Enhancing early-stage chilling tolerance [*Sorghum bicolor* (L.) Moench] by integrating physiological and genetic approaches

by

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B.S., Kansas State University, 2016

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2020

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Abstract

Grain sorghum (*Sorghum bicolor* L. Moench) is an important grain crop in the United States. Early planting can help to extend sorghum's growing season and increase planting area and production, but is poorly adapted to chilling temperatures (<15°C) because of its tropical origin. Earlier planting could have additional advantages, which include effective utilization of early spring soil moisture as well as earlier canopy cover to reduce weed pressure and evaporation losses. Developing sorghum hybrids with early-stage chilling tolerance has been one of the major goals for the private and public sectors in the U.S. and elsewhere. However, with sorghum's tropical adaptation, chilling temperatures pose serious challenges to obtain uniform emergence, maintaining good plant stand, and early seedling vigor. Therefore, it is important to understand the inheritance of seedling and agronomic traits and to select the most appropriate parental lines and hybrids with the highest degree of early-stage chilling tolerance. The objectives of this project were: (i) to estimate the general and specific combining ability of 27 newly developed sorghum hybrids and their 12 parental lines over two years in the field; and (ii) to phenotype a subset of the original population, including the four most promising chilling tolerant hybrids, their five parental lines and three checks in field and controlled environment chamber conditions. To impose chilling stress in the field, the genotypes were planted 1.5 months ahead (mid-April) of current agronomic practices of sorghum production in Kansas followed with a regular planting (end of May) at the Agricultural Research Center, Hays, Kansas in 2018 and 2019. In the controlled environment chambers, the genotype subset were exposed to three temperature treatments, which included a constant chilling stress (current chilling tolerance screening practice), a field-like gradual increase in temperature (improved chilling tolerant screening) and a control treatment (optimal temperatures). Plants grown under early-stage chilling stress showed a significant decrease in

seedling emergence and vigor. Chilling stress also delayed time until flowering and maturity compared to control. However, it did not negatively affect the final yield or grain quality. From this study, we observed that early planting has the potential to increase vegetative growth and grain filling duration. The tannin-free hybrid, ARCH11192A/ARCH12012R, had the highest potential for early planting. Overall, heterosis was shown to be beneficial in plant stand and seedling vigor as well as final grain yield for early planting. Developing early-stage chilling tolerant hybrids with the ability to emerge, develop, and out-yield currently available hybrids will pave the way to increasing grain sorghum productivity.

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Acknowledgements

I would like to thank my co-major professors, Dr. Ramasamy Perumal and Dr. S.V. Krishna Jagadish with their guidance and motivation throughout the last two years. Their wisdom and expertise allowed me to complete this project while being a full-time employee of the Agricultural Research Center, Hays (ARCH). I would also like to make a special thanks to Dr. Robert Gillen, the department head of the Western Kansas Agricultural Research Centers. Without his support, this project would not have been possible. I would also like to thank my committee member, Dr. Christopher Little with the support and guidance he handed down to me throughout this journey. In addition, I would like to thank the Kansas Grain Sorghum Commission for the funding and contribution to make this project possible.

I am also extremely grateful for all of the help and support from the employees of ARCH, specifically Jeff Ackerman and Kashli Holthaus. The amount of time and effort in assisting me in this project did not happen without notice, and I am truly grateful. Additionally, I would like to thank the Crop Eco-physiology Lab (CEL) on campus for the generous support and making available the best tools available for the project. Additionally, a special thanks to Raju Bheemanahalli for the most generous guidance of the chamber experiments and data analysis. Lastly, I am forever grateful of the generous support and love received from my family and friend through the duration of the project.

Chapter 1 - Literature Review

Introduction on chilling tolerance in sorghum

Sorghum production

Grain sorghum [*Sorghum bicolor* (L.) Moench] is a warm-season crop grown in arid and semi-arid regions of the world. Sorghum originated in Africa and is one of the most resilient crops to abiotic stresses such as heat and drought, which makes it an ideal fit for hot and arid regions of the world (Doggett, 1988; Smith and Frederiksen, 2000; Blum, 2004; Pennisi, 2009). Sorghum is one of the major agricultural commodities that has a significant impact on the economy of the Great Plains of the USA, India, and African countries (Leff et al., 2004; Nagaraj et al., 2013; Hariprasanna and Rakshit, 2016). In addition, high water-use efficiency, ability to maintain productivity under low input levels, and suitability for cropping rotation makes sorghum as one of the major dryland cereal crops in the US Great Plains region (Saballos, 2008; Assefa et al., 2010). The grain sorghum production in the United States from 2010 to 2019 was 377.6 million bushels per year, which makes it the largest producer in the world (USDA-NASS, 2019). In 2017, Kansas grain sorghum production was 7.5 million tons across 2.6 million acres, followed by Texas' 3.2 million tons over 1.7 million acres. These two states account for about 80% of the annual grain sorghum production and 76% of the total sorghum acreage (5.6 million acres) in the United States, with an average yield of 72.1 bushels/acre (USDA-NASS, 2019).

Importance

In the United States, low temperatures in late spring and early fall has limited sorghum production to a narrow period from May to September. The sensitivity to early-stage chilling stress limits sorghum planting to warmer months as well as its overall area (Maulana et al., 2017).

Likewise, chilling temperatures in the fall may undermine grain development and lead to low yield. Hence, unlike tropical sorghum, hybrids adapted to temperate environments are bred to fit this short time window at the expense of higher yield. A number of previous studies have shown positive associations between grain yield and the maturity period in sorghum, showing that efforts to breed early and extra-early maturing hybrids can compromise yield potential under chilling stress (Franks et al., 2006; Maulana and Tesso, 2013). However, the presence of genotype × environment (GxE) interactions complicate field evaluation for chilling tolerance, and proper characterization requires repeated multi-environment testing (Knoll and Ejeta, 2008; Marla et al., 2019). Controlled environment facilities could substitute for early field sowings as a controlled selection method, or at least as a preliminary test to discriminate weak from vigorous lines before spring planting (Kapanigowda et al., 2013). Therefore, screening sorghum lines under controlled environments is a continuous and parallel process with field screening to develop chilling tolerant parental lines and hybrids for early planting. This would be beneficial for sorghum cultivation in Kansas and its extension into northern regions of the United States.

Screening approaches

Screening for chilling tolerance was studied in multiple different settings and conditions across research studies. Researchers have studied the temperature and germination interaction within laboratory settings using incubators and petri dishes (Pinthus and Rosenblum, 1961; Mendoza-Onofre et al., 1979; Brar and Stewart, 1994; Cisse and Ejeta, 2003; Franks et al., 2006). Others have studied using controlled environment chambers with the ability to set the exact temperature, relative humidity and light (Majora et al., 1982; Anda and Pinter, 1994; Tiryaki and Andrews, 2001; Cisse and Ejeta, 2003; Chiluwal et al., 2018). Greenhouse studies have been used to screen for chilling tolerance, which provides opportunities to better replicate field conditions

(Majora et al., 1982; Harris et al., 1987; Tiryaki and Andrews, 2001). Field conditions are, as expected, the most pragmatic and reliable phenotyping environments (Pinthus and Rosenblum, 1961; Singh, 1985; Tiryaki and Andrews, 2001; Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Franks et al., 2006; Chiluwal et al., 2018), but inherently presents a number of challenges confounded by other environmental factors. Despite these challenges, using natural field conditions provides a realistic environment to test genetically diverse germplasm for early-stage chilling tolernace, but an integrated approach using controlled environment facilities and field-testing provides a more robust phenotyping approach (Chiluwal et al., 2018).

Impact of chilling tolerance

Germination and emergence

Chilling temperatures (<15°C) are a major constraint to increase the growing area and length of season for sorghum. With its tropical adaptation, sorghum is highly sensitive to early-stage chilling stress (Peacock, 1982; Rooney, 2004; Burow et al., 2011; Chiluwal et al., 2018). Currently, corn is significantly more early-stage chilling tolerant compared to sorghum. Wherein the minimum average soil temperature for corn emergence is 10°C (Warrington and Kanemasu, 1983; Gesch and Archer, 2005) while the recommended minimum average soil temperature for sorghum planting is 18°C (Doggett, 1970). This difference in temperature threshold allows corn to be planted up to two months prior to sorghum. Early-stage chilling stress on sorghum is known to negatively affect seedling traits such as germination and emergence which results in poor plant stand and establishment (Pinthus and Rosenblum, 1961; Singh, 1985; Harris et al., 1987; Anda and Pinter, 1994; Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Franks et al., 2006; Kapanigowda et al., 2013; Maulana and Tesso, 2013; Fernandez et al., 2015; Chiluwal et al., 2018). Introducing early-stage chilling tolerance and planting earlier under the U.S. Great Plains conditions can help

reduce the cumulative impact of heat and drought stresses during the sorghum-growing period (Chiluwal et al., 2018). Shifting to earlier planting of sorghum can have other benefits such as efficient utilization of spring residual soil moisture and early canopy cover for improved water conservation by reducing evaporation (Burow et al., 2011; Moghimi et al., 2019). Some major challenges that are faced when trying to induce chilling tolerance in sorghum is the crop's ability to germinate and emerge by protecting the tender developing coleoptile due to its tropical adaptation (Peacock, 1982; Rooney, 2004; Chiluwal, 2018).

Seedling vigor and physiology

Early seedling vigor is an essential component in plant development under a wide range of environments (Ludlow and Muchow, 1990; Cisse and Ejeta, 2003). Seedlings are damaged by temperatures of <15°C and eventually dies when the chilling stress turns into freezing conditions of <0°C (Peacock, 1982). In areas where rainfall is less abundant, early seedling vigor and plant establishment can potentially take advantage of the limited soil moisture resulting in greater biomass and grain yield (Cisse and Ejeta, 2003). Seedling emergence associated with plant stand and seedling vigor as a measure of growth and development defines early-stage chilling tolerance in sorghum (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Kapanigowda et al., 2013; Maulana and Tesso, 2013; Fernandez et al., 2015). A major challenge of chilling stress on seedlings in the field is their ability to maintain sufficient vigor to allow photosynthetic activity to grow normally without dying (Chiluwal et al., 2018). Chlorophyll content could be decreased in seedlings (Maulana and Tesso, 2013; Chiluwal et al., 2018), which also causes a decrease in the photosynthetic rate (Ortiz et al., 2017; Chiluwal et al., 2018). The photosynthetic rate is key in the growth and development of the emerged seedlings. Taylor and Rowley, (1971) documented that sorghum is more sensitive to chilling stress compared to similar C4 crops like corn. Under the

same study, the authors reported that chilling stress of 10°C for two days while exposed to sunny conditions or three days of 10°C while exposed to cloudy conditions permanently damaged the photosynthetic machinery to the point that the plants could not recover under normal conditions. Developing sorghum hybrids that can overcome or tolerate this chilling stress more efficiently is crucial to advance sorghum production with regard to higher yield or increasing planting areas.

Phenological, reproductive growth, grain yield and quality

Limited work has been done on following the impacts of early-stage chilling stress on later agronomic traits such as flowering time and grain yield and quality. Quinby et al., (1973) hypothesized that early-stage chilling stress can delay time from emergence to flowering. This hypothesis is feasible in that with the slower early growth of the seedlings, the entire life cycle of the plant is delayed. Maulana and Tesso, (2013) found that with a short spell of chilling stress of 15/13°C day/night temperatures (10 days in length) could cause a delay on days to flower by 8 days. Kapanigowda et al., (2013) reported a 12 to 15 day delay in days to flower with early planting compared to regular. Chiluwal et al., (2018) has also documented a delay in days to flower with early planting in grain sorghum. A delay in flowering or maturity may not be a negative effect, as stated previously; a longer maturing genotype generally yields more grain than that of an early maturing genotype. With a longer vegetative growth period, the plants have the ability to accumulate more assimilates which can be converted into additional grain numbers or weight. Not only does the longer vegetative stage have benefits, a longer grain-filling period would too. It is believed that there is no genetic variance in grain-fill period among grain sorghum. This may be true across genotypes, but those genotypes could act different across environments where GxE interaction can play a significant role in manipulating the grain-fill duration. Even a small increase in grain fill has the possibility to lead to an increase in grain yield.

