2014). All six genotypes were stagger planted on four planting dates in 2016 and two planting dates in 2017. In 2016, the first two i.e. April 01 (planting I), April 15 (planting II) were considered as early while May 13 (planting III) and June 02 (planting IV) were considered as regular planting based on sorghum production practices in Kansas. In 2017, first planting on April 21 (planting I) was considered as early and second on May 25 (planting II) was considered as regular planting. Planting I and II in 2016 and planting I in 2017 were aimed at inducing chilling stress while regular plantings were aimed to expose sorghum to optimal growing conditions. All experiments were laid out in split plot randomized complete block design with three replications. Planting dates were considered as whole plot and genotypes were considered as sub-plots. Each genotype was planted in four rows plot, replicated thrice for each planting. Each row was 3.6 m (12 ft) long accommodating 48 plants per row and with an inter-row spacing of

0.75 m. The maximum/minimum soil temperature at 5 cm depth for different planting dates were recorded at 15 min intervals using soil temperature data loggers (Onset Computer Corporation, Bourne, MA, USA). Details on soil and air minimum and maximum temperature, planting, emergence and sampling in both 2016 and 2017 are presented in Table 2.1. Daily increments in average minimum and maximum soil and air temperature between planting and 30 days sampling across both the years is presented in Fig. 2.1.

2.2.3 Observations

Several above and below ground parameters were measured including (i) emergence index and emergence percentage that determine plant stand; (ii) seedling above ground (leaf area, shoot weight, effective quantum yield of photosystem II, and chlorophyll index) and below ground (root weight, total root length, root surface area, average root diameter, and root volume) parameters that define seedling growth and vigor; (iii) Yield per plant, 200 seed weight and related (days to 50% flowering, plant height, and panicle length) parameters; and (iv) aerial imagery capturing reflectance based Normalized Difference Vegetation Index (NDVI) and Normalized Difference RedEdge index (NDRE).

2.2.3.1 Seedling emergence

Emergence index and emergence percentage were recorded to measure seedling emergence rate and number, respectively. In the field experiments, they were recorded only under early plantings as no differences in seedling emergence between the genotypes are expected under regular plantings. To estimate emergence differences across genotypes in both field and growth chambers - number of emerged seedlings were counted on alternate days until no change in seedling emergence was observed. Emergence percentage of each genotype was calculated as the ratio of the total number of emerged seedlings to the total number of planted seeds, times 100. Emergence index was calculated as a measure of rate of emergence as described by Yu et al. (2004) using the formula - Emergence Index = $\sum(Ej \times Dj)/E$, where Ej = total emergence on day j, Dj = days after planting and E = total seedlings emerged.

2.2.3.2 Seedling vigor and root morphology

Across all growth chamber and field experiments, plants were harvested 30 days after emergence (Table 2.1). Six replicate plants were sampled for both shoot and root related parameters to quantify chilling stress induced responses in above and below ground traits. Roots were carefully extracted from plants from growth chamber and all plantings in 2016 and 2017. In growth chamber and both planting I and II in 2016 and planting I in 2017 which were the chilling stress treatments, 100% of the root volume were obtained as they were small and easily extractable. However, with later plantings III and IV in 2016 and planting II in 2017 approximately 80% of the roots were extractable (Fig. 2.2). Roots were separated from the shoot and stored in 20% ethanol for WinRhizo analysis. The stored root samples were scanned with Epson Perfection 7000 scanner at a resolution of 600 dots per inch. The digitized images were used to determine root morphological traits such as total root length (RL, cm plant^{-1}), root surface area (RA, cm^2), average root diameter (RD, mm) and root volume (RV, cm^3) using the root system analyzer (WinRhizo 2004b, Regent Instruments, Inc., Québec, Canada). Leaf area was estimated by a leaf area meter (LI-3100 area meter, LI-COR. Inc., Lincoln, NE, USA) before oven drying. Shoot and roots were separately oven dried at 60°C for 72 h and shoot biomass (g plant^{-1}) and root biomass (g plant^{-1}) were recorded after reaching constant weight. In 2017, approximately one month after planting, effective quantum yield of photosystem II (QY) in the light adapted state and chlorophyll index were recorded using FluorPen (FluorPen FP 100, Photon System Instruments, Ltd., Brno, Czech Republic) and Dualex (Dualex 4 Scientific, Force-A, Orsay, France), respectively. The data were collected from 30 fully expanded leaves (10 per replication) per genotype in each planting

2.2.3.3 Yield and related parameters

Phenology, that is days to 50% flowering (defined as the number of days from planting to 50% flowering) was obtained on a plot basis. Plant height was recorded from the base of the plant to the tip of panicle at the time of harvest. In 2016, after 100% flowering, twenty plants from each replication were selected and panicles were bagged with mesh bags to avoid damage by birds. At physiological maturity, the bagged heads were harvested at approximately 12 to 14% moisture content and dried at 40°C for a week, threshed and grain yield (g plant^{-1}) was recorded. In 2017, all plants in middle two rows in each plot were bagged and grain yield per plant and grain yield

per m^2 were recorded. Panicle length was recorded from the neck of the panicle to the tip before threshing. Test weight (200 seed weight) was recorded in both the field experiments.

2.2.3.4 Aerial phenotyping

Aerial imagery were collected via small unmanned aircraft systems (sUAS). A DJI Matrice 100 quadcopter outfitted with a DJI X3 RGB 12- megapixel camera, MicaSense RedEdge with a Downwelling Light Sensor. The MicaSense RedEdge is a five-channel multispectral imager that captures discrete spectral data at Blue (475 nm), Green (560), Red (668), RedEdge (717), and Near-Infrared (840) wavelengths. The MicaSense RedEdge was calibrated to percent reflectance using a Downwelling Light Sensor in addition to calibration targets of known reflectance to normalize the spectral data across time and variable sky conditions. All optical sensors were equipped with GPS units for geotagging each image throughout flight operations. In 2016, flight operations began in May and were conducted through September during solar noon under full sun sky conditions. Electronic speed control failures occurred during July that prevented flight operations from July to August 22 in 2016. Flights were conducted using autonomous flight mode with altitude set to 40 m above ground level, 85% sidelap for flight lines, and a forward flight speed of four meters per second. Ground control points were established at the corners and throughout the study area using tiles approximately one meter per square for imagery registration. In 2017, the frequency of flights was increased to once a week beginning May 30 until November 26, following the same procedure as described above. Imagery were processed to create orthomosaics using MicaSense Atlas processing service (2016, Seattle, WA, USA) and Agisoft Photoscan (ver 1.35; Agi- soft LLC, St. Petersburg, Russia). After the orthomosaics were created, all imagery dates were aligned to the ground control points to correct for GPS drift and allow for automated plot extraction across all imagery dates. NDVI was calculated based on the reflectance in the Red and Near-Infrared (NIR) part of the spectrum using the formula - NDVI = NIR - RED/ NIR + RED (Rouse et al., 1974). NDRE was calculated based on the reflectance in the RedEdge and Near-Infrared (NIR) part of the spectrum using the formula - NDRE = NIR - RedEdge/NIR + RedEdge (Gitelson and Merzlyak, 1994). An Index-Based Method outlined by An et al. (2016) was applied to the RGB imagery captured by the DJI X3 camera to create a soil background segmented binary mask. The soil binary mask was applied to the MicaSense RedEdge imagery, thus allowing NDVI and NDRE to be extracted from the plants after removing soil background.

ArcMap 10.4.1 and ArcGis Pro was utilized for image processing of orthomosaics for spectral data extraction (ESRI, 2016, Redlands, CA, USA) (Fig. 2.3). To minimize the noise associated with scale due to differences in plant stand, fishnet grid was created on each plot in orthomosaics image and spectral data were extracted from the grided region. Grid areas were adjusted based on plant/row or a part of the row based on plant stand.

2.2.4 Statistical analysis

Statistical analysis was done using SAS (ver 9.4; SAS Institute, 2013). Analysis of variance (ANOVA) was performed using GLM procedure. Genotypes and temperature/planting date were considered as fixed effect while block and block × temperature/planting were considered as random effects. Means were separated using least significant difference (LSD), when treatments and interactions were significant at $P \leq 0.05$. Correlation between destructive (one time point) with aerial measurements (multiple time points) were tested to ascertain the strength of association between destructive ground measurements with aerial phenotyping indices. Similar approach has been followed in previous studies involving different crops including sorghum (Todd et al., 1998; Boelman et al., 2003; Moges et al., 2005; Gracia-Romero et al., 2017).

2.3 Results

2.3.1 Soil and air temperature

Night-time soil and air temperatures in the growth chambers were consistently close to the target, while the day-time maximum air temperatures were slightly higher with cooler temperature treatments due to the light load (Table 2.1). In the field conditions, significant differences in soil and air temperatures were observed across the planting dates. Average daily minimum and maximum soil and air temperatures were higher in later plantings compared to early plantings in both years (Table 2.1). Although, the soil and air day-time maximum temperature were similar, significantly lower night-time air temperatures were recorded compared to soil temperature, in both the field experiments.

2.3.2 Seedling emergence and vigor in growth chambers

In the first growth chamber experiment, no seedling emergence was observed in any of the tested genotypes with continuous 6/6°, 8/8°, and 10/10°C day/night temperature treatments. Seedlings emerged only in 15/15°C day/night temperature treatment wherein about 80% seedling emergence was recorded (Fig. 2.4). In the second growth chamber experiment, emergence index and shoot weight were affected significantly by genotype ($P < 0.05$), temperature ($P < 0.0001$) and their interaction ($P < 0.05$) (Table 2.2). However, all other shoot and root related parameters differed significantly with temperature ($P < 0.0001$) but not with temperature and genotype interaction. No significant difference in emergence index among genotypes was observed with higher temperature (20/10°, 20/15° and 32/22°C) treatments. However, with minimum temperature treatment (15/15°C), ARCH10747-1 and ARCH12045 had significantly lower emergence index than other genotypes (Fig. 2.5A). Averaged across all genotypes, emergence index was significantly ($P < 0.0001$) higher under 15/15° and 20/10°C compared to 20/15° and 32/22°C (Table 2.3), because of the longer duration to emerge under chilling conditions. Similarly, no significant difference in shoot biomass was observed among genotypes under higher temperature (20/10°, 20/15° and 32/22°C) treatments. However, in minimum temperature treatment (15/15°C), ARCH10747-1 produced significantly higher shoot weight than other genotypes (Fig. 2.6A). Averaged across all genotypes, control treatment (32/22°C) resulted in significantly ($P < 0.0001$) higher leaf area, shoot weight, root weight, root length, root area, and root volume compared to moderate (20/10°C and 20/15°C) and severe chilling temperature (15/15°C) treatments (Table 2.3).

2.3.3 Seedling emergence and vigor under field conditions

Emergence percentage was significantly affected by genotype ($P < 0.0001$) in both years and by planting date ($P < 0.0001$) and their interaction ($P < 0.0001$) in 2016 (Table 2.2). In contrast to the growth chamber study, emergence index did not differ significantly across genotypes in both years, genotypes and planting date interaction in 2016 but varied significantly ($P < 0.0001$) between planting dates in 2016 (Table 2.2). In 2016, under both early plantings I and II, ARCH lines on average had superior emergence percentage than SQR and RTx430 (Fig. 2.5B and C). ARCH10747-2 and ARCH12045 had significantly higher emergence percentage than SQR (9 and

12%, respectively) and RTx430 (36 and 39%, respectively) in first planting (Fig. 2.5B). Similarly, ARCH10747-2 had significantly higher emergence percentage than SQR (12%) and RTx430 (51%) in second planting (Fig. 2.5C). In 2017, all ARCH lines had significantly higher emergence percentage than RTx430 (18–57%) and three ARCH lines (except ARCH12012) with SQR (26–46%) in early planting (Fig. 2.5D). In 2016, all the root and shoot related traits differed significantly between planting dates and genotypes ($P < 0.05$ to $P < 0.0001$) except root length and shoot weight among genotypes (Table 2.2). Significant interaction between genotype and planting date was recorded with leaf area, root weight ($P < 0.05$), root diameter and root volume ($P < 0.01$) (Table 2.2). ARCH10747-2 produced significantly higher leaf area than other genotypes in first planting (Fig. 2.6B), with 105% higher leaf area than the average of the remaining genotypes. It recorded significantly higher root weight (Fig. 2.6C) and root volume (Fig. 2.6D) than ARCH12012, SQR and RTx430 in second planting. In 2017, all the recorded traits differed significantly ($P < 0.0001$) between planting dates but not with genotypes and their interactions (Table 2.2). Averaged across all genotypes in both the years, regular planting resulted in significantly ($P < 0.0001$) higher leaf area, shoot weight, root weight, length, area, diameter and root volume compared to early planting (Table 2.3).

2.3.4 Yield and related parameters

In 2016, days to 50% flowering and plant height were significantly affected by genotype, planting date, and their interaction ($P < 0.0001$) (Table 2.2). Panicle length was significantly affected by genotype and it's interaction with plantings dates ($P < 0.0001$) but not with planting date (Table 2.2). Yield per plant and test weight differed significantly between planting dates ($P < 0.0001$) but did not interact significantly with genotypes (Table 2.2). Averaged across all the genotypes first planting produced significantly ($P < 0.0001$) higher grain yield per plant than other plantings (Table 2.3, Fig. 2.7). RTx430 and SQR had significantly higher test weight and shorter panicle length ($P < 0.0001$) than other genotypes, respectively, averaged across plantings (Fig. 2.7, Table 2.3). In 2017, days to flowering, plant height, panicle length, yield per plant, and test weight differed significantly among genotypes ($P < 0.001$ to $P < 0.0001$) but only days to flowering differed significantly among planting dates ($P < 0.0001$). Panicle length, yield per plant and test weight recorded a significant interaction between genotype and planting date ($P < 0.05$ to $P < 0.01$). Yield per meter square differed significantly ($P < 0.05$) between planting dates but was not

affected by genotype and their interaction (Table 2.2). Averaged across all planting dates, from both the years, SQR and RTx430 were significantly ($P < 0.0001$) taller and took longer duration to flower, respectively. On the other hand, ARCH10747-2 was significantly shorter and needed lesser duration to flower (Fig. 2.7, Table 2.3).

2.3.5 Aerial phenotyping

The strength of the correlation between destructive measurements and HTP indices and the effectiveness of the latter in differentiating early season chilling tolerance can be classified into three phases, Phase I – emergence till 30 DAE (days after emergence); Phase II – 30–60 DAE and Phase III – after 60 DAE. Aerial phenotyping indices (NDVI and NDRE) had poor correlation with most of the destructive phenotyping measurements when they were not subjected to chilling stress which includes regular plantings III and IV in 2016 and planting II in 2017 (Supplementary Table 1). However, both NDVI and NDRE had significant correlation with many of the destructive measurements recorded under chilling conditions with early plantings I and II in 2016 (Table 2.4). Temporal correlations between NDVI and NDRE with emergence, leaf area, shoot weight, root weight, root length, root area, and root volume were significantly associated with planting I and II in 2016, when plants were exposed to chilling conditions (Table 2.4, Supplementary Table 1). The strong association between the aerial and destructive phenotyping approaches captured during June 2016 was between 30 and 60 DAE i.e., Phase II (Table 2.4, Fig. 2.3, Supplementary Table 1). However, correlation between aerial phenotyping indices including NDVI and NDRE with destructive measurements was weak and non-significant during early seedling stages i.e., emergence till 30 DAE (Phase I). This indicates that aerial phenotyping may not be effective under conditions where the seedling foliage is still in the process of development. In 2017, effective quantum yield of photosystem II (QY) and chlorophyll index recorded one month after planting with early planting was very low compared to regular planting (Fig. 2.8), supporting the poor correlation seen during Phase I compared to Phase II in 2016. Similarly, weak and non-significant correlation was observed between aerial phenotyping indices with physical measurements during later growth stages (Phase III) and with temperatures closer to optimum conditions (Supplementary Table 1).

2.4 Discussion

Based on multiple advantages mentioned in the introduction, developing sorghum with greater early season chilling tolerance in well adapted genetic background is a high priority target among the US and other sorghum improvement programs (Maulana et al., 2017). Our findings demonstrated that ARCH lines (ARCH10747-1, ARCH10747-2 and ARCH12045) recorded superior seedling emergence and early seedling vigor compared to popular chilling tolerant check SQR under both growth chamber and field conditions. The differential response of genotypes to chilling was more distinct under field conditions than with growth chambers, which supports the findings of Franks et al. (2006). The soil temperature profile presented in Fig. 2.1 supports this differential response from growth chamber and field-based chilling stress conditions. Temperatures imposed under growth chamber, although follow a diurnal pattern, were uniform and constant at 20/10°C air temperature throughout the period. However, a gradual increase in day/night temperature during early season plantings under field conditions (Fig. 2.1), provides the rationale for the above lack of association. Hence, a direct translation of the response from chambers to field conditions needs to be dealt with caution, unless the comparisons are made at relatively similar increments in temperatures in growth chambers as discussed below.

Optimum air temperature for sorghum growth and development is known to range between 21° and 35°C, with a minimum of 7° and 10°C required for germination and emergence, respectively (Peacock, 1982; Rooney, 2004). However, the minimum air temperature for inducing ≥50% sorghum germination and emergence is around 15°C (Peacock, 1982; Mann et al., 1985). Hence, 15°C is considered an ideal temperature to study chilling stress response in sorghum (Tiryaki and Andrews, 2001). Results from our growth chamber study supports this threshold (Fig. 2.2, Table 2.1). Many previous chilling stress studies have recorded either air temperature (Yu et al., 2004; Franks et al., 2006), soil temperature (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Maulana and Tesso, 2013), or both (Kapanigowda et al., 2013; Fernandez et al., 2015). However, the above studies have not highlighted the need to account for large differences between soil and air temperature under chilling conditions. In addition, having thresholds based on average day and night temperature masks the stressful impact induced by minimum night-time temperature compared to maximum day-time temperature, wherein the latter is generally close to or over the required optimum even under chilling conditions in the field (Table 2.5). Hence, to effectively compare genotypic responses between growth chamber and field conditions, we propose a day-

time maximum/night-time minimum of 20°/6°C (severe) and 20/9°C (moderate stress). In addition, increasing day and night air temperature by 0.5°C once every three days (Table 2.6), is an improvement over the generic 15°C threshold. The above revision to the phenotyping approach needs to be validated to help improve comparison between chambers and field conditions. However, when the objective is to identify true chilling tolerant donors from large populations a 20/10°C constant day/night temperature is justified.

Previous studies have reported Chinese kaoliang lines including SQR, to have high chilling tolerance but also possess undesirable agronomic traits like tall phenotype, high tannin content (Kapanigowda et al., 2013). On the contrary, ARCH lines in addition to higher chilling tolerance than SQR, are bred with desired agronomic traits including farmer desired height, compact to semi-compact panicle with complete panicle exertion, long panicle (ARCH12045), and lesser days to flower (ARCH10747-2 took 8 and 17 days lesser than SQR and RTx430, respectively, in 2016). The results support our first hypothesis that ARCH lines along with good agronomic background also have comparable early stage chilling tolerance as SQR. Except ARCH10747-2, other three ARCH lines are tannin-free. Tannin reduces protein digestibility in sorghum and hence considered undesirable in animal feed or human consumption (Proietti et al., 2015). Hence, both public and private grain sorghum breeding programs focus on developing tannin free hybrids. Thus, ARCH lines have shown promise to be used as novel donors for developing improved early season chilling tolerant sorghum hybrids for the US Great Plains and other locations having similar challenges (Maulana et al., 2017). There are reports indicating similar or even higher yields per plant from seedlings surviving the chilling temperature (Maulana and Tesso, 2013), which could be an artifact with few surviving seedlings receiving more than adequate sunlight and resources. This was also observed with our study (Fig. 2.7) and the above phenomena has been demonstrated in rice, soybean and other plants by altering the planting geometry (Kumagai et al., 2015; Kikuchi et al., 2017; Chiluwal et al., 2018). Hence, significantly higher grain yield per plant under challenging chilling stress exposure with planting I (Table 2.3, Fig. 2.7) is attributed to poor plant stand with the few surviving seedlings receiving abundant resources. Hence, advances made in early stage chilling tolerance needs to be evaluated on area basis, which has not been the case so far, primarily because of the lack of a genotype that can maintain normal plant population under such chilling conditions, limiting our ability to make progress in this direction. Hence, genotypes that can maintain good plant stand and seedling - both shoot and root vigor are key traits essential for

developing sorghum for chilling environments. In addition, occurrence of post emergent chilling spells is not unusual (Yu and Tuinstra, 2001; Kapanigowda et al., 2013; Maulana and Tesso, 2013). Although this was not tested in our studies, ARCH lines with improved chilling tolerance may be better placed to ward of such chilling spells during early vegetative stage. Further, improved ARCH lines recorded grain yield similar to the US elite cultivar RTx430, which is an additional benefit. Developing hybrids using these promising inbreds is seen as a way forward to enhance genetic potential for chilling tolerance and efforts in this direction are ongoing.

Previous studies dealing with early season chilling tolerance have used either growth chambers or field conditions, with a few of them validating the findings across both conditions (Tiryaki and Andrews, 2001; Yu et al., 2004; Franks et al., 2006; Kapanigowda et al., 2013; Maulana and Tesso, 2013). This is the first report connecting sorghum chilling temperature responses under growth chambers and field conditions, integrated with aerial high throughput phenotyping. Our findings demonstrate that promising ARCH lines were consistently superior over the checks across growth chambers and field conditions, with aerial phenotyping indices including NDVI and NDRE capturing the same response (Fig. 2.3). Although aerial phenotyping indices were able to capture genotypic differences within a few weeks (30–60 DAE; Phase II), but not earlier (emergence to 30 DAE; Phase I) which can be associated with photosynthetically less active green foliage. Significantly lower effective quantum yield of photosystem II (QY) and chlorophyll index one month after planting under chilling stress conditions supports the above hypothesis. In addition, chilling stress at the vegetative stage significantly reducing the photosynthetic rate has been documented (Ortiz et al., 2017). Since, leaves during Phase I were not able to perform normal photosynthetic activity, effectiveness of aerial phenotyping also diminishes as vegetation indices like NDVI and NDRE depends on absorbance and reflectance of visible and near-infrared light by the plant leaves. The ability of the aerial phenotyping to differentiate genotypic response at a later date (30–60 DAE; Phase II) was supported by strong relationships between NDVI and NDRE with almost all the destructively measured parameters with planting I and II in 2016 (Table 2.4). In summary, it is most effective to have aerial HTP measurements for determining chilling stress responses in sorghum between 30 and 60 DAE and not earlier or later. The findings provide evidence in support of our second hypothesis, that aerial phenotyping during the above-mentioned period can effectively differentiate chilling stress resilience in sorghum.

2.5 Conclusions

This study has captured the potential of advanced breeding lines with desirable agronomic characteristics accompanied by seedling stage chilling tolerance, compared to currently available checks. A strong correlation between destructive measurements and aerial phenotyping indices under chilling conditions facilitated differentiating genotypic responses. The results from this study extends the possibility of utilizing aerial high throughput phenotyping to explore large populations for genetic analysis and segregating populations for advancing breeding efforts to develop early season chilling tolerant sorghum.

2.6 References

An, N., C.M. Palmer, R.L. Baker, R.J.C. Markelz, J. Ta, M.F. Covington, J.N. Maloof, S.M. Welch, and C. Weinig. 2016. Plant high-throughput phenotyping using photogrammetry and imaging techniques to measure leaf length and rosette area. Comput. Electron. Agric. 127:376–394.

Assefa, Y., S.A. Staggenborg, and V.P.V. Prasad. 2010. Grain sorghum water requirement and responses to drought stress: a review. Crop. Manage. 9. https://doi.org/10.1094/CM2010-1109-01-RV.

Bekele, W.A., K. Fiedler, A. Shiringani, D. Schnaubelt, S. Windpassinger, R. Uptmoor, W. Friedt, and R.J. Snowdon. 2014. Unravelling the genetic complexity of sorghum seedling development under low-temperature conditions. Plant Cell Environ. 37:707–723.

Bendig, J., K. Yu, H. Aasen, A. Bolten, S. Bennertz, J. Broscheit, M.L. Gnyp, and G. Bareth. 2015. Combining UAV-based plant height from crop surface models, visible, and near infrared vegetation indices for biomass monitoring in barley. Int. J. Appl. Earth Obs. Geoinf. 39:79–87.

Blum, A. 2004. Sorghum physiology. In: H.T. Nguyen, and A. Blum (Eds.), Physiology and biotechnology integration for plant breeding. Marcel Dekker Inc., New Nork, pp. 141–223.

Boelman, N.T., M. Stieglitz., H.M. Rueth, M. Sommerkorn, K.L. Griffin, G.R. Shaver, and J.A. Gamon. 2003. Response of NDVI, biomass, and ecosystem gas exchange to long-term warming and fertilization in wet sedge tundra. Oecologia 135:414–421.

Brar, G.S., J.L. Steiner, P.W. Unger, and S.S. Prihar. 1992. Modeling sorghum seedling establishment from soil wetness and temperature of drying seed zones. Agron. J. 84:905–910.

Burow, G., J.J. Burke, Z. Xin, and C.D. Franks. 2011. Genetic dissection of early-season cold tolerance in sorghum (*Sorghum bicolor* (L.) Moench). Mol. Breed. 28:391–402.

Chiluwal, A., H.P. Singh, U. Sainju, B. Khanal, W.F. Whitehead, and B.P. Singh. 2018. Spacing effects on energycane growth and biomass yield. Crop Sci. 58:1371-1384.

Cisse, N., and G. Ejeta. 2003. Genetic variation and relationships among seedling vigor traits in sorghum. Crop Sci. 43:824–828.

Dash, J.P., M.S. Watt, G.D. Pearse, M. Heaphy, and H.S. Dungey. 2017. Assessing very high resolution UAV imagery for monitoring forest health during a simulated disease outbreak. ISPRS J. Photogramm. Remote Sens. 131:1–14.

Daughtry, C.S.T., C.L. Walthall, M.S. Kim, E.B. De Colstoun, and J.E. McMurtrey III. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sens. Environ. 74:229–239.

Doggett, H. 1988. Sorghum, second ed. Wiley, New York, NY.

Fernandez, M.G.S., G.R. Schoenbaum, and A.S. Goggi. 2015. Novel germplasm and screening methods for early cold tolerance in sorghum. Crop Sci. 54:2631–2638.

Fiedler, K., W.A. Bekele, W. Friedt, R. Snowdon, H. Stützel, A. Zacharias, and R. Uptmoor. 2012. Genetic dissection of the temperature dependent emergence processes in sorghum using a cumulative emergence model and stability parameters. Theor. Appl. Genet. 125:1647–1661.

Franks, C.D., G.B. Burow, and J.J. Burke. 2006. A comparison of US and Chinese sorghum germplasm for early season cold tolerance. Crop Sci. 46:1371–1376.

Gitelson, A., and M.N. Merzlyak. 1994. Spectral reflectance changes associated with autumn senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. leaves. Spectral features and relation to chlorophyll estimation. J. Plant Physiol. 143:286–292.

Gracia-Romero, A., S.C. Kefauver, O. Vergara-Diaz, M.A. Zaman-Allah, B.M. Prasanna, J.E. Cairns, and J.L. Araus, 2017. Comparative performance of ground versus aerially assessed RGB and multispectral indices for early-growth evaluation of maize performance under phosphorus fertilization. Front. Plant Sci. 8:2004.

Kapanigowda, M.H., R. Perumal, R.M. Aiken, T.J. Herald, S.R. Bean, and C.R. Little. 2013. Analyses of sorghum (*Sorghum bicolor* (L.) Moench) lines and hybrids in response to early-season planting and cool conditions. Can. J. Plant Sci. 93:773–784.

Kikuchi, S., R. Bheemanahalli, K.S.V. Jagadish, E. Kumagai, Y. Masuya, E. Kuroda, C. Raghavan, M. Dingkuhn, A. Abe, and H. Shimono, 2017. Genome-wide association mapping for phenotypic plasticity in rice. Plant Cell Environ. 40:1565–1575.

Knoll, J., N. Gunaratna, and G. Ejeta. 2008. QTL analysis of early-season cold tolerance in sorghum. Theor. Appl. Genet. 116:577–587.

Kumagai, E., N. Aoki, Y. Masuya, and H. Shimono. 2015. Phenotypic plasticity conditions the response of soybean seed yield to elevated atmospheric CO_2 concentration. Plant Physiol. 169:2021–2029.

Mann, J.A., E.E. Gbur, and F.R. Miller. 1985. A screening index for adaptation in sorghum cultivars. Crop Sci. 25:593–598.

Maulana, F., D. Weerasooriya, and T. Tesso. 2017. Sorghum landrace collections from cooler regions of the world exhibit magnificent genetic differentiation and early season cold tolerance. Front. Plant Sci. 8:756.

Maulana, F., and T.T. Tesso. 2013. Cold temperature episode at seedling and flowering stages reduces growth and yield components in sorghum. Crop Sci. 53:564–574.

Moges, S.M., W.R. Raun, R.W. Mullen, K.W. Freeman, G.V. Johnson, and J.B. Solie. 2005. Evaluation of green, red, and near infrared bands for predicting winter wheat biomass, nitrogen uptake, and final grain yield. J. Plant Nutr. 27:1431–1441.

Ortiz, D., J. Hu, and M.G. Salas Fernandez. 2017. Genetic architecture of photosynthesis in *Sorghum bicolor* under non-stress and cold stress conditions. J. Exp. Bot. 68:4545–4557.

Peacock, J.M., 1982. Response and tolerance of sorghum to temperature stress. In: Sorghum in the Eighties: Proceedings of the International Symposium on Sorghum. Patancheru, A.P, India. pp. 143–159.

Pennisi, E., 2009. How sorghum withstands heat and drought. Science 323:573.

Prasad, P.V.V., M. Djanaguiraman, R. Perumal, and I.A. Ciampitti. 2015. Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Front. Plant Sci. 6:820.

Proietti, I., C. Frazzoli, and A. Mantovani. 2015. Exploiting nutritional value of staple foods in the world's semi-arid areas: risks, benefits, challenges and opportunities of sorghum. Healthcare 3:172–193.

Rooney, W.L., 2004. Sorghum improvement-integrating traditional and new technology to produce improved genotypes. Adv. Agron. 83:37–109.

Rouse Jr., J.W., R.H. Haas, J.A. Schell, and D.W. Deering. 1974. Monitoring Vegetation Systems in the Great Plains With ERTS. (Accessed March 2018). http://ntrs.nasa. gov/search.jsp?R=19740022614.

SAS Institute Inc, 2013. SAS ver 9.4. SAS Institute Inc., Cary, NC, USA.

Smith, C.W., and R.A. Frederiksen. 2000. History of cultivar development in the United States: From "memoirs of A.B. Maunder - sorghum breeder'. In: Smith, C.W., and R.A. Frederiksen (Eds.), Sorghum: Origin, history, technology, and production. John Wiley & Sons, New York, NY, pp. 191–223.

Tiryaki, I., and D.J. Andrews, 2001. Germination and seedling cold tolerance in sorghum. Agron. J. 93:1391–1397.

Todd, S.W., R.M. Hoffer, and D.G., Milchunas. 1998. Biomass estimation on grazed and ungrazed rangelands using spectral indices. Int. J. Remote Sens. 19:427–438.

Yu, J., and M.R., Tuinstra. 2001. Genetic analysis of seedling growth under cold temperature stress in grain sorghum. Crop Sci. 41:1438–1443.

Yu, J., M.R. Tuinstra, M.M. Claassen, W.B. Gordon, and M.D. Witt. 2004. Analysis of cold tolerance in sorghum under controlled environment conditions. Field Crops Res. 85:21–30.

Table 2.1 Planting, emergence, and sampling dates and daily average soil and air temperature from the growth chamber and field studies in 2016 and 2017.