The longer a crop is in the field; higher is the likelihood of exposure to negative environmental factors. Along with the reproductive traits, extending observation until grain yield is very minimally studied while quantifying the impact of early-stage chilling stress. With the reduced number of plants due to the chilling damage on germination and emergence, it can be hypothesized that yield would be reduced. Chiluwal et al., (2018) reported that sorghum was able to maintain grain yield per plant, or at times recorded higher yield per plant but the reduced plant stand was not able to make up for total grain yield. On the contrary, a fewer number of surviving plants growing in the field after chilling stress is released, would allow for capturing additional resources (nutrients, water, light, etc.) due to lesser competition. To our knowledge, there has been no work done on the impact that early planting has on final grain quality in grain sorghum. Tremblay et al., (2006) and Kumar et al., (2006) found that oil content decreased with delayed planting dates and decreasing temperatures during maturity in soybeans. A study on sugar yield in sweet sorghum and planting date suggests that the earlier the planting, the greater the sugar yield (Almodares and Mostafafi Darany, 2006). These studies suggest that an earlier planting would influence grain quality and hence an interesting aspect to consider with earlier planting in sorghum.

Importance of hybrids for early planting

The goal of the United Sorghum Checkoff Program is to increase the average national yield from 62 to 100 bushels per acre by 2025 (https://www.sorghumcheckoff.com/newsroom/2016/03/28/sorghum-industry-establishes-coordinated-research-and-marketing-program/). To achieve this, genetic yield improvements as well as improving the agronomic traits related to drought, heat and chilling tolerance, herbicide and sugarcane aphid resistance are essential. Chilling tolerant sorghum hybrids could be used to take advantage of early season moisture, minimum tillage and a longer growing period. Adapted,

chilling-tolerant sorghum hybrids would increase competitiveness of sorghum in semi-arid cropping systems (Kapanigowda et al., 2013). Identification of new sources and hybrids for chilling tolerance with desirable agronomic and quality traits is of paramount importance to improve the overall productivity and acreage of grain sorghum.

New sources and hybrid development

The chilling tolerance traits are expected to stabilize and increase yield by establishing excellent crop stand and maintaining high plant density starting at the critical early season planting period (mid-April to first week of May) in the Great Plains of the United States. Variation for chilling tolerance were identified within sorghum germplasm (Yu and Tuinstra, 2001) and these sources are landraces that have evolved in the temperate regions of China (Soujeole and Miller, 1984; Nordquist, 1971; Singh, 1985; Lu and Dahlberg, 2001). These Chinese landraces are called ‘‘kaoliangs’’ and exhibit higher seedling emergence and improved seedling vigor under cool conditions compared to select US hybrids and elite inbred lines (Franks et al., 2006). However, most of these landraces are accompanied with poor or undesirable agronomic traits including high tannin content. Due to these practical constraints and challenges, research focusing on exploring and developing non-tannin chilling tolerant resources/inbred lines/hybrids with other desirable agronomic traits has been limited. Recently, many new sources for chilling tolerance in sorghum were integrated with elite breeding materials, resulting in advanced non-tannin breeding lines with desirable agronomic traits. These new inbred lines and hybrids were developed at Kansas State University, Agricultural Research Center, Hays, Kansas and are involved in the current combing ability and heterosis study for chilling tolerance.

Yu and Tuinstra, (2001) first studied combining ability and high parent heterosis for chilling tolerance related seedling traits in sorghum. The study suggested for developing vigorous

pollinators that contribute to enhanced heterotic seedling growth under chilling stress. Windpassinger et al., (2017) studied the *per se* performance, heterosis and combining ability for seedling traits like emergence, early shoot and root development under chilling stress exposure in sorghum. Schaffasz et al., (2019) focused on later stage reproductive chilling tolerance in sorghum with a conclusion that robust and efficient enhancement of reproductive chilling tolerance is feasible via hybrid breeding in sorghum. These limited studies on heterosis and combining ability warrants more detailed research focusing on seedling and agronomic traits for improving chilling tolerance in sorghum. This would allow developing potential parents and hybrids for early season planting in sorghum.

Opportunities and challenges

The increasing world population, demand for more food and frequent occurrences of heat and drought stress is becoming more of a reality each day. With the enhancement and implementation of early-stage chilling tolerant sorghum, the previously mentioned issues could be minimized. Chilling tolerance has the potential to increase the overall cultivated area as well as the grain yield and quality of sorghum. The increase of genetic variation among sorghum hybrids that are commercially available for producers along with improved agronomic practices, those higher yields can be ascertained. In the first study, identification of a promising grain sorghum hybrid with enhanced early-stage chilling tolerance that can be planted earlier in harsher chilling conditions without affecting final yield and grain quality was carried out. In the second study, were able to implement a research strategy on how chilling tolerance traits are carried on from inbred parental lines to hybrids to help in developing more chilling tolerant hybrids for future use. Further research focus on seed spacing and population studies as well as exact planting dates for certain

regions are needed to optimize good plant stand and to improve the chilling tolerance yield potential.

Chapter 2 - Quantifying the agronomic performance of new grain sorghum hybrids for enhanced early-stage chilling tolerance

Abstract

Enhancing chilling tolerance to attain uniform emergence with better seedling vigor with temperatures < 15°C will enable earlier planting of sorghum. Early-stage chilling tolerant sorghum has the potential to better utilize residual soil moisture and enhance yield through extended vegetative and grain-filling periods. This study comprised of 12 genotypes, including four new hybrids developed from different parental combinations (two A-lines and three R-lines), parents and three checks (two inbred lines - RTx430 and SQR; and one commercial hybrid). The above genotypes were phenotyped over two years under field conditions and in controlled environment chambers. An improvised phenotyping approach was devised for controlled environment chambers to better replicate temperature conditions prevailing under field conditions. Systematic testing resulted in enhanced early-stage seedling vigor with the improvised field-like treatment and better represented field-grown seedlings, compared to those maintained under constant chilling conditions. Averaged across genotypes, early planting resulted in longer duration to flower and accumulated a higher number of growing degree units (GDU) during grain filling. Our most promising tannin free hybrid (ARCH11192A/ARCH12012R) with enhanced chilling tolerance took 23 days (707 GDU) longer to reach flowering compared to regular planting, with the grain-filling period extended by three days (66 GDU). Reduction in grain protein and an increase in starch content with early planting indicated extended vegetative stage possibly providing additional carbon for the developing grains. In summary, developing tannin free early-stage chilling tolerant hybrids and optimizing an appropriate planting window would allow for an extended vegetative and grain-filling duration, paving the way to enhance grain sorghum productivity.

Introduction

Grain sorghum (*Sorghum bicolor* (L.) Moench) is a warm-season crop and is grown in arid and semi-arid regions of the world. Sorghum originated from semi-arid tropics of Africa (Smith and Frederiksen, 2000) and is known for its tolerance to drought and heat stress, which makes it an ideal crop to be grown in hot and arid regions of the world (Doggett, 1988; Blum, 2004; Pennisi, 2009). Sorghum is one of the major agricultural commodities that has a significant impact on the economy of the Great Plains of the USA, India, and African countries (Leff et al., 2004; Nagaraj et al., 2013; Hariprasanna and Rakshit, 2016). Due to sorghum's high water-use efficiency, ability to maintain productivity under low input levels, and suitability for cropping rotation in the US Great Plains region (Saballos, 2008), 76% of US grain sorghum area is in Kansas and Texas (USDA-NASS, 2019). In spite of sorghum's tolerance, extreme environmental conditions occurring during pre- and post-flowering phases were shown to cause significant yield losses (Assefa et al., 2010; Prasad et al., 2015; Tack et al., 2017). Introducing early-stage chilling tolerance and planting earlier under US Midwestern conditions can help reduce the cumulative impact of heat and drought stresses during the sorghum growing period (Chiluwal et al., 2018). Shifting to earlier planting of sorghum can have other benefits such as efficient utilization of spring residual soil moisture and early canopy cover for improved water conservation by reducing evaporation (Burow et al., 2011; Moghimi et al., 2019). However, due to sorghum's tropical adaptation, the crop is highly sensitive to chilling stress (Peacock, 1982; Rooney, 2004).

Sorghum in Kansas is currently planted during late May or early June since soil temperatures >18^{o}C are required for optimum seed germination and emergence (Stoffer and Riper, 1963; Chiluwal et al., 2018). Sorghum when planted early (soil temperatures <15^{o}C) is associated with challenges which include poor seedling emergence and seedling vigor which negatively

affects yield (Yu and Tuinstra, 2001; Cisse and Ejeta, 2003; Burow et al., 2011; Kapanigowda et al., 2013; Maulana and Tesso, 2013; Chiluwal et al., 2018). Developing sorghum hybrids that can maintain good plant stand with improved growth under early-stage chilling temperatures is critical to advance the growing period forward and to provide opportunities for expanding sorghum cultivation into more temperate and higher elevated regions of the US and the world. Using natural field conditions provides realistic conditions to test genetically diverse germplasm for early-stage chilling, but an integrated approach using controlled environment facilities and field-testing provides a more robust phenotyping approach (Chiluwal et al., 2018). However, a persistent challenge faced by the research community is to devise an acceptable methodology that can better connect the findings from controlled environments to field conditions (Poorter et al., 2016), which also applies to early-stage chilling response in sorghum. Recently, Chiluwal et al., (2018) proposed an approach to improve the relevance of controlled environment findings to field conditions by varying chamber temperature settings to replicate actual field conditions, which is further modified and systematically tested in this study.

It is well known that by producing F_1 hybrids, heterosis can be exploited to enhance the effectiveness of the traits of interest. Yu and Tuinstra, (2001) have reported that sorghum hybrids were generally more vigorous than that of the inbred parental lines, justifying the need for developing sorghum hybrids with enhanced early-stage chilling tolerance. In the US, all sorghum grain production is with hybrids, which further adds to the relevance of developing early-stage chilling tolerant hybrids. An additional challenge posed with improving chilling tolerance in sorghum is related to the tight linkage between tannins and chilling tolerance (Rooney et al., 2004). Tannins are known to reduce the protein digestibility of the grain, thereby lowering its value for human or animal feed (Wu et al., 2012; Proietti et al., 2015).

Many years of screening recently developed germplasm, both under controlled environments and the field conditions, allowed us to identify inbred lines that are early-stage chilling tolerant and some without tannins (Chiluwal et al., 2018). This progress presented a unique opportunity for developing chilling tolerant tannin free grain sorghum hybrids. Although extensive efforts have been invested in the past to capture early-stage chilling tolerance in many different inbred lines (Franks et al., 2006; Kapanigowda et al., 2013; Maulana and Tesso, 2013), diversity panels or mapping populations (Knoll et al., 2008; Knoll and Ejeta, 2008; Chopra et al., 2017; Ortiz et al., 2017; Moghimi et al., 2019), there has been no attempt to develop chilling tolerant hybrids by utilizing the diversity captured. Keeping these knowledge gaps in mind the objectives of this study were to (i) Identify sorghum hybrids with enhanced early-stage chilling stress tolerance with stable agronomic performance; (ii) Develop, test and establish an improvised phenotyping approach to better relate controlled environment chamber findings to field conditions and (iii) Quantify the degree of early-stage chilling stress tolerance of newly developed grain sorghum hybrids and the impact on flowering time, yield and quality characteristics.