	Planting	Emergence	Sampling	Soil temperature (°C)*		Air temperature (°C)*	
				Max	Min	Max	Min
Growth Chamber							
15/15°C	15-Nov-16	29-Nov-16	30-Dec-16	16.8 ± 0.7	14.7 ± 0.1	17.8 ± 0.2	15.0 ± 0.1
20/10°C	15-Nov-16	28-Nov-16	28-Dec-16	20.7 ± 0.1	9.7 ± 0.04	22.0 ± 0.3	9.9 ± 0.05
20/15°C	15-Nov-16	26-Nov-16	24-Dec-16	21.4 ± 0.2	15.1 ± 0.1	22.4 ± 0.4	15.2 ± 0.04
32/22°C	18-Nov-16	26-Nov-16	26-Dec-16	35.4 ± 0.6	21.3 ± 0.3	35.1 ± 0.6	21.6 ± 0.7
Field 2016							
Planting I	1-Apr-16	15-Apr-16	18-May-16	22.5 ± 4.7	10.8 ± 2.8	20.3 ± 5.8	5.8 ± 3.9
Planting II	15-Apr-16	26-Apr-16	29-May-16	21.2 ± 4.8	14.2 ± 3.2	20.9 ± 6.2	8.6 ± 3.9
Planting III	13-May-16	25-May-16	28-Jun-16	30.6 ± 6.3	20.1 ± 4.1	28.7 ± 7.2	14.8 ± 4.9
Planting IV	2-Jun-16	8-Jun-16	11-Jul-16	31.4 ± 2.7	24.8 ± 1.9	33.1 ± 3.6	18.3 ± 3.2
Field 2017							
Planting I	21-Apr-17	9-May-17	8-Jun-17	22.5 ± 5.9	14.5 ± 4.7	22.7 ± 6.9	8.2 ± 5.2
Planting II	25-May-17	3-Jun-17	3-Jul-17	30.9 ± 3.6	21.2 ± 3.0	30.9 ± 4.5	15.0± 3.5

*Average daily minimum and maximum temperatures **(**°C ± standard deviation**)** during early seedling growth (until 30 days after emergence).

Table 2.2 Summary of analysis of variance for growth chamber and field experiments.

Traits	Growth Chamber			Field 2016			Field 2017		
	Genotype (G)	Temperature (T)	G×T	Genotype (G)	Planting date (D)	G×D	Genotype (G)	Planting date (D)	G×D
EP	<0.05	NS	NS	<0.0001	<0.0001	<0.0001	<0.0001		
EI	<0.05	<0.0001	<0.05	NS	<0.0001	NS	NS		
LA	NS	<0.0001	NS	<0.01	<0.0001	<0.05	NS	<0.0001	NS
SW	<0.05	<0.0001	<0.05	NS	<0.0001	NS	NS	<0.0001	NS
RW	NS	<0.0001	NS	<0.001	<0.0001	<0.05	NS	<0.0001	NS
RL	NS	<0.0001	NS	NS	<0.0001	NS	NS	<0.0001	NS
RA	NS	<0.0001	NS	<0.05	<0.0001	NS	NS	<0.0001	NS
RD	NS	<0.0001	NS	<0.05	<0.0001	<0.01	NS	<0.0001	NS
RV	NS	<0.0001	NS	<0.01	<0.0001	<0.01	NS	<0.0001	NS
DTF				<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS
PHT				<0.0001	<0.0001	<0.0001	<0.0001	NS	NS
PL				<0.0001	NS	<0.0001	<0.0001	NS	<0.01
GY/Plant				NS	<0.0001	NS	<0.001	NS	<0.01
200 SW				<0.0001	<0.0001	NS	<0.0001	NS	<0.05
GY/m^2							NS	<0.01	NS

EP - Emergence percentage, EI - Emergence index (days), LA - Leaf area (cm^2), SW- Shoot weight (g), RW - Root weight (g), RL - Root length (cm), RA - Root area (cm^2), RD - Average root diameter (mm), RV -Root volume (cm^3), DTF -Days to 50% flowering, PHT - Plant height (cm), PL - Panicle length (cm), GY/Plant - Grain yield (g/plant), GY/m^2 - Grain yield (g/m^2), 200 SW - 200 seed weight (g), Significant at the 0.05 to < 0.0001 probability level; NS - Non-significant.

Table 2.3 Summary of phenotypic differences among genotypes and temperatures/planting dates across all treatments.

Growth chamber study										
Traits	Mean across temperature/planting dates				Mean across genotype					
	15/15°C	20/10°C	20/15°C	32/22°C	ARCH 10747-1	ARCH 10747-2	ARCH 12012	ARCH 12045	SQR	RTx430
EP	77.78 a	77.78 a	72.22 a	83.33 a	72.92 bc	81.25 ab	58.33 c	79.17 ab	85.42 ab	89.58 a
EI	14.5 a	13.85 a	11.33 b	7.89 c	11.67 bc	11.58 bc	13.25 a	10.85 c	11.42 bc	12.58 ab
LA	3.11 b	14.63 b	55.29 b	727.99 a	244.51 a	251.42 a	166.65 a	179.35 a	182.87 a	176.71 a
SW	0.02 b	0.10 b	0.34 b	7.27 a	2.218 ab	2.68 a	1.23 c	1.99 abc	1.89 abc	1.57 bc
RW	0.01 c	0.08 bc	0.18 b	0.71 a	0.2125 a	0.30 a	0.2498 a	0.223 a	0.2325 a	0.25 a
RL	34.80 c	304.8 bc	908.53 b	6990.9 a	1958.2 a	2103.94 a	1924.5 a	2357.8 a	2148.0 a	1866.04 a
RA	4.72 c	52.94 bc	138.84 b	897.64 a	242.54 a	313.16 a	277.73 a	290.31 a	275.36 a	242.12 a
RD	0.45 c	0.59 a	0.51 b	0.41 d	0.47 a	0.53 a	0.49 a	0.48 a	0.45 a	0.52 a
RV	0.05 c	0.74 bc	1.72 b	9.55 a	2.51 a	3.79 a	3.30 a	2.89 a	2.98 a	2.61 a
Field study 2016										
Traits	P I	P II	P III	P IV	ARCH 10747-1	ARCH 10747-2	ARCH 12012	ARCH 12045	SQR	RTx430
EP	33.78 b	50.23 a			48.81 a	46.25 a	46.16 a	46.29 a	39.77 b	8.75 c
EI	15.06 b	17.34 a			12.70 a	12.52 a	12.70 a	12.44 a	13.03 a	12.98 a
LA	5.17 b	23.21 b	1655.3 a	1834.5 a	825.6 bc	884.48 b	600.21 c	928.2 ab	882.58 b	1156.17 a
SW	0.02 b	0.16 b	15.13 a	17.03 a	7.95 a	8.76 a	6.31 a	9.12 a	7.68 a	8.68 a
RW	0.01 c	0.05 c	1.32 b	2.14 a	0.65 b	0.99 a	0.71 b	0.92 a	1.05 a	0.96 a
RL	83.18 c	369.34 c	2846.9 b	3706.0 a	1817.0 a	1642.9 a	1803.4 a	1815.6 a	1932.7 a	1496.43 a
RA	27.50 c	163.77 b	1564.1 a	1623.8 a	746.12 c	848.3 abc	773.2 bc	902.1 ab	966.75 a	832.3 abc
RD	1.04 c	1.46 b	1.88 a	1.49 b	1.36 b	1.55 a	1.39 b	1.40 b	1.54 a	1.54 a
RV	0.74 c	5.93 c	60.00 b	73.63 a	26.72 b	36.72 a	28.56 b	37.39 a	42.32 a	38.74 a
DTF	108.2 a	89.28 b	70.83 c	60.28 d	80.75 c	73.08 d	82.42 c	85.50 b	81.08 c	90.08 a
PHT	103.5 b	112.55 a	113.98 a	111.04 a	104.55 c	94.75 d	98.27 d	114.02 b	151.39 a	98.74 d

PL	26.6 a	27.15 a	26.96 a	27.42 a	26.31 bc	25.61 c	26.40 bc	32.95 a	23.90 d	27.02 b
GY/Plant	79.21 a	38.90 c	43.77 c	60.64 b	62.46 a	54.60 a	50.21 a	65.19 a	56.26 a	45.05 a
200 SW	4.89 bc	4.63 c	5.13 ab	5.4 ab	4.58 c	4.31 c	4.32 c	5.10 b	5.05 b	6.73 a

Field study 2017

Traits	P I	P II	P III	P IV	ARCH 10747-1	ARCH 10747-2	ARCH 12012	ARCH 12045	SQR	RTx430
EP					65.78 ab	61.98 ab	52.97 bc	71.00 a	43.75 c	39.77 c
EI					12.90 b	12.75 b	13.25 b	12.98 b	12.93 b	13.92 a
LA	43.27 b	689.30 a			299.12 a	378.18 a	309.83 a	347.00 a	504.62 a	358.95 a
SW	0.31 b	6.43 a			2.57 a	3.42 a	3.46 a	3.33 a	4.35 a	3.08 a
RW	0.05 b	0.44 a			0.21 a	0.21 a	0.26 a	0.22 a	0.24 a	0.32 a
RL	40.01 b	359.54 a			165.72 a	174.41 a	211.23 a	177.63 a	230.75 a	237.77 a
RA	4.66 b	66.21 a			25.53 a	33.21 a	35.99 a	32.70 a	40.62 a	44.44 a
RD	0.36 b	0.57 a			0.43 a	0.51 a	0.45 a	0.47 a	0.45 a	0.49 a
RV	0.05 b	1.00 a			0.32 a	0.52 a	0.52 a	0.51 a	0.59 a	0.68 a
DTF	94.33 a	66.61 b			80.83 bc	71.50 d	79.33 bc	82.67 b	78.17 c	90.33 a
PHT	102.2 a	103.90 a			93.80 c	83.35 d	91.17 c	109.88 b	131.78 a	108.40 b
PL	25.89 a	25.35 a			24.45 cd	21.93 e	23.65 de	30.78 a	25.62 bc	27.28 b
GY/Plant	52.42 a	57.25 a			50.68 bc	45.53 cd	43.80 d	55.97 bc	75.03 a	58.00 b
200 SW	4.64 a	4.75 a			4.43 c	4.37 c	4.34 c	4.57 bc	5.50 a	4.95 b
GY/m^2	348.5 b	475.54 a			422.62 a	408.15 a	274.12 a	512.25 a	424.75 a	430.28 a

EP - Emergence percentage, EI - Emergence index (days), LA - Leaf area (cm^2), SW- Shoot weight (g), RW - Root weight (g), RL - Root length (cm), RA - Root area (cm^2), RD - Average root diameter (mm), RV -Root volume (cm^3), DTF -Days to 50% flowering, PHT - Plant height (cm), PL - Panicle length (cm), GY/Plant - Grain yield (g/plant), GY/m^2 - Grain yield (g/m^2), 200 SW - 200 seed weight (g). Numbers followed by the different letter within a row of the same trait are significantly different at P = 0.05.

Table 2.4 Significance of NDVI and NDRE and correlation with measured phenotypic traits in selected dates and plantings focusing on planting I and II in 2016.

Traits	Planting I, 2016								Planting II, 2016							
	NDVI June 17, 2016		NDRE June 17, 2016		NDVI June 28, 2016		NDRE June 28, 2016		NDVI June 17,2016		NDRE June 17, 2016		NDVI June 28, 2016		NDRE June 28, 2016	
	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r
EP	<0.01	0.75	<0.01	0.76	<0.0001	0.90	<0.0001	0.87	<0.01	0.66	<0.01	0.70	<0.05	0.53	<0.05	0.56
LA	0.0006	0.78	<0.001	0.82	<0.01	0.72	<0.01	0.76	<0.001	0.80	<0.0001	0.81	<0.01	0.64	<0.01	0.66
SW	<0.01	0.72	<0.001	0.77	<0.01	0.68	<0.01	0.76	<0.001	0.75	<0.0001	0.81	<0.01	0.67	<0.001	0.77
RW	NS	NS	NS	NS	NS	NS	NS	NS	<0.01	0.65	<0.01	0.70	<0.01	0.68	<0.001	0.77
RL	<0.01	0.68	<0.01	0.72	<0.05	0.60	<0.05	0.63	NS	NS	NS	NS	NS	NS	<0.05	0.49
RA	<0.01	0.67	<0.01	0.71	<0.05	0.60	<0.05	0.61	<0.05	0.49	<0.05	0.57	<0.01	0.61	<0.01	0.69
RD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RV	<0.05	0.63	<0.01	0.65	<0.05	0.56	<0.05	0.55	<0.01	0.63	<0.01	0.70	<0.01	0.70	<0.001	0.78
GY/Plant	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
200 SW	NS	NS	NS	NS	<0.01	-0.65	<0.05	-0.59	NS	NS	NS	NS	NS	NS	NS	NS

P – probability of significance, r – correlation coefficient, EP - Emergence percentage, LA - Leaf area (cm^2), SW- Shoot weight (g), RW - Root weight (g), RL - Root length (cm), RA - Root area (cm^2), RD - Average root diameter (mm), RV -Root volume (cm^3), GY/Plant - Grain yield (g/plant), 200 SW - 200 seed weight (g).

Table 2.5 Daily average soil and air temperature during first to 50% seedling emergence under early plantings in 2016.

Genotypes	Planting I				Planting II			
	Soil Temp (°C) *		Air Temp (°C) *		Soil Temp (°C) *		Air Temp (°C) *	
	Min	Max	Min	Max	Min	Max	Min	Max
ARCH10747-1	12.4	24.0	7.3	23.4	12.6	24.8	8.0	21.5
ARCH10747-2	11.1	23.4	4.3	21.2	12.6	24.8	8.0	21.5
ARCH12012	11.1	23.4	4.3	21.2	12.4	23.3	7.8	18.9
ARCH12045	12.4	24.0	7.3	23.4	12.6	24.8	8.0	21.5
RTx430	12.4	24.0	7.3	23.4	12.4	25.0	9.9	21.2
SQR	11.1	23.4	4.3	21.2	12.4	25.0	9.9	21.2
Mean	**11.6**	**23.7**	**5.8**	**22.3**	**12.5**	**24.6**	**8.6**	**21.0**

*Average daily temperatures.

Table 2.6 Slope of soil and air minimum and maximum temperature under early plantings in 2016 from planting to sampling dates.

	Planting I				Planting II			
	Soil Temperature		Air Temperature		Soil Temperature		Air Temperature	
	Min	Max	Min	Max	Min	Max	Min	Max
slope	0.09	0.04	0.12	0.02	0.13	0.12	0.14	0.15

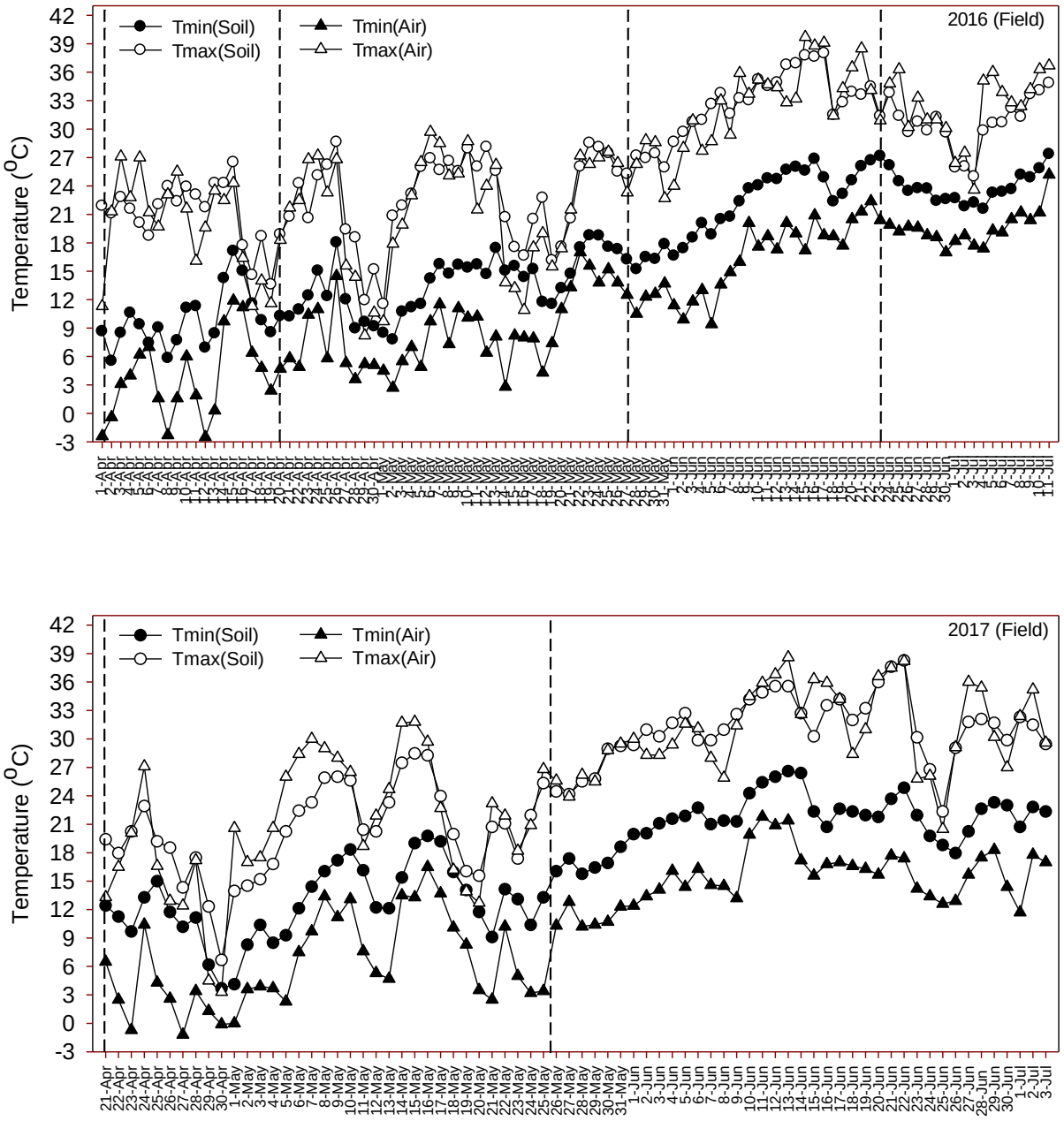

Figure 2.1 Soil and air minimum and maximum temperature in different planting dates and through the period till 30 days sampling in 2016 and 2017.

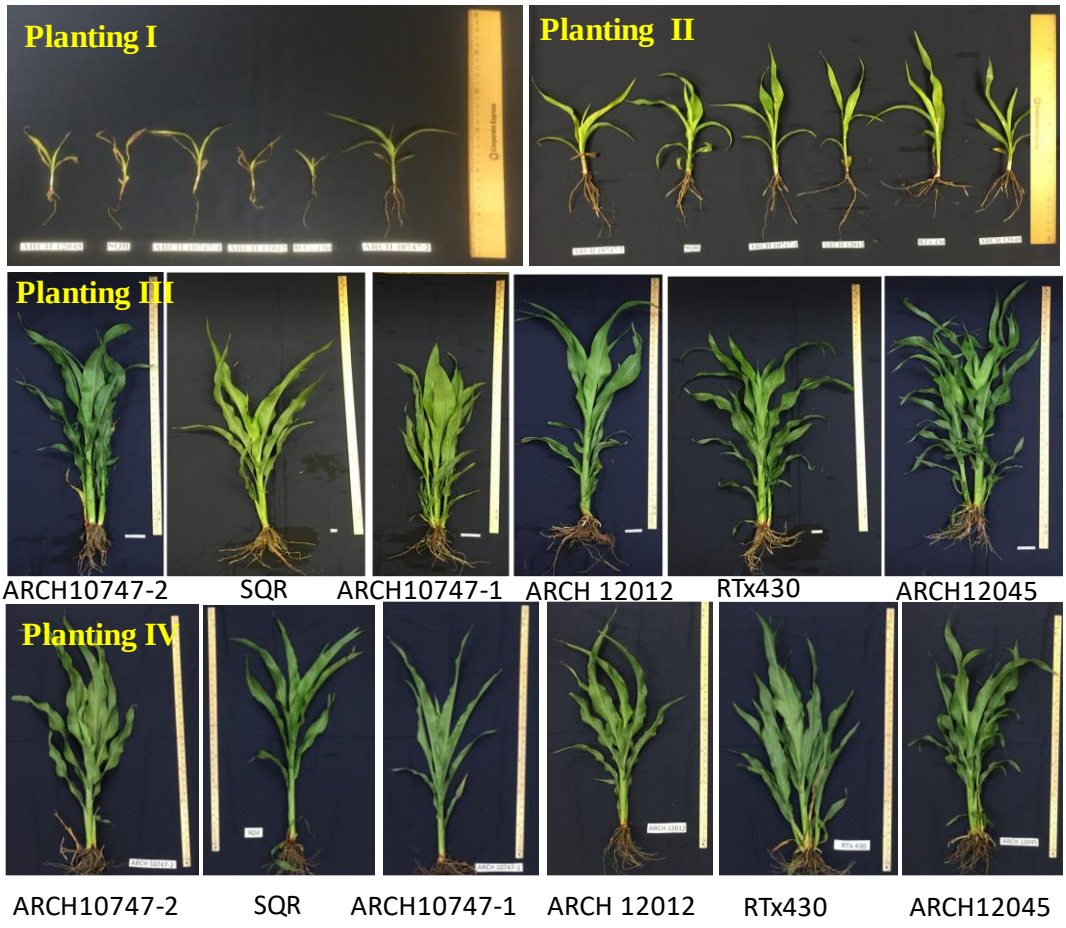

Figure 2.2 Whole plant images from all four plantings in 2016 field experiment.

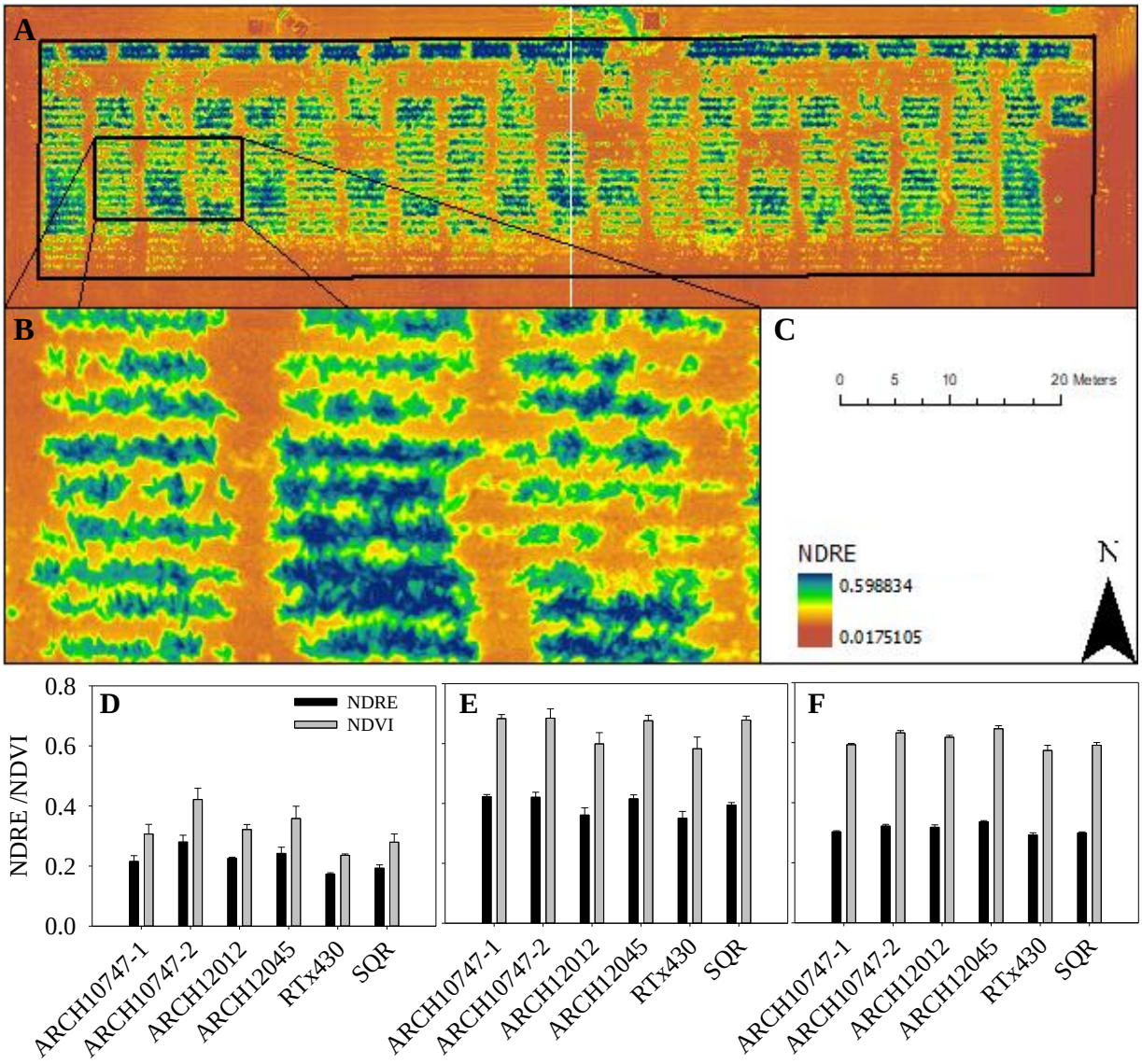

Figure 2.3 Summary of aerial phenotyping procedure followed in the study.

Aerial view of early season chilling stress experiments. Red-edge based field view of the study across different planting dates (planting I to IV, A) and regular planting (planting IV, B) captured on June 28, 2016. NDRE as the efficiency of photosystem (C). Average NDRE and NDVI of 6 genotypes: under planting I from aerial image captured on June 17, 2016 (D), in planting II from aerial image captured on June 28, 2016 (E), in planting I from aerial image captured on June 26, 2017 (F).

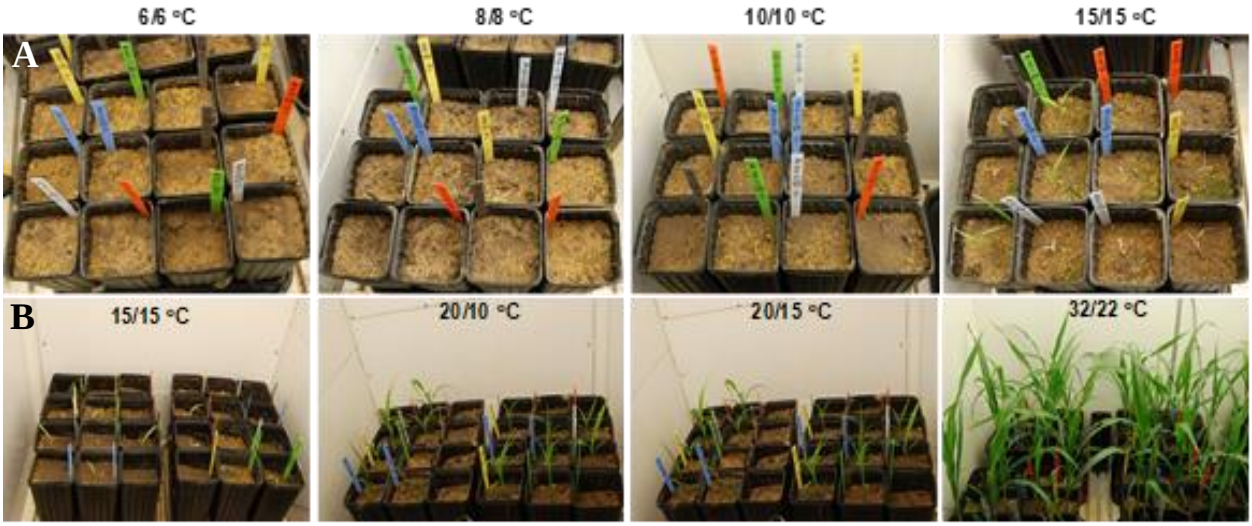

Figure 2.4 Sorghum response under different chilling temperature in growth chamber.
Sorghum chilling response to constant day and night chilling temperature (A). Combination of different day and night temperature inducing chilling and control 33/22ºC day/night temperature (B).

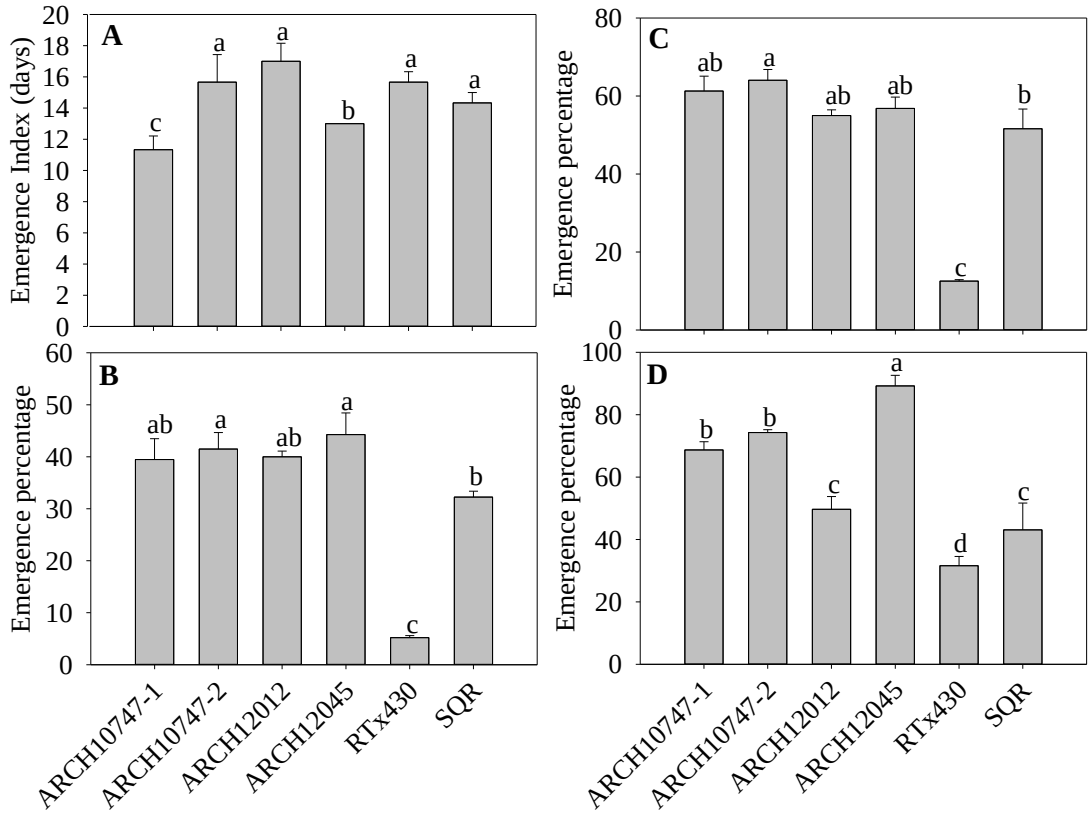

Figure 2.5 Chilling stress induced genotypic response on seedling emergence traits.

A - emergence index under 15/15°C in growth chamber, B - emergence percentage in planting I - 2016, C - emergence percentage in planting II - 2016, D - emergence percentage in planting I - 2017. Bars indicate standard error of the mean ± 3 replications. Bars with different letters within a figure are significantly different at P = 0.05. Response of all genotypes for these traits that determine plant stand across years and planting dates are presented in Table 2.3.