Materials and Methods

Plant materials

Efforts over the past few years have helped to develop advanced breeding lines that were tested under field and controlled environment chamber conditions for tannin content and early-stage chilling tolerance at the Agriculture Research Center, Hays (ARCH) and their performance was revalidated for chilling response (Chiluwal et al., 2018). Using adapted-inbred lines KS116B, ARCH11192B, ARCH10747-1R, ARCH10747-2R, and ARCH12012R with promising levels of chilling stress tolerance, crosses were made at the winter nursery in Puerto Vallarta, Mexico to produce four unique hybrid combinations. A, B and R lines are used in sorghum hybrid development. The A-lines used in this study are cytoplasmic male sterile (CMS) and are crossed with an R-line, which restores the fertility of their offspring (F_1 hybrid). The B-lines are used as maintainer lines to increase the seed of the CMS A-lines and are genetically identical as the A-line besides the CMS. The hybrids developed included H1 (ARCH11192A/ARCH12012R), H2 (ARCH11192A/ARCH10747-2R), H3 (KS116A/ARCH10747-1R), and H4 (KS116A/ARCH10747-2R). These four hybrids along with their five parental lines, a well-known donor for early-stage chilling tolerance (SQR; Chiluwal et al., 2018 and references within), US elite chilling sensitive inbred line (RTx430; Chiluwal et al., 2018), and one commercial hybrid check (with known chilling tolerance), with a total of 12 genotypes were used to study early-stage chilling stress tolerance in the field and controlled environment chambers. The seed source for RTx430 in the 2018 field study was poor even under normal conditions and hence no data were collected on this line.

Crop Husbandry

Controlled environment chamber experiment

An experiment was conducted in controlled environment chambers (Conviron Model CMP6050, Manitoba, Canada) at the Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas, USA. The chambers used for this experiment were automated to control temperature, light, and relative humidity following the diurnal changes in temperature similar to field conditions.

The experiment was conducted using three different temperature treatments (20/10°C, 30/20°C day/night temperatures, as well as an hourly changing temperature setting [field-like]), using the same set of 12 genotypes mentioned in the plant materials. The first constant chilling treatment follows a standard approach of imposing chilling stress (20/10°C, day/night) in controlled environment chamber conditions (Chiluwal et al., 2018). However, the second field-like treatment is an improved approach wherein the temperature conditions were programmed to change dynamically (an hourly raise of 0.5°C after every three days) to represent the field conditions (see Figure 2.1), and the third treatment was used as a control (30/20°C, day/night). Air temperatures recorded over three years (2016-2018) from our chilling stress field experiments, including Chiluwal et al., (2018), were used as a reference for the field-like treatment. The temperatures were adjusted on an hourly basis throughout the 24 hours once every three days, based on the change estimated from the field conditions. A preliminary recommendation of this approach has been mentioned in our previous study (see Supplementary Table. 4 in Chiluwal et al., 2018), and the same was extensively tested in this experiment (visual illustration of the chamber settings are presented in Figure 2.1). The three treatments were provided with the same 12 h photoperiod (06:00 to 18:00 h), around 850 µmol $m^{-2}s^{-1}$ light intensity and 60% relative

humidity. The microclimatic conditions (both soil and air temperatures) were recorded at 15-minute intervals using soil temperature HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA) in each chamber. The soil data logger was placed at 5 cm depth in an independent pot without plants. The genotypes were sown in 1.6 L pots (24 cm length and 10 cm width; MT49 Mini-Treepot) filled with about 3.5 kg of a 2:1 proportion of sand and farm soil, respectively. After seedling emergence, each pot was fertilized with Osmocote, controlled-release fertilizer at 3 g per pot (14:14:14% N: P: K, respectively; Hummert International, Topeka, KS, USA). The genotypes were grown under the three temperature conditions mentioned above, with two independent chambers for each treatment to account for the chamber effect. Each chamber had three replicate pots for each of the genotypes and randomized in a Split Plot Design. The plants were grown until 30 days after seedling emergence, after which they were harvested for biomass and root analysis.

Field experiments (2018 and 2019)

A field experiment was conducted at the Kansas State University, Agricultural Research Center, Hays, Kansas, USA in 2018. The same 12 genotypes were planted on two different dates; April 16, which is considered as early planting and May 27 as regular planting based on current sorghum cultivation practices in Kansas. The early planting was conducted to impose early-stage chilling stress, while the regular planting was to have a comparative response with the same set of sorghum genotypes grown under optimal conditions. Each genotype was planted in a four-row plot and replicated twice for each planting. Each row was 3.6 m long, accommodated 48 seeds and with an inter-row spacing of 0.75 m. The seeding depth was ~3.8 cm. Each experiment followed a Randomized Complete Block Design.

The maximum/minimum soil temperature at 5 cm depth for each planting date was recorded at 15-minute intervals using soil temperature HOBO data loggers. The air temperature at 15-minute intervals was obtained from the weather station located on the Agricultural Research Center (https://mesonet.k-state.edu/). The same set of 12 genotypes was grown in 2019 similar to 2018 following two planting dates and management strategies. In both 2018 and 2019, the temporal soil and air temperatures from the time of planting until sampling (~30 DAE, days after planting) are presented in Figure 2.2 and the averages for this period are provided in Table 2.1.

Observations

Above and below ground plant parameters were recorded from both field experiments in 2018 and 2019 including (i) seedling emergence percentage and emergence index, (ii) seedling vigor (per plant - shoot and root biomass, total root length, root surface area), (iii) growth and yield parameters (days to 50% flowering, individual plant yield, two-hundred kernel weight and plot yield), (iv) seed quality parameters (starch content, protein content, tannin presence, average kernel hardness index, average kernel diameter and average kernel weight).

Seedling emergence

Number of seedlings that emerged were counted temporally and used to measure the seedling emergence index and the final emergence counts were used to determine the emergence percentage. In both the field experiments, emergence was documented on a Monday-Wednesday-Friday schedule, starting from planting until no further change was obtained with seedling emergence percentage. In the chamber experiment, the emergence was documented on a daily basis. Emergence percentage and emergence index were calculated using the following equations described by Yu et al., (2004):

$$Emergence\ Percent\ (EP) = \left(\frac{TSE}{TSP}\right) \text{ x } 100\%$$

$$Emergence\ Index\ (EI)\ = \sum \frac{(n_i\ \times \text{DAP})}{TSE}$$

Where TSE; total seedlings emerged, TSP; total seeds planted, n_i; number of emerged seedlings on ith days after planting (DAP).

Seedling vigor

In the chamber experiment, three seeds of each genotype were planted in a triangular pattern and the location of each seed was recorded so that each plant could be followed. The date of emergence of each plant was recorded, so the exact 30-day biomass could be harvested for each of the emerged seedlings. Once emergence was completed, the pots in both control and chilling stress were thinned down to one plant to overcome competition within pots. In the field experiments, the biomass (above and belowground) was harvested, on average 30 days after 50% emergence in the early planting and just the aboveground biomass with the regular planting. Shoot and root dry weights were recorded after oven drying at 60 °C after a constant weight was achieved.

Root morphology

In both the field (early planting only) and chamber experiments (field-like and constant stress treatments only), individual plants were harvested and the roots were separated from the shoot and stored in 20% ethanol after cleaning thoroughly. Roots were floated in 6 mm of water in a 0.5 x 0.2 m glass tray and were separated and untangled using plastic forceps to minimize any root overlap. The roots were scanned with an Epson Perfection 7000 scanner at a resolution of 600 dots per inch and images were analyzed using WinRhizo Pro 2009C software (Regent Instruments, Inc., Québec, QC, Canada). Obtaining whole roots under regular planting with huge aboveground

biomass was not reliable under field conditions (Chiluwal et al., 2018). Besides, the difference in root biomass between the regular and early-planting conditions is always incomparably large and less meaningful. Hence, detailed rooting characteristics were obtained only under chilling conditions and compared between genotypes.

Yield and yield related parameters

In both the field experiments, days to 50% flowering was measured on a plot average basis. In the 2018 field experiment, after 100% flowering had occurred, twenty panicles in the middle two rows from each plot in each replication were covered with a mesh bag to prevent any bird damage. In the 2019 field experiment, a bird deterrent system (Guardian 2 Single Rotary Propane Cannon) was used to prevent bird damage. At physiological maturity, twenty panicles were harvested to estimate yield. To account for a broad range in the plant stand, the obtained twenty-panicle grain weight was normalized by multiplying the yield with plant stand, using the following formula:

$$Yield = \frac{\left(\left(\frac{TGW}{H_n}\right) TSE)\right)}{PA}$$

Where TGW; total grain weight (g), H_n; number of heads harvested, TSE; total seedlings emerged, PA; plot area (m^2).

Grain quality parameters

Grain protein and starch content were estimated using near infrared (NIR) spectroscopy method, as described in Peiris et al. (2019). Physical grain traits, including average kernel hardness, kernel diameter and kernel weight were determined using the single kernel characterization system (SKCS) as described in Bean et al., (2006), with each of the three replicates

having 100 kernels. The presence of tannins was determined using the "bleach test" as described in Dykes, (2019).

Statistical analysis

Both the field experiments followed a Randomized Complete Block Design with two factors and two replications. In the chambers, the experimental layout was a Split-Plot with the Randomized Complete Block Design, considering temperature treatment as the main plot and genotypes as the subplot. Genotypes and planting date/temperature were considered a fixed effect while replication and replication x planting date/temperature were considered as random effects. An ANOVA was performed for seedling emergence and vigor, physiological, biomass, yield and yield-related and quality parameters to estimate the significance of treatment (planting date in field experiments), genotype, and their interaction across experiments using RStudio 3.6.1 (https://rstudio.com/). Library (dplyr) was used to perform Tukey's test. Means were separated using the least significant difference (LSD), when treatments and interactions were significant at P ≤0.05. To compare the phenology differences between genotypes, growing degree units (GDU) was calculated using the following formula:

$$GDU = \left(\frac{Daily\ maximum\ air\ temperature + \ Daily\ minimum\ air\ temperature}{2}\right) - 10$$

Results

Soil and air temperature

Soil and air temperatures were different with field experiments in years 2018 and 2019 (Table 2.1, Figure 2.2). Although planted at the same time of the year, the air temperature with early plantings was highly contrasting for the first 25 days of field experiments between the two years (Figure 2.2). Overall, the 2019 early planting treatment was significantly ($P<0.05$) cooler than that of 2018 early planting with both soil and air temperature, averaged over the duration from planting until sampling.

Day-time maximum air and soil temperatures in the chamber constant stress (20/10°C) treatment was cooler than the early planting in both 2018 and 2019 field experiments. However, the field-like chamber treatment was more consistent with the daytime maximum soil and air temperatures with the field studies with early planting. The nighttime minimum soil temperature in both chilling induced treatments (20/10^{o} and field-like) in the chamber experiment were significantly cooler than the nighttime temperatures of both field experiments with early planting but was opposite with air temperature (Table 2.1). On average, air temperature of the constant (20/10°C) treatment in the chambers was higher than the field-like treatment until emergence (Figure 2.1). However, a gradual increase in air temperature by 0.5°C once every three days with the field-like treatment (Figure 2.1) followed a day/night temperature pattern similar to early plantings under field conditions (Figure 2.2).

Seedling emergence percentage and index

Emergence percentage and emergence index were both affected significantly by genotype and treatment across all experiments except for the 2018 field experiment where the emergence index was not affected significantly by genotype (Table 2.2 and Table 2.3). The interaction of

treatment by genotype for emergence percent was only significant in the 2019 field and chamber constant stress treatments, while for emergence index the interaction was significant in the 2019 field and field-like chamber stress treatments (Table 2.3).

Emergence percentage across both field experiments with the early planting treatment ranged from 12% to 71% and ranged between 57 (in field-like) and 97% (in constant) in the chamber stress treatments. In the 2018 field experiment, the commercial hybrid emergence percentage was significantly greater than KS116B, while the performance of all other genotypes were statistically the same. In 2019, all hybrids were significantly better than KS116B and RTx430 in the stress treatment while the commercial hybrid significantly outperformed all parental lines (except ARCH10747-2R) and checks. In the chamber study, all 12 genotypes responded similarly to chilling conditions under field-like treatments (Table 2.2).

Emergence index ranged between 11 and 25 days in the field experiments. On average, in 2018, it took about 8 days longer for the seedlings to emerge compared to 2019 early planting due to the difference in early season chilling conditions, as shown in Figure 2.2. Emergence index ranged from 12 to 17 days in the chamber stress treatments. The field-like treatment on average, took roughly 7 days longer to emerge than the constant stress treatment, as the temperatures were significantly lower than the constant temperature at the start (Figure 2.2). In the 2019 field experiment, H2 emerged significantly quicker than ARCH12012R and ARCH10747-2R in the stress treatment. Both H4 and the commercial hybrid emerged significantly earlier than KS116B and RTx430 in the field-like chamber treatment (Table 2.3).

Seedling shoot biomass

In the field experiments, seedling shoot biomass was significantly affected by planting dates. However, the genotype difference was only significant in 2018 field experiment (Table 2.4),

with no significant interaction across both years. On average, shoot biomass across genotypes was reduced by 67% in 2018 and 94% in 2019 with early planting over regular planting. In the 2019 early planting, the overall shoot biomass was significantly lower (90%) than in 2018 due to the severe chilling stress during early growth (Figure 2.2). Shoot biomass ranged from 1 to 4 g with H2, H3 and the commercial hybrid having significantly higher shoot biomass than KS116B and ARCH10747-1R in the 2018 early planting (Table 2.4).

Under controlled chamber experiments, shoot dry weight was significantly affected by treatment, genotype and their interaction. A significant reduction in the shoot biomass was observed in the constant temperature treatment (94%) followed by field-like (87%) compared with control. The shoot biomass in the field-like treatment ranged from 0.07 to 0.2 g, wherein H3 and the commercial hybrid accumulated significantly more shoot biomass than inbred line RTx430, while H3 was also significantly greater than KS116B. Within the constant stress, only H2 was significantly greater than KS116B for shoot biomass (Table 2.4).

Seedling root morphology

Seedling root biomass was significantly affected by treatment, genotype and their interaction for both stress treatments in a controlled chamber experiment, with constant temperature on average inducing 84% reduction in root biomass over control (Table 2.5). H3 and H4 accumulated a significantly higher amount of root biomass than that of RTx430, while H3 outperformed KS116B in the field-like chamber treatment. The constant stress treatment was similar, except that H2 and H4 had significantly more root biomass than RTx430 (Table 2.5). The root biomass of H3 was significantly higher than that of KS116B and ARCH10747-2R from the 2018 field early planting treatment (Table 2.5).

Total root length ranged from 235 to 697 cm in the 2018 field stress treatment while the range was 55 to 139 cm in the 2019 field stress treatment. In 2018 stress treatment, H1 had significantly greater total root length than KS116B, but all genotypes did not differ significantly in 2019 (Table 2.6). Within the chamber stress treatments, H3, H4 and the commercial hybrid all had significantly longer total root length than that of KS116B and ARCH10747-1R in the field-like treatment. While in the constant stress treatment, H2 significantly outperformed RTx430 in total root length (Table 2.6). With root surface area, there were no significant changes among genotypes in the field experiments and constant stress treatment in the controlled environment chambers. The field-like treatment in the chambers ranged from 21 to 47 cm^2, wherein H3 significantly outperformed H1 as well as KS116B (Table 2.7).

Yield and maturity parameters

In the field experiment, GDU to flowering was significantly affected by genotype, treatment, and their interaction in 2019 (Figure 2.3), while only genotype was significant in 2018 (Table 2.8). On average, GDU for flowering across genotypes was extended by 182.3 GDUs in 2019 under early planting compared to regular planting (Figure 2.3). In 2018, H4 and the commercial hybrid flowered at a significantly earlier than KS116B, while the commercial hybrid also flowered significantly earlier than ARCH11192B (Table 2.8). In 2019, all hybrids flowered significantly earlier than RTx430, while H2, H4, and the commercial hybrid flowered significantly earlier than KS116B (Figure 2.3A). Grain-filling duration in 2019 was affected significantly by genotype and genotype and treatment interaction. Grain filling duration of H2, H3, H4, commercial hybrid, ARCH12012R and ARCH10747-2R significantly increased with early planting compared to regular planting (Figure 2.3B).

Two hundred seed weight was affected significantly by the genotype in both years but not treatment and their interaction (Table 2.9). The commercial hybrid had a significantly higher two hundred seed weight than all other genotypes in 2018 under early planting except for H3 and SQR, while in 2019 RTx430 had the significantly highest two hundred seed weight.

Grain weight per panicle and plot yield were both significantly affected by genotype and genotype by treatment interaction in both years, but not with the treatment (Figure 2.4). Grain weight per panicle ranged from 15 to 65 g in both years of early planting. In 2018, H1, commercial hybrid, ARCH11192B, KS116B, and ARCH10747-1R yielded significantly more grain per panicle in the early planting treatment than the regular planting. In 2019, H1, H2 and SQR yielded significantly more grain per panicle in the early planting treatment compared to the regular planting. Overall plot yield ranged from 52 to 965 g/m^2 across both years in early planting treatments. In 2018, H1 and the commercial hybrid yielded significantly more in the early planting, while H1, H2 and SQR yielded more in the early planting compared to the regular in 2019 (Figure 2.4)

Grain quality parameters

Grain protein content was significantly affected by genotype and treatment by genotype interaction in both 2018 and 2019 field experiments (Figure 2.5). A greater reduction in grain protein content was recorded in 2019 early planting, with an average decrease of 16% across genotypes compared to the regular planting (Fig 2.55B). In 2018, KS116B had the highest protein content compared to all other genotypes. In 2019, KS116B and RTx430 had the highest amount of protein content and was substantially greater than all other genotypes with early planting (Figure 2.5B).

Grain starch content was significantly affected by genotype in both years, while the treatment by genotype interaction was significant only in 2018 (Fig 2.5). In the 2018 stress treatment, the commercial hybrid had a significantly higher amount of starch content compared to all the other genotypes except ARCH10747-1R (Figure 2.5C). Similarly, in 2019, the commercial hybrid had the highest amount of starch content compared to all other genotypes, although not varying significantly (Figure 2.5D).

Concerning tannin presence, ARCH10747-2R is the only parental line that contains tannins; hence, any hybrid developed using ARCH10747-2R as a male parent (H2 and H4) subsequently displayed presence of tannins. SQR is the only other inbred line or check included in this study that contains tannins, presented in Table 2.10.

Grain physical characteristics

Average kernel hardness, diameter and weight were all significantly affected by genotype and treatment by genotype interaction across both years (Tables 2.11, 2.12 and 2.13).

In both 2018 and 2019, KS116B had the significantly highest average kernel hardness index (i.e. harder kernels) compared to all other genotypes, including hybrids, parents and checks, while H3 had the hardest kernels among the hybrids (Table 2.11).

The commercial hybrid had significantly larger average kernel diameter compared to all other hybrids and parents, except ARCH10747-1R and SQR in the 2018 early planting. In 2019, RTx430 had the significantly largest average kernel diameter compared to all other genotypes, except the commercial hybrid, with seeds obtained from early-planted plants. (Table 2.12). Single kernel weight followed similar trends as that of kernel diameter. In 2018 and 2019, the commercial hybrid and RTx430 had significantly higher kernel weight than all other genotypes, respectively (Table 2.13).

Discussion

Enhancing early-stage chilling tolerance in grain sorghum has been identified as an important breeding target for improving sorghum productivity in the US Great Plains (Knoll et al., 2008; Knoll and Ejeta, 2008; Fernandez et al., 2015; Chiluwal et al., 2018; Moghimi et al., 2019) and other parts of the world facing a similar challenges (Bekele et al., 2014; Zegada-Lizarazu et al., 2016). Planting early-stage chilling tolerant grain sorghum is hypothesized to provide flexibility in the planting window, reduce water loss due to quicker ground cover and extend grain-filling duration (Moghimi et al., 2019), and potentially increase productivity. Testing some of these hypotheses have not been possible due to the lack of hybrids developed specifically with enhanced early-stage chilling tolerance. For the first time, hybrids were developed utilizing promising chilling-tolerant inbred lines identified by Chiluwal et al., (2018) and were tested under field and controlled environment conditions to investigate and address the knowledge gaps highlighted in the introduction section.

A disconnect with findings between controlled environment chambers and field conditions can be attributed to the changing environmental conditions over time in the field compared to the constant conditions maintained throughout the experimental period in the chambers. A potential improvement with phenotyping was recommended by Chiluwal et al., (2018), i.e., to establish similar field-based dynamic changing conditions in the chambers to obtain findings that could be better applicable to the field. This study investigated this systematically by imposing a gradual increase in temperature in chambers replicating the field conditions and demonstrated that key chilling traits such as the shoot, root and total biomass, which defines overall seedling vigor, was more closely related to the field conditions (Figure 2.6). The visuals presented in Figure 2.6 were supported by a higher seedling biomass under field-like conditions (average ranging from 0.12 g

to 0.14 g for hybrids) and were closer to the field experiments (average between 1.2 g and 1.9 g for hybrids [averaged across both years]) than with the constant chilling conditions (average between 0.05 g and 0.07 g for hybrids). Hence, integrating constant chilling conditions in chambers during the very early stage and shifting to field-like settings after a week past emergence appears to be an appropriate phenotyping method compared to the constant chilling conditions throughout (Figure 2.2). By following this improved approach, researchers would be in a position to better capture both tolerance (under constant chilling) and recovery (shifting to field-like conditions), as indicated by Chiluwal et al., (2018). Although extensive attention has been paid by the scientific community on quantifying the impact of stress *per se* across crops, incorporating a higher recovery rate following a stress event needs more emphasis. This has been recently demonstrated in rice exposed to combined drought and heat stress (Lawas et al., 2019). Likewise, recovery responses have been used as a key trait under temperature stress in *Pisum sativum* (Srikanthbabu et al., 2002), *Arabidopsis thaliana* (Larkindale and Vierling, 2008), *Gossypium hirsutum* (Kheir et al., 2012), *Helianthus annuus* (Senthil-Kumar et al., 2003) and *Triticum aestivum* (Wang et al., 2012).

Maintaining comparable plant stand under early planting similar to regular planting is an important consideration to avoid deriving confounding conclusions. For example, almost all field-based studies dealing with early-stage chilling stress tolerance have presented findings on a per plant basis (Yu and Tuinstra, 2001; Knoll et al., 2008; Chiluwal et al., 2018). However, with a significantly lower overall plant stand under chilling conditions (Knoll and Ejeta, 2008), the per plant comparison will exemplify the yield performance of a few plants that survived under chilling conditions, which would have lesser competition for resources including sunlight and nutrients (Craine and Dybzinski, 2013; Villalobos et al., 2016). This has been highlighted in sorghum grown under chilling stress (Chiluwal et al., 2018), limited water conditions (Blum, 1970), and extreme

heat stress in rice (Bahuguna et al., 2015). Hence, studies dealing with extreme stresses, where the discrepancy in plant population is inevitable, expressing yield per plot and normalizing the yield to plant stand is considered to minimize the artifacts and facilitate realistic comparison across studies.

On the other hand, the aim of developing chilling-tolerant sorghum hybrids is to plant early, achieve earlier flowering and harvest early to reduce the additional period that the crop is in the field, which can increase the chances of damage or yield loss due to uncontrolled events. On the other hand, an extended vegetative growth could accumulate additional assimilates and better support the extended grain-filling period. In sorghum, previous reports have all concluded that the grain-filling period is relatively fixed with a very narrow opportunity for extending under US Midwest growing conditions (Quinby, 1972). These conclusions have been drawn by using diverse genetic material but were all planted in the regular planting window. However, the earlier planting of sorghum did extend the vegetative period as anticipated but also had an extended grain-filling duration (Fig 2.3b), indicating that earlier planted sorghum provides an opportunity to extend grain fill and thereby enhance productivity. Interestingly, grains from early planted inbred lines and hybrids (Figure 2.5 B and D from 2019; 2018 seeds were withered due to rains at grain fill) had a significantly lower protein content but a non-significant increase in grain starch content, which can be attributed to additional carbon assimilated during the extended vegetative stage.