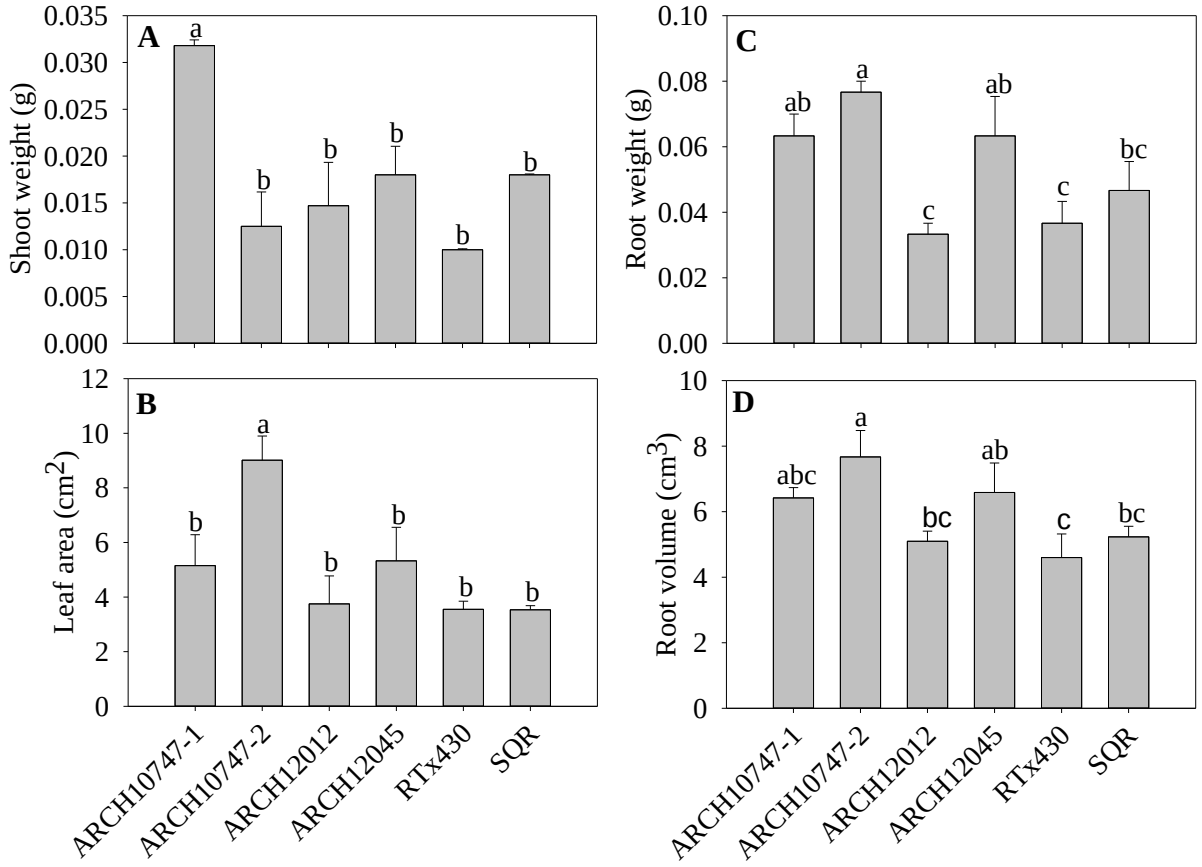

Figure 2.6 Chilling stress induced genotypic responses on traits related to seedling vigor.

A - shoot weight under 15/15°C in growth chamber, B - leaf area in planting I - 2016, C - root weight in planting II - 2016, D - root volume in planting II - 2016. Destructive sampling was done 30 days after seedling emergence. Bars indicate standard error of the mean ± 3 replications. Bars with different letters within a figure are significantly different at P = 0.05. Response of all genotypes for these traits and other above and below ground traits that determine seedling growth and vigor across years and planting dates are presented in Table 2.3.

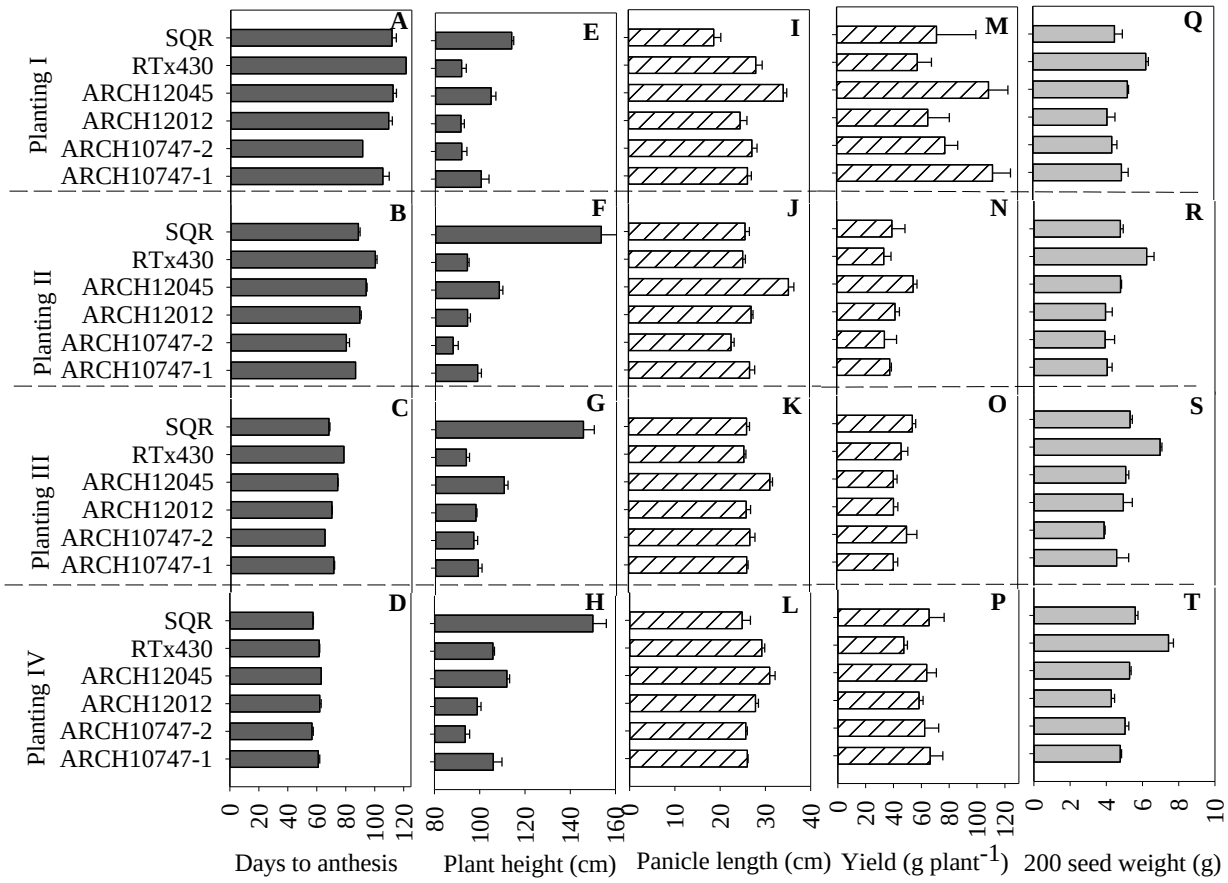

Figure 2.7 Effect of planting dates on anthesis, plant height, panicle length, grain yield and 200 seed weight.

Variations in days to anthesis (A-D), plant height (E-H), panicle length (I-L), grain yield (M-P) and 200 seed weight (Q-T) under four different planting environments in 2016 field study. Bars indicate standard error of the mean ± 3 replications.

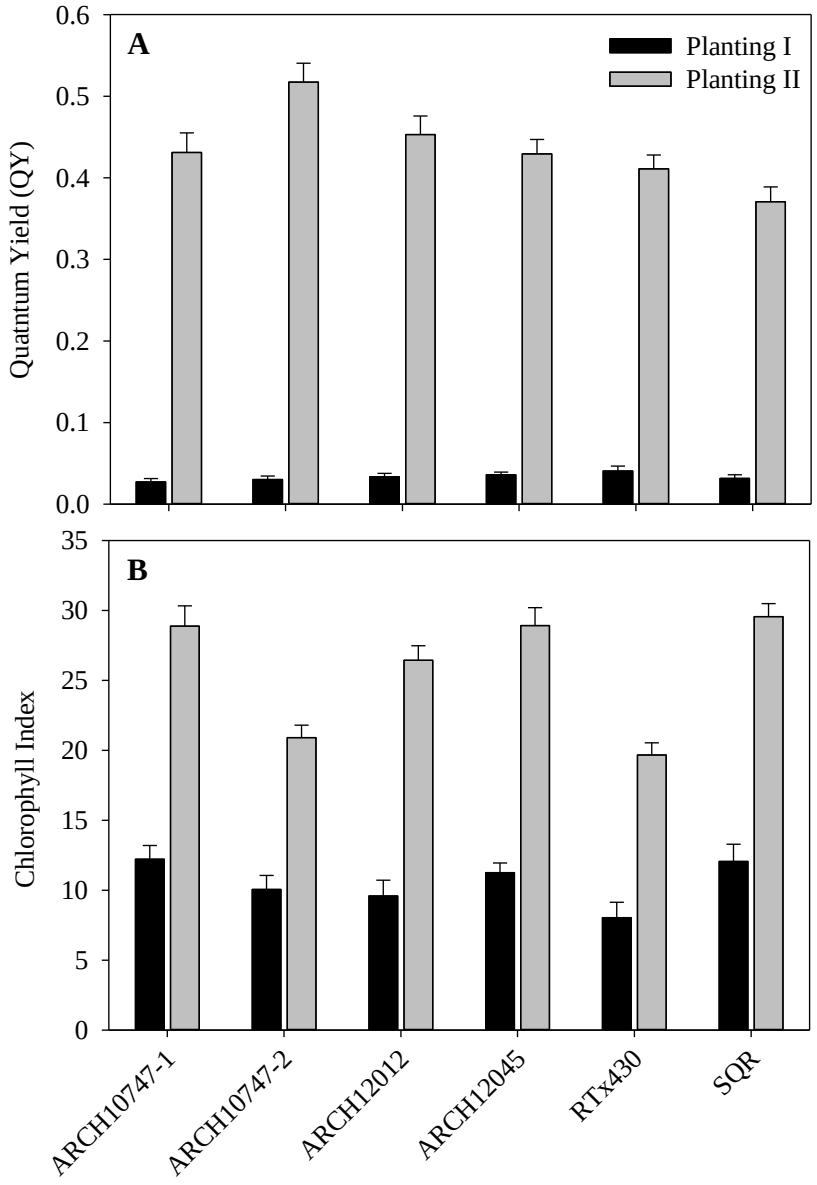

Figure 2.8 Chilling stress impact on effective quantum yield of photosystem II and chlorophyll index.

Effective quantum yield of photosystem II (A) and chlorophyll index (B) recorded one month after planting under Planting I and II in 2017. Bars indicate standard error of the mean ± 3 replications.

Chapter 3 - Unraveling physiological mechanisms inducing heat stress resilience in sorghum during flowering

Abstract

Sorghum is often considered as one of the hardier cereal to heat stress exposure during anthesis but the mechanisms that induce resilience are not known. A diverse set of sorghum inbreds and selected hybrids were tested under greenhouse, growth chamber facilities and field conditions. A highly conserved early-morning-flowering mechanism was recorded across all the inbreds and hybrids, with the peak anthesis with >90% of florets flowering within 30 min after dawn. The conserved response was consistent even under heat stress exposure imposed at different times of the day. Our findings report a novel heat escaping early-morning-flowering mechanism effectively employed by sorghum to minimize heat stress impact at anthesis. Another experiment was conducted to unravel heat stress impact on sensitive processes during flowering with a control (32/22°C) and 3 daytime temperature treatments (35/22°C, 38/22°C, 40/22°C), using 4 contrasting genotypes. *In vitro* pollen germination analysis revealed heat stress affects pollen viability resulting in lower pollen germination. Significant correlation between *in vitro* pollen germination and grain number suggest reduced pollen germination to be one of the factors for heat stress induced reduction in seed-set, grain number and consequently grain yield. However, despite >25% pollen germination in some of the genotypes, nearly 100% floret sterility was recorded under 40/22°C indicating heat stress also impacting post-pollen-germination processes such as fertilization, embryo formation and development reducing seed-set and grain yield in sorghum. All genotypes recorded almost no grain under 40/22°C with 38/22°C clearly differentiating the tolerant from the susceptible cultivars. We identified a heat tolerant genotype (Macia) from the study which appears to be a promising donor for developing improved heat tolerant sorghum hybrids.

3.1 Introduction

Climate models predict a steady increase in global mean temperature by up to 3.7°C by the end of the 21st Century (IPCC, 2013). Historically, plants have evolved to mitigate such increases in mean temperatures extending over long time-frame, but the increase in temperature variability around the mean is a significant new dimension that most crops are not programmed to cope with. This increase in variability will lead to more intense but short episodes of heat stress, which when coincided with critical reproductive stage such as flowering leads to negative impact on yield and productivity in field crops (Jagadish et al., 2010; Prasad et al., 2008, 2015, 2017; Siebers et al., 2017; Aiqing et al., 2018; Djanaguiraman et al., 2018). Even under current environmental conditions, heat stress coinciding with key developmental stages have resulted in significant economic losses in field crops grown in different geographical locations (rice - Ishimaru et al., 2016; wheat - Tack et al., 2015; sorghum - Tack et al., 2017). Such scenarios are predicted to increase further under future warmer climate, potentially leading to increased agricultural losses and jeopardizing ongoing efforts to sustain global food security. Hence, significant efforts are ongoing to explore large genetic diversity to identify donors that can induce greater resilience to such short episodes of stress affecting physiological processes during flowering, fertilization and early embryo development (Shi et al., 2018).

Within the reproductive stage, flowering is identified as the most vulnerable stage, wherein a series of heat stress sensitive physiological processes including anther dehiscence, pollination and pollen germination, fertilization and embryo formation occur in a short time span (For example, rice – 1-4 h [Cho et al., 1956; Shi et al., 2018], sorghum – as early as 2 h [Stephens and Quinby, 1934; Bandyopadhyay et al., 1998]). Extensive investigations across multiple crops have led to the conclusion that temperature coinciding with the flower opening (anthesis) to be key determining criteria for off-setting normal fertilization and seed-set, but minimally with similar temperatures occurring even an hour after anthesis (Yoshida et al., 1981; Shi et al., 2018). Considering the extreme sensitivity of anthesis and specifically pollen viability, sources of early-morning-flowering (EMF) trait from wild rice (*Oryza officinalis*) has been successfully transferred to heat sensitive local popular varieties (Hirabayashi et al., 2014). This introgression has resulted in significant reduction in spikelet sterility at highly challenging temperatures under growth chamber conditions (Ishimaru et al., 2010; Hirabayashi et al., 2014) and recently under field

conditions (Bheemanahalli et al., 2017). Similarly, field and growth chamber studies have indicated that sorghum like rice and other crops are sensitive to heat stress during anthesis, reducing pollen viability and in turn lowering seed-set and grain yields (Prasad et al., 2008, 2015; Nguyen et al., 2013; Djanaguiraman et al., 2014; Singh et al., 2015). However, a systematic analysis of the flower opening time-of-day using genetically diverse lines has never been attempted in sorghum to ascertain if inducing earlier flowering in sorghum would help minimize the impact of heat stress under current and more importantly under anticipated future hotter climate. Hence, using 24 highly diverse genotypes (Sunoj et al., 2017), hybrids grown in field, greenhouse and growth chamber facility, imposing different temperature conditions we posed the question – Is sorghum an escaper or truly tolerant to heat stress during flowering? We hypothesized that sorghum primarily follows escaping mechanism to avoid heat stress impact during flowering. To address this, four different experiments were conducted to – i) Ascertain the time-of-day of anthesis and its variability under different environmental conditions among diverse sorghum cultivars and hybrids; ii) Investigate the quantitative impact of heat stress on pollen germination, floret sterility and grain yield.

3.2 Materials and Methods

All the four experiments were conducted in greenhouse, growth chamber facilities at Throckmorton Plant Science Center or the North Experimental Farm at Kansas State University, Manhattan, Kansas, USA.

3.2.1 Experiment 1

Twenty-four genetically and geographically diverse sorghum genotypes which are parents of two independent Nested Association Mapping (NAM) populations (Casa et al., 2008; Yu et al., 2013), were selected for recording the time-of-day of flower opening. Plants were grown in 15 L pots (top diameter = 27.5 cm and bottom diameter = 26 cm; pot height = 25 cm). Metro mix 380 (Hummert International Topeka, KS, USA) was used as growing medium which was fertilized with 26 grams of Osmocote (controlled-release fertilizer, 14:14:14% N:P:K, respectively; Hummert International) and 4 g of Micro-max (micronutrient granules; Hummert International). Four seeds were sown at 4 cm depth and one plant per pot was maintained by thinning, 2 weeks

after emergence. Four grams of Marathon (Systemic insecticide; OHP Inc, Maryland, PA, USA) per pot was applied a week after seedling emergence for insect control. Plants were grown under natural environmental conditions in greenhouse. The experiment was laid out in a completely randomized design with two treatments i.e. control and drought stress during flowering. There were four replications in each treatment. Plants under control treatment were irrigated at regular interval as per requirements and care was taken to ensure that there was no water-deficit stress during flowering. Irrigation was stopped after 80% panicle emergence in drought stress treatment to impose drought stress during flowering.

3.2.2 Experiment 2

Flower opening pattern in three commercial sorghum hybrids (Pioneer 84G62, Dekalb [DKS51-01] and Dekalb [DKS38-88]) were observed under field conditions using the plants grown for 2016 grain sorghum performance tests, by Kansas Agricultural Experiment Station. Fifteen flowering panicles in each hybrid (5 plants per replication) were tagged a day before the observation.

3.2.3 Experiment 3

Three contrasting sorghum NAM founder lines (RTx430, P898012, Macia) identified for their contrasting response to heat stress under field conditions (Sunoj et al., 2017) and a commercial popular sorghum hybrid (Dekalb-DKS51-50) were selected for recording the time-of-day of flower opening under heat stress imposed during different times of the day. Crop husbandry was followed similar to Experiment 1. The experiment was laid out in a split-plot completely randomized design with 3 replications, where temperature was the main-plot and genotypes the sub-plot. There were 9 pots/plants per genotype (3 for each treatment) with a total of 36 pots. Plants were grown in controlled environment growth chambers under control conditions (32/22°C maximum day/minimum night temperature) until 80% panicle emergence (about 4 d before anthesis). Three plants each were then transferred to 3 different temperatures treatments lasting 12 days (i) control (32/22°C day/night temperature; actual inside chambers: 31.3±0.3°C day/21.9±0.5°C night temperature), (ii) heat stress during early morning (40/22°C; 6 to 11 AM actual: 39.9±0.9°C day/21.7±0.3°C night temperature), coinciding with peak flowering time and

(iii) heat stress during the day (40/22°C; 9 AM to 4 PM actual: 40.5±0.3°C day/22.7±0.3°C night temperature). Light intensity of 1000–1200 µmol m^{-2} s^{-1}, 12 h photoperiod (0600 h to 1800 h) and 70% relative humidity was maintained in all growth chambers. After the heat stress treatments, plants were moved to greenhouse and grown under natural environmental conditions until physiological maturity.

3.2.4 Experiment 4

In this experiment, the same four genotypes (RTx430, P898012, Macia and Dekalb [DKS51-50]) used in experiment 3 were used to assess the quantitative impact of heat stress on pollen germination, floret sterility and grain yield. The experiment was conducted in greenhouse and controlled environment growth chamber facility and crop husbandry was followed similar to previous experiments. The experiment was laid out in split-plot complete randomized design where temperature was the main-plot and genotype the sub-plot. Incubation temperature (for *in vitro* pollen germination), day of flowering (for floret sterility) and day of observation (for effective quantum yield of PSII and chlorophyll index) were considered as sub-sub plot factor. Twenty-eight pots per genotype with a total of 112 pots (for 4 genotypes) were maintained under control conditions in greenhouse (32/22°C, day/night temperatures) until 80% panicle emergence (about 4 d before anthesis). A set of seven plants each were then transferred from greenhouse to growth chamber facility maintained at four different temperature treatments including control condition (control - 32/22°C; actual inside chamber: 32.8±0.3/22.2±0.2°C, Heat Stress - 35/22°C, actual: 35.3±0.5/21.9±0.1°C; 38/22°C, actual: 38.5±0.6/22.7±0.4°C and 40/22°C actual: 40.5±0.9/22.7±0.3°C day/night temperature; 9AM to 4PM) for 12 days. Out of the seven plants from each genotype in each treatment, three were used to collect pollen for the pollen germination studies (destructive measurements). The remaining 4 plants were used for recording quantitative impact of heat stress on leaf chlorophyll content, light adapted effective quantum yield of photosystem II, floret sterility, grain number and grain weight per panicle. Spikelets that flowered on first, second, third and after third day of flowering were separated by marking last flowering branches on each day around 5PM. Acrylic paint with different colors (black - first day, blue - second day, red - third day) were used for marking flowering branches. Floret sterility for each flowering day (first, second, third and remaining) was estimated separately to quantify the

quantitative impact of heat stress as determined in rice (Rang et al., 2011). After 12 days of heat stress, plants from each treatment were returned to greenhouse and were maintained under non-stress condition until physiological maturity.

Standard liquid pollen germination media (H_3BO_3 [150 mg], $Ca(NO_3)_2.4H_2O$ [500 mg], $MgSO_4.7H_2O$ [200 mg], KNO_3 [100 mg] and sucrose [300 g] dissolved in 1 L of deionized water) used by Prasad et al. (2006) was used as *in vitro* pollen germination media for RTx430 and Dekalb. Standard pollen germination media was slightly modified with different concentrations of $Ca(NO_3)$ as recommended by Sunoj et al. (2017) for Macia (540mg $Ca(NO_3)_2.4H_2O$) and P898012 (460 mg $Ca(NO_3)_2.4H_2O$). Germination media was prepared fresh and stored at 4°C a day before pollen collection. The media was warmed using microwave just before pollen collection and then poured into the chamber slides. *In vitro* pollen germination was estimated by incubating glass chamber slides having pollen grains collected from plants grown at 32/22°C in four different incubation temperatures (32°C, 35°C, 38°C and 40°C) using four incubators. Similarly, pollen grains collected from plants grown under other heat stress temperature treatments (35/22°C, 38/22°C and 40/22°C) were incubated at all four incubation temperatures (32°C, 35°C, 38°C and 40°C). Microclimatic conditions within the incubators were monitored at 15-min intervals during incubation using a HOBO data logger (Onset Computer Corporation, Bourne, MA, USA). Actual temperature of 32.2±0.3°C, 35.3 ±0.6°C, 37.6±0.8°C and 41.0±0.9°C were recorded in those incubators.

3.2.5 Observations

Total number of newly opened flowers on the main panicles were counted at half an hour interval starting from 6 AM till noon for three to four consecutive flowering days in experiment 1, 2 and 3. Number of flowers that opened at every half an hour interval were recorded cumulatively to avoid any manual stimuli, which is known to alter the flowering pattern (Kobayasi et al., 2010). Anthers were carefully removed from the panicles around 4 PM on the preceding day of the observation, to ensure only newly opened flowers were recorded on the observation day. In the third experiment, panicles were harvested after physiological maturity. The harvested panicles were oven dried at 40°C for 7 days. Panicles were hand threshed and grain weight per panicle was recorded.

In experiment 4, pollen grains from each treatment were collected from spikelets that flowered on the second day just before anthesis (0600–0630 h) and placed in glass chamber slides containing germination media and immediately incubated at different temperatures as mentioned above for 2 hours. After the incubation, glass slides were stored in refrigerator at 4°C to arrest any further pollen germination after the incubation treatments. Images of the pollens (at least 6 pictures per glass slides) were taken randomly using compound light microscope with a resolution of 100X (BX 51, Olympus fitted with DP 70 camera). Pollen germination was calculated as the proportion of total number of pollen germinated to total number of pollens in each field of view. Pollen was considered germinated if pollen tube was longer than the pollen diameter (Prasad et al., 2006). Effective quantum yield of photosystem II (QY) was measured in the flag leaf on 6 and 12 days after temperature treatment using the portable fluorometer FluorPen (FluorPen FP 100, Photon System Instruments, Ltd., Brno,Czech Republic). Leaf chlorophyll index was also measured on the same day in the same flag leaf using Dualex senor (Dualex ScientificTM). At maturity, panicles were harvested from all the treatments. Panicles were separated into 4 parts and harvested separately based on the branches that flowered on first, second, third and after third day of flowering. Floret sterility for each day of flowering (first, second, third and after third day) and in whole panicles were estimated. Panicles were oven dried at 40°C for 7 days, then hand threshed to check the number of filled and unfilled florets to determine floret sterility. Floret sterility was estimated as the proportion of the unfilled florets to the total number of florets (sum of sterile florets and total number of seeds). Grain number and grain weight per panicle were recorded. 200 grains from each treatment was weighed and 1000-grain weight was estimated.

3.2.6 Statistical analysis

Statistical analysis was done using SAS (ver 9.4; SAS Institute, 2013). Analysis of variance (ANOVA) was performed using GLM procedure. Genotypes, temperature, incubation, day of flowering and observation were considered as fixed effect while replication was considered as random effects. Means were separated using least significant difference (LSD), when treatments and interactions were significant at $P \leq 0.05$.

3.3 Results

3.3.1 Experiment 1, 2 and 3

All 24 genotypes showed very early morning peak flowering i.e. >90% of the florets on the panicles flowered before 7 AM, and consistently across different flowering days (Fig. 3.1a). Similarly, all three hybrids in field conditions also had >90% of flowering completed by 6:30 AM, with a large proportion flowering right at the onset of dawn (Fig. 3.1b and 3.1c). These observations confirmed the consistent expression of early-morning-flowering (EMF) trait across very diverse sorghum inbreds and tested hybrids across controlled environments and field conditions. All four genotypes, with contrasting response to heat stress under control, heat stress during early morning (40/22°C, 6 to 11 AM), heat stress during day (40/22°C, 9 AM to 4 PM) and drought stress (Fig. 3.2) had similar flower opening pattern as the 24 genotypes i.e. majority of florets opening (>90%) before 7AM. On exposure to 40^{o}C either during early morning (6 to 11 AM) or during the day (9 AM to 4 PM) resulted in all four genotypes having nearly complete sterility (Fig. 3.3a and Fig. 3.3b).

3.3.2 Experiment 4

3.3.2.1 Pollen germination

Pollen germination was significantly affected by temperature ($P < 0.0001$), incubation ($P < 0.0001$) but not by genotype (Table 3.1). Interactions were significant for temperature × genotype ($P < 0.01$), temperature × incubation ($P < 0.05$), genotype × incubation ($P < 0.0001$) and temperature × genotype × incubation ($P < 0.0001$) (Table 3.1). Pollen germination was negatively affected by higher temperature in all the genotypes. Across all genotypes and incubation temperatures, pollen germination decreased gradually with sequential increase in temperature treatments (Fig. 3.4 and Fig. 3.5). On average, across all four cultivars, 63.1%, 53.9%, 38.0 % and 17.5% pollen germination were recorded under 32/22°C, 35/22°C, 38/22°C and 40/22°C respectively. Across all genotypes and temperature treatments, no significant differences in pollen germination was observed with 32°C, 35°C and 38°C incubation temperatures however pollen germination was found significantly lower under 40°C compared with other incubation temperatures (Fig. 3.4).

3.3.2.2 Floret sterility

Floret sterility was significantly affected by temperature ($P < 0.0001$), genotype ($P < 0.0001$) and day of the flowering ($P < 0.0001$) and all two way and three-way interactions between these factors ($P < 0.0001$) (Table 3.1). Across all genotypes, floret sterility increased with sequential increase in temperature from 32/22°C to 40/22°C (Fig. 3.6a). Similar to the previous experiment, plants under 40/22°C recorded 100% floret sterility (Fig. 3.6a). Significant differences in floret sterility among the genotypes were observed. Heat stress with 35/22°C resulted in 5%, 13%, 22% and 25% and with 38/22°C resulted in 18%, 40%, 41% and 48% floret sterility in Macia, Dekalb, RTx430 and P898012, respectively. Across all temperature treatments, and on different days of flowering Macia recorded significantly lower floret sterility than other genotypes (Fig 3.7a). Across all temperature treatments in all the genotypes, flowers that opened after third day of flowering had significantly higher floret sterility compared with flowers that opened during first few days (Fig. 3.7a).

3.3.2.3 Yield and yield components

Grain weight, grain number and 1000 grain weight were significantly affected by temperature, genotypes, and their interaction ($P < 0.0001$) (Table 3.1). Across all genotypes, grain weight and grain number decreased significantly as the level of temperature increased from 32/22°C to 38/22°C (Fig. 3.6b and Fig 3.6c). Across all temperature treatments, Macia produced significantly higher grain weight and grain number and P898012 had significantly lowest grain weight and grain number compared to other genotypes. Heat stress with 40/22°C resulted in complete floret sterility in all genotypes and no grain yield was recorded (Fig. 3.6a). Grain weight in P898012 was significantly reduced under both 35/22°C and 38/22°C compared with control treatment, while Dekalb and RTx430 recorded significantly lower grain yield only under 38/22°C compared with control (Fig 3.6c). However, no significant reduction in grain weight was observed in Macia under both intermediate heat stress treatments (35/22°C and 38/22°C) compared with control (Fig. 3.6c). Grain number in Dekalb reduced significantly under both intermediate heat stress treatments (35/22°C and 38/22°C) while P898012, RTx430 and Macia recorded significant reduction in grain number only under 38/22°C (Fig. 3.6b). No significant differences in 1000 grain weight were found in Macia, RTx430 and Dekalb across all the temperature treatments however

it reduced significantly in P898012 under 38°C/22° compared with 32/22°C and 35/22°C (Fig. 3.6d). Significant positive relationship between *in vitro* pollen germination and grain number (R^2 = 0.30 to 0.66, with different incubation temperatures) was observed (Fig. 3.8a). Significant negative correlation between pollen germination with floret sterility (R^2 = 0.80 to 0.89, with different incubation temperatures) was observed (Fig. 3.8b).

3.3.2.4 Photosynthetic efficiency

Effective quantum yield of photosystem II (QY) differed significantly among temperature ($P < 0.01$), genotype ($P < 0.0001$) and day of observation ($P < 0.0001$) (Table 3.1). Interactions were significant for temperature × genotype ($P < 0.0001$), genotype × day ($P < 0.0001$) and temperature × genotype × day ($P < 0.0001$) but not with temperature and day of observation (Table 3.1). No significant difference in QY among the temperature treatments were observed in Dekalb, Macia and RTx430 on 6 and 12 days after stress imposition (Fig. 3.9a and Fig. 3.9b). However, in P898012, QY decreased significantly under 40/22°C compared with 32/22°C and 35/22°C treatments on 6 days after stress imposition and further decreased significantly than all other treatments (32/22°C, 35/22°C and 38/22°C) when recorded on 12 days after 40/22°C stress imposition (Fig. 3.9a and Fig. 3.9b).

3.3.2.5 Chlorophyll index

Chlorophyll index differed significantly among temperature ($P < 0.001$), genotypes ($P < 0.0001$) and day of observation ($P < 0.0001$) (Table 3.1). Interactions were significant for temperature × genotype ($P < 0.0001$), genotype × day ($P < 0.05$) and temperature × genotype × day ($P < 0.001$) but not with temperature and day of observation (Table 3.1). Across all genotypes, with observations on 6 and 12 days after stress imposition, no differences in chlorophyll index were observed under 32/22°C, 35/22°C and 38/22°C while chlorophyll index was significantly reduced under 40/22°C compared to the other three treatments. No significant differences in chlorophyll index among temperature treatments were observed in Macia after 6 and 12 days after stress imposition (Fig. 3.9c and Fig. 3.9d). In P898012 chlorophyll index decreased significantly under 40/22°C than other treatments on 12 days after stress imposition (Fig. 3.9d). Chlorophyll

index in Dekalb and RTx430 were significantly lower under 40/22°C compared to other temperatures on both 6 and 12 days after stress imposition (Fig. 3.9c and Fig.3.9d).

3.4 Discussion

The general assumption is that hardy dryland cereals such as sorghum and millets have been equipped to overcome harsh and marginal environments that they are currently cultivated and houses the potential to sustain productivity even under future harsher climate. However, a significant knowledge gap exists on mechanisms that operate in these species to ensure their potential to adapt to constantly changing climate. Investigating highly diverse sorghum accessions assembled across Asia, Africa and the United States (Bouchet et al., 2017; Sunoj et al., 2017) and popular hybrids in both controlled environments and the field conditions, a highly conserved early-morning-flowering (EMF) mechanism was consistently documented (Fig. 3.1). Interestingly, to overcome heat stress induced increase in spikelet sterility in rice, *Oryza officinalis* a wild rice accession was used as a donor to introduce EMF trait (Ishimaru et al., 2010; Hirabayashi et al., 2014). The introduction of this trait facilitated reduction in spikelet sterility by 71% compared to 289 other rice cultivars in hotter climate under field conditions (Bheemanahalli et al., 2017), by effectively demonstrating the heat stress escaping phenomenon. Sorghum naturally equipped with EMF, even under heat (Fig. 3.1b and Fig. 3.1c) and drought stress (Fig. 3.2), indicates an effective mechanism that has made it possible to sustain productivity even under severe heat and drought stress conditions.

Previous studies on heat stress impact at flowering in sorghum have concluded that significant reduction in pollen viability to be the major driver for stress induced reduction in seed-set (Prasad et al., 2006; Jain et al., 2007; Prasad et al., 2011; Nguyen et al., 2013; Djanaguiraman et al., 2014; Singh et al., 2015; Djanaguiraman et al., 2018). Recently, the role of the female reproductive organ partially determining heat stress impact at flowering has been documented (Djanaguiraman et al., 2018). In all the above studies, stress has been imposed following a diurnal increase in temperature without a thorough understanding of the flowering behavior. Our findings provide an alternate view highlighting the inherent ability or a result of natural selection in sorghum to flower early. Sorghum's ability to flower immediately following dawn has facilitated sorghum to escape heat stress and protect pollination related events in nearly 100% of florets,

without exposing them to hotter temperatures during late morning or early noon. Arguably, drought stress during flowering can also have a major impact on sorghum productivity (Assefa and Staggenborg, 2010; Assefa et al., 2010). An extension of EMF phenomenon is that it provides sorghum the ability to minimize both heat and drought stress impact during flowering. Drought stress induced reduction in transpiration leads to increased tissue temperature further enhancing the impact of drought or combined heat and drought stress (Gholipoor et al., 2010; Mutava et al., 2011). However, with the ability to flower early sorghum escapes not just heat stress but combined heat and drought stress, wherein both these stresses increase in intensity towards noon. Hence, based on our findings it can be reliable interpreted that sorghum's ability to sustain productivity under current and future harsh conditions could be attributed to this highly conserved flowering mechanism helping escape both heat and drought stress impact during flowering.

Despite the highly conserved EMF phenomenon in sorghum, heat stress had a significant quantitative reduction in pollen germination and seed-set, respectively. There are two possibilities that can lead to such an impact. Firstly, although, florets flower early in the morning the hotter temperature imposed from 9 to 4 PM replicating real-world conditions, can potentially impact pollen viability negatively in spikelets that are yet to flower. We found pollen collected from stressed plants recording reduced germination even under control incubation temperature which support the above argument. Macia, Dekalb, P898012 and RTx430 grown under 40/22°C for 5 days, with pollen collected on subsequent (6^{th} day) morning recorded only 14.1%, 23.4%, 26.3%, 11.6% germination, respectively, under control incubation temperature of 32°C (Fig. 3.4). In contrast, pollen from control plants demonstrated satisfactory germination even under higher incubation temperatures. Macia, Dekalb, P898012 and RTx430 grown under control conditions (32/22°C) recorded 41.1%, 67%, 50.6%, 52.9% pollen germination, respectively, when exposed to 40°C incubation temperature (Fig. 3.4). This indicates that heat stress exposure affects pollen viability in florets that open on subsequent flowering days, wherein pollen loses its germination ability in spite of the conserved EMF mechanism. A similar impact of previous days heat stress impacting pollen viability in spikelet's yet to flower has been documented in rice (Jagadish et al., 2007) and recently in field grown sorghum (Sunoj et al., 2017). Furthermore, we found florets that opened after third day of flowering had more sterile florets compared with earlier opened florets (first and second day of flowering) (Fig. 3.7a) suggesting heat stress during pre-pollination hinders pollen germination potential.

A counter argument to the above discussed scenario i.e. pollen germination to be the major driver for heat stress induced seed-set reduction, is that 10% pollen germination is considered sufficient for seed-set in sorghum (Nguyen et al., 2013; Singh et al., 2015). Similarly, about 10 to 20 germinating pollen are considered sufficient to maintain floret fertility in heat stress sensitive rice (Jagadish et al., 2010). However, despite showing more than 17% (some up to 25%) pollen germination under 40/22°C across all genotypes and incubation temperatures, none of the genotypes had any seeds formed under 40/22°C temperature treatment. Although we observed significant positive correlation between pollen germination with grain number (R^2 = 0.30 to 0.66) (Fig. 3.8a), the relationship was not very strong compared with previous studies (R^2 = 0.93 in Nguyen et al., 2013 and R^2 = 0.91 in Singh et al., 2015). Hence retaining fertility in sorghum at more extreme temperatures such as 40^{o}C may require tolerance in processes beyond pollen germination. The second aspect which has been relatively less studied is the impact of heat stress on the early embryo development and abortion under heat stress exposure, which may be an equally or more vulnerable stage, with a conserved EMF mechanism operating effectively in sorghum. The results from this study suggest that EMF mechanism can help sorghum to minimize some of the heat stress damage during anthesis. However, the mechanism alone couldn't protect the day-time heat stress impact on other sensitive processes during seed set and early grain development such as fertilization, embryo formation and development. Hence, we reject our hypothesis that sorghum primarily follows escaping mechanism to avoid heat stress impact during flowering.

With an exception (P898012 under 40/22°), photochemical efficiency of photosystem II was not affected by heat stress in the study (Fig. 3.9a and Fig. 3.9b). Similar results of heat stress not significantly reducing photosynthetic efficiency have been reported in previous studies (Jain et al., 2007; Prasad et al., 2008). A study (Prasad et al., 2006) conducted with one genotype reported photosynthesis rate reduced by only 4% even under severe season long heat stress (44/34°C) exposure. However, other studies using one or multiple genotypes reported decrease in photosynthesis rate (up to 48%) under heat stress (Djanaguiraman et al., 2010; Prasad and Djanaguiraman, 2011; Djanaguiraman et al., 2014; Sunoj et al., 2017). We found chlorophyll index to be unaffected up to 38/22°C in all genotypes, while 40/22°C resulted in a significant reduction, except in Macia. Reduced chlorophyll content under heat stress in sorghum have also been reported

in several previous studies (Prasad et al., 2008; Djanaguiraman et al., 2010; Prasad and Djanaguiraman, 2011; Djanaguiraman et al., 2014; Sunoj et al., 2017).

Grain number decreased significantly with the increase in heat stress severity (Fig. 3.6b). However, with an exception of P898012 under 38/22°C, individual grain weight was not affected by heat stress treatments. The result agrees with previous findings wherein heat stress during flowering reduced grain number and consequently grain yield but not individual grain size (Prasad et al., 2008; Prasad et al., 2015; Singh et al., 2015). Across all and within each temperature treatments, Macia produced significantly higher grain weight and grain number than other genotypes. It also showed no significant reduction in grain yield even at 38/22°C. The results indicate Macia possessing novel heat tolerant alleles that helped to minimize heat stress damage. This study suggest Macia can be used as a donor in heat tolerance breeding in sorghum to develop improved heat stress tolerant sorghum hybrids.

3.5 Conclusions

Our conclusions provide evidence of a highly conserved EMF phenomena in sorghum under different environmental conditions including heat and drought stress. This is the first report documenting sorghum's unique ability to escape heat stress induced damage on pollen viability. Findings suggests post pollen-germination events such as embryo formation and its development could be other critical processes along with pollen viability when sorghum is exposed to heat stress at flowering. Macia was identified to be a promising donor for developing improved heat tolerant sorghum hybrids. Breeding efforts to enhance tolerance during early embryo formation can help sustain sorghum productivity even under predicted hotter and drier environments.

3.6 References

Aiqing, S., I. Somayanda, S.V. Sebastian, K. Singh, K. Gill, P.V.V. Prasad, and S.V.K. Jagadish. 2018. Heat stress during flowering affects time of day of flowering, seed set, and grain quality in spring wheat. Crop Sci. 58:380-392.

Assefa, Y., and S.A. Staggenborg. 2010. Grain sorghum yield with hybrid advancement and changes in agronomic practices from 1957 through 2008. Agron. J. 102:703-706.

Assefa, Y., S.A. Staggenborg, and V.P.V. Prasad. 2010. Grain sorghum water requirement and responses to drought stress: a review. Crop. Manage. 9. https://doi.org/10.1094/CM2010-1109-01-RV.

Bandyopadhyay, R., D.E. Frederickson, N.W. McLaren, G.N. Odvody, and M.J. Ryley. 1998. Ergot: a new disease threat to sorghum in the Americas and Australia. Plant Dis. 82:356-367.

Bheemanahalli, R., R. Sathishraj, M. Manoharan, H.N. Sumanth, R. Muthurajan, T. Ishimaru, and J.S.V. Krishna. 2017. Is early morning flowering an effective trait to minimize heat stress damage during flowering in rice? Field Crops Res. 203:238-242.

Bouchet, S., M.O. Olatoye, S.R. Marla, R. Perumal, T. Tesso, J. Yu, M. Tuinstra, and G.P. Morris. 2017. Increased power to dissect adaptive traits in global sorghum diversity using a nested association mapping population. Genetics 206:573-585.

Casa, A.M., G. Pressoir, P.J. Brown, S.E. Mitchell, W.L. Rooney, M.R. Tuinstra, C.D. Franks, and S. Kresovich. 2008. Community resources and strategies for association mapping in sorghum. Crop Sci. 48:30-40.

Cho, J. 1956. Double fertilization in *Oryza sativa* L. and development of the endosperm with special reference to the aleuron layer. Bull. Natl. Agric. Sci. D 6:61-101.

Djanaguiraman, M., P.V.V. Prasad, M. Murugan, R. Perumal, and U.K. Reddy. 2014. Physiological differences among sorghum (*Sorghum bicolor* L. Moench) genotypes under high temperature stress. Environ. Exp. Bot. 100:43-54.

Djanaguiraman, M., P.V.V. Prasad, and M. Seppanen. 2010. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol. Bioch. 48:999-1007.

Djanaguiraman, M., R. Perumal, S.V.K. Jagadish, I.A. Ciampitti, R. Welti, and P.V.V Prasad. 2018. Sensitivity of sorghum pollen and pistil to high-temperature stress. Plant Cell Environ. 41:1065-1082.

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

Hirabayashi, H., K. Sasaki, T. Kambe, R.B. Gannaban, M.A. Miras, M.S. Mendioro, E.V. Simon, P.D. Lumanglas, D. Fujita, Y. Takemoto-Kuno, Y. Takeuchi, R. Kaji, M. Kondo, N. Kobayashi, T. Ogawa, I. Ando, K.S.V. Jagadish, and T. Ishimaru. 2014. *qEMF3*, a novel QTL for the early-morning flowering trait from wild rice, *Oryza officinalis*, to mitigate heat stress damage at flowering in rice, *O. sativa*. J. Exp. Bot. 66:1227-1236.

IPCC. 2013. Summary for policymakers. In: T.F. Stocker et al., editors, Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, Cambridge, UK, and New York. p. 1–27.

Ishimaru, T., H. Hirabayashi, M. Ida, T. Takai, Y.A. San-Oh, S. Yoshinaga, I. Ando, T. Ogawa, and M. Kondo. 2010. A genetic resource for early-morning flowering trait of wild rice *Oryza officinalis* to mitigate high temperature-induced spikelet sterility at anthesis. Ann. Bot. 106:515-520.

Ishimaru, T., S. Xaiyalath, J. Nallathambi, R. Sathishraj, M. Yoshimoto, L. Phoudalay, B. Samson, T. Hasegawa, K. Hayashi, G. Arumugam, R. Muthurajand, and K.S.V. Jagadish. 2016. Quantifying rice spikelet sterility in potential heat-vulnerable regions: Field surveys in Laos and Southern India. Field Crops Res. 190:3-9.

Jagadish, S.V.K., P.Q. Craufurd, and T.R. Wheeler. 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). J. Exp. Bot. 58:1627-1635.

Jagadish, S.V.K., R. Muthurajan, R. Oane, T.R. Wheeler, S. Heuer, J. Bennett, and P.Q. Craufurd. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). J. Exp. Bot. 61:143-156.

Jain, M., P.V.V. Prasad, K.J. Boote, A.L. Hartwell Jr., and P.S. Chourey. 2007. Effects of season-long high temperature growth conditions on sugar-to-starch metabolism in developing microspores of grain sorghum (*Sorghum bicolor* L. Moench). Planta 227:67-79.

Kobayasi, K., T. Matsui, M. Yoshimoto, and T. Hasegawa. 2010. Effects of temperature, solar radiation, and vapor-pressure deficit on flower opening time in rice. Plant Prod. Sci. 13:21-28.

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

Nguyen, C.T., V. Singh, E.J. van Oosterom, S.C. Chapman, D.R. Jordan, and G.L. Hammer. 2013. Genetic variability in high temperature effects on seed-set in sorghum. Funct. Plant. Biol. 40:439-448

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

Prasad, P.V.V, K.J. Boote, and L.H. Allen Jr. 2011. Longevity and temperature response of pollen as affected by elevated growth temperature and carbon dioxide in peanut and grain sorghum. Environ. Exp. Bot. 70:51-57.

Prasad, P.V.V., and M. Djanaguiraman. 2011. High night temperature decreases leaf photosynthesis and pollen function in grain sorghum. Funct. Plant. Biol. 38:993-1003.

Prasad, P.V.V., M. Djanaguiraman, R. Perumal, and I.A. Ciampitti. 2015. Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Front. Plant Sci. 6:820.

Prasad, P.V.V., R. Bheemanahalli, and S.V.K. Jagadish. 2017. Field crops and the fear of heat stress-opportunities, challenges and future directions. Field Crops Res. 200:114-121.

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

Rang, Z.W., S.V.K. Jagadish, Q.M. Zhou, P.Q. Craufurd, and S. Heuer. 2011. Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. Environ. Exp. Bot. 70:58-65.

SAS Institute Inc, 2013. SAS ver 9.4. SAS Institute Inc., Cary, NC, USA.

Shi, W., X. Li, R.C. Schmidt, P.C. Struik, X. Yin, and S.V.K. Jagadish. 2018. Pollen germination and in vivo fertilization in response to high-temperature during flowering in hybrid and inbred rice. Plant Cell Environ. 41:1287-1297.

Siebers M.H., R.A. Slattery, C.R. Yendrek, A.M. Locke, D. Drag, E.A. Ainsworth, C.J. Bernacchi, and D.R. Ort. 2017. Simulated heat waves during maize reproductive stages alter reproductive growth but have no lasting effect when applied during vegetative stages. Agr. Ecosyst. Environ. 240:162-170.

Singh, V., C.T. Nguyen, E.J. van Oosterom, S.C. Chapman, D.R. Jordan, and G.L. Hammer. 2015. Sorghum genotypes differ in high temperature responses for seed set. Field Crops Res. 171:32-40.

Stephens, J.C., and J.R. Quinby. 1934. Anthesis, pollination, and fertilization in sorghum. J. Agric. Res. 49:123-136.

Sunoj, V.S.J., I.M. Somayanda, A. Chiluwal, R. Perumal, P.V.V. Prasad, and S.V.K. Jagadish. 2017. Resilience of pollen and post-flowering response in diverse sorghum genotypes exposed to heat stress under field conditions. Crop Sci. 57:1658-1669.

Tack, J., A. Barkley, and L.L. Nalley. 2015. Effect of warming temperatures on US wheat yields. Proc. Natl. Acad. Sci. USA. 112:6931-6936.

Tack, J., J. Lingenfelser, and S.V.K. Jagadish. 2017. Disaggregating sorghum yield reductions under warming scenarios exposes narrow genetic diversity in US breeding programs. Proc. Natl. Acad. Sci. USA. 114:9296-9301.

Yoshida, S. 1981. Fundamentals of rice crop science. Int. Rice Res. Inst. Manila, Philippines.

Yu, J., M.T. Hamblin, and M.R. Tuinstra. 2013. Association genetics strategies and resources. p. 187-203. In Association genetics strategies and resources. Genomics of the Saccharinae. Springer, New York, NY.

Table 3.1 Summary of analysis of variance for the recorded traits in both experiments.

	Traits	Temperature (T)	Genotype (G)	T×G				
Experiment 3	Grain weight	< .0001	< .01	< .001				
Experiment 4	Grain number	<.0001	<.0001	<.0001				
	Grain weight	<.0001	<.0001	<.0001				
	1000 grain weight	<.0001	<.0001	<.0001				
		Temperature (T)	Genotype (G)	Incubation (I)	T×G	T×I	G×I	T×G×I
	Pollen germination	<.0001	NS	<.0001	< .01	< .05	<.0001	<.0001
		Temperature (T)	Genotype (G)	Day (D)	T×G	T×D	G×D	T×G×D
	Floret sterility	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Quantum yield	< .01	<.0001	<.0001	<.0001	NS	<.0001	< .001
	Chlorophyll index	<.0001	<.0001	<.0001	<.0001	NS	< .05	< .001

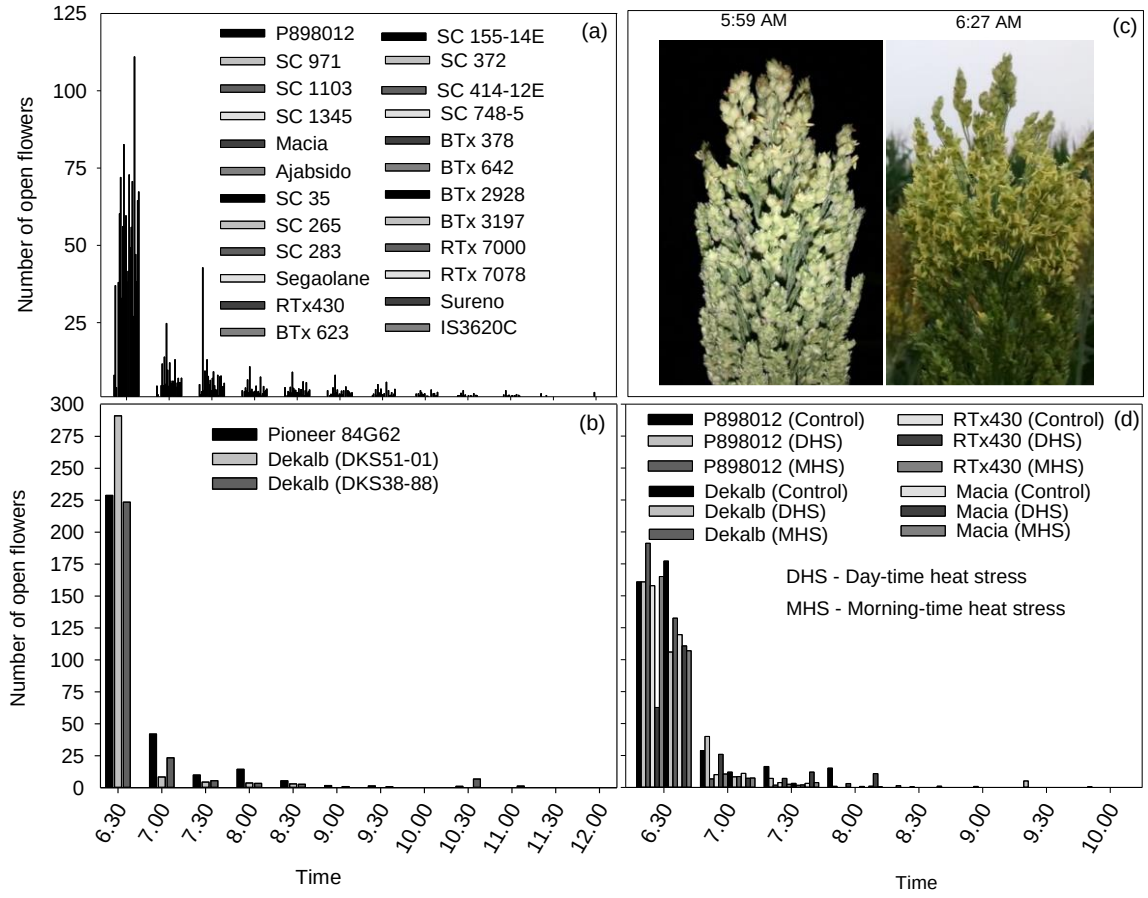

Figure 3.1 Time-of-day of flower opening in sorghum under diverse environments.

Twenty-four diverse sorghum genotypes (a; in greenhouse), three sorghum hybrids (b and c; in field) and response of four sorghum genotypes (d) to heat stress (40/22^{o}C- day/night temperatures) under different time of the day (MHS- morning-time [6:00AM to 11:00AM]; DHS- day-time [9:00AM to 4:00PM]) during flowering under controlled-environmental conditions.

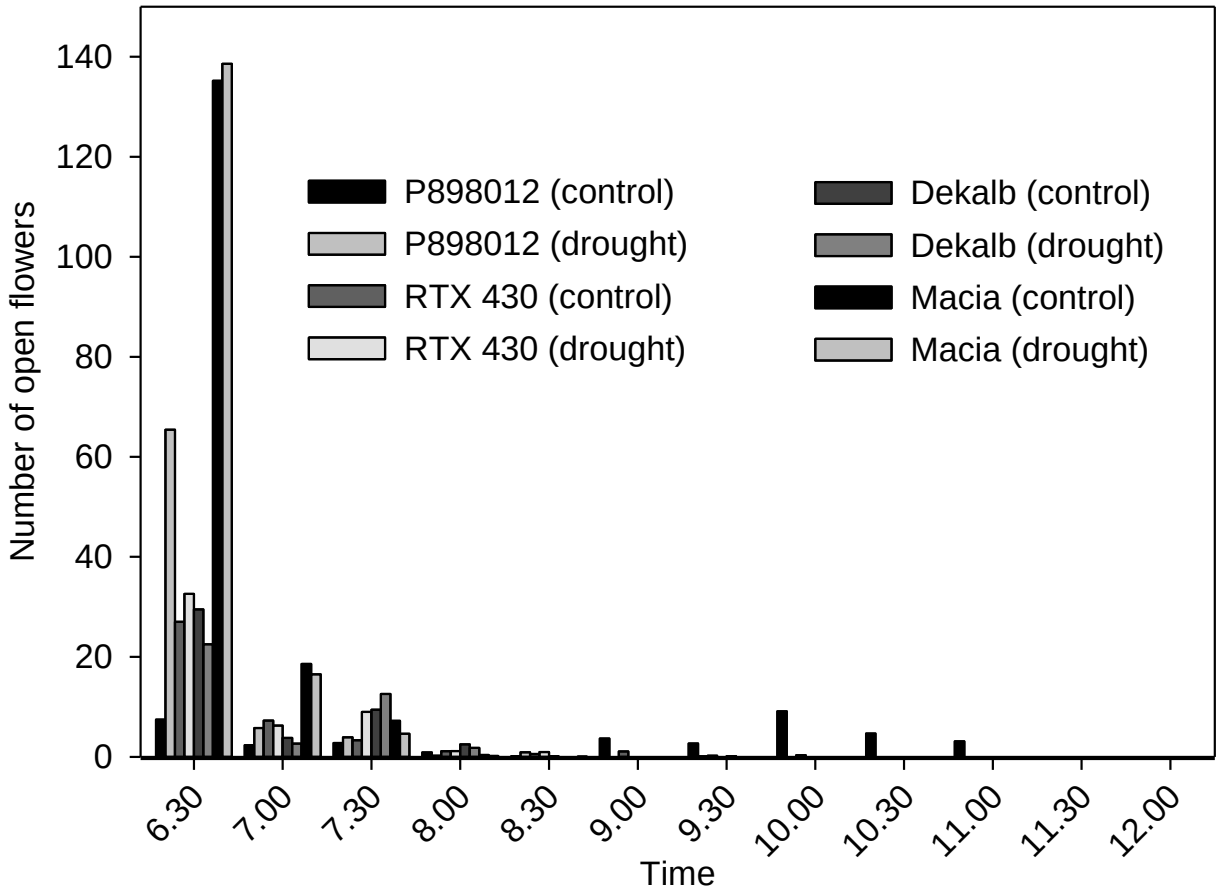

Figure 3.2 Time-of-day of flower opening in four sorghum genotypes under control and drought stress during flowering in green house.

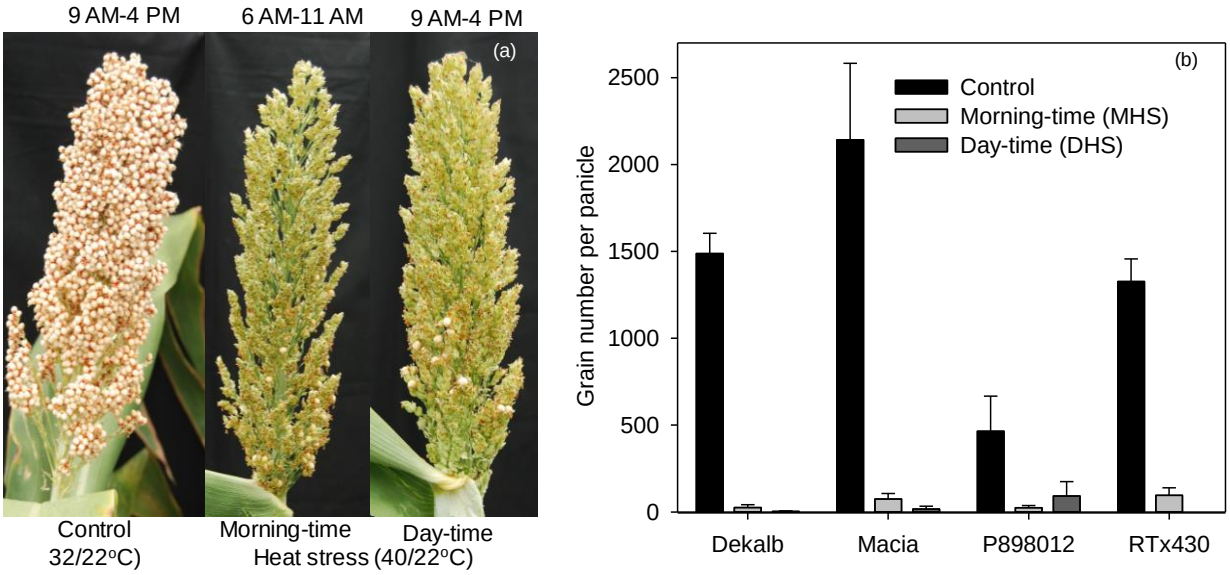

Figure 3.3 Heat stress impact on grain number during flowering in four sorghum genotypes. Impact of morning-time heat stress (MHS- morning-time [6:00AM to 11:00AM]) and day-time heat stress (DHS- day-time [9:00AM to 4:00PM]) during flowering on grain number among four sorghum genotypes.

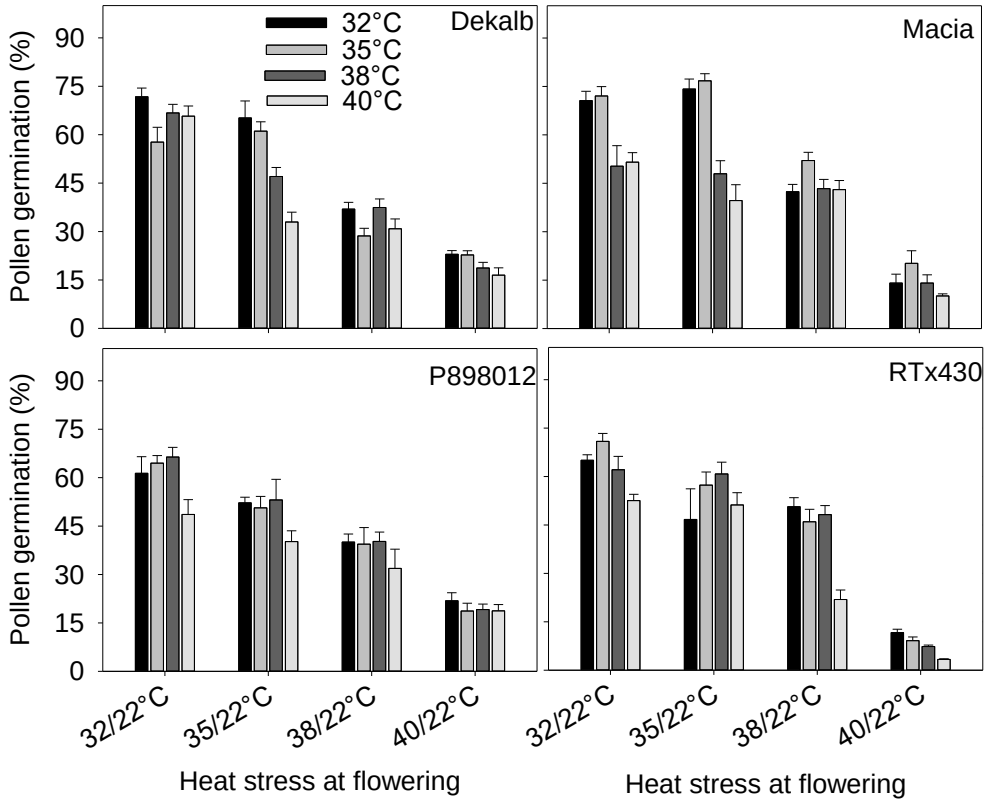

Figure 3.4 Heat stress impact on *in vitro* pollen germination in four sorghum genotypes.

In vitro pollen germination (%) of four sorghum genotypes exposed to different regimes of day-time heat stress for 12 days during flowering under controlled-environmental conditions. Pollen grains collected from plants grown under control (32/22^{o}C) and different (day-time) heat stress (35/22^{o}C, 38/22^{o}C and 40/22^{o}C) treatments were germinated at temperatures of 32°C, 35°C, 38°C and 40°C for 2 hours.

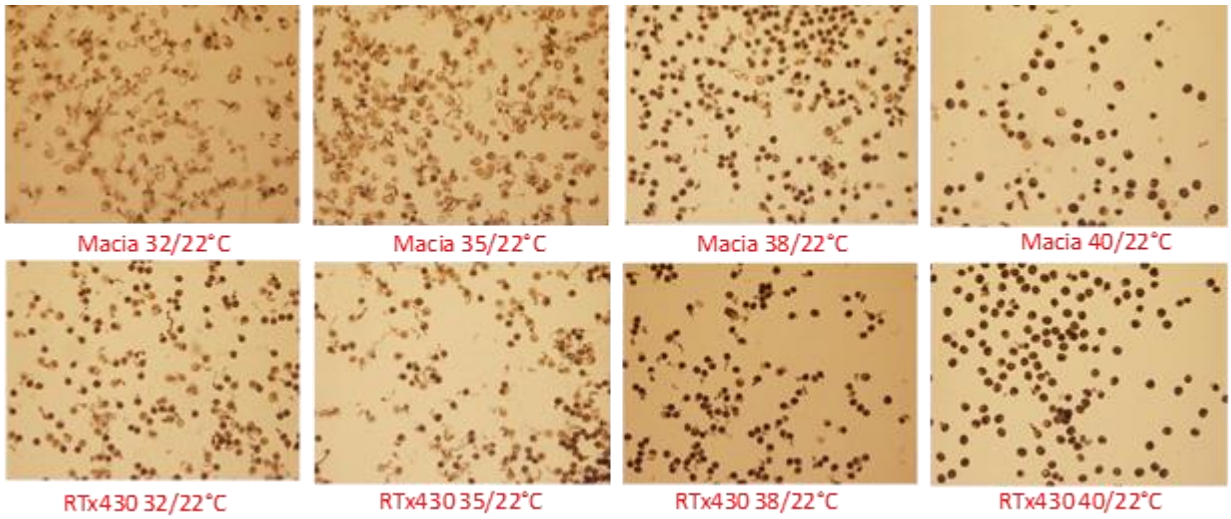

Figure 3.5 Heat stress impact on *in vitro* pollen germination in two contrasting sorghum genotypes. *In vitro* pollen germination in Macia and RTx430 under different (day-time) temperature treatments (32/22^{o}C, 35/22^{o}C, 38/22^{o}C and 40/22^{o}C) during flowering. Pollen grains were incubated at 32°C for 2 hours.