Selection of sorghum hybrids with longer grain-filling period coupled with an increase in grain number per panicle would provide opportunities for enhancing productivity in the US Midwest. Interestingly, our most promising tannin free hybrid (ARCH11192A/ARCH12012R) with enhanced early-stage chilling tolerance, with early planting recorded 23 additional days (707 GDU) to reach flowering compared to regular planting and extended the grain-filling period by 3

days (66 GDU). This finding reiterates the need for optimizing an appropriate earlier planting window for the chilling tolerant hybrids wherein the reduction in plant stand is minimized to leverage the advantage highlighted here i.e., extended vegetative and grain-filling duration. Such a scenario would help provide a solution to an otherwise narrow planting window for sorghum, and at the same time, break the notion of limited opportunity to extend grain-filling duration in sorghum, with both complementing to enhance productivity (McMaster et al., 2016). However, this aspect cannot be addressed from our current data set, as that would require multiple plantings within the range used in this study to identify the appropriate temperature/week of the year. As mentioned above, an extended grain-filling duration also resulted in an increase in the grain weight per panicle (Figure 2.4A and B) and plot yield (Figure 2.4C and D) in the most promising hybrid (ARCH11192A/ARCH12012R). This supports the rationale for extending the vegetative period to provide additional assimilates for meeting the demand from the extended grain-filling period.

The seedlings that survive after exposure to severe chilling stress essentially have a significantly higher recovery rate and attain comparable levels of growth with regular planting. This observation is supported by aerial phenotyping (NDVI values), wherein the differences in chilling response was captured between 30 and 60 days following emergence but did not differ during the later growth stages (Chiluwal et al., 2018). A functional aspect of the chilling tolerant response in hybrids may be due to the heterotic vigor but can partly be due to the physiological priming that enhances the robustness of the plant. Priming due to early-stage chilling stress can possibly enhance the plants ability to address subsequent stresses during the life cycle of a crop. A number of studies have used the priming approach to demonstrate the increased ability of plants to protect from oxidative damage during subsequent exposure of the same stress or different stresses (reviewed in Shi et al., 2016; Kerchev et al., 2019). Hence, with sorghum grown under the

harshest summers from planting until harvest in the US (Tack et al., 2017) and elsewhere (Zegada-Lizarazu et al., 2016; Windpassinger et al., 2017), there will be multiple waves of heat and drought stress exposures during its crop cycle. Therefore, the acquired tolerance during the early-stage chilling stress exposure could prime the tolerant hybrids and equip them with robust reactive oxygen scavenging mechanisms to help tackle subsequent heat and drought waves that occur during the crop cycle. The extent to which the early chilling can equip the plant to take on other stresses under varying field conditions is an interesting hypothesis that would require further systematic investigation.

Conclusion

In conclusion, we have been successful in developing a tannin free early-stage chilling tolerant sorghum hybrid (ARCH11192A/ARCH12012R) that took a longer duration to reach flowering compared to normal but had significantly longer grain-filling period. We tested and recommended an improved chilling stress phenotyping method in chambers, with constant chilling stress to start with and switching to field-like variable conditions to obtain findings that can be better related to the field conditions. Our findings indicate that the long perceived notion of a lack of diversity in grain-filling duration in sorghum can be partially addressed by optimizing an appropriate window for earlier planting of the newly developed or other chilling tolerant hybrids. Extended vegetative stage, grain-filling period and the potential early-stage chilling stress priming presents a pragmatic framework to help enhance sorghum productivity and expand sorghum production into areas where sorghum is currently not grown.

Table 2.1 Planting, emergence and sampling dates and daily average soil and air temperature from the field and controlled environment chamber studies in 2018 and 2019.

	Planting	Emergence	Sampling	Soil temperature (°C)		Air temperature (°C)	
				Max	Min	Max	Min
Field 2018							
Planting I	16-Apr-18	6-May-18	4-Jun-18	25.4 ± 6.9	15.4 ± 5.0	26.1 ± 6.7	9.5 ± 6.3
Planting II	26-May-18	30-May-18	2-Jul-18	32.8 ± 3.1	22.1 ± 2.5	33.2 ± 3.8	16.9 ± 3.4
Field 2019							
Planting I	17-Apr-19	1-May-19	7-Jun-19	23.0 ± 5.6	14.4 ± 4.2	22.9 ± 6.3	8.8 ± 5.0
Planting II	5-Jun-19	12-Jun-19	9-Jul-19	31.1 ± 3.2	21.7 ± 3.1	30.4 ± 4.3	15.8 ± 3.5
Controlled environment chamber							
20/10°C	11-Jan-19	24-Jan-19	23-Feb-19	21.8 ± 0.2	10.1 ± 0.1	21.1 ± 0.3	10.1 ± 0.1
Field-like	11-Jan-19	27-Jan-19	26-Feb-19	24.9 ± 2.9	11.7 ± 2.6	24.9 ± 3.0	11.5 ± 2.6
30/20°C	11-Jan-19	16-Jan-19	15-Feb-19	30.8 ± 0.6	19.4 ± 0.2	31.1 ± 0.3	19.6 ± 0.3

Data presented is the average daily maximum and minimum temperatures (°C ± standard deviation) during early seedling growth (from planting until sampling).

Table 2.2 Emergence percentage of sorghum hybrids, parents and checks in field and chamber experiments.

Pedigree	Genotype	Emergence (%)						
		Field 2018		Field 2019		Chamber		
		Regular	Early	Regular	Early	Control	Field-like	Constant
Hybrids								
ARCH11192A/ARCH12012R	H1	58.9a	52.1ab	63.0abc	50.5ab	96.7a	83.3a	86.7ab
ARCH11192A/ARCH10747-2R	H2	47.9a	38.0ab	51.3abcde	55.0ab	83.3ab	73.3a	86.7ab
KS116A/ARCH10747-1R	H3	53.1a	53.1ab	74.2ab	47.1ab	90.0ab	66.7a	80.0ab
KS116A/ARCH10747-2R	H4	62.5a	54.2ab	77.3ab	54.9ab	93.3a	73.3a	96.7a
Commercial Hybrid	Check	76.6a	71.4a	85.9a	69.3a	100.0a	90.0a	90.0ab
Parents/checks								
ARCH11192B	Parent	66.7a	52.6ab	29.7cde	38.5bc	80.0ab	70.0a	83.3b
KS116B	Parent	50.5a	11.5b	37.0bcde	13.3c	96.7a	63.3a	63.3b
ARCH12012R	Parent	49.5a	25.0ab	63.5abc	31.3bc	90.0ab	56.7a	90.0ab
ARCH10747-1R	Parent	50.5a	24.0ab	16.7de	34.6bc	83.3ab	66.7a	66.7b
ARCH10747-2R	Parent	59.9a	37.0ab	52.1abcd	56.5ab	96.7a	80.0a	73.3ab
SQR	Check	50.5a	44.8ab	8.1e	27.6bc	63.3b	56.7a	60.0b
RTx430	Check	-	-	41.9bcde	17.2c	80.0ab	90.0a	96.7a
	Treatment (T)	< 0.05		< 0.05			< 0.01	< 0.05
	Genotype (G)	< 0.001		< 0.001			< 0.001	< 0.001
	T x G	NS		< 0.01			NS	< 0.01

Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within a respective treatment. "-": data was not collected on this line.

Table 2.3 Emergence index of sorghum hybrids, parents and checks in field and chamber conditions.

Pedigree	Genotype	Emergence index (days)						
		Field 2018		Field 2019		Chamber		
		Regular	Early	Regular	Early	Control	Field-like	Constant
Hybrids								
ARCH11192A/ARCH12012R	H1	7.9a	23.3a	8.1a	15.8ab	4.8a	16.3abc	11.6a
ARCH11192A/ARCH10747-2R	H2	8.0a	19.7a	7.8a	11.9a	5.2a	14.4abc	11.6a
KS116A/ARCH10747-1R	H3	7.9a	19.2a	7.7a	12.8ab	4.6a	15.6abc	12.0a
KS116A/ARCH10747-2R	H4	8.1a	18.7a	7.1a	13.1ab	4.4a	13.9a	11.8a
Commercial Hybrid	Check	7.2a	20.0a	7.3a	15.1ab	4.6a	13.9a	12.1a
Parents/checks								
ARCH11192B	Parent	7.2a	17.7a	7.2a	10.8a	5.5a	14.1ab	13.1a
KS116B	Parent	7.4a	23.4a	7.6a	10.6a	5.6a	16.9c	13.3a
ARCH12012R	Parent	8.2a	21.4a	7.6a	24.5c	4.9a	15.4abc	13.1a
ARCH10747-1R	Parent	7.4a	20.0a	7.4a	15.7ab	5.1a	14.1ab	12.4a
ARCH10747-2R	Parent	8.2a	21.8a	7.9a	19.6bc	4.9a	15.2abc	12.2a
SQR	Check	8.2a	20.4a	8.1a	11.9a	5.9a	13.9a	12.9a
RTx430	Check	-	-	8.0a	16.5ab	4.8a	16.6bc	13.6a
	Treatment (T)	< 0.05		< 0.05			< 0.001	< 0.001
	Genotype (G)	NS		< 0.001			< 0.001	< 0.001
	T x G	NS		< 0.001			< 0.001	NS

Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line.

Table 2.4 Shoot biomass of sorghum hybrids, parents and checks in field and chamber experiments.

Pedigree	Genotype	Shoot biomass (g)						
		Field 2018		Field 2019		Chamber		
		Regular	Early	Regular	Early	Control	Field-like	Constant
Hybrids								
ARCH11192A/ARCH12012R	H1	8.9ab	2.6ab	4.5a	0.28a	0.93abc	0.12abc	0.06ab
ARCH11192A/ARCH10747-2R	H2	11.4a	3.8a	5.4a	0.36a	1.17ab	0.11abc	0.08a
KS116A/ARCH10747-1R	H3	9.7ab	3.8a	5.5a	0.46a	1.40a	0.18a	0.07ab
KS116A/ARCH10747-2R	H4	6.5ab	2.6ab	6.5a	0.47a	1.15ab	0.13abc	0.06ab
Commercial Hybrid	Check	11.4a	4.3a	5.1a	0.50a	1.00abc	0.17ab	0.07ab
Parents/checks								
ARCH11192B	Parent	7.0ab	2.3ab	4.9a	0.30a	0.63bc	0.13abc	0.05ab
KS116B	Parent	5.1b	1.1b	4.3a	0.17a	0.59c	0.08bc	0.04b
ARCH12012R	Parent	7.7ab	2.6ab	2.2a	0.23a	0.85abc	0.12abc	0.05ab
ARCH10747-1R	Parent	6.4ab	1.4b	2.8a	0.23a	0.92abc	0.15ab	0.05ab
ARCH10747-2R	Parent	10.5ab	2.8ab	4.8a	0.13a	0.88abc	0.10abc	0.05ab
SQR	Check	8.3ab	3.3ab	4.4a	0.18a	1.40a	0.18a	0.05ab
RTx430	Check	-	-	2.4a	0.16a	0.53c	0.07c	0.05ab
	Treatment (T)	< 0.05		< 0.01			< 0.01	< 0.01
	Genotype (G)	< 0.001		NS			< 0.001	< 0.001
	T x G	NS		NS			< 0.001	< 0.001

Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within a respective treatment. "-": data was not collected on this line.

Table 2.5 Root biomass of sorghum hybrids, parents and checks in field and chamber conditions.

Pedigree	Genotype	Root biomass (g)				
		Field 2018	Field 2019	Chamber		
		Early	Early	Control	Field-like	Constant
Hybrids						
ARCH11192A/ARCH12012R	H1	0.34ab	0.047a	0.31abcd	0.070abc	0.054abc
ARCH11192A/ARCH10747-2R	H2	0.41ab	0.069a	0.49ab	0.059abc	0.075a
KS116A/ARCH10747-1R	H3	0.50a	0.084a	0.53a	0.094a	0.065abc
KS116A/ARCH10747-2R	H4	0.40ab	0.077a	0.38abcd	0.089ab	0.068ab
Commercial Hybrid	Check	0.39ab	0.072a	0.31abcd	0.080abc	0.062abc
Parents/checks						
ARCH11192B	Parent	0.25ab	0.055a	0.24cd	0.067abc	0.040bc
KS116B	Parent	0.16b	0.033a	0.20cd	0.049bc	0.047abc
ARCH12012R	Parent	0.22ab	0.036a	0.25bcd	0.065abc	0.045abc
ARCH10747-1R	Parent	0.21ab	0.039a	0.30abcd	0.068abc	0.039bc
ARCH10747-2R	Parent	0.34b	0.018a	0.28bcd	0.055abc	0.044abc
SQR	Check	0.29ab	0.027a	0.45abc	0.083abc	0.037bc
RTx430	Check	-	0.030a	0.20d	0.046c	0.034c
	Treatment (T)				< 0.01	< 0.01
	Genotype (G)				< 0.001	< 0.001
	T x G				< 0.001	< 0.001

Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Entries with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line.

Table 2.6 Total root length of sorghum hybrids, parents and checks in field and chamber conditions.

Pedigree	Genotype	Total root length (cm)			
		Field 2018	Field 2019	Chamber	
		Early	Early	Field-like	Constant
Hybrids					
ARCH11192A/ARCH12012R	H1	696.8a	104.9a	287.5ab	258.2ab
ARCH11192A/ARCH10747-2R	H2	611.8ab	128.5a	462.5ab	318.3a
KS116A/ARCH10747-1R	H3	515.8ab	120.0a	500.3a	266.7ab
KS116A/ARCH10747-2R	H4	526.3ab	100.6a	478.1a	253.4ab
Commercial Hybrid	Check	581.9ab	138.5a	470.9a	261.5ab
Parents/checks					
ARCH11192B	Parent	440.0ab	105.1a	401.8ab	198.5ab
KS116B	Parent	234.5b	66.0a	214.4b	166.1ab
ARCH12012R	Parent	358.8ab	82.6a	360.0ab	238.5ab
ARCH10747-1R	Parent	285.1ab	93.1a	372.9b	182.1ab
ARCH10747-2R	Parent	364.0ab	55.4a	306.0ab	213.8ab
SQR	Check	558.6ab	70.1a	431.7ab	169.6ab
RTx430	Check	-	72.6a	258.6ab	122.5b

Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line.

Table 2.7 Root surface area of sorghum hybrids, parents and checks in field and chamber conditions.

Pedigree	Genotype	Root surface area (cm²)			
		Field 2018	Field 2019	Chamber	
		Early	Early	Field-like	Constant
Hybrids					
ARCH11192A/ARCH12012R	H1	108.3a	14.8a	20.9b	23.8a
ARCH11192A/ARCH10747-2R	H2	113.1a	19.3a	35.2ab	27.1a
KS116A/ARCH10747-1R	H3	105.3a	22.5a	46.8a	26.7a
KS116A/ARCH10747-2R	H4	94.9a	22.0a	43.8ab	25.7a
Commercial Hybrid	Check	109.8a	20.8a	41.1ab	23.6a
Parents/checks					
ARCH11192B	Parent	79.2a	17.3a	36.0ab	16.0a
KS116B	Parent	45.1a	10.7a	21.5b	17.3a
ARCH12012R	Parent	65.7a	12.0a	30.0ab	24.6a
ARCH10747-1R	Parent	48.8a	13.1a	34.6ab	16.3a
ARCH10747-2R	Parent	77.4a	6.9a	27.0ab	18.7a
SQR	Check	94.6a	9.1a	39.9ab	14.9a
RTx430	Check	-	12.5a	22.6b	12.7a

Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "- ": data was not collected on this line.

Table 2.8 Accumulated growing degree units from planting until 50% flowering of sorghum hybrids, parents and checks in 2018 field conditions.

Pedigree	Genotype	Flowering (GDU)	
		2018 Field	
		Control	Stress
Hybrids			
ARCH11192A/ARCH12012R	H1	1795.0bc	1863.6abc
ARCH11192A/ARCH10747-2R	H2	1665.0cd	1685.8abc
KS116A/ARCH10747-1R	H3	1796.2bc	1685.3abc
KS116A/ARCH10747-2R	H4	1744.6bc	1645.2bc
Commercial Hybrid	Check	1544.0d	1533.9c
Parents/checks			
ARCH11192B	Parent	1900.1ab	1937.7ab
KS116B	Parent	2057.6a	2057.6a
ARCH12012R	Parent	1819.1bc	1834.5abc
ARCH10747-1R	Parent	1795.0bc	1834.5abc
ARCH10747-2R	Parent	1665.0cd	1632.1bc
SQR	Check	1795.0bc	1720.2abc
RTx430	Check	-	-
	Treatment (T)	NS	
	Genotype (G)	< 0.001	
	T x G	NS	

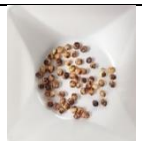
Significance at < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line**.**

Table 2.9 Two hundred seed weight of sorghum hybrids, parents and checks in field conditions.

Pedigree	Genotype	200 seed weight (g)			
		Field 2018		Field 2019	
		Regular	Early	Regular	Early
Hybrids					
ARCH11192A/ARCH12012R	H1	4.25ab	4.27bc	3.95a	3.77cde
ARCH11192A/ARCH10747-2R	H2	5.22ab	4.60bc	4.08a	4.15bcd
KS116A/ARCH10747-1R	H3	4.95ab	5.08abc	3.77a	4.33bc
KS116A/ARCH10747-2R	H4	4.93ab	4.32bc	3.63a	4.38bc
Commercial Hybrid	Check	5.97a	6.03a	4.30a	4.68c
Parents/checks					
ARCH11192B	Parent	3.98b	4.22bc	3.95a	3.58de
KS116B	Parent	3.77b	3.87c	2.70a	3.37e
ARCH12012R	Parent	4.77ab	4.17bc	3.72a	4.03bcd
ARCH10747-1R	Parent	4.22ab	4.60bc	4.30a	3.73cde
ARCH10747-2R	Parent	4.73ab	4.03bc	3.60a	3.85cde
SQR	Check	4.65ab	5.17ab	4.13a	4.30bc
RTx430	Check	-	-	4.35a	5.53a
	Treatment (T)	NS		NS	
	Genotype (G)	< 0.001		< 0.001	
	T x G	NS		NS	

Significance ranged between < 0.05 to < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line.

Table 2.10 Grain tannin content of hybrids, parents and checks.

Pedigree	Tannin	Before bleach test	After bleach test
(H1) ARCH11192A/ARCH12012R	-		
(H2) ARCH11192A/ARCH10747-2R	+		
(H3) KS116A/ARCH10747-1R	-		
(H4) KS116A/ARCH10747-2R	+		
Commercial Hybrid	-		
ARCH11192B	-		
KS116B	-		
ARCH12012R	-		
ARCH10747-1R	-		
ARCH10747-2R	+		

SQR	+		

A genotype with a “+” symbol (black after bleach test) in the tannin column illustrates that the genotype contains tannins while a “-“ symbol illustrates that the genotype does not contain tannins.

Table 2.11 Single kernel hardness index of sorghum hybrids, parents and checks in field conditions.

Pedigree	Genotype	Single kernel hardness index			
		Field 2018		Field 2019	
		Regular	Early	Regular	Early
Hybrids					
ARCH11192A/ARCH12012R	H1	63.8cd	68.5bc	76.7a	80.5cd
ARCH11192A/ARCH10747-2R	H2	49.9e	58.8c	72.7a	68.4f
KS116A/ARCH10747-1R	H3	76.3ab	77.5b	90.5a	88.3b
KS116A/ARCH10747-2R	H4	63.9cd	68.6bc	68.1a	73.5def
Commercial Hybrid	Check	64.3cd	61.3c	77.0a	77.5cde
Parents/checks					
ARCH11192B	Parent	67.4bc	63.6c	81.3a	78.8cdef
KS116B	Parent	82.3a	89.4a	76.3a	106.5a
ARCH12012R	Parent	59.9cd	61.1c	80.2a	69.2f
ARCH10747-1R	Parent	63.9cd	61.1c	77.7a	75.0cdef
ARCH10747-2R	Parent	56.3de	62.5c	98.8a	72.1ef
SQR	Check	63.1cd	66.6bc	73.3a	77.2cde
RTx430	Check	-	-	95.0a	81.4bc
	Treatment (T)	NS		NS	
	Genotype (G)	< 0.001		< 0.01	
	T x G	< 0.05		< 0.01	

Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line.

Table 2.12 Single kernel diameter of sorghum hybrids, parents and checks in field conditions.

Pedigree	Genotype	Single kernel diameter (mm)			
		Field 2018		Field 2019	
		Regular	Early	Regular	Early
Hybrids					
ARCH11192A/ARCH12012R	H1	2.89bcd	2.92cd	2.54abc	2.37bcde
ARCH11192A/ARCH10747-2R	H2	3.18ab	3.11bc	2.58abc	2.49bcd
KS116A/ARCH10747-1R	H3	2.79cd	2.99bcd	2.28bc	2.27de
KS116A/ARCH10747-2R	H4	2.92bcd	2.84d	2.57abc	2.41bcde
Commercial Hybrid	Check	3.29a	3.35a	2.61ab	2.58ab
Parents/checks					
ARCH11192B	Parent	2.83cd	2.98bcd	2.30bc	2.31cde
KS116B	Parent	2.68d	2.80d	2.44abc	2.20e
ARCH12012R	Parent	3.18ab	3.05bc	2.53abc	2.52bc
ARCH10747-1R	Parent	2.89bcd	3.16abc	2.71a	2.33cde
ARCH10747-2R	Parent	3.04abc	3.06bc	2.23c	2.42bcde
SQR	Check	3.01abc	3.17ab	2.52abc	2.47bcd
RTx430	Check	-	-	2.33bc	2.79a
	Treatment (T)	NS		NS	
	Genotype (G)	< 0.001		< 0.001	
	T x G	< 0.01		< 0.001	

Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line.

Table 2.13 Single kernel weight of sorghum hybrids, parents and checks in field experiments.

Pedigree	Genotype	Single kernel weight (mg)			
		Field 2018		Field 2019	
		Regular	Early	Regular	Early
Hybrids					
ARCH11192A/ARCH12012R	H1	22.1bc	23.3cd	24.4a	20.2de
ARCH11192A/ARCH10747-2R	H2	26.9ab	26.2bc	25.0a	23.2bcd
KS116A/ARCH10747-1R	H3	24.3abc	27.4b	22.5a	21.7bcde
KS116A/ARCH10747-2R	H4	25.5abc	24.8bcd	23.8a	25.4ab
Commercial Hybrid	Check	30.2a	31.7a	26.2a	24.9abc
Parents/checks					
ARCH11192B	Parent	22.3bc	23.6cd	22.3a	20.5cde
KS116B	Parent	19.7c	21.5d	23.0a	18.5e
ARCH12012R	Parent	24.0abc	22.4d	21.3a	22.5bcde
ARCH10747-1R	Parent	22.4bc	27.5b	28.6a	21.0bcde
ARCH10747-2R	Parent	23.8abc	24.6bcd	20.0a	20.1de
SQR	Check	23.5abc	27.4b	23.0a	22.5bcde
RTx430	Check	-	-	20.8a	29.2a
	Treatment (T)	NS		< 0.05	
	Genotype (G)	< 0.001		< 0.01	
	T x G	< 0.05		< 0.01	

Significance ranged between < 0.05 and < 0.001 probability level; NS, non-significant. Genotypes with different letters are significantly different at P = 0.05 within respective treatment. "-": data was not collected on this line.