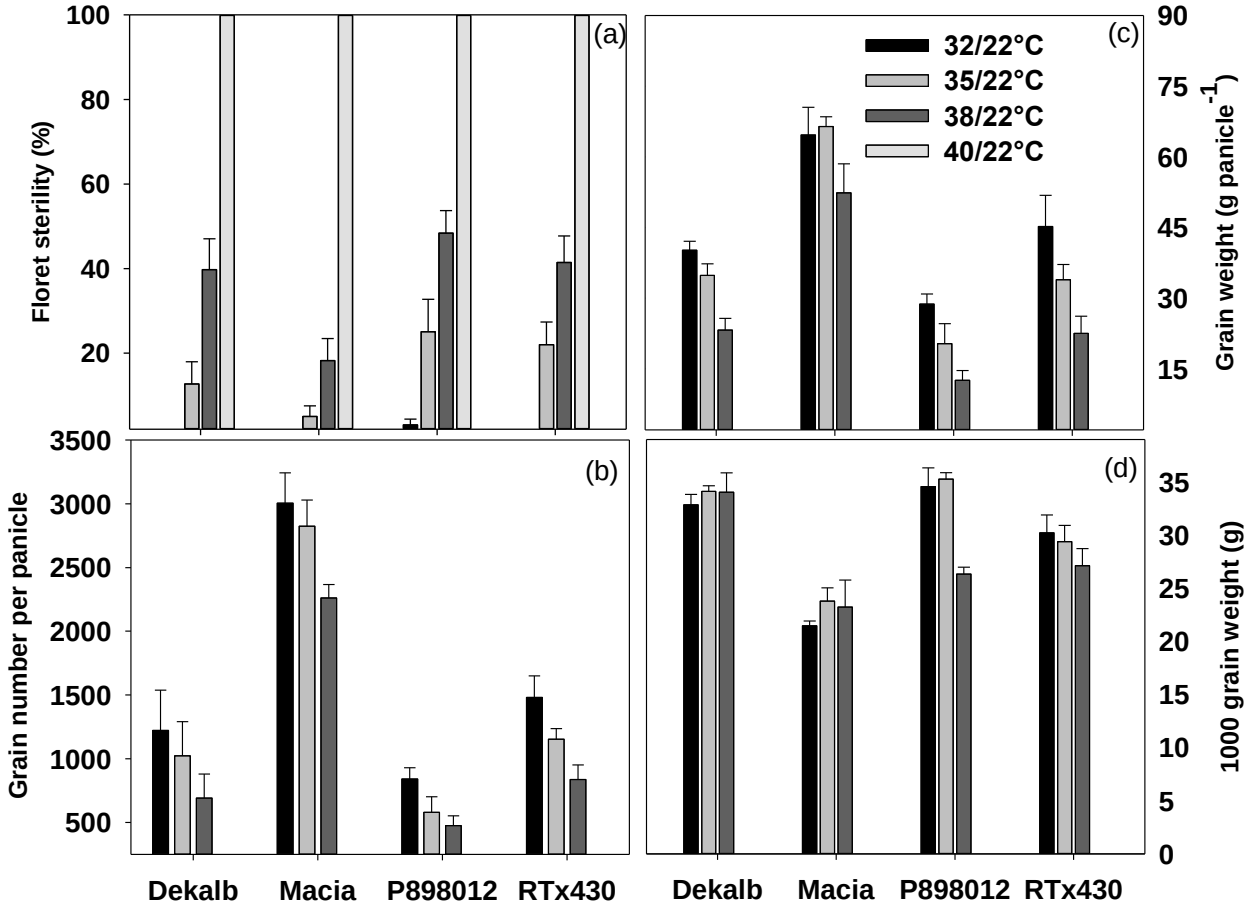

Figure 3.6 Heat stress impact on yield and related component in four sorghum genotypes. Effect of gradient day-time temperature treatments (32/22ºC, 35/22ºC, 38/22ºC and 40/22ºC) on floret sterility (%; a), grain number (b) 1000-grain weight (g; c) and grain yield (g panicle^{-1}; d) among four sorghum genotypes under controlled-environmental conditions.

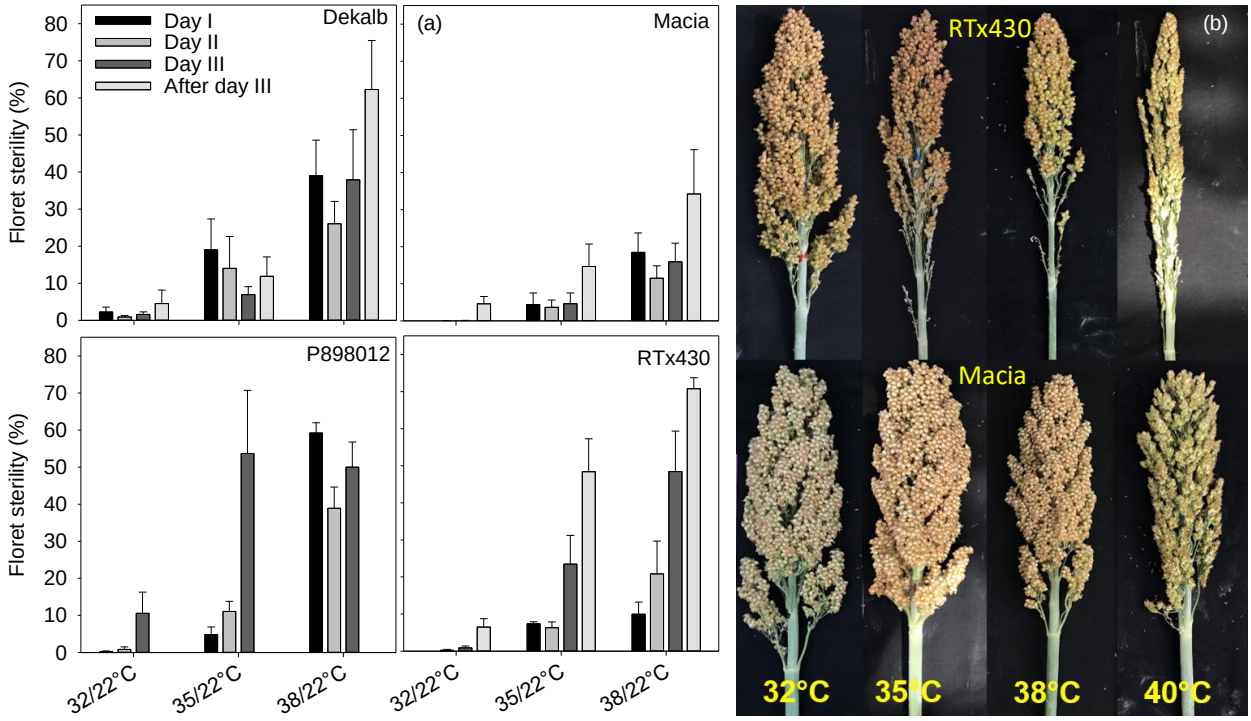

Figure 3.7 Heat stress impact on floret sterility in four sorghum genotypes.

Effect of day-time heat stress on floret sterility, a - across different flowering days (day I, II, III and after day III) among four sorghum genotypes, b - response of two contrasting sorghum genotypes.

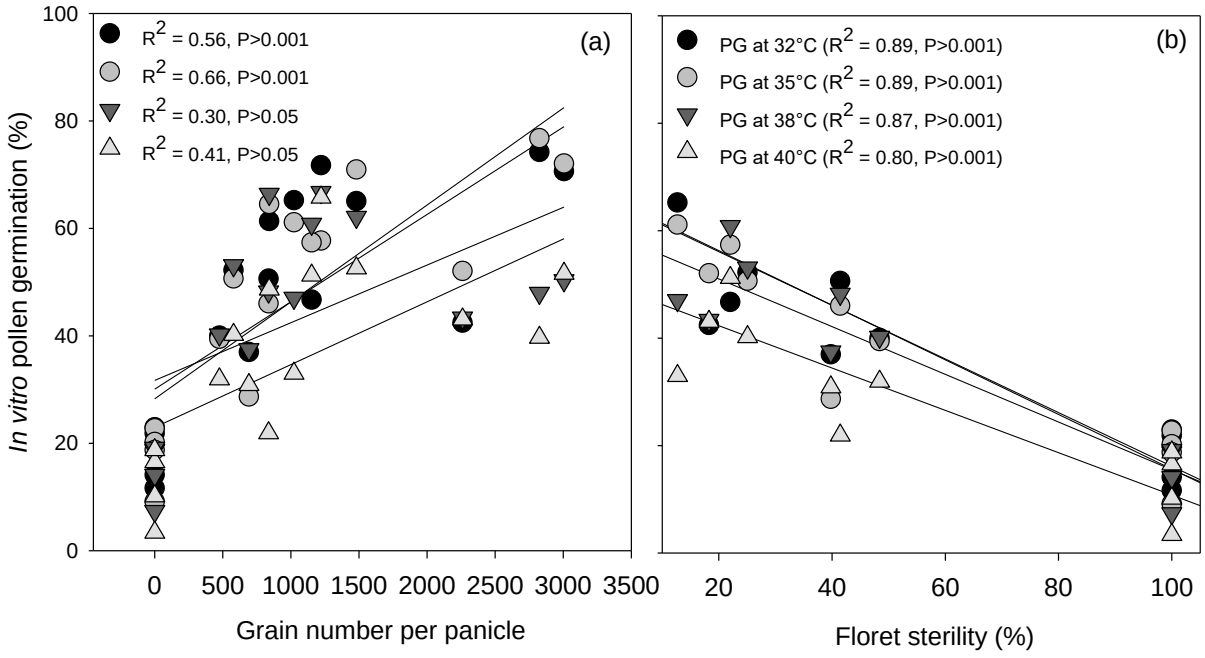

Figure 3.8 Relationship of *in vitro* pollen germination with grain number and floret sterility in four sorghum genotypes.

Relationship of *in vitro* pollen germination with grain number (a) and floret sterility (b) under different day-time heat stress among sorghum genotypes. Pollen grains collected from plants grown under different (day-time) temperature treatments (32/22^{o}C, 35/22^{o}C, 38/22^{o}C and 40/22^{o}C) during flowering and were germinated at temperatures of 32°C, 35°C, 38°C and 40°C for 2 hours in incubators.

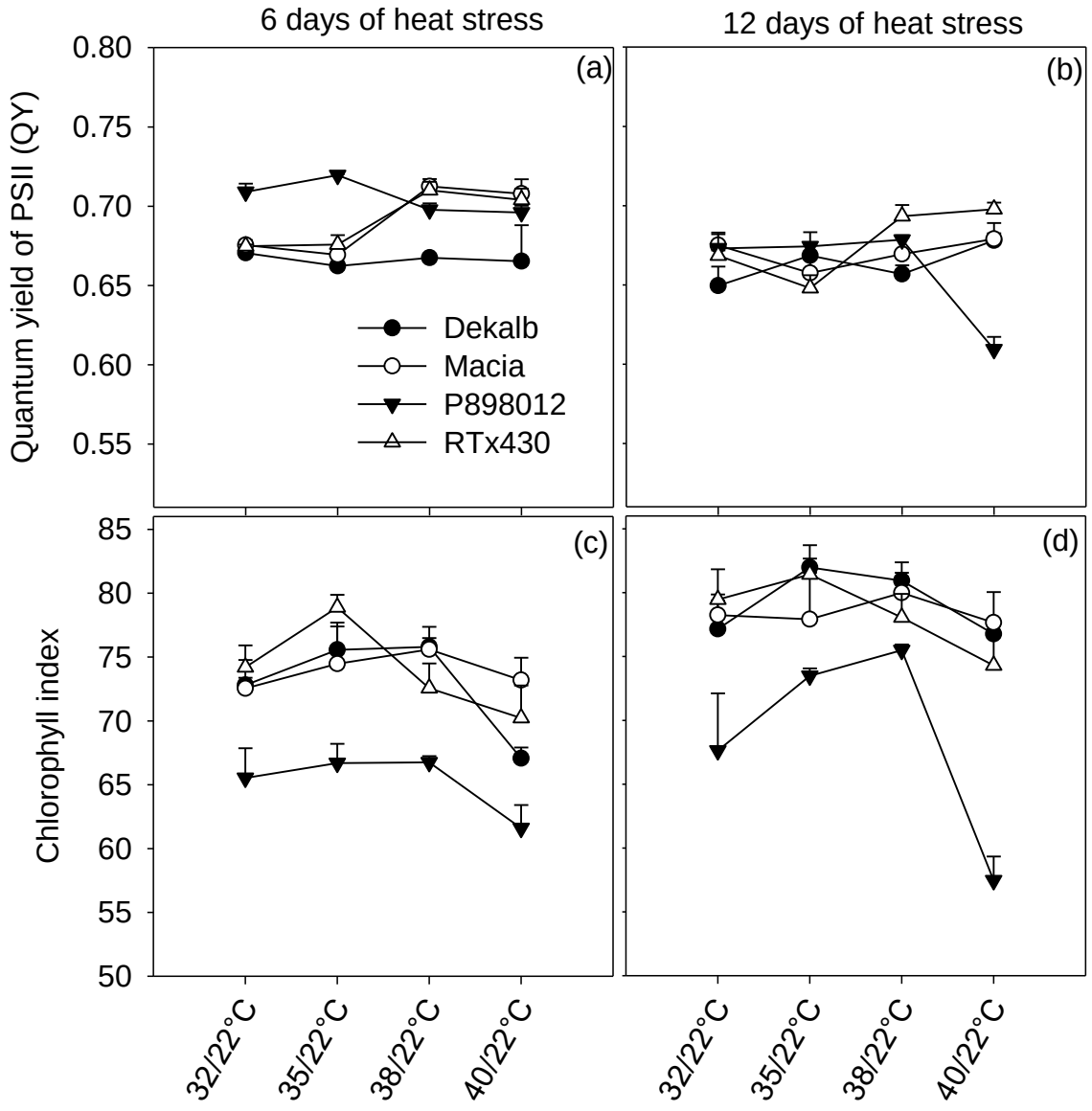

Figure 3.9 Heat stress impact on effective quantum yield of PSII and chlorophyll index in four sorghum genotypes.

Effective quantum yield of photosystem II (a and b) and leaf chlorophyll index (c and d) of sorghum genotypes after 6 days (a and c) and 12 days (b and d) of heat stress during flowering under different day-time temperature regimes.

Chapter 4 - Physiological and genetic characterization of source, sink and panicle neck diameter in sorghum

Abstract

The understanding of genetic architecture of source and sink dynamics under wide range of environments would be useful to optimize sorghum yield under diverse environments. Hence, this study was conducted to identify genomic regions associated with source, sink and related traits in sorghum in different environments. A bi-parental mapping population consisting of 210 recombinant inbred lines (RILs) developed from US elite post flowering drought susceptible cultivar RTx430 and a known post flowering drought tolerant cultivar (SC35) along with their parents were evaluated in two environments (irrigated throughout season and irrigated until heading) at Hays, Kansas, USA in 2016. The same set of RILs and parents were evaluated in Hays and also in Manhattan, Kansas in 2017. Experiment at Manhattan was carried out under natural environment while other experiments (irrigated throughout season, irrigated until heading and natural dryland environment) were conducted at Hays. In total, the population was tested under six different environments with considerable range in temperature and soil moisture conditions. Physiological traits related to source such as effective quantum yield of photosystem II and chlorophyll index were measured on the flag leaf or the next fully expanded leaf at three to four time-points during grain-filling stages. Grain weight per panicle and respective panicle-neck diameter were recorded at two time-points i.e. mid grain-filling stage (15 days after complete flowering) and physiological maturity. QTL analysis was conducted with composite interval model in R/QTL package. Six QTL for final grain yield (PVE [Phenotypic Variation Explained] - 3.1 to 11.8%), 9 QTL for grain yield at mid grain-filling stage (PVE - 1.9 to 18.8%), 9 QTL for final panicle neck diameter (PVE - 2.5 to 15.4%), 8 QTL for panicle neck diameter at mid grain-filling stage (PVE - 4.9 to 16.6%), 15 QTL for effective quantum yield of photosystem II (PVE - 2.1 to 15.4%) and 15 QTL for chlorophyll content (PVE - 2.7 to 13.4%) were detected. The genomic regions and the candidate genes within these regions can potentially help in improving source and sink dynamics in sorghum under diverse environments.

4.1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), with its origin from tropical and subtropical regions of Africa, is well adapted to arid, semi-arid, sub humid and humid environments (Smith and Frederiksen, 2000). Sorghum is considered one of the hardiest heat and drought tolerant cereal crop in the world. (Doggett, 1988; Blum, 2004; Smith and Frederiksen, 2000). Eighty percent of sorghum in the world is grown in dryland environments (Assefa et al., 2010). Currently, the average yield of sorghum in the world is only 1.2 t ha^{-1} (Jordan et al., 2012). Low productivity of sorghum compared with other grain crops is attributed majorly to its growing environments, wherein most of the sorghum in the world is grown under rainfed conditions with minimal inputs, hence prone to many abiotic stresses and nutritional deficiencies. However, various studies have demonstrated its high production potential under non-limiting conditions. A study in Australia after analyzing 23 field trials in 12 locations for 5 years with varying moisture conditions reported mean sorghum yield ranging from 2.3 to 10.5 t ha^{-1} (Jordan et al., 2012). Another study using hybrid grain sorghum performance trials at Kansas from 1957 to 2008 documented sorghum production of up to 9.3 Mg ha^{-1} under well irrigated conditions (Assefa and Staggenborg, 2010). Hence, looking at traits that contribute towards this wide range in productivity spanning across multiple environments is an interesting research undertaking.

Grain yield is an integrated measure of multiple traits and is dependent on both genetic and environmental factors and their interactions. Polygenic traits such as grain yield are very complex to understand compared to traits that are controlled by a single or a few gene (Mackay et al., 2009). Quantitative Trait Loci (QTL) mapping helps to dissect those complex traits to each QTL level (Tanksley, 1993). Identification of QTL and significant markers associated with the trait, followed by marker-trait association validation provides the foundation for marker assisted selection and breeding for trait improvement (Collard et al., 2005).

Production and transport of photoassimilates from the source (leaf) to sink (grain) is a key physiological process that determines crop grain yield. The understanding of genetic architecture of source and sink dynamics is important for improving yield under different environments. Several studies have been conducted to identify and validate QTL for chlorophyll content in sorghum and some of them were co-localized with stay-green loci under post-flowering drought stress (Xu et al., 2000; Gelli et al., 2016; Sukumaran et al., 2016). Recently, some studies (Fiedler

et al., 2016; Sukumaran et al., 2016; Ortiz et al., 2017) have identified few genomic regions associated with chlorophyll fluorescence and gas exchange traits in few environments. Hence, identifying novel QTL associated with photosynthetic efficiency of sorghum in different genetic backgrounds and in different growth stages under diverse environments will help towards improving productivity.

Several studies have been conducted to identify QTL related to panicle architecture in sorghum such as panicle length, panicle neck length, panicle exsertion and peduncle diameter (Pereira et al 1995; Zou et al., 2012; Fakrudin et al., 2013; Reddy et al., 2013). However, none of them have included panicle neck diameter trait. With an exception of an association mapping study (Hmon et al., 2013), there are no other studies on identifying genomic regions associated with panicle neck diameter in sorghum. Positive association between panicle neck diameter and grain yield in sorghum (Hmon et al., 2013) suggests a key role played by the source-sink connecting panicle neck for photoassimilate transport from leaf to grain. Hence, identifying QTL associated with panicle neck diameter during different panicle maturity stages may be useful to improve source and sink dynamics in sorghum under diverse environments.

Several QTL related to grain yield and yield related parameters have been identified in sorghum in different genetic backgrounds. However, none of them have attempted to identify and compare QTL associated with grain weight during grain development (mid grain filling) and at maturity. Most of these studies have been conducted in field conditions under natural environments (Rami et al., 1998; Zou et al., 2012; Fakrudin et al., 2013; Reddy et al., 2013; Reddy et al., 2014; Gelli et al., 2016; Tao et al., 2018). Only some studies have been conducted under well irrigated conditions and compared with drought stress environment (Tuinstra et al., 1997; Sabadin et al., 2012; Phuong et al., 2013). Some have divided their experiments into different moisture level treatments based on precipitation received in the locations (Sukumaran et al., 2016). There are no studies which have attempted to map QTL for source, sink and panicle neck diameter during different growth stages under wide environment and moisture conditions including full irrigation throughout the crop duration, partial irrigation and no irrigation (see Materials and Methods for details) under field conditions. Identifying QTL associated with source, sink and related traits under wide spectrum of environments varying with moisture regimes will provide additional complementary information that is already existing but skewed towards challenging and harsh environments.

SC35 is an important stay-green sorghum line converted from durra landrace IS12555 (Stephens et al., 1967). IS12555 is a stay-green source originated in Ethiopia and its derivative line, BTx642 (formerly known as B35) has been used in most of the previous studies as post-flowering drought tolerant stay-green check line (Tuinstra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu et al., 2000; Sanchez et al., 2002). These genotypes have comparatively low yield potential than RTx430 (Xu et al., 2000; Subudhi et al., 2000). RTx430 is a widely adapted elite line with high yield potential and has been commonly used in many breeding programs in the United States (Miller, 1984; Yu et al., 2013). RTx430 is a non-senescent line however is susceptible to post flowering drought stress (Miller, 1984; Crasta et al., 1999). It is also the common parent of a sorghum Nested Association Mapping (NAM) population (Yu et al., 2013; Bouchet et al., 2017).

Based on the above information and existing knowledge, following hypotheses were tested in the study

1) Large variation in grain yield will be observed in the population across environments leading to identification of major QTL for grain yield.

2) SC35 having higher panicle neck diameter than RTx430 will result in wider phenotypic variation in the population and consequently QTL accounting for higher phenotypic variation.

3) Variation in effective quantum yield of photosystem II will lead to identifying QTL which operate independently from the known stay green QTL.

4) Chlorophyll content related QTL identified under different environments will lead to identifying QTL that co-localize with known stay-green loci.

Specific objectives of this study are to (i) identify QTL associated with source and sink related traits and panicle neck diameter in different growth stages in a SC35 and RTx430 biparental recombinant inbred line population under wide spectrum of environments and moisture conditions and (ii) identify QTL co-localizing for the above traits.

4.2 Materials and Methods

4.2.1 Plant material and experimental design

A bi-parental mapping population consisting of 210 recombinant inbred lines (RILs) developed from the cross between two sorghum inbred lines RTx430 and SC35, along with their parents were used in the study.

The mapping population was tested in two environments [(irrigated throughout season - (environment 1) and irrigated until heading (environment 2)] at Hays, Kansas, USA in 2016. The same set of RILs and parents were studied in two locations in Kansas in 2017. Three experiments [irrigated throughout season (environment 3), irrigated until heading (environment 4) and natural dryland environment (environment 5)] were conducted at Hays and one experiment was carried out at Manhattan under natural environment (environment 6). In total, the population was tested under six different environments with considerable range in temperature and soil moisture conditions (Table 4.1). The populations were planted on June 2 in 2016 and on May 25 and on June 15 in Hays and Manhattan respectively in 2017. All experiments were set up in Randomized Complete Block Design (RCBD) with two replications. The experiment had one row plot in 2016 and 2 rows plot in 2017. Each row was 3.6-meter (12 ft) and seeds were planted with inter-row spacing of 0.75-meter. Air temperature and precipitation in the experimental location were recorded using WatchDog data loggers (1000 Series Micro Station, Spectrum Technologies, Aurora, IL, USA). Volumetric soil moisture content was measured in environment 3 and environment 5 around 10 cm below soil surface using soil moisture sensors (WaterScout SM 100 moisture sensor, Spectrum Technologies, Aurora, IL, USA).

4.2.2 Phenotypic observations

The populations were evaluated for a few key physiological traits related to source (effective quantum yield of PSII and chlorophyll index), sink (grain yield) and panicle neck diameter across the environments. Effective Quantum Yield of photosystem II in the light adapted state were measured at three to four time-points during grain filling using FluorPen (Photon System Instruments, Ltd., Brno, Czech Republic). Chlorophyll index was also measured on the same day using Dualex (Dualex ScientificTM) in 2016 and SPAD meter (SPAD-502 Plus, Konica

Minolta) in 2017. Both these measurements were recorded on the flag leaf or the next fully expanded leaf within a single day between 9:30 am and 3:30 pm in the bright sunny days. Grain weight per panicle and respective panicle neck diameter were recorded at two-time points i.e. mid grain-filling stage and physiological maturity. Three uniform heads from the main tillers were tagged at the time of anthesis. Mid grain-filling stage was considered as 15 days *exactly* after complete flowering. Panicles were manually harvested to record grain yield (g panicle^{-1}) at mid grain-filling stage and respective panicle neck diameter was recorded using digital vernier calipers. After physiological maturity, three uniform panicles from main tillers were harvested from each plot to record final grain yield (g panicle^{-1}) and respective panicle neck diameter were recorded as mentioned above. Panicles were dried at 40^{o}C for a week before threshing.

4.2.3 Phenotypic data analysis

Analysis of variance was conducted with PROC GLM using SAS 9.4 (SAS Institute, 2013). Phenotypic correlation coefficients between the traits were calculated in each and averaged across all the environments. Broad-sense heritability was calculated from estimated variance components of genotype (Vg) and prediction error variance (PEV) as H = 1 - (PEV/Vg) (Clark et al., 2012) which is equivalent to genotype-mean heritability. The variance components were calculated using random-effects linear model in R package lme4 (Bates et al., 2014).

4.2.4 QTL analysis

Linkage map constructed by Bouchet et al. (2017) for the same biparental populations were used for QTL analysis. QTL analysis was conducted for all traits in R using the composite interval mapping (CIM) with 8 covariates and 15-SNP sliding window as implemented in R/qtl package (Broman et al., 2003). QTL scans were performed considering normal model and Haley-Knot regression method on a dense 1-cM grid using the cal.genoprob function. The LOD threshold value for each trait was calculated based on 1000 permutations. The additive effect was calculated using the formula, additive effect = (A-B)/2, where A indicates the average value of individuals with SC35 allele, B indicate the average value of individuals with RTx430 allele. QTL explaining more than or equal to 10% phenotypic variation was considered as major QTL.

4.3 Results

4.3.1 Environmental variations

Considerable variations in soil texture, soil nutrient status, temperature, precipitation and soil moisture were observed among the environments (Table 4.1, Fig. 4.1). Precipitation during crop growing season (May to October) was highest in Manhattan 2017 (445.48 mm) followed by Hays 2016 (395.46 mm) and Hays 2017 (355.82 mm) (Fig. 4.1D). Soil moisture sensor recorded 19.4%, 18.6% and 3.3% average volumetric soil moisture in July, August and September respectively in environment 5, and 28.3%, 29.1% and 25% in July, August and September in environment 3. Total number of days when daily maximum temperature exceeded 35°C during crop growing season was 34, 33 and 19 days in Hays 2016, Hays 2017 and Manhattan 2017, respectively (Table 4.1).

4.3.2 Phenotypic trait variation

All the studied traits showed normal distribution with skewness <1.0 suggesting all traits following polygenic or continuous inheritance (Fig. 4.2). Analysis of variance (ANOVA) revealed significant effect of genotype, environment and genotype × environment interactions for all the traits (Table 4.2). The average phenotypic measures for both the parents, range and the mean phenotypic values of the RILs population in each environment and averaged across all the environments are presented in Table 4.3A to Table 4.3G. Averaged across all the environments, parents varied significantly only with final panicle neck diameter (FND) and panicle neck diameter at mid grain-filling stage (MND) but not with other traits (Table 4.3A). SC35 recorded significantly greater final panicle neck diameter (FND) and panicle neck diameter at mid grain-filling stage (MND) in all the environments than RTx430 except in environment 5 and in environment 6 where final panicle neck diameter (FND) was higher but not significantly different than RTx430 (Table 4.3B – 4.3G). Differences between the parents in other traits were also observed in some of the environments (Table 4.3B – 4.3G). RTx430 had significantly higher final grain yield (FGY) and grain yield at mid grain-filling stage (MGY) in environment 1 (Table 4.3B). Significant differences in effective quantum yield of photosystem II (QY) between the parents were observed in environment 1 and 2 (Table 4.3B - 4.3C). Similarly, significant differences in

chlorophyll index (Ch) between the parents was observed in environment 6 (Table 4.3G). RIL population showed transgressive segregation resulting in a wide variation for all the studied traits in each environment and averaged across all the environments (Table 4.3A – 4.3G).

The average phenotypic value within population (parents + RILs) in each environment is presented in Table 4.3H. The population grown under natural dryland environment in Hays (environment 5) recorded lowest grain yield and panicle neck diameter during both final and at mid grain-filling stage among the environments. The population in environment 5 recorded significantly lower final grain yield (FGY) than all other environments, significantly lower grain yield at mid grain-filling stage (MGY) than environment 1 and 2, significantly lower final panicle neck diameter (FND) than environment 1, 2, 3 and 6, and significantly lower panicle neck diameter at mid grain-filling stage (MND) than environment 1 and 2 (Table 4.3H).

Moderate to high broad sense heritability (h^2) was observed for all studied traits. The broad sense heritability (h^2) for all studied traits in each environment and averaged across all the environments is presented in Table 4.3A to 4.3G. Averaged across all the environment, broad sense heritability (h^2) for the traits ranged from 0.58 to 0.85 (Table 4.3A). Final panicle neck diameter had highest heritability (0.85) followed by panicle neck diameter at mid grain-filling stage (0.84), final grain yield (0.80), chlorophyll index (0.78), grain yield at grain-filling stage (0.72) and effective quantum yield of photosystem II (0.58).

Correlation among all the studied traits in each environment and averaged across all the environments is presented in Table 4.4A to Table 4.4G. Averaged across all environments, final grain yield was significantly ($P < 0.01$) and positively correlated with grain yield at mid grain-filling stage ($r = 0.68$) and also with panicle neck diameter at mid grain-filling stage ($r = 0.36$) and more strongly with final panicle neck diameter ($r = 0.52$). Grain yield at mid grain-filling stage was also significantly ($P < 0.01$) and positively correlated with panicle neck diameter at mid grain-filling stage ($r = 0.42$) and final panicle neck diameter ($r = 0.45$). Significant, positive and very strong correlation ($P < 0.01$, $r = 0.83$) was observed between panicle neck diameter at final and mid grain-filling stage. Chlorophyll index had significant correlation ($P < 0.01$) with panicle neck diameter at mid grain-filling stage ($r = 0.26$) and final panicle neck diameter ($r = 0.19$) but not with other traits.

4.3.3 QTL Mapping

4.3.3.1 Final grain yield

Six QTL for final grain yield were detected on chromosome 1, 3, 5, 6 and 7 (Table 4.5 and Fig. 4.3). Phenotypic variation explained by these QTL ranged from 3.1 to 11.8% (Table 4.5). SC35 and RTx430 each contributed favorable alleles for 3 QTL. (Table 4.5). Only one QTL on chromosome 5 was detected in two environments (Table 4.5 and Fig. 4.3).