Time	Light	Days after planting																	
		0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51
0:00	0	10	10	11	11	12	12	13	14	14	15	15	16	17	17	18	18	19	20
1:00	0	9	10	10	11	11	12	12	13	14	14	15	15	16	17	17	18	18	19
2:00	0	9	9	10	10	11	12	12	13	13	14	15	15	16	16	17	18	18	19
3:00	0	8	9	9	10	11	11	12	12	13	13	14	15	15	16	16	17	18	18
4:00	0	8	9	9	10	10	11	12	12	13	13	14	14	15	16	16	17	17	18
5:00	0	8	8	9	10	10	11	11	12	12	13	14	14	15	15	16	16	17	18
6:00	400	7	8	8	9	10	10	11	11	12	12	13	13	14	15	15	16	16	17
7:00	425	7	8	8	9	10	10	11	12	12	13	14	14	15	16	16	17	18	18
8:00	520	9	9	10	11	11	12	13	13	14	15	15	16	17	17	18	19	19	20
9:00	630	10	11	12	12	13	14	14	15	16	16	17	18	18	19	20	20	21	22
10:00	630	12	13	13	14	15	15	16	17	17	18	19	19	20	21	21	22	23	23
11:00	630	14	14	15	16	16	17	18	19	19	20	21	21	22	23	23	24	25	25
12:00	630	15	16	17	17	18	19	20	20	21	22	22	23	24	24	25	26	26	27
13:00	630	17	18	18	19	20	20	21	22	22	23	24	24	25	26	26	27	28	28
14:00	630	18	19	19	20	21	21	22	23	23	24	25	25	26	27	27	28	29	29
15:00	520	19	20	21	21	22	23	23	24	24	25	26	26	27	28	28	29	30	30
16:00	425	20	20	21	22	22	23	24	24	25	26	26	27	28	28	29	30	30	31
17:00	400	20	20	21	21	22	23	23	24	25	25	26	27	27	28	29	29	30	30
18:00	0	19	20	20	21	22	22	23	24	24	25	25	26	27	27	28	29	29	30
19:00	0	18	18	19	19	20	21	21	22	23	23	24	24	25	26	26	27	28	28
20:00	0	16	16	17	17	18	19	19	20	21	21	22	22	23	24	24	25	26	26
21:00	0	14	14	15	15	16	16	17	17	18	19	19	20	20	21	21	22	23	23
22:00	0	13	13	14	14	15	15	16	17	17	18	18	19	20	20	21	21	22	23
23:00	0	12	12	13	13	14	15	15	16	16	17	18	18	19	19	20	21	21	22
	Minimum	7	8	8	9	10	10	11	11	12	12	13	13	14	15	15	16	16	17
	Maximum	20	20	21	22	22	23	24	24	25	26	26	27	28	28	29	30	30	31
	Mean	13	13	14	15	15	16	17	17	18	19	19	20	20	21	22	22	23	23

Figure 2.1 Temperature (°C) settings in the field-like chamber treatment conditions.

Temperature was set at an hourly increment and then changed accordingly on a three-day basis. The temperature settings and the changes were based on the average air temperature obtained from three years of early planting field trials in Hays, Kansas. Light settings were held constant throughout the experiment. Light level at different times of the day was measured in µmol/m^2/second.

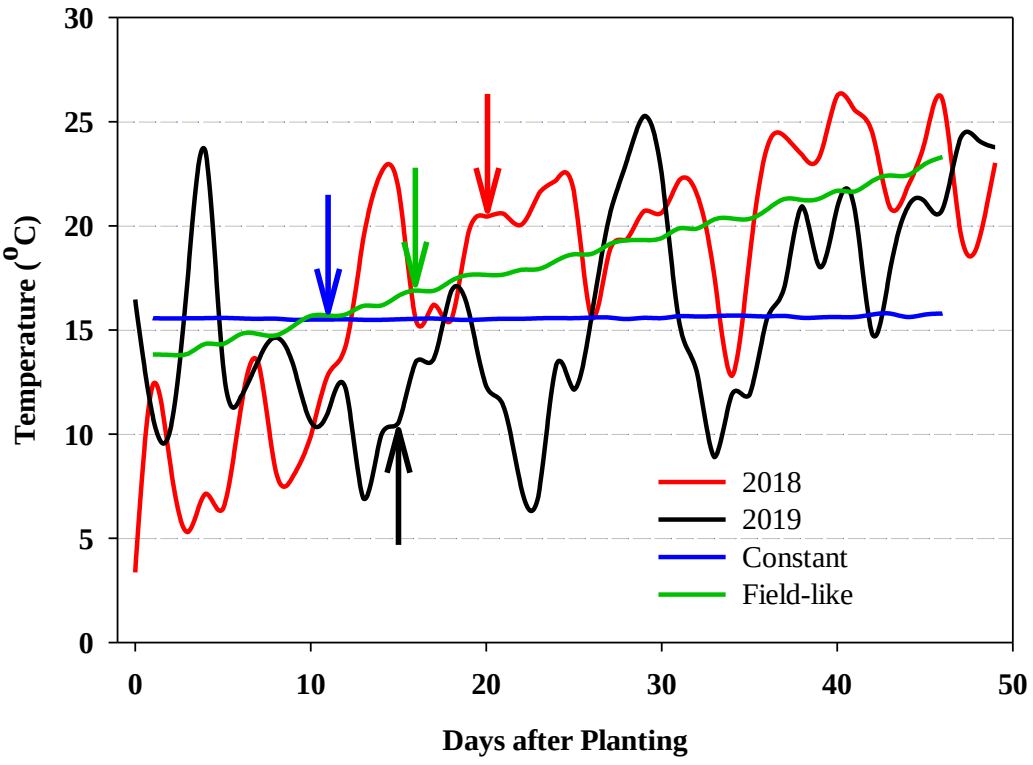


Figure 2.2 Average daily air temperature in field and chamber conditions during stress period (0 to 50 days after planting).

2018 and 2019 lines are from field conditions, while constant and field-like lines are from controlled environment chamber conditions. Arrows represent the average emergence timing of entries in each treatment.

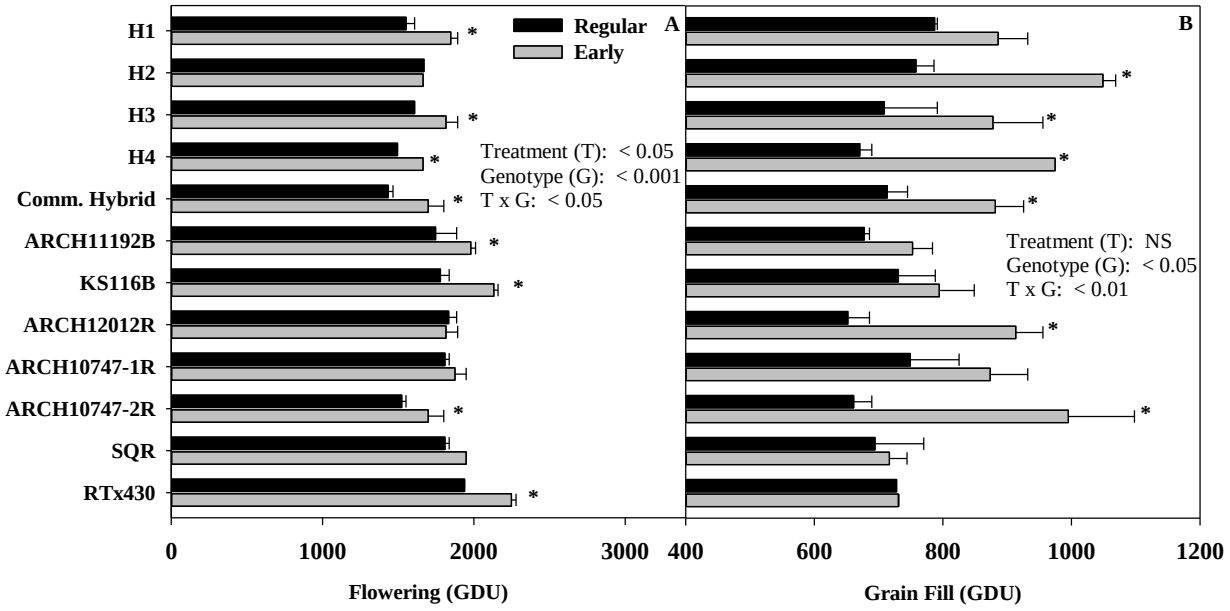


Figure 2.3 Growing degree units (GDU) for flowering (A) and grain fill (B) in early and regular planting of the 2019 field experiment.

A - Accumulated growing degree units from planting until 50% flowering in early and regular planting. B - Accumulated growing degree units required from 50% flowering until black layer formation or reaching physiological maturity. Significant ranged between < 0.05 to < 0.001 probability level; NS, non-significant. Entries with an asterisks (*) are significantly different between treatments at P = 0.05.

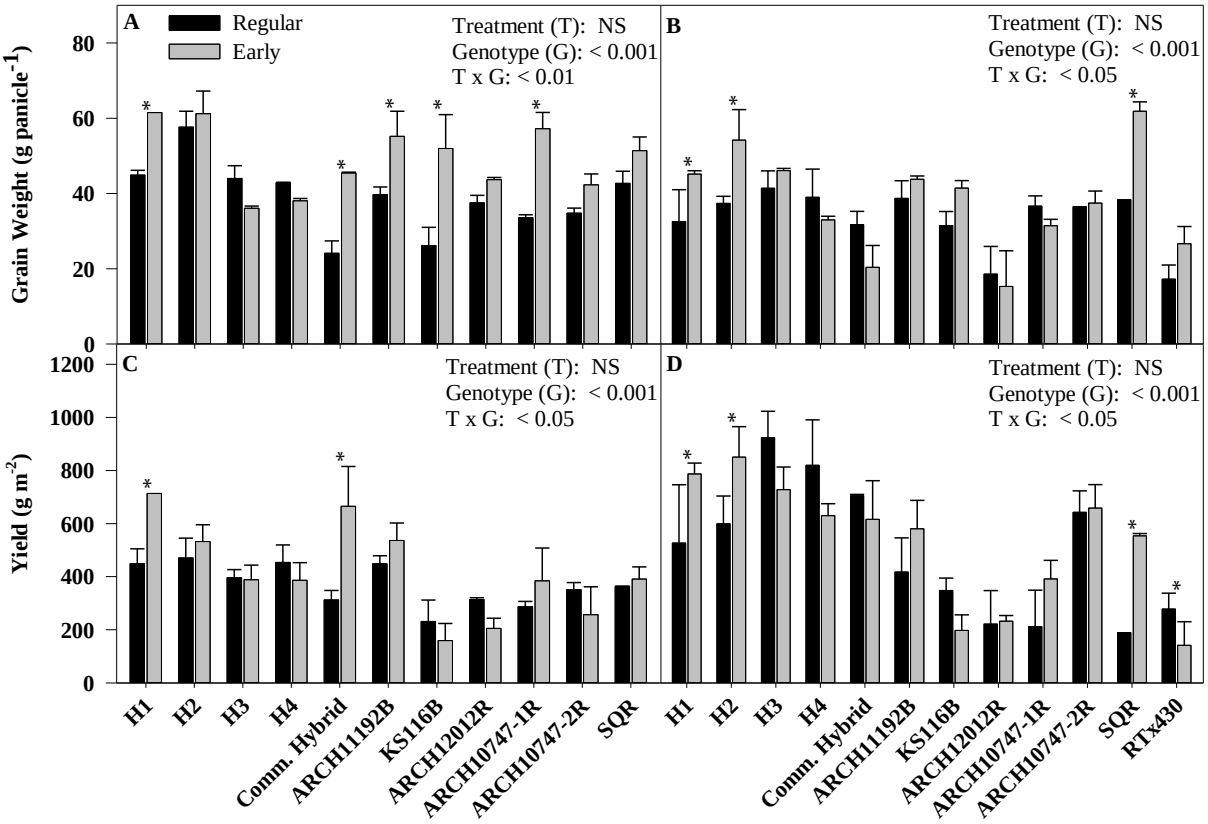


Figure 2.4 Yield parameters in early and regular planting in 2018 (A and C) and 2019 (B and D) under field conditions.