4.4.3.2 Grain yield at mid grain-filling stage

Nine QTL for grain yield at mid grain-filling stage were detected on chromosome 1, 2, 3, 4 and 5 (Table 4.6 and Fig. 4.4). Phenotypic variation explained by these QTL ranged from 1.9 to 18.8% (Table 4.6). SC35 and RTx430 possessed favorable alleles for 5 and 4 QTL respectively. (Table 4.6). One QTL each on chromosome 1, 2 and 3 were detected in multiple environments (Table 4.6 and Fig. 4.4), with the one on chromosome 1 detected in three environments.

4.4.3.3 Final panicle neck diameter

Nine QTL for final panicle neck diameter were detected on chromosome 1, 2 and 3 (Table 4.7 and Fig. 4.5). Phenotypic variation explained by these QTL ranged from 2.5 to 15.4% (Table 4.7). SC35 and RTx430 contributed favorable alleles for 7 and 2 QTL respectively (Table 4.7). One QTL on chromosome 2 and two QTL on chromosome 3 were detected in two environments (Table 4.7 and Fig. 4.5).

4.4.3.4 Panicle neck diameter at mid grain-filling stage

Eight QTL for panicle neck diameter at mid grain-filling stage were detected on chromosome 2, 3, 6, 7 and 9 (Table 4.8 and Fig. 4.6). Phenotypic variation explained by these QTL ranged from 4.9 to 16.6% (Table 4.8). SC35 and RTx430 possessed favorable alleles for 6 and 2 QTL, respectively (Table 4.8). One QTL on chromosome 3 was detected in two environments (Table 4.8 and Fig. 4.6).

4.4.3.5 Effective quantum yield of photosystem II

Fifteen QTL for effective quantum yield of photosystem II were detected across all 10 chromosomes (Table 4.9 and Fig. 4.7). Phenotypic variation explained by these QTL ranged from 2.1 to 15.4% (Table 4.9). SC35 and RTx430 contributed favorable alleles for 10 and 5 QTL respectively (Table 4.9).

4.4.3.6 Chlorophyll content

Fifteen QTL for chlorophyll content were detected on chromosomes 2, 4, 5, 6, 8, 9 and 10 (Table 4.10 and Fig. 4.8). Phenotypic variation explained by these QTL ranged from 2.7 to 13.4% (Table 4.10). SC35 and RTx430 possessed favorable alleles for 5 and 10 QTL respectively. (Table 4.10). One QTL on chromosome 2, two QTL on chromosome 8, one QTL on chromosome 9 and one QTL on chromosome 10 were detected in multiple environments (Table 4.10 and Fig. 4.8).

4.4.3.7 QTL co-localization between growth stage within same trait

QTL detected on chromosome 3 for final grain yield (qFGY3.1) colocalized with QTL for grain yield at mid grain-filling stage (qMGY3.1). Similarly, QTL detected on chromosome 5 for final grain yield (qFGY5.1) was co-located with QTL for grain yield at mid grain-filling stage (qMGY5.1) (Fig. 4.9). Four QTL for final panicle neck diameter and panicle neck diameter at mid grain-filling stage were overlapped (Fig. 4.9). Two major QTL detected chromosome 2 (qFND2.1 with qMND2.1; qFND2.3 with qMND2.2) were overlapped which explained up to 16.6% and 15.4% of the phenotypic variation for panicle neck diameter. Similarly, two QTL detected on chromosome 3 (qFND3.2 with qMND3.1; qFND3.3 with qMND3.2) were overlapped with each other (Fig. 4.9).

4.4.3.8 QTL co-localization across traits

QTL for grain yield at mid grain-filling stage (qMGY1.3), final panicle neck diameter (qFND1.1) and effective quantum yield of photosystem II (qQY1.1) were co-localized on chromosome 1 (Fig. 4.9). QTL for final panicle neck diameter (qFND2.1), panicle diameter at mid grain-filling stage (qMND2.1) and chlorophyll content (qCh2.1) were co-localized on chromosome 2 (Fig. 4.9). Similarly, QTL for final panicle neck diameter (qFND2.4) and effective

quantum yield of photosystem II (qQY2.2) on chromosome 2; QTL for final panicle neck diameter (qFND3.1) and effective quantum yield of photosystem II (qQY3.1) on chromosome 3; QTL for panicle neck diameter at mid grain-filling stage (qMND6.1) and effective quantum yield of photosystem II (qQY6.2) on chromosome 6 and QTL for panicle neck diameter at mid grain-filling stage (qMND9.1) and chlorophyll content (qCh9.2) on chromosome 9 overlapped with each other (Fig. 4.9). There was no overlap between any QTL for effective quantum yield of Photosystem II with QTL for chlorophyll content.

4.4 Discussion

Experimental sites in both the years received some rainfall in all the months throughout the growing season, hence population grown under environments 2, 3 and 6 were not exposed to anticipated level of water-deficit conditions (Fig. 4.1D). However, population grown under natural dryland environment in Hays (environment 5) experienced water-limited conditions during its terminal stage as no additional irrigation was provided during entire growing season and precipitation was less in Hays compared with Manhattan in 2017 (Fig 4.1D). Average volumetric soil moisture content, 10 cm below the soil surface between July to September in environment 5 was 13.8% (July - 19.4%, August - 18.6%, September - 3.3%), which was very less compared with 27.4% (July - 28.3%, August - 29.1%, September - 25%) in environment 3 (Table 4.1). The results also indicate that natural dryland environment in Hays (environment 5) received moderate level of water-deficit conditions at the terminal stage. Furthermore, population grown in environment 5 recorded lowest grain yield and panicle neck diameter during both final and at mid grain-filling stage among the environments (Table 4.3H) providing additional evidence of moderate terminal water-deficit stress compared with other environments. Temperature above 32°C during the reproductive stage is known to damage sorghum yield as even short episodes of those temperature could cause significant grain yield reduction (Prasad et al., 2015; Tack et al., 2017). Differences in total number of days when daily maximum temperature exceeded 35°C during crop growing season (Table 4.1) suggest plants grown in Hays experienced significantly more days under hotter conditions compared to Manhattan.

All the QTL identified for final grain yield and grain yield at mid grain-filling stage were co-localized with earlier reported QTL for grain yield and yield related parameters in sorghum

(Rami et al., 1998; Feltus et al., 2006; Srinivas et al., 2009; Sabadin et al., 2012; Takai et al., 2012; Phuong et al., 2013; Reddy et al., 2013; Shehzad and Okuno, 2015; Gelli et al., 2016; Spagnolli et al., 2016; Sukumaran et al., 2016; Boyles et al., 2017). One major QTL detected for grain yield at mid grain-filling stage on chromosome 1 (qMGY1.1) explained 18.8% of the phenotypic variation. It was co-localized with previously identified QTL for grain yield (Srinivas et al., 2009; Reddy et al., 2013; Sukumaran et al., 2016). Sukumaran et al. (2016) reported it as a robust QTL for grain yield as it was consistently detected in multiple environments and accounted for 16% of the phenotypic variation in their study. Another major QTL detected for grain yield at mid grain-filling stage on chromosome 1 (qMGY1.3) explained 14.2% of the phenotypic variation which was also co-localized with a previously reported QTL for grain yield (Gelli et al., 2016). One major QTL detected for final grain yield on chromosome 1 (qFGY1.1) accounted for 11.8% of the phenotypic variation. The QTL was co-localized with earlier reported QTL for grain yield in sorghum (Gelli et al., 2016; Sukumaran et al., 2016). Another major QTL identified on chromosome 3 for both final grain yield (qFGY3.1) and grain yield at mid grain-filling stage (qMGY3.1) explained upto 10.6% and 10.1% of the phenotypic variation for final grain yield and grain yield at mid grain-filling stage respectively. This QTL was also co-located with previously reported QTL for grain yield (Shehzad and Okuno, 2015). Grain yield is a very complex trait and is controlled by several genes and their interaction with environments. Our findings provide further support that these identified major QTL have potential for further consideration to improve grain yield under a range of moisture and environmental conditions.

Significant positive relationship between panicle neck diameter and grain yield was observed in this study (Table 4.4). Similar relationship has been reported in a previous study (Hmon et al., 2013). These results further strengthen the relationship between grain yield and the role played by panicle neck diameter, which acts as a route for assimilate transfer from source to the sink. All the identified QTL for final panicle neck diameter and panicle neck diameter at mid grain-filling stage in sorghum were reported for the first time, which adds novelty to the findings. These QTL after further validation, or the trait can be a useful target for improving source and sink dynamics and productivity in sorghum.

SC35 is a stay-green post flowering drought stress tolerant line. Four major QTL (*Stg1, Stg2*, *Stg3* and *Stg4*) associated with stay-green have been consistently identified in BTx642 background in several studies (Tuinstra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu

et al., 2000; Sanchez et al., 2002). Stay green rating and chlorophyll index are highly correlated under post flowering drought stress and many chlorophyll content QTL were co-localized with stay-green QTL (Crasta et al., 1999; Xu et al., 2000). Favorable alleles for chlorophyll content and stay-green QTL in these studies were contributed by stay-green lines. The source of stay green is a durra landrace, IS12555 for both SC35 and BTx642. However, QTL for chlorophyll content identified in our study were not co-localized with any of these four major stay-green loci. Similar results were reported in a study wherein one of the parents had same stay-green source but none of the chlorophyll content QTL overlapped with stay-green QTL (Sukumaran et al., 2016). Another parent in this study (RTx430) is also a non-senescent line (Miller, 1984). Hence both the parents maintain green leaf throughout its growth that could be one of the reason for no stay-green QTL detection in this study. However, stay-green QTL have been identified earlier in B35×RTx430 RILs population under post flowering drought stress environment (Crasta et al., 1999). They reported chlorophyll index of stay-green line (B35) was higher than RTx430 under post flowering drought environment but not under well irrigated conditions. Our experiments in many cases were not exposed to the level of water limited conditions needed to identify stay-green QTL. Previous studies have also reported detecting stay-green loci and its association with grain yield diminishes under non-water limiting conditions (Tuinstra et al., 1997; Jordan et al., 2012). Two major QTL for chlorophyll content were detected one each on chromosome 2 (qCh2.1) and chromosome 8 (qCh8.1) which explained 13.4% and 10.2% of the phenotypic variation respectively. QTL on chromosome 8 (qCh8.1) was colocalized with previously reported QTL for chlorophyll content (Fiedler et al., 2014). However, QTL on chromosome 2 (qCh2.1) was not reported in earlier studies. These QTL could be a potential region to target for improving chlorophyll content in sorghum and to complement other known stay green QTL.

Two major QTL for effective quantum yield of photosystem II were detected one each on chromosome 2 and 7 which explained 13.8% and 15.4% of the phenotypic variation respectively. These QTL were reported for the first time in sorghum. Very few studies have reported QTL associated with chlorophyll fluorescence and gas exchange traits in sorghum (Fiedler et al., 2016; Sukumaran et al., 2016; Ortiz et al., 2017). Some of the minor QTL identified in this study (qQY1.1, qQY3.1, qQY4.1, qQY5.1, qQY9.1) were co-localized with previously reported loci from the above association mapping studies (Fiedler et al., 2016; Ortiz et al., 2017). However, those were identified at seedling stage measurement under chilling tolerance studies. Majority of

the identified QTL including 2 major QTL were novel. These genomic regions after further validation can be targeted to improve photosynthetic efficiency in sorghum.

Despite no significant differences between the parents in most of the environments, QTL were identified for final grain yield, grain yield at mid grain-filling stage, effective quantum yield of photosystem II and chlorophyll content. Transgressive segregation was observed on both directions for all studied traits which suggests both the parents possessed favorable alleles for the measured traits. Favorable allele in detected QTL for all traits were contributed by both parents which further support favorable alleles distribution from both parents. Similar results of transgressive segregation in sorghum with or without differences between the parents have been reported in several previous studies (Rami et al., 1998; Ritter et al., 2008; Reddy et al., 2013; Reddy et al., 2014; Gelli et al., 2016; Sukumaran et al., 2016).

Although RTx430 significantly produced higher grain yield than SC35 in only one environment (Table 4.3B), large variation in the RIL population was observed due to transgressive segregation (Table 4.3A – 4.3G) allowing detection of major QTL supporting our hypothesis. SC35 recorded significantly higher panicle neck diameter than RTx430 in many of the environments and large variation in panicle neck diameter was observed in the population, both at final and mid grain-filling stage (Table 4.3A – 4.3G). This facilitated detection of major QTL related to panicle neck diameter, providing evidence in support of our second hypothesis. Similar to the first hypothesis, no significant difference in effective quantum yield of photosystem II and chlorophyll content was detected between the parents in most of the environments (Table 4.3A – 4.3G). Nevertheless, transgressive segregation for both the traits in RILs population allowed identification of novel QTL for effective quantum yield of PSII. Lack of intended level of post flowering water-deficit stress in our experiments did not provide us with the opportunity to test the fourth hypothesis.

4.5 Conclusions

Large phenotypic variation in the mapping population allows QTL detection for all studied traits. Some major QTL were detected for grain yield, panicle neck diameter, effective quantum yield of photosystem II and chlorophyll content. Some novel QTL for panicle neck diameter, effective quantum yield of photosystem II and chlorophyll content were identified for the first time in sorghum and adds to the existing knowledge. The genomic regions identified from the study would be useful for improving source and sink dynamics in sorghum to improve its productivity under diverse environments.

4.6 References

Assefa, Y., and S.A. Staggenborg. 2010. Grain sorghum yield with hybrid advancement and changes in agronomic practices from 1957 through 2008. Agron. J. 102:703-706.

Assefa, Y., S.A. Staggenborg, and V.P.V. Prasad. 2010. Grain sorghum water requirement and responses to drought stress: a review. Crop. Manage. 9. https://doi.org/10.1094/CM2010-1109-01-RV.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using eigen and S4. R Package Version 1:1-23.

Blum, A. 2004. Sorghum physiology. In: H.T. Nguyen, and A. Blum (Eds.), Physiology and biotechnology integration for plant breeding. Marcel Dekker Inc., New Nork, pp. 141–223.

Bouchet, S., M.O. Olatoye, S.R. Marla, R. Perumal, T. Tesso, J. Yu, M. Tuinstra, and G.P. Morris. 2017. Increased power to dissect adaptive traits in global sorghum diversity using a nested association mapping population. Genetics 206:573-585.

Boyles, R.E., B.K. Pfieffer, E.A. Cooper, K.J. Zielinski, M.T. Myers, W.L. Rooney, and S. Kresovich. 2017. Quantitative trait loci mapping of agronomic and yield traits in two grain sorghum biparental families. Crop Sci. 57:2443-2456.

Broman, K.W., H. Wu, Ś. Sen, and G.A. Churchill. 2003. R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889-890.

Clark, S.A., J.M. Hickey, H.D. Daetwyler, and J.H.J. van der Werf. 2012. The importance of information on relatives for the prediction of genomic breeding values and the implications for the makeup of reference data sets in livestock breeding schemes. Genet. Sel. Evo. 44:4.

Collard, B., M. Jahufer, J. Brouwer, and E. Pang. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142:169-196.

Crasta, O.R., W.W. Xu, D.T. Rosenow, J. Mullet, and H.T. Nguyen. 1999. Mapping of post-flowering drought resistance traits in grain sorghum: Association between QTLs influencing premature senescence and maturity. Mol. Gen. Genet. 262:579-588.

Doggett, H. 1988. Sorghum, second ed. Wiley, New York, NY.

Fakrudin, R.B., S.P. Kavil, Y. Girma, S.S. Arun, D. Dadakhalandar, B.H. Gurusiddesh, A.M. Patil, M. Thudi, S.B. Bhairappanavar, and Y.D Narayana. 2013. Molecular mapping of genomic regions harbouring QTLs for root and yield traits in sorghum (*Sorghum bicolor* L. Moench). Physiol. Mol. Biol. Pla. 19:409-419.

Feltus, F.A., G.E. Hart, K.F. Schertz, A.M. Casa, S. Kresovich, S. Abraham, P.E. Klein, P.J. Brown, and A.H. Paterson. 2006. Alignment of genetic maps and QTLs between inter-and intra-specific sorghum populations. Theor. Appl. Genet. 112:1295.

Fiedler, K., W.A. Bekele, C. Matschegewski, R. Snowdon, S. Wieckhorst, A. Zacharias, and R. Uptmoor. 2016. Cold tolerance during juvenile development in sorghum: a comparative analysis by genomewide association and linkage mapping. Plant Breeding 135:598-606.

Fiedler, K., W.A. Bekele, R. Duensing, S. Gründig, R. Snowdon, H. Stützel, A. Zacharias, and R. Uptmoor. 2014. Genetic dissection of temperature-dependent sorghum growth during juvenile development. Theor. Appl. Genet. 127:1935-1948.

Gelli, M., S.E. Mitchell, K. Liu, T.E. Clemente, D.P. Weeks, C. Zhang, D.R. Holding, and I.M. Dweikat. 2016. Mapping QTLs and association of differentially expressed gene transcripts for multiple agronomic traits under different nitrogen levels in sorghum. BMC Plant Biol. 16:16.

Hmon, K.P.W., T. Shehzad, and K. Okuno. 2013. Variation in inflorescence architecture associated with yield components in a sorghum germplasm. Plant Genet. Resour. 11:258-265.

Jordan, D.R., C.H. Hunt, A.W. Cruickshank, A.K. Borrell, and R.G. Henzell. 2012. The relationship between the stay-green trait and grain yield in elite sorghum hybrids grown in a range of environments. Crop Sci. 52:1153-1161.

Mackay, T.F.C., E.A. Stone, and J.F. Ayroles. 2009. The genetics of quantitative traits: challenges and prospects. Nat. Rev. Genet. 10:565-577.

Miller, R.F. 1984. Registration of RTx430 parental line. Crop Sci. 24:1224-1224.

Ortiz, D., J. Hu, and M.G. Salas Fernandez. 2017. Genetic architecture of photosynthesis in *Sorghum bicolor* under non-stress and cold stress conditions. J. Exp. Bot 68:4545–4557.

Pereira, M.G., D. Ahnert, M. Lee, and K. Klier. 1995. Genetic mapping of quantitative trait loci for panicle characteristics and seed weight in sorghum. Rev. Bras. Genet. 18:249-249.

Phuong, N., H. Stützel, and R. Uptmoor. 2013. Quantitative trait loci associated to agronomic traits and yield components in a *Sorghum bicolor* L. Moench RIL population cultivated under pre-flowering drought and well-watered conditions. Agric. Sci. 4:781-791.

Prasad, P.V.V., M. Djanaguiraman, R. Perumal, and I.A. Ciampitti. 2015. Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Front. Plant Sci. 6:820.

Rami, J.F., P. Dufour, G. Trouche, G. Fliedel, C. Mestres, F. Davrieux, P. Blanchard, and P. Hamon. 1998. Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). Theor. Appl. Genet. 97:605-616.

Reddy, N.R.R., M. Ragimasalawada, M.M. Sabbavarapu, S. Nadoor, and J.V. Patil. 2014. Detection and validation of stay-green QTL in post-rainy sorghum involving widely adapted cultivar, M35-1 and a popular stay-green genotype B35. BMC Genomics 15:909.

Reddy, R.N., R. Madhusudhana, S.M. Mohan, D.V.N. Chakravarthi, S.P. Mehtre, N. Seetharama, and J.V. Patil. 2013. Mapping QTL for grain yield and other agronomic traits in post-rainy sorghum [*Sorghum bicolor* (L.) Moench]. Theor. Appl. Genet. 126:1921-1939.

Ritter, K.B., D.R. Jordan, S.C. Chapman, I.D. Godwin, E.S. Mace, and C.L. McIntyre. 2008. Identification of QTL for sugar-related traits in a sweet × grain sorghum (*Sorghum bicolor* L. Moench) recombinant inbred population. Mol. Breed. 22:367-384.

SAS Institute Inc, 2013. SAS ver 9.4. SAS Institute Inc., Cary, NC, USA.

Sabadin, P.K., M. Malosetti, M.P. Boer, F.D. Tardin, F.G. Santos, C.T. Guimaraes, R.L. Gomide, C.L.T. Andrade, P.E.P. Albuquerque, F.F. Caniato, M. Mollinari, G.R.A. Margarido, B.F. Oliveira, R.E. Schaffert, A.A.F. Garcia, F.A. van Eeuwijk, and J.V. Magalhaes. 2012. Studying the genetic basis of drought tolerance in sorghum by managed stress trials and adjustments for phenological and plant height differences. Theor. Appl. Genet. 124:1389-1402.

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

Shehzad, T., and K. Okuno. 2015. QTL mapping for yield and yield-contributing traits in sorghum (*Sorghum bicolor* (L.) Moench) with genome-based SSR markers. Euphytica 203:17-31.

Smith, C.W., and R.A. Frederiksen. 2000. History of cultivar development in the United States: From "memoirs of A.B. Maunder - sorghum breeder'. In: Smith, C.W., and R.A. Frederiksen (Eds.), Sorghum: Origin, history, technology, and production. John Wiley & Sons, New York, NY, pp. 191–223.

Spagnolli, F.C., E. Mace, D. Jordan, L. Borrás, and B.L. Gambin. 2016. Quantitative trait loci of plant attributes related to sorghum grain number determination. Crop Sci. 56:3046-3054.

Srinivas, G., K. Satish, R. Madhusudhana, R.N. Reddy, S.M. Mohan, and N. Seetharama. 2009. Identification of quantitative trait loci for agronomically important traits and their association with genic-microsatellite markers in sorghum. Theor. Appl. Genet. 118:1439-1454.

Stephens, J.C., F.R. Miller, and D.T. Rosenow. 1967. Conversion of alien sorghums to early combine genotypes. Crop Sci. 7:396–396.

Subudhi, P.K., D.T. Rosenow, and H.T. Nguyen. 2000. Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. Theor. Appl. Genet. 101:733-741.

Sukumaran, S., X. Li, X. Li, C. Zhu, G. Bai, R. Perumal, M.R. Tuinstra, P.V.V. Prasad, S.E. Mitchell, T.T. Tesso, and J. Yu. 2016. QTL mapping for grain yield, flowering time, and stay-green traits in sorghum with genotyping-by-sequencing markers. Crop Sci. 56:1429-1442.

Tack, J., J. Lingenfelser, and S.V.K. Jagadish. 2017. Disaggregating sorghum yield reductions under warming scenarios exposes narrow genetic diversity in US breeding programs. Proc. Natl. Acad. Sci. USA. 114:9296-9301.

Takai, T., J. Yonemaru, H. Kaidai, and S. Kasuga. 2012. Quantitative trait locus analysis for days-to-heading and morphological traits in an RIL population derived from an extremely late flowering F_1 hybrid of sorghum. Euphytica 187:411-420.

Tanksley, S.D. 1993. Mapping polygenes. Annu. Rev. Genet. 27:205-233.

Tao, Y., E. Mace, B. George-Jaeggli, C. Hunt, A. Cruickshank, R. Henzell, and D. Jordan. 2018. Novel grain weight loci revealed in a cross between cultivated and wild sorghum. Plant Genome 11:170089.

Tuinstra, M.R., E.M. Grote, P.B. Goldsbrough, and G. Ejeta. 1997. Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench. Mol. Breed. 3:439-448.

Xu, W., P.K. Subudhi, O.R. Crasta, D.T. Rosenow, J.E. Mullet, and H.T. Nguyen. 2000. Molecular mapping of QTLs conferring stay-green in grain sorghum (*Sorghum bicolor* L. Moench). Genome 43:461-469.

Yu, J., M.T. Hamblin, and M.R. Tuinstra. 2013. Association genetics strategies and resources. p. 187-203. In Association genetics strategies and resources. Genomics of the Saccharinae. Springer, New York, NY.

Zou, G., G. Zhai, Q. Feng, S. Yan, A. Wang, Q. Zhao, J. Shao, Z. Zhang, J. Zou, B. Han, and Y. Tao. 2012. Identification of QTLs for eight agronomically important traits using an ultra-high-density map based on SNPs generated from high-throughput sequencing in sorghum under contrasting photoperiods. J. Exp. Bot. 63:5451-5462.

Table 4.1 Details of different environments of the study where the population was tested.

Env.	Treatment	Location	Year	Soil characteristics									
				pH	Mehlich P ppm	K ppm	NH4-N ppm	NH3-N ppm	Sand %	Silt %	Clay %	Days >35C*	Moisture %[#]
1	Irrigated throughout season	Hays	2016	8.28	26.73	408.00	2.35	27.55	20.67	60.67	18.67	34	
2	Irrigated until heading	Hays	2016	8.19	32.43	468.67	2.04	33.33	21.33	60.67	18.00	34	
3	Irrigated throughout season	Hays	2017	8.01	70.67	466.00	2.05	17.47	21.33	62.00	16.67	33	27.4
4	Irrigated until heading	Hays	2017	8.02	25.67	435.00	1.84	24.56	20.00	62.67	17.33	33	
5	Natural dryland environment	Hays	2017	7.68	16.60	557.67	3.02	18.14	18.00	48.00	34.00	33	13.8
6	Natural environment	Manhattan	2017	5.49	27.77	259.67	22.52	51.25	12.00	57.33	30.67	19	

* Number of days when daily maximum temperature exceeded 35°C.

[#] Average volumetric soil moisture content 10 cm below the soil surface between July to September.

Table 4.2 The P value and F value of ANOVA for genotype, environment and genotype x environment interaction for all the studied traits.

Traits	Genotype		Environment		Genotype x Environment	
	F-value	P-value	F-value	P-value	F-value	P-value
FGY	7.9	<.0001	298.29	<.0001	1.57	<.0001
MGY	5.24	<.0001	417.78	<.0001	1.48	<.0001
FND	9.05	<.0001	650.04	<.0001	1.31	<.0001
MND	9.07	<.0001	391.17	<.0001	1.37	<.0001
QY	2.97	<.0001	113.01	<.0001	1.29	<.0001
Ch	8.11	<.0001	883.33	<.0001	1.82	<.0001

FGY - Final grain yield (g); MGY - Grain yield at mid grain-filling stage (g); FND - Final panicle neck diameter (mm); MND - Panicle neck diameter at mid grain-filling stage (mm); QY - Effective quantum yield of photosystem II, Ch - Chlorophyll index.

Table 4.3 Summary statistics for all studied traits

A) Parents and RILs averaged across all environments

Traits	Parental lines [#]				RILs			SEM	h^2
	SC35	SEM	RTx430	SEM	Min	Max	Mean		
FGY	53.61 [a]	3.39	54.95 [a]	4.48	22.19	74.20	49.18	0.68	0.80
MGY	17.70 [a]	1.09	19.95 [a]	1.96	7.48	34.83	17.32	0.33	0.72
FND	11.91 [a]	0.71	10.55 [b]	0.49	7.48	14.04	11.18	0.07	0.85
MND	12.62 [a]	0.62	10.64 [b]	0.43	9.06	15.02	11.91	0.08	0.84
QY	0.54 [a]	0.01	0.54 [a]	0.01	0.47	0.63	0.55	0.00	0.58
Ch	54.01[a]	1.27	56.31[a]	1.49	48.85	64.74	58.07	0.21	0.78

B) Parents and RILs in environment 1

Traits	Parental lines [#]				RILs			SEM	h^2
	SC35	SEM	RTx430	SEM	Min	Max	Mean		
FGY	59.08 [b]	5.26	73.09 [a]	3.14	25.97	100.51	63.64	1.15	0.60
MGY	20.03 [b]	2.92	26.76 [a]	0.03	8.67	54.49	24.83	0.54	0.46
FND	14.71 [a]	0.05	11.93 [b]	0.49	7.32	17.55	13.29	0.12	0.58
MND	14.65 [a]	0.20	11.86 [b]	0.35	8.65	18.29	13.45	0.12	0.60
QY	0.47 [b]	0.02	0.57 [a]	0.02	0.34	0.69	0.54	0.00	0.54
Ch	60.33 [a]	1.75	64.96 [a]	2.11	34.29	77.17	65.57	0.41	0.75

C) Parents and RILs in environment 2

Traits	Parental lines [#]				RILs			SEM	h^2
	SC35	SEM	RTx430	SEM	Min	Max	Mean		
FGY	55.30 [a]	1.75	57.92 [a]	1.65	23.90	90.78	57.12	1.01	0.49
MGY	19.29 [a]	1.30	21.98 [a]	2.45	9.78	44.05	24.24	0.52	0.47
FND	13.02 [a]	0.37	11.29 [b]	0.39	8.13	16.59	12.69	0.10	0.66
MND	13.41 [a]	0.21	11.25 [b]	0.01	7.94	16.65	12.96	0.11	0.61
QY	0.55 [a]	0.02	0.49 [b]	0.03	0.38	0.71	0.53	0.00	0.50
Ch	62.28 [a]	2.15	62.61 [a]	1.63	43.50	76.18	64.15	0.38	0.83

D) Parents and RILs in environment 3

Traits	Parental lines #				RILs			SEM	h^2
	SC35	SEM	RTx430	SEM	Min	Max	Mean		
FGY	61.82 [a]	7.31	56.19 [a]	0.18	19.20	101.00	54.38	0.96	0.65
MGY	19.05 [a]	0.17	17.61 [a]	0.08	2.13	40.75	15.57	0.45	0.61
FND	11.37 [a]	0.45	9.83 [b]	0.22	7.09	14.78	10.82	0.09	0.64
MND	11.96 [a]	0.19	10.69 [b]	0.23	8.04	16.04	11.64	0.10	0.71
QY	0.56 [a]	0.03	0.54 [a]	0.03	0.41	0.67	0.54	0.00	0.49
Ch	42.63 [a]	1.32	41.90 [a]	1.51	48.68	69.41	57.77	0.24	0.64

E) Parents and RILs in environment 4

Traits	Parental lines #				RILs			SEM	h^2
	SC35	SEM	RTx430	SEM	Min	Max	Mean		
FGY	52.22 [a]	3.58	49.57 [a]	0.95	16.95	83.52	45.11	0.92	0.66
MGY	15.10 [a]	1.84	16.76 [a]	1.53	1.37	30.70	11.63	0.45	0.71
FND	10.68 [a]	0.01	9.61 [b]	0.13	7.25	18.36	10.21	0.09	0.63
MND	11.41 [a]	0.17	9.87 [b]	0.02	8.27	14.86	11.03	0.09	0.76
QY	0.55 [a]	0.03	0.57 [a]	0.02	0.44	0.65	0.56	0.00	0.21
Ch	54.56 [a]	1.76	55.17 [a]	2.12	46.92	65.73	56.31	0.25	0.66

F) Parents and RILs in environment 5

Traits	Parental lines #				RILs			SEM	h^2
	SC35	SEM	RTx430	SEM	Min	Max	Mean		
FGY	38.08 [a]	2.20	39.75 [a]	5.62	14.30	64.72	36.53	0.77	0.66
MGY	15.02 [a]	3.73	16.64 [a]	1.46	1.19	31.70	12.33	0.41	0.64
FND	9.82 [a]	0.39	9.04 [a]	0.06	7.22	12.79	9.33	0.08	0.62
MND	11.66 [a]	0.51	9.56 [b]	0.52	7.97	14.72	10.74	0.09	0.73
QY	0.60 [a]	0.04	0.58 [a]	0.03	0.45	0.70	0.58	0.00	0.50
Ch	47.62 [a]	2.35	50.59 [a]	1.90	36.83	61.60	51.08	0.31	0.68

G) Parents and RILs in environment 6

Traits	Parental lines [#]				RILs			SEM	h^2
	SC35	SEM	RTx430	SEM	Min	Max	Mean		
FGY	55.16 [a]	3.86	53.20 [a]	0.13	3.86	80.28	41.37	1.05	0.76
FND	11.83 [a]	0.22	11.62 [a]	0.10	7.48	15.84	11.25	0.09	0.67
QY	0.51 [a]	0.03	0.50 [a]	0.02	0.33	0.62	0.50	0.00	0.18
Ch	56.65 [b]	1.76	62.63 [a]	1.37	47.00	71.43	59.10	0.38	0.69

H) Population (parents + RILs) in each environment

Env	FGY	MGY	FND	MND	QY	Ch
1	66.08 [a]	23.39 [a]	13.32 [a]	13.25 [a]	0.49 [d]	59.85 [a]
2	56.61 [b]	20.63 [ab]	12.15 [b]	12.33 [a]	0.51 [cd]	58.23 [a]
3	59.00 [ab]	18.33 [bc]	10.60 [c]	11.33 [b]	0.55 [bc]	42.27 [c]
4	50.90 [b]	15.93 [c]	10.14 [cd]	10.64 [b]	0.56 [ab]	54.87 [a]
5	38.91 [c]	15.83 [c]	9.43 [d]	10.61 [b]	0.59 [a]	49.11 [b]
6	54.18 [b]		11.73 [b]		0.50 [d]	55.43 [a]

[#] Number followed by different letter within a row of same trait are significantly different at P = 0.05.