A and B – grain weight per panicle, C and D – yield. Significant ranged between < 0.05 to < 0.001 probability level; NS, non-significant. Entries with an asterisks (*) are significantly different between treatments at P = 0.05.

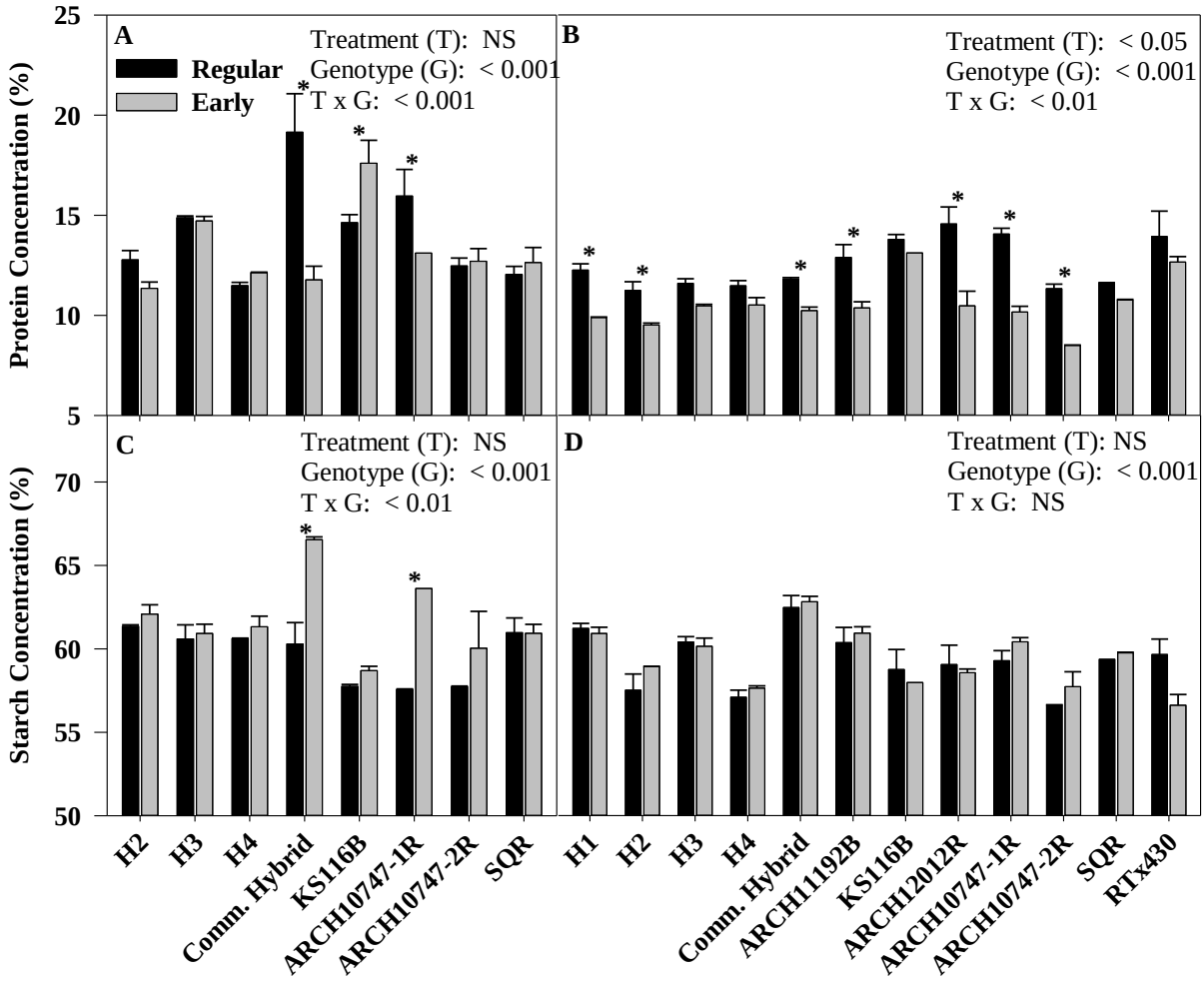
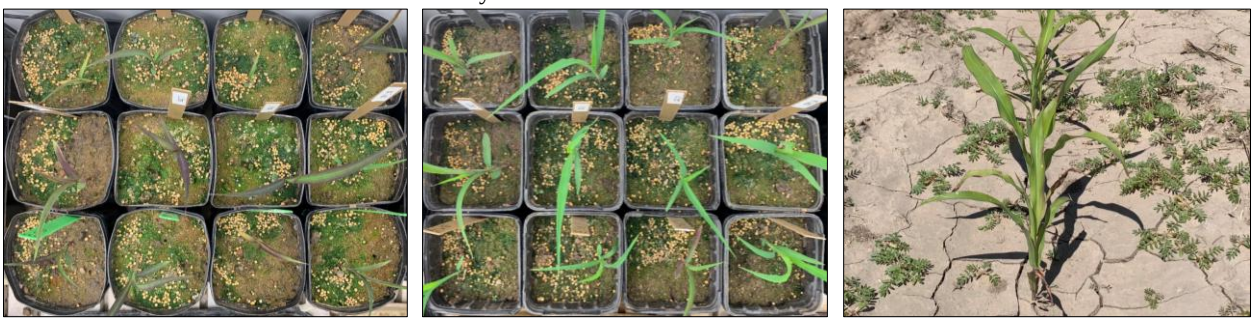


Figure 2.5 Protein and starch concentration among genotypes in early and regular planting in 2018 (A and C) and 2019 (B and D) under field conditions.

A and B – protein content, C and D – starch content. Grains from entries H1, ARCH11192A and ARCH12012R were too wethered to analyze with NIR and therefore left out of the figure. Significance ranged between < 0.05 to < 0.001 probability level; NS, non-significant. Entries with an asterisks (*) are significantly different between treatments at P = 0.05.

Chamber Study 2019 Field



A - Constant stress treatment B - Field-like stress treatment C - Early planting stress treatment

Figure 2.6 Visual images of seedlings grown under constant chilling (A – 39 days after planting), field-like settings in the growth chambers (B – 39 days after planting) and the actual field (0 – 51 days after planting).

Chapter 3 - Heterosis and combining ability analysis over environments on chilling tolerance in grain sorghum (*Sorghum bicolor* L. Moench)

Abstract

Early planting has the potential to help extend the grain sorghum growing [*Sorghum bicolor* (L.) Moench] season into the higher latitude temperate regions. However, average minimum soil temperatures of <15°C and average minimum air temperature of <10°C adversely affects germination, emergence and early seedling growth. Hybrids form the backbone of sorghum production in the US. Therefore, it is important to understand the inheritance of seedling and agronomic traits related to chilling tolerance and to select the most appropriate parental lines and hybrids with superior heterotic combinations for early planting. Field experiments with early and regular plantings were conducted in 2018 and 2019 at the Kansas State University, Agricultural Research Center, Hays, Kansas, USA. Another study took place at the Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas, USA within controlled environment chambers with two treatments including chilling stress and control conditions. The studies involved twelve cytoplasmic male sterile (CMS) seed parental line (A-Lines), four male pollinator lines (R-lines) as well as 27 newly developed hybrids and one know chilling tolerant check (SQR – Shan Qui Red). The above genotypes were used to estimate general and specific combining ability (GCA, SCA) as well as better-parent heterosis (BPH). Significant differences were observed among parents, hybrids and their interactions for most of the key chilling tolerant traits in both early and regular plantings. Multiple parental lines indicated potential evidence of desirable gene flow of additive genes to their offspring at high intensity as well as higher heritability with less environmental effects and gene interactions. Hybrid ARCH11201A/ARCH10747-1R proved to have high *per se* performance, favorable SCA and heterosis estimates as well had at least one parent with high GCA in multiple traits crucial for chilling tolerance.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is inherently a drought and heat tolerant crop that is water-use efficient and a low input cereal (Doggett, 1988; Saballos 2008). However, most of the available sorghum lines and hybrids under cultivation are vulnerable to chilling stress at early-season planting (April to May) in many growing areas of the US sorghum belt (Burow et al., 2011). Specifically, sorghum's stand establishment and seedling vigor are adversely affected by soil and air temperatures during germination and emergence (< 15°C) and early seedling growth (< 20°C) (Yu and Tuinstra, 2001; Upadhyaysa et al., 2016). Heterotrophic growth (i.e. germination and growth relying mainly on seed reserves) and autotrophic growth (i.e. photosynthesis-based growth after the exhaustion of seed reserves) appear to require different minimum limiting temperatures, which may be attributed to different genetic control (Brandolini et al., 2000; Bhosale et al., 2007). In addition, poor chilling tolerance reduces early-season growth, lowers biomass and limits this crop to more semi-tropical regions across the globe (Peacock and Heinrich, 1982; Knoll et al., 2008; Saballos, 2008; Burow et al., 2011). However, it is advantageous to avail spring moisture, minimum tillage, and a longer growing period following early planting. With these challenges in mind, sorghum with early stage chilling tolerance is expected to stabilize and increase yield by establishing significant crop stand and maintaining high plant density starting at the critical planting period (Maulana and Tesso, 2013; Kapanigowda et al., 2013).

Natural variation and chilling tolerance in sorghum are identified in the worldwide germplasm collection (Upadhyaya et al., 2009; Salas-Fernandez et al., 2014). Chinese kaoliangs, such as Shan Qui Red (SQR), are the identified potential chilling tolerant parental sources but with poor or undesirable agronomic traits including high tannin content (Knoll and Ejeta, 2008). Recently, many new sources of chilling tolerance in sorghum have been integrated with elite

breeding materials, resulting in advanced non-tannin breeding lines with adaptable traits. These new inbred lines and hybrids developed at Kansas State University, Agricultural Research Center, Hays, Kansas are used in the current study. However, due to the polygenic complex nature, the results from field screening are highly unpredictable for the traits related to chilling tolerance. The presence of genotype × environment (GxE) interactions also complicates the inheritance of these traits and warrants multi-environments testing for reliable results (Leon-Valesco et al., 2009; Kapanigowda et al., 2013). A parallel growth chamber screening validates for early field planting as a controlled selection method or at least as a preliminary test to discriminate between weak and vigorous lines before spring planting (Kapanigowda et al., 2013). Hence, the current study on seedling and other agronomic traits was aimed to evaluate parents and hybrids under controlled and field environments over two years following early and regular plantings. The study would help to understand the underlying genetic mechanisms and discovery of genes affecting the chilling tolerance to develop elite parents and hybrids for earlier planting.

It is known that heterotic vigor improves the overall grain yield due to cross breeding between two diverse inbred lines (Shull, 1948). Yu and Tuinstra, (2001) reported that sorghum hybrids were generally more vigorous than that of the inbred parents, justifying the need for developing sorghum hybrids with enhanced early-stage chilling tolerance. The exploitation of heterosis can be used in early-stage chilling tolerance, as the tolerance could be amplified to overcome the chilling damage or reduced seedling growth. General combining ability (GCA) was described by Falconer, (1989) as the mean performance of a genotype when crossed with a series of other genotypes. Research focus on GCA helps to select genetically diverse parents to maximize heterosis rather than on selection among lines