FGY - Final grain yield (g); MGY - Grain yield at mid grain-filling stage (g); FND - Final panicle neck diameter (mm); MND - Panicle neck diameter at mid grain-filling stage (mm); QY - Effective quantum yield of photosystem II, Ch - Chlorophyll index. SEM - standard error of mean. h^2 - broad sense heritability, Env - Environment.

Table 4.4 Phenotypic correlation coefficient among the studied traits

A) Averaged across all environments

	MGY	FND	MND	QY	Ch
FGY	0.68**	0.52**	0.36**	NS	NS
MGY		0.45**	0.42**	NS	NS
FND			0.83**	NS	0.19**
MND				NS	0.26**
QY					NS

B) Environment 1

	MGY	FND	MND	QY	Ch
FGY	0.47**	0.44**	NS	- 0.15*	NS
MGY		0.26**	0.27**	NS	NS
FND			0.54**	NS	0.25**
MND				NS	0.19**
QY					NS

C) Environment 2

	MGY	FND	MND	QY	Ch
FGY	0.47**	0.37**	0.24**	NS	NS
MGY		0.15*	0.26**	NS	NS
FND			0.64**	NS	0.28**
MND				NS	0.22**
QY					NS

D) Environment 3

	MGY	FND	MND	QY	Ch
FGY	0.49**	0.55**	0.33**	NS	NS
MGY		0.34**	0.35**	NS	NS
FND			0.68**	0.14*	0.21**
MND				NS	0.20**
QY					NS

E) Environment 4

	MGY	FND	MND	QY	Ch
FGY	0.49**	0.55**	0.39**	NS	NS
MGY		0.24**	0.29**	NS	- 0.16*
FND			0.71**	NS	NS
MND				NS	0.24**
QY					0.17*

F) Environment 5

	MGY	FND	MND	QY	Ch
FGY	0.54**	0.55**	0.35**	NS	0.14*
MGY		0.29**	0.33**	NS	NS
FND			0.69**	0.17*	0.15*
MND				NS	0.19**
QY					NS

G) Environment 6

	FND	QY	Ch
FGY	0.44**	NS	0.24**
FND		NS	0.25**
QY			NS

FGY - Final grain yield (g); MGY - Grain yield at mid grain-filling stage (g); FND - Final panicle neck diameter (mm); MND - Panicle neck diameter at mid grain-filling stage (mm); QY - Effective quantum yield of photosystem II, Ch - Chlorophyll index. *P < 0.05; **P < 0.01.

Table 4.5 List of QTL detected for final grain yield.

QTL	Env	Chr	Position	QTL interval (cM)	LOD	Additive effect[#]	PVE	Peak SNP
qFGY1.1	2	1	107.1	104.2-109.5	6.29	5.8	11.81	S1_55108738
qFGY1.2	4	1	129.8	128.1-131.1	6.01	3.56	7.25	S1_60415581
qFGY3.1	2	3	3.9	3.2-5.9	8.37	5.37	10.64	S3_1325223
qFGY5.1	6	5	23.2	20.1-26.0	5.37	-3.88	8	S5_4149301
qFGY5.1	5	5	26	25.2-27.0	5.17	-1.9	3.11	S5_4506725
qFGY6.1	1	6	48.3	47.9-50.0	5.4	-4.78	9.41	S6_43211995
qFGY7.1	5	7	41	38.8-44.0	4.78	-3.23	8.38	S7_6703572

Env - environment, Chr – chromosome, LOD - logarithm of odds, PVE - phenotypic variation explained.

[#] Additive effect - positive values indicate contribution by SC35 and negative values by RTx430.

Table 4.6 List of QTL detected for grain yield at mid grain-filling stage.

QTL	Env	Chr	Position (cM)	QTL interval (cM)	LOD	Additive effect [#]	PVE	Peak SNP
qMGY1.1	2	1	25.9	23.1-27.7	8.92	3.06	18.84	S1_10857585
qMGY1.2	1	1	31.3	28.6-32.0	5.55	2.12	7.58	S1_11595657
qMGY1.3	3	1	119	117.3-120.9	5.72	2.11	14.23	S1_57171649
qMGY1.3	4	1	120.3	119.9-120.9	6.68	1.62	11.07	S1_57442199
qMGY1.3	5	1	120.3	117.9-120.6	5.76	1.62	8.12	S1_57442199
qMGY2.1	4	2	92.3	89.5-93.8	5.62	0.81	4.56	S2_61697565
qMGY2.1	3	2	93.2	92.6-95.5	5.90	0.46	1.88	S2_61935681
qMGY3.1	1	3	3.2	0.3-5.3	4.98	2.32	10.10	S3_1178864
qMGY3.1	5	3	5.7	3.2-8.3	4.21	1.66	5.97	S3_1605688
qMGY4.1	5	4	43.7	43.1-44.0	4.68	-0.96	5.15	S4_8693838
qMGY4.2	7	4	123.1	120.0-123.7	4.91	-1.30	6.82	S4_62961638
qMGY4.3	8	4	129.1	127.0-131.0	9.89	-1.48	8.97	S4_64139837
qMGY5.1	9	5	24.5	22.7-27.4	6.14	-1.40	6.90	S5_4305004

Env - environment, Chr – chromosome, LOD - logarithm of odds, PVE - phenotypic variation explained.

[#] Additive effect - positive values indicate contribution by SC35 and negative values by RTx430.

Table 4.7 List of QTL detected for final panicle neck diameter.

QTL	Env	Chr	Position (cM)	QTL interval (cM)	LOD	Additive effect [#]	PVE	Peak SNP
qFND1.1	4	1	117.9	115.9-119.3	4.44	0.25	5.07	S1_56913169
qFND2.1	2	2	15.5	14.1-17.7	4.97	0.45	8.41	S2_3340593
qFND2.2	3	2	30.2	28.0-32.7	7.47	0.37	9.31	S2_5638382
qFND2.2	1	2	32	28.0-32.7	4.73	0.41	6.87	S2_6039782
qFND2.3	4	2	35.9	32.8-37.5	9.39	0.50	15.40	S2_6795698
qFND2.4	6	2	75.8	75.1-78.3	6.55	-0.16	2.49	S2_58293965
qFND3.1	1	3	13.3	12.5-19.0	5.37	0.41	9.58	S3_2926948
qFND3.2	6	3	80.9	78.1-83.0	5.10	-0.27	5.69	S3_53593376
qFND3.2	1	3	87.4	79.8-90.2	5.10	-0.35	8.09	S3_56144986
qFND3.3	3	3	104.1	101.9-106.6	7.52	0.33	7.12	S3_59466158
qFND3.4	2	3	120	115.0-121.1	5.82	0.46	9.44	S3_65279115
qFND3.4	1	3	124.7	112.7-128.0	6.86	0.36	6.99	S3_66747535

Env - environment, Chr – chromosome, LOD - logarithm of odds, PVE - phenotypic variation explained.

[#] Additive effect - positive values indicate contribution by SC35 and negative values by RTx430.

Table 4.8 List of QTL detected for panicle neck diameter at mid grain-filling stage.

QTL	Env	Chr	Position (cM)	QTL interval (cM)	LOD	Additive effect [#]	PVE	Peak SNP
qMND2.1	5	2	16.3	13.1-16.8	6.52	0.53	16.6	S2_3434743
qMND2.2	3	2	34.7	31.7-36.8	5.15	0.50	10	S2_6534053
qMND2.3	1	2	43.7	42.1-44.7	5.92	0.47	9.02	S2_8937679
qMND3.1	3	3	82.1	60.3-90.7	5.87	-0.32	6.51	S3_54111110
qMND3.1	4	3	81	79.8-83.7	4.96	-0.28	4.87	S3_53606611
qMND3.2	3	3	102.5	94.3-107.8	4.83	0.23	5.91	S3_58929569
qMND6.1	1	6	85.7	57.6-85.9	4.99	0.37	5.53	S6_52105090
qMND7.1	2	7	115.8	113.1-116.2	6.62	-0.42	7.8	S7_63859329
qMND9.1	4	9	93.8	92.4-97.0	6.41	0.36	7.68	S9_56035773

Env - environment, Chr – chromosome, LOD - logarithm of odds, PVE - phenotypic variation explained.

[#] Additive effect - positive values indicate contribution by SC35 and negative values by RTx430.

Table 4.9 List of QTL detected for effective quantum yield of photosystem II.

QTL	Env*	Chr	Position (cM)	QTL interval (cM)	LOD	Additive effect [#]	PVE	Peak SNP
qQY1.1	1.2	1	117.9	116-120.2	6.34	0.03	7.96	S1_56913169
qQY2.1	6.2	2	26.8	26.3-27.4	6.93	0.05	5.29	S2_5132605
qQY2.2	5.3	2	80.3	69.1-81.2	4.77	-0.02	4.14	S2_59167708
qQY2.3	6.3	2	119.7	119.1-122	7.70	0.07	13.72	S2_70457570
qQY3.1	4.2	3	14.6	12-16.9	5.96	-0.02	7.28	S3_3063134
qQY4.1	2.2	4	2.5	1.6-4.5	4.96	-0.01	7.03	S4_572956
qQY4.2	2.1	4	48.1	47.3-49.8	8.35	0.02	5.91	S4_9429397
qQY5.1	2.2	5	36	33-36.9	6.03	0.01	5.7	S5_6796145
qQY6.1	5.1	6	67.7	64.7-69.4	5.53	0.02	4.89	S6_46614418
qQY6.2	2.2	6	72.7	70.3-74.6	5.99	0.02	9.21	S6_47671002
qQY7.1	6.3	7	88.7	88.4-91.3	7.99	1.19	15.4	S7_58871733
qQY8.1	4.3	8	101.1	98.6-102.5	5.11	-0.02	8.88	S8_53842917
qQY9.1	2.1	9	19.9	17.2-20.7	5.51	-0.01	2.12	S9_2899073
qQY10.1	3.1	10	94.5	89.6-97.4	6.90	0.01	9.15	S10_56982210
qQY10.2	3.1	10	104.4	95.5-106.2	6.14	0.00	5.84	S10_58653694

Env - environment, Chr – chromosome, LOD - logarithm of odds, PVE - phenotypic variation explained.

* Digit after decimal represent time of the measurement i.e. Env 1.1 - first measurement in environment 1.

[#] Additive effect - positive values indicate contribution by SC35 and negative values by RTx430.

Table 4.10 List of QTL detected for chlorophyll content.

QTL	Env*	Chr	Position (cM)	QTL interval (cM)	LOD	Additive effect #	PVE	Peak SNP
qCh2.1	2.1	2	15.5	14.7-15.9	10.31	2.42	13.44	S2_3340593
qCh2.1	2.2	2	15.5	14-17.3	6.41	2.08	9.54	S2_3340593
qCh4.1	5.3	4	10	9.9-10.7	5.16	1.15	4.53	S4_1622454
qCh4.2	4.4	4	70	67.4-70.7	5.23	-1.17	5.1	S4_49353588
qCh5.1	5.1	5	66.1	63.2-68.2	5.65	-1.59	8.65	S5_50907543
qCh6.1	3.4	6	106.1	104.1-108.3	4.52	-0.98	4.12	S6_56897503
qCh8.1	3.2	8	83.1	79.2-84.7	6.12	-1.45	10.21	S8_51052733
qCh8.1	4.2	8	84	83.1-87.6	7.18	-1.25	5.95	S8_51334618
qCh8.2	6.1	8	86.3	83.7-88.5	5.05	-1.71	7.37	S8_51608345
qCh8.2	3.4	8	87.9	86.6-90.2	5.36	-1.36	7.34	S8_51902533
qCh8.2	1.1	8	89.4	87.8-91.2	5.02	-1.59	6.9	S8_52180160
qCh9.1	2.1	9	88.9	86.2-91.4	5.39	1.76	6.65	S9_54256760
qCh9.2	1.1	9	94	90.3-96	5.03	1.35	5.3	S9_56112754
qCh9.2	2.2	9	95.1	93.8-97	5.58	1.54	6.22	S9_56392269
qCh10.1	6.2	10	9.2	6.6-11.6	4.80	-1.67	6.16	S10_1835738
qCh10.2	3.2	10	29.3	29.2-34.4	4.73	-1.30	7.22	S10_5679456
qCh10.3	6.1	10	37.8	34.4-51.4	4.88	-2.02	8.33	S10_7540643
qCh10.4	4.3	10	46.2	45.8-50.7	5.60	-1.60	7.35	S10_9731650
qCh10.4	2.3	10	47.3	45.7-49	7.43	-2.01	8.98	S10_10169620
qCh10.4	2.2	10	50.9	48.5-52.5	7.31	-2.40	6.55	S10_11641904
qCh10.5	3.3	10	64.1	60.9-71	4.69	0.37	2.67	S10_50458891
qCh10.6	3.3	10	82.7	70.6-84.3	4.81	-1.09	7.57	S10_55223808

Env - environment, Chr – chromosome, LOD - logarithm of odds, PVE - phenotypic variation explained.

* Digit after decimal represent time of the measurement i.e. Env 2.1 - first measurement in environment 2.

Additive effect - positive values indicate contribution by SC35 and negative values by RTx430.

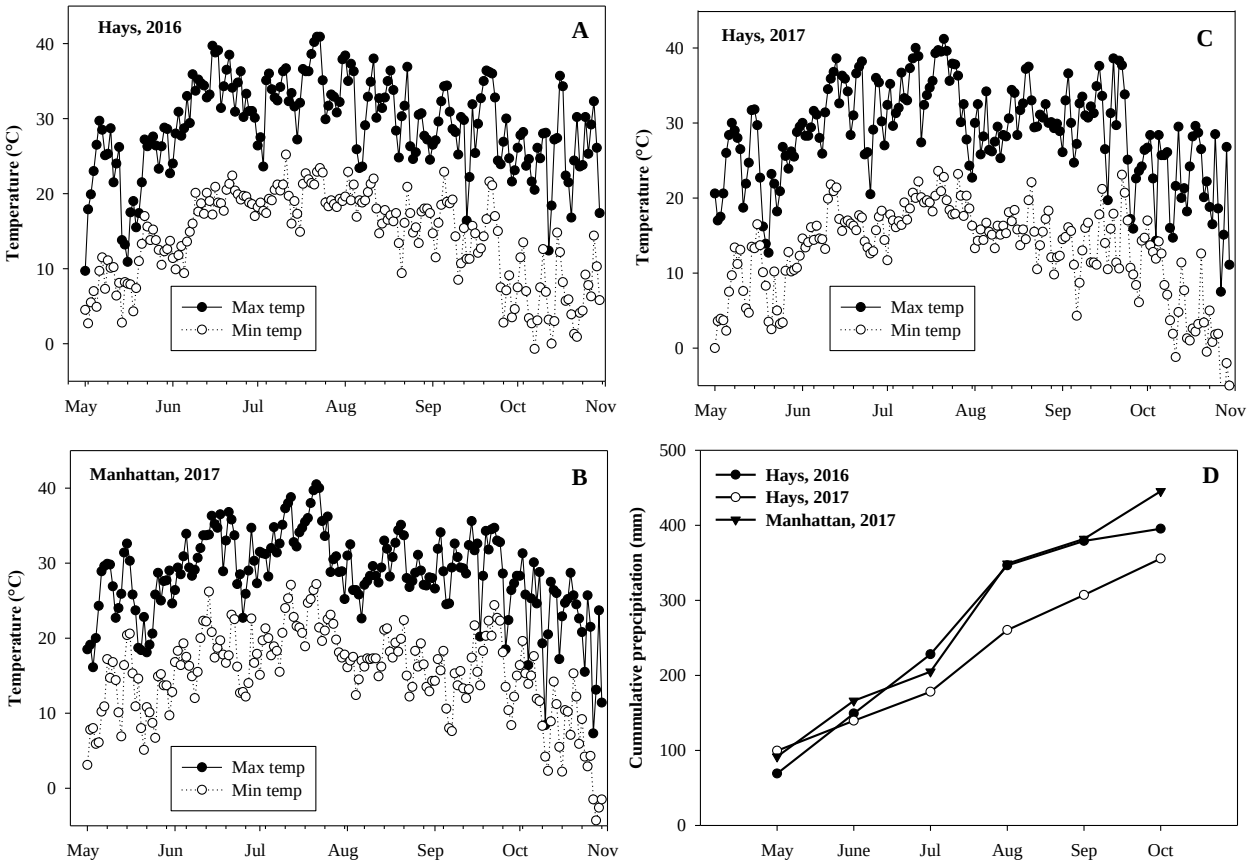

Figure 4.1 Daily (minimum and maximum) air temperature and cumulative precipitation during the plant growing season in 2016 and 2017 at the experimental sites.

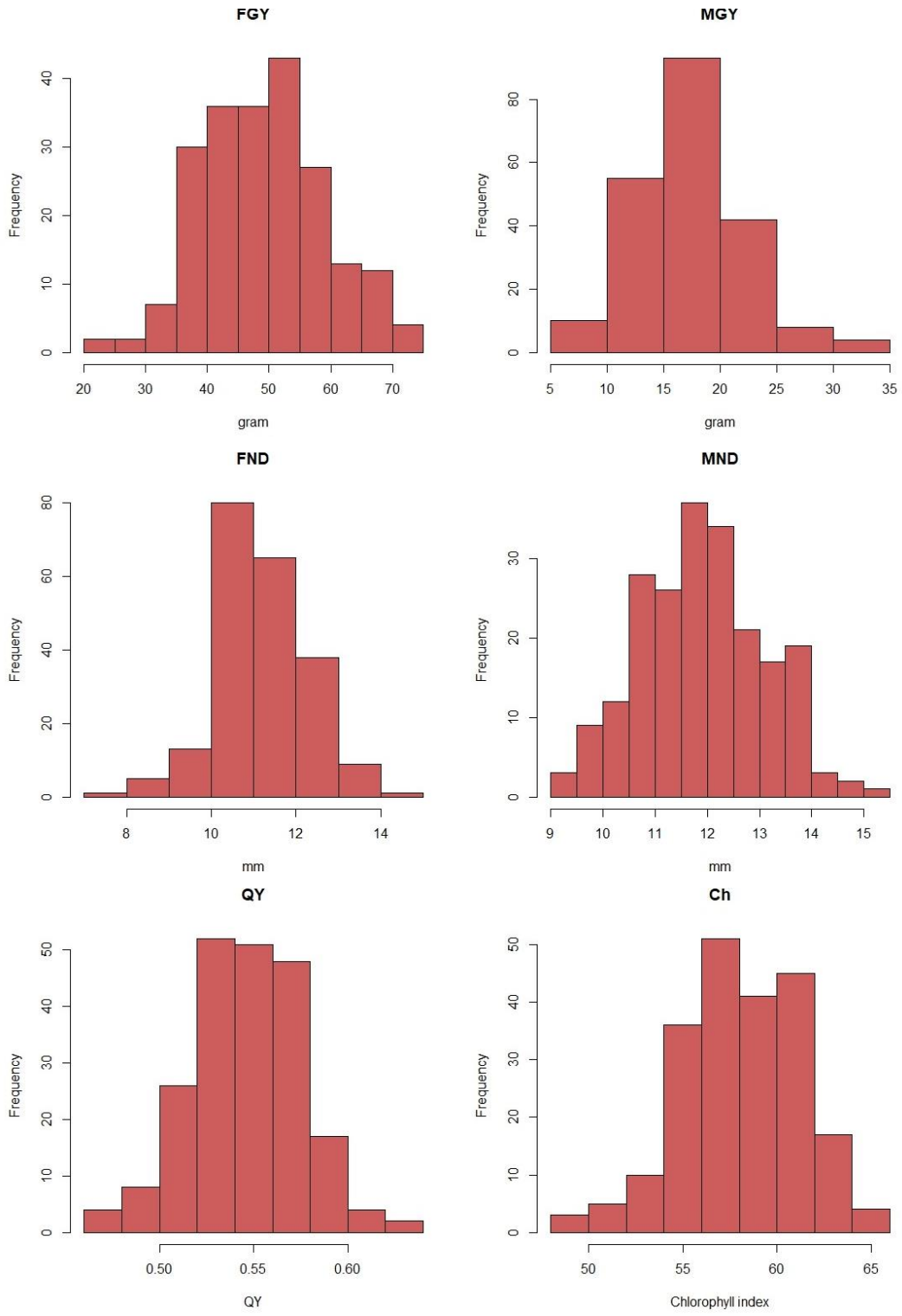

Figure 4.2 Histogram showing frequency distribution of the traits averaged across all the environments.

FGY - Final grain yield (g); MGY - Grain yield at mid grain-filling stage (g); FND - Final panicle neck diameter (mm); MND - Panicle neck diameter at mid grain-filling stage (mm); QY - Effective quantum yield of photosystem II, Ch - Chlorophyll index.

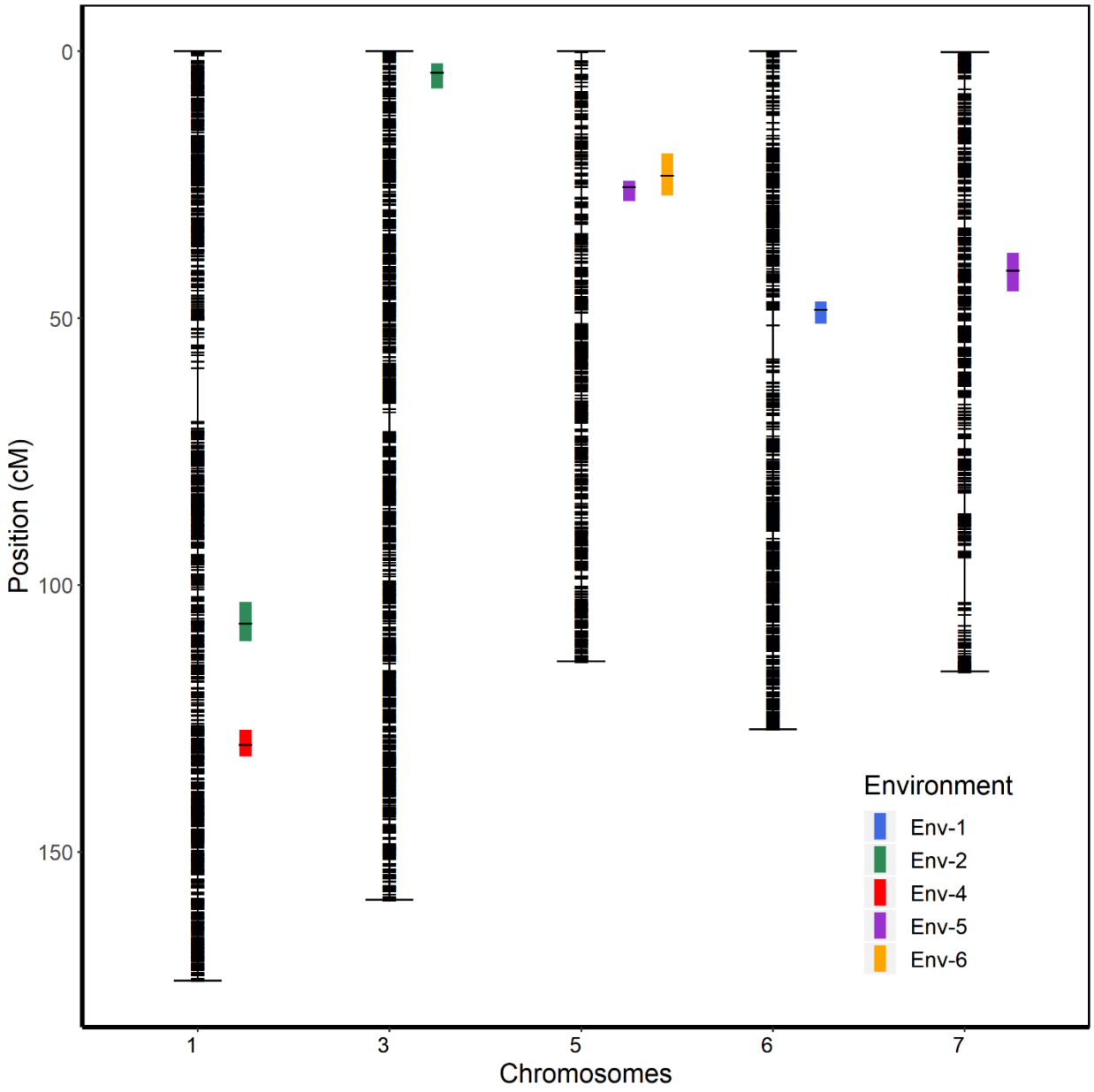

Figure 4.3 Position of detected QTL for final grain yield.

Only chromosomes where the QTL was detected has been included. Chromosomes that are not included did not house any significant QTL affecting final grain yield.

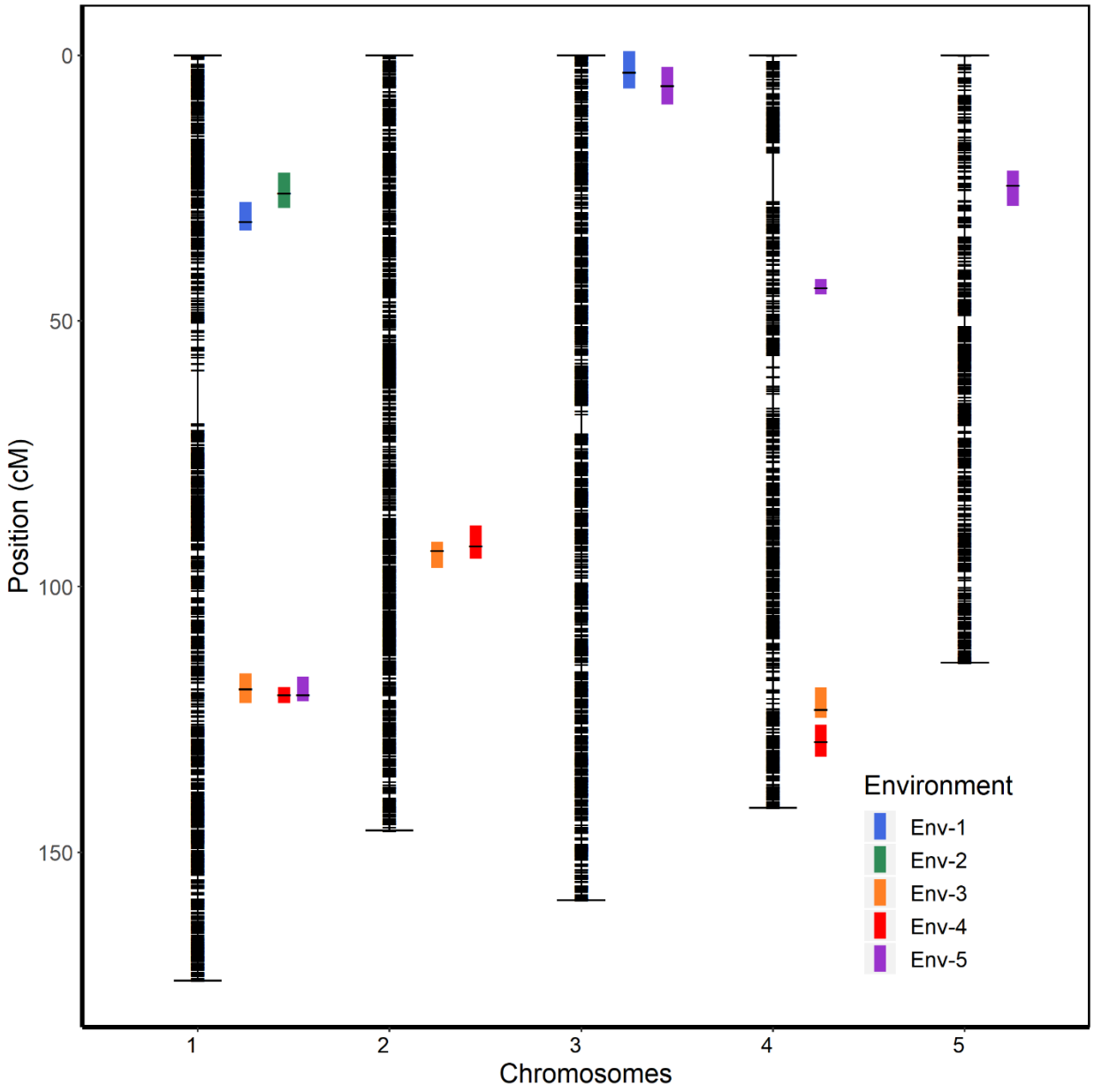

Figure 4.4 Position of detected QTL for grain yield at mid grain-filling stage.

Only chromosomes where the QTL was detected has been included. Chromosomes that are not included did not house any significant QTL affecting grain yield at mid grain-filling stage.

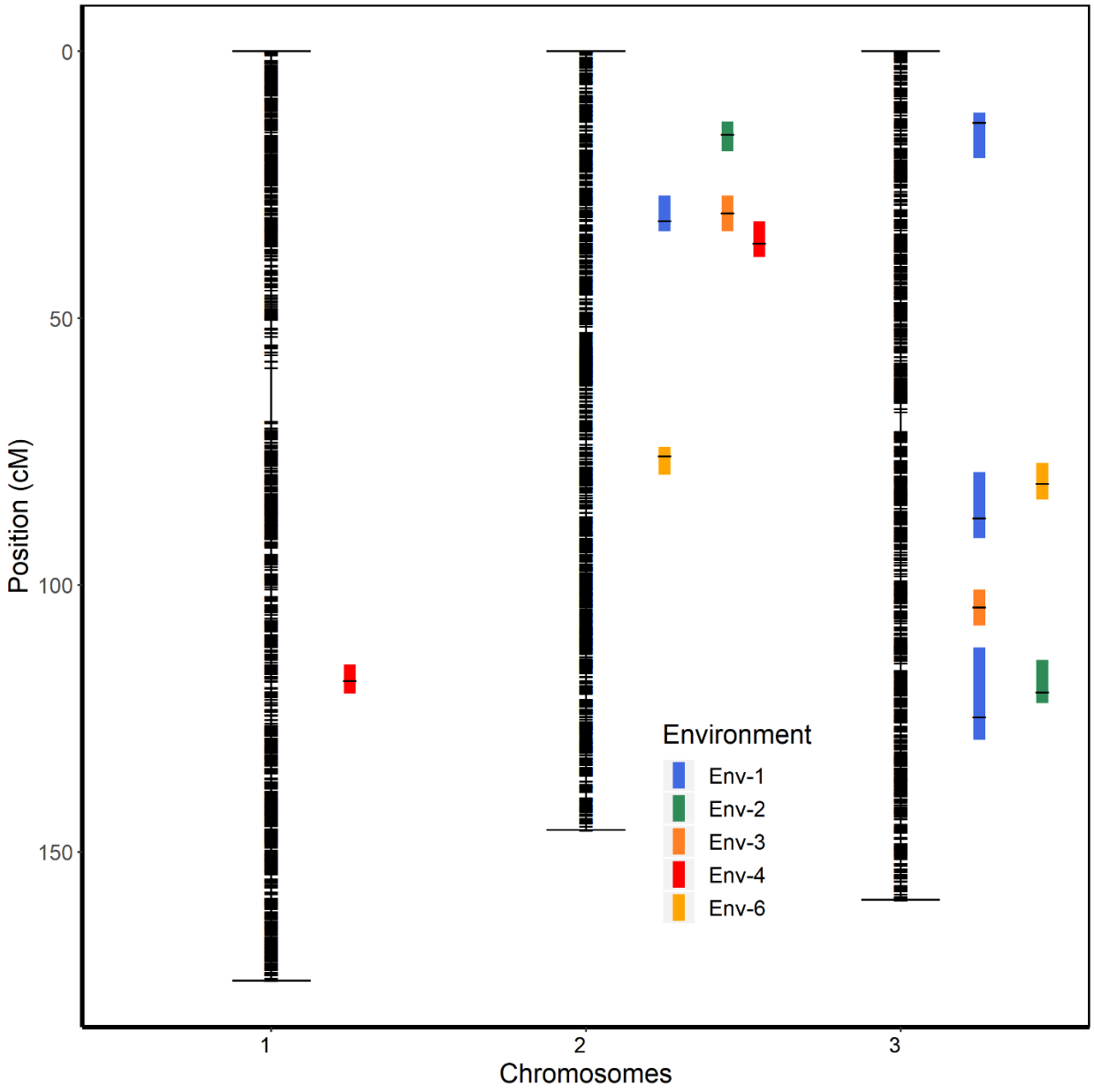

Figure 4.5 Position of detected QTL for final panicle neck diameter.

Only chromosomes where the QTL was detected has been included. Chromosomes that are not included did not house any significant QTL affecting final panicle neck diameter.

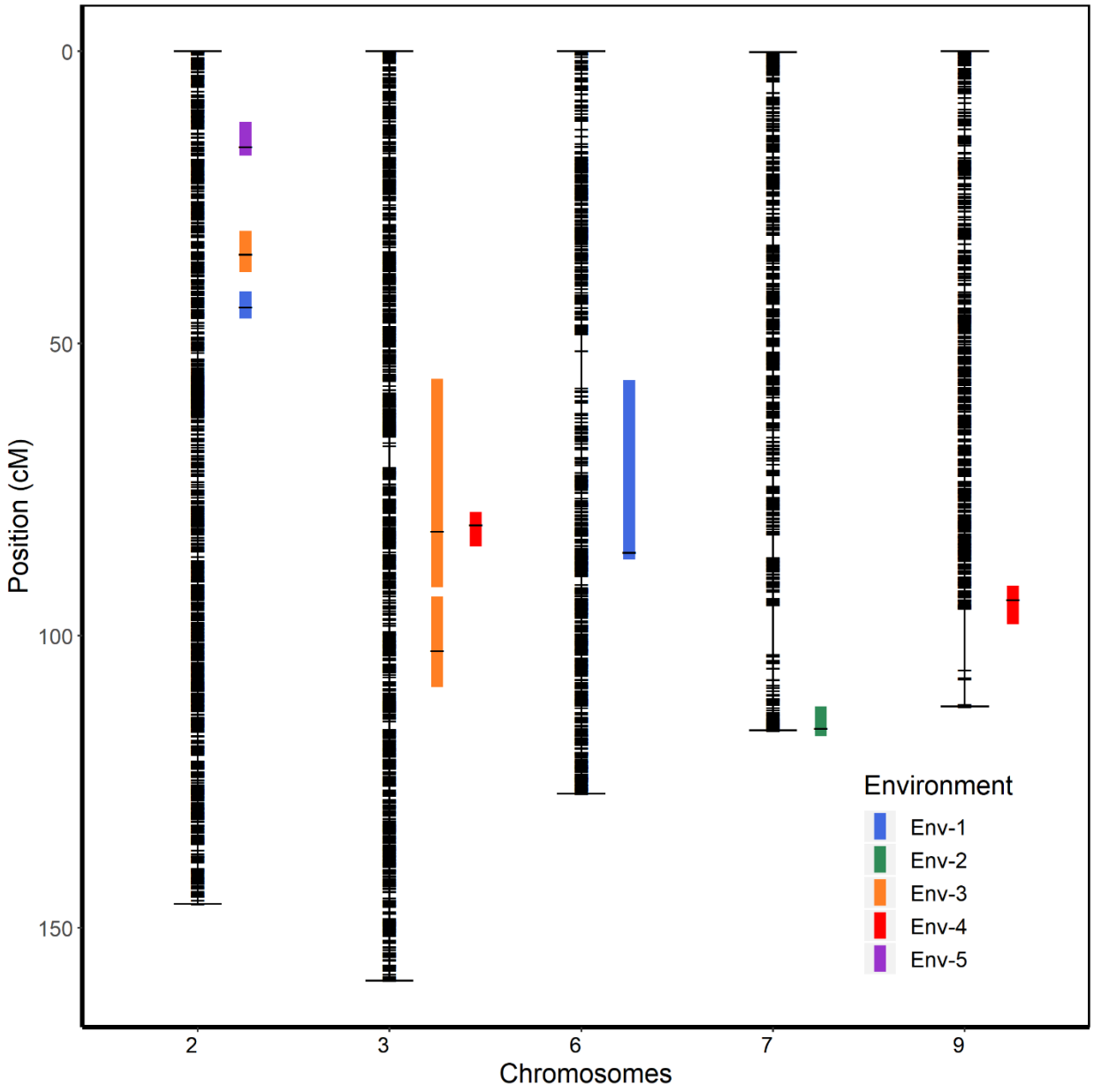

Figure 4.6 Position of detected QTL for panicle neck diameter at mid grain-filling stage.

Only chromosomes where the QTL was detected has been included. Chromosomes that are not included did not house any significant QTL affecting panicle neck diameter at mid grain-filling stage.

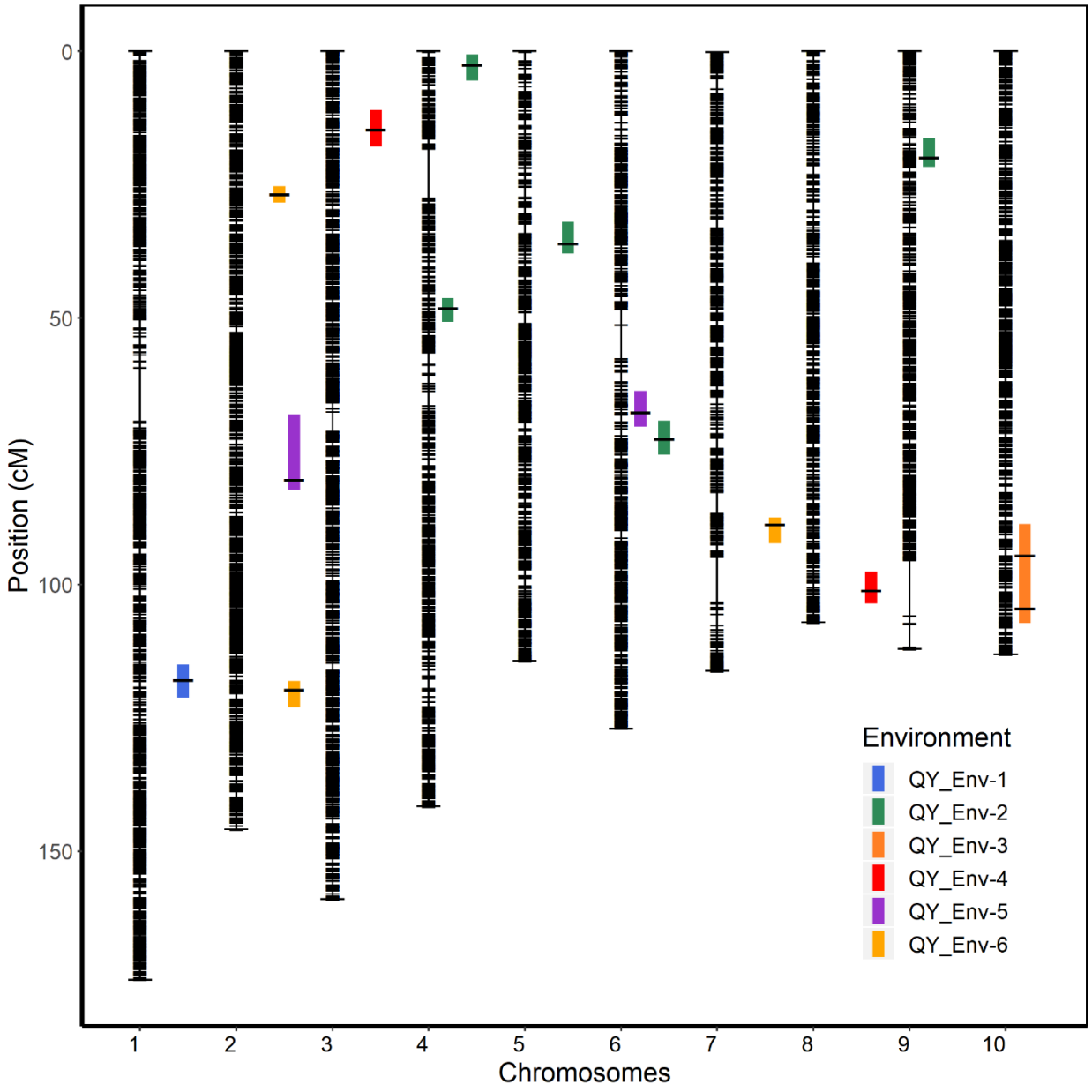

Figure 4.7 Position of detected QTL for effective quantum yield of photosystem II.

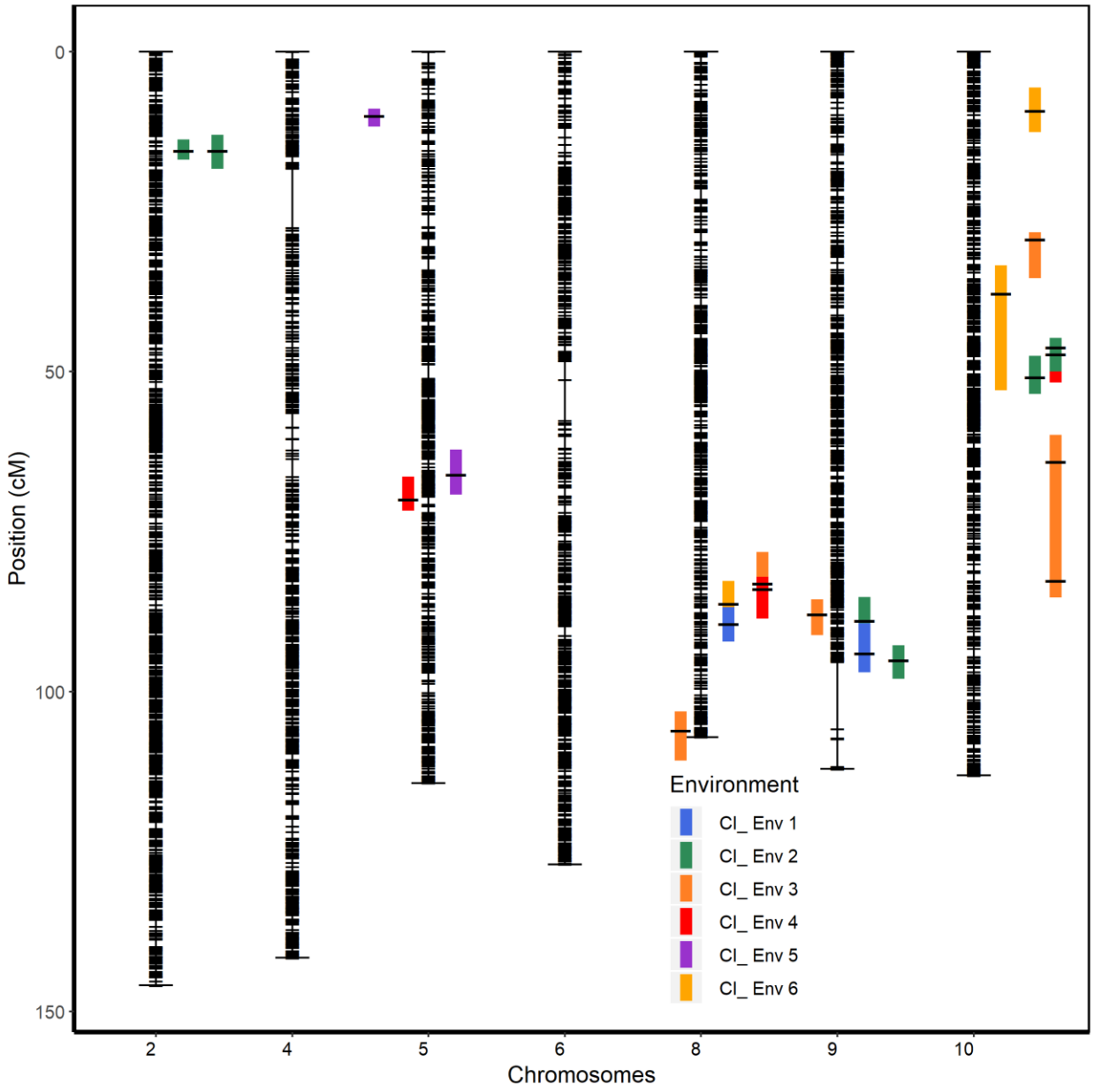

Figure 4.8 Position of detected QTL for chlorophyll content.

Only chromosomes where the QTL was detected has been included. Chromosomes that are not included did not house any significant QTL affecting chlorophyll content.

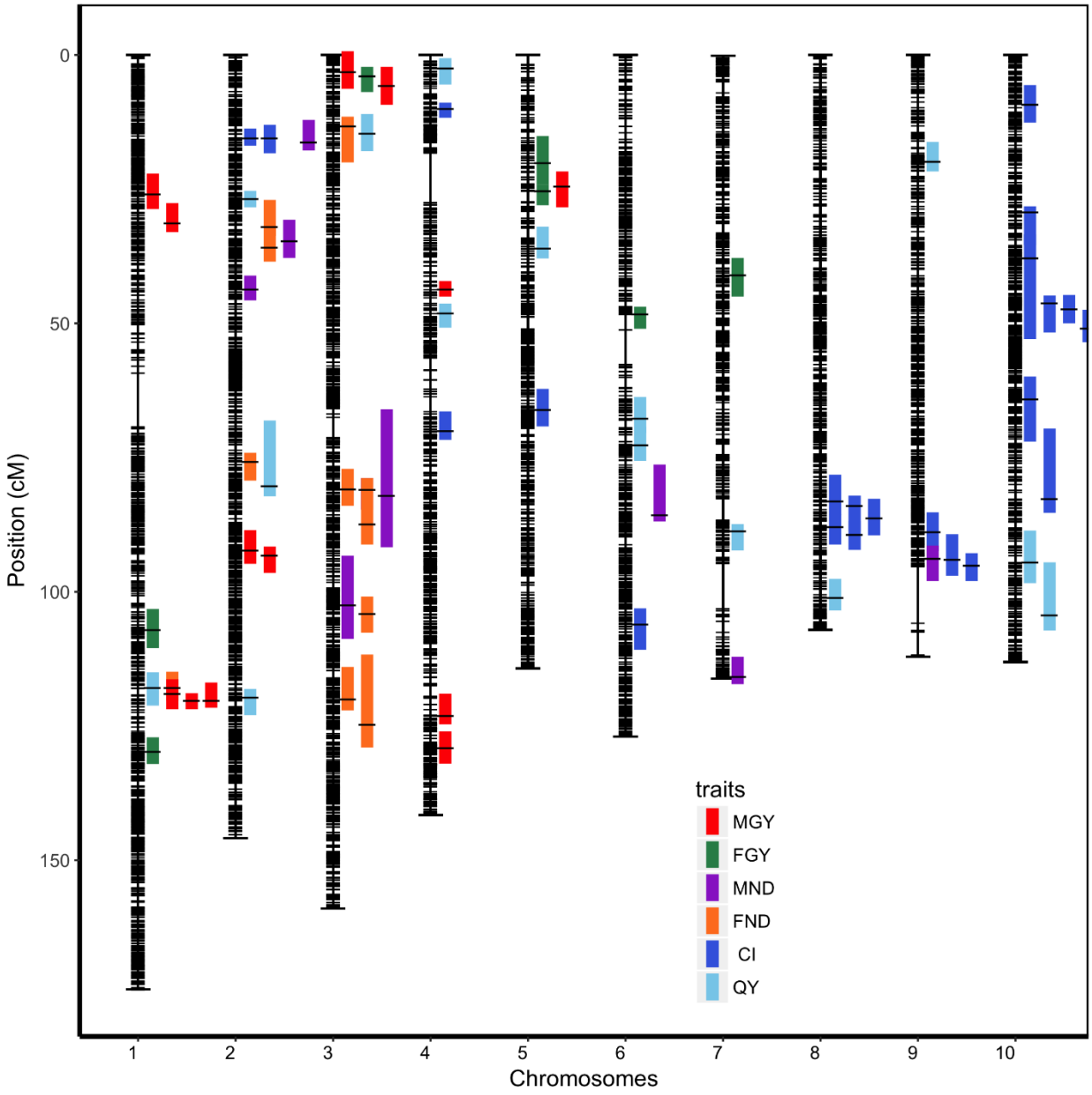

Figure 4.9 Position of detected QTL for all studied traits.

MGY - Grain yield at mid grain-filling stage (g); FGY - Final grain yield (g); MND - Panicle neck diameter at mid grain-filling stage (mm); FND - Final panicle neck diameter (mm); Cl - Chlorophyll content; QY - Effective quantum yield of photosystem II.

Chapter 5 - General discussion and future line of work

Sorghum is considered as one of the most resilient grain crop to abiotic stresses, however early stage chilling, terminal heat and drought stress continues to challenge sorghum productivity in the United States and other locations with similar climatic conditions. Enhancing tolerance to these abiotic stresses in sorghum is increasingly important specially under predicted harsher climate in the future. During the sorghum growing cycle either one or more of these stresses can occur at different growth stages. The occurrence of one stress can induce other (for example - drought and heat stress) and resilience to one of these stresses can potentially help minimize or avoid damage caused by other stresses. Hence, following an integrated approach aimed at improving tolerance to different stresses at key growth and developmental stages can be more effective in enhancing overall sorghum resilience during its crop cycle.

Figure 5.1 shows the current sorghum cultivation practice in many locations in the Great Plains of the United States such as Kansas. Sorghum is generally planted between last week of May to early June, when soil temperatures is above 15°C (Shroyer et al., 1996). Temperature start to increase rapidly after second week of May, leading to quicker evaporation of residual soil moisture. Hence, even a short episode without precipitation would lead to soil drying. Inadequate soil moisture at the time of planting and during emergence and early seedling growth, can affect the overall vigor of the plant and consequently impact the actual achievable yield. Since most of the sorghum in the United States is grown under rainfed conditions, the overall productivity or the genetic potential of the hybrids has not been completely realized.

Gametogenesis, flowering and grain filling are considered as most sensitive stages in sorghum to heat stress. Temperature above 32°C during gametogenesis and flowering increases floret sterility leading to reduced grain number and consequently grain yield. With current sorghum planting between last week of May to first week of June, gametogenesis and flowering in sorghum begins around last week of July and early August, respectively. July and August are two hottest months of the year in the United States. For examples in Hays, Kansas, average daily maximum air temperature over 10 years (2006 to 2015) was highest in July (34°C) followed by August (33°C) (Fig. 5.1 and Fig. 5.2). Hence, these key reproductive stages occur at the hottest time of the year and will be further challenged under increasingly hotter climate predicted for the future.

Similarly, major proportion of the grain-filling phase in sorghum occurs between Mid-August to September which is also considered not so optimal for normal grain filling. Firstly, precipitation starts to decrease after September. Temperature on the other hand continues to be relatively high until mid-September, to further evaporate remaining moisture present in the soil, consequently increasing the chances of severe water-deficit stress during late grain-filling stage. Hence, sorghum grown in United States is highly prone to drought stress during grain-filling stage. Drought stress decreases grain-filling duration and reduces final grain size and consequently grain weight. Secondly, temperature starts decreasing rapidly after mid-September. Low temperature during late grain-filling period can also affect grain-filling dynamics and reduce its grain size and in turn grain yield, and grain quality. Although not many studies have investigated grain yield or quality loss in sorghum due to chilling stress during its grain-filling stage, Maulana and Tesso (2013) showed that even short episodes (10 days) of low temperature during flowering can cause significant grain yield reduction. The impact of late season cooler temperature on grain yield and quality is an interesting and poorly understood research areas and hence warrants detailed investigation in the future.

As discussed above, sorghum in the United States and in other locations with similar challenges is not being grown under optimum conditions. Earlier sorghum planting between Mid-April to early May when soil temperature is above 10°C as shown in Figure 5.2 is seen as an effective strategy to minimize the above-mentioned disadvantages and to provide more conducive environment during its seedling establishment, early vegetative and reproductive stages. Potential hypothesis that needs further testing with this change in the cropping pattern are (i) Does earlier planting by a month provide more conducive growth environment during its seedling establishment and help reduce some of the heat and drought stress during its terminal growth stage? (ii) By planting early, can sorghum effectively utilize early season residual moisture present in the field due to late spring and early summer rainfall. Temperature during early planting window is not high to result in high evaporative loss of residual soil moisture hence higher amount of moisture is preserved in the soil. But can sorghum have the vigor under chilling conditions to utilize this residual soil moisture for better seedling emergence and early seedling growth. Another dimension for the early planting to be effective and to be considered by the growers is the ability of the sorghum to flower early and mature early, which is key for this shift to occur. Some of these questions are highly complex and change dynamically and is not possible to test all these scenarios

with classical experiments and hence a crop modelling approach is recommended to understand and design sorghum that can effectively allow for earlier planting in these conditions.

This paragraph below needs to be read in the light of some of the hypothesis above are favorable resolved through integrated modelling and breeding efforts. By adopting early planting, gametogenesis and flowering could begin around middle of June and last week of June, respectively which are comparatively cooler than current months when those sensitive stages occur. Hence, early sorghum planting in United States can help reduce the impact of more severe heat stress in future, complemented by the early-morning-flowering mechanism that operates effectively in sorghum. Furthermore, sorghum can potentially complete its grain filling by August when planted early. The period between May to August received most of the rainfall in mid-west region of the United States. For example, more than 55% rainfall occurred between May to August from 2006 to 2015 in Hays, Kansas (Fig 5.1 and Fig. 5.2). Hence, early sorghum planting is also seen as an opportunity to escape terminal drought stress in sorghum which is one of the major reason for grain yield reduction under current cultivation practices.

As discussed above, early planting offers numerous advantages compared with regular planting and minimize some of the risks associated with current planting dates. However, sorghum is highly sensitive to chilling stress that remains as the bottleneck to implement early planting. Minimum temperature requirement for sorghum emergence is 15°C hence sorghum can only be planted after May 15 in Kansas (Shroyer et al., 1996). Corn is comparatively more chilling tolerant. The minimum temperature requirement for corn emergence is 10°C (Warrington and Kanemasu, 1983; Gesch and Archer, 2005) which allows corn to be planted about a month or more ahead than sorghum, which tends to coincide with the proposed early sorghum planting date. Hence, enhancing sorghum's chilling tolerance is the first major research challenge for making early planting of sorghum feasible and to potentially test the list of hypotheses listed above, that come along with this change.

Although early stage chilling tolerance would be helpful to minimize some of the terminal heat and drought stress, it can't completely escape those stresses. Hence, increasing sorghum tolerance to both heat and drought stress is considered to complement early stage chilling tolerance to enhance sorghum productivity under current and future harsher climatic conditions. We have discovered a novel heat escaping early-morning-flowering mechanism, which is effectively employed by sorghum to minimize heat stress impact at anthesis. We have also identified a heat

tolerant genotype “Macia” which appears to be a promising donor for developing improved heat tolerant sorghum hybrids. We believe these findings will be useful in the context of designing breeding strategies to tackle heat stress impact during flowering in sorghum. We have also identified some novel QTL associated with source, sink and transport (panicle neck diameter) in sorghum under wide range of environments including some terminal heat and drought stress. In summary, we have discovered some novel findings from this dissertation research and some of which will be useful for incorporating into ongoing sorghum breeding or for further investigation to help develop future sorghum with enhanced abiotic stress resilience.

5.1 References

Gesch, R.W., and D.W. Archer. 2005. Influence of sowing date on emergence characteristics of maize seed coated with a temperature-activated polymer. Agron. J. 97:1543-1550.

Maulana, F., and T.T. Tesso. 2013. Cold temperature episode at seedling and flowering stages reduces growth and yield components in sorghum. Crop Sci. 53:564–574.

Shroyer, J.P., C. Thompson, R. Brown, P. Ohlenbach, D. Fjell, S. Staggenborg, S. Duncan, and G. Kilgore. 1996. Kansas crop planting guide. L-818. Manhattan, Kansas: Kansas State University Cooperative Extension Service.

Warrington, I.J., and E.T. Kanemasu. 1983. Corn growth response to temperature and photoperiod I. seedling emergence, tassel initiation, and anthesis. Agron. J. 75:749-754.

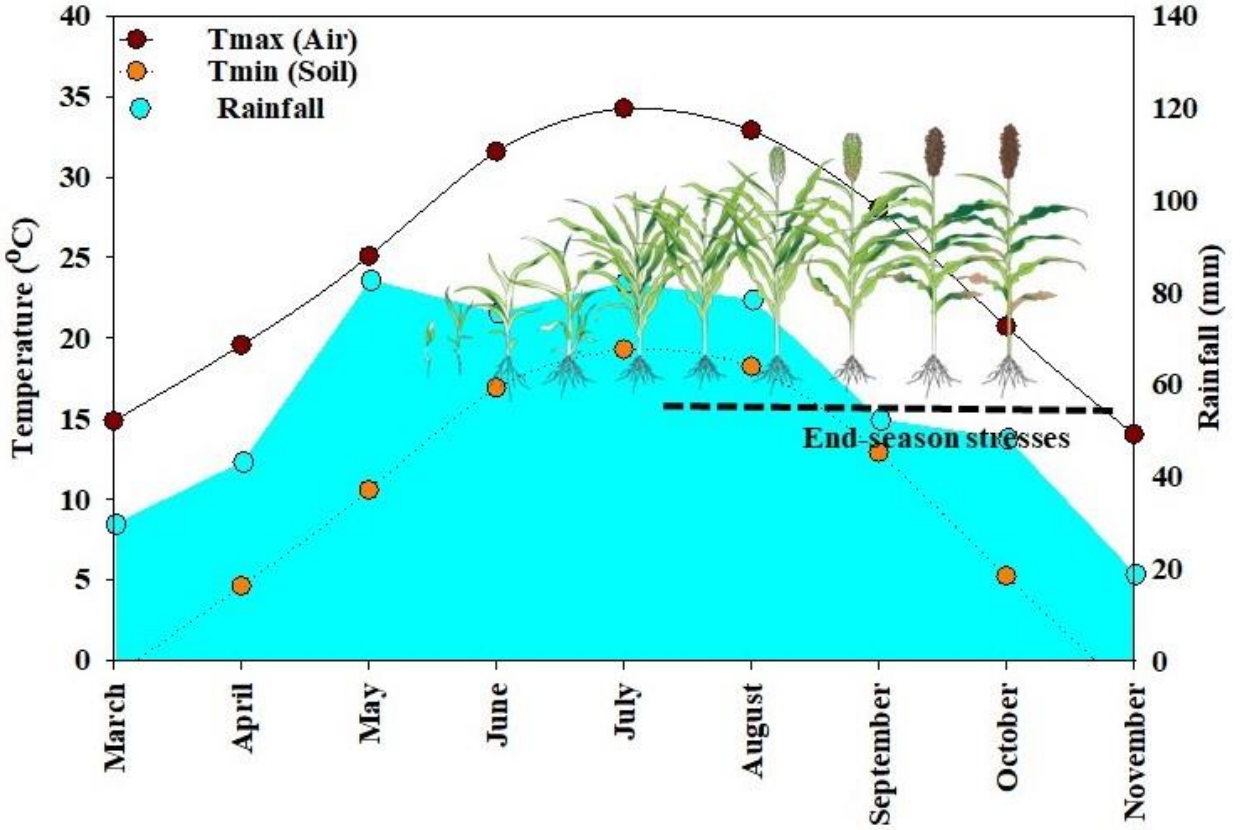

Figure 5.1 Timeline of current sorghum cultivation practices in Kansas, United States.

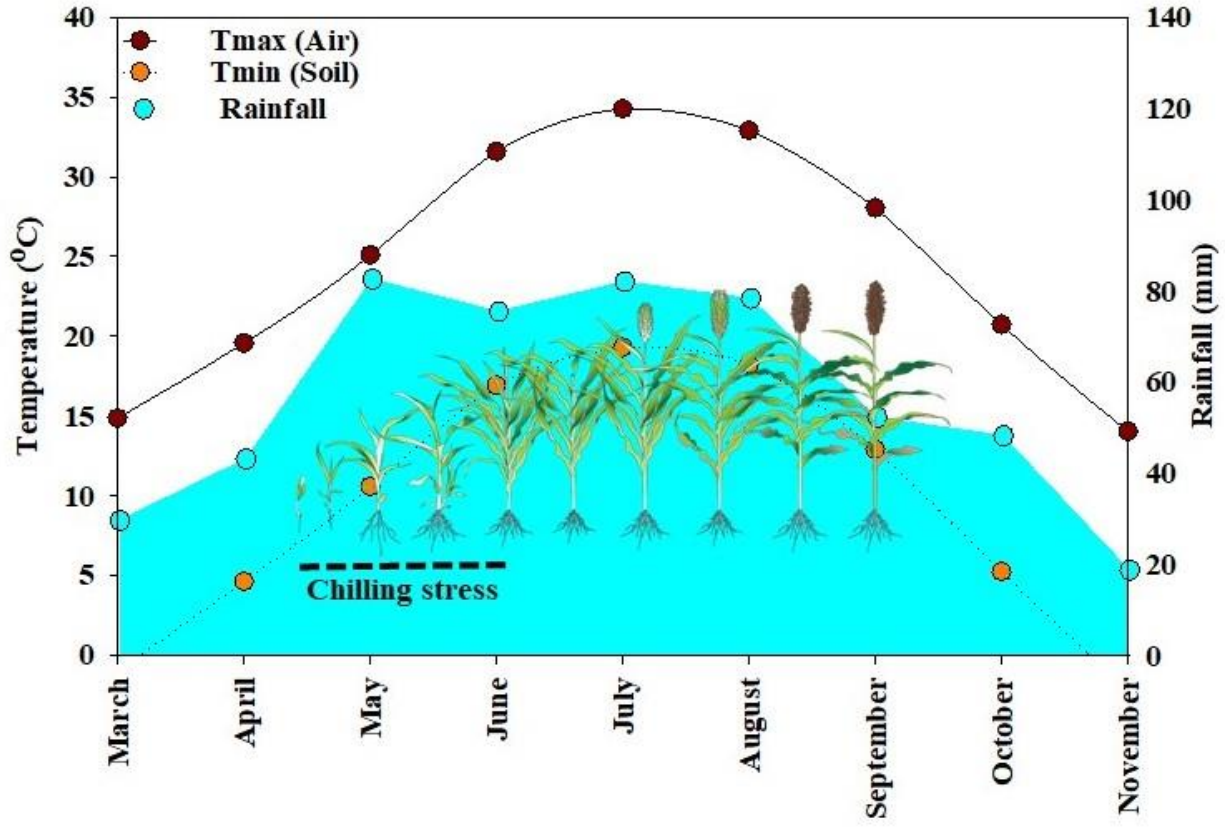

Figure 5.2 Timeline of proposed sorghum cultivation practices with early planting in Kansas, United States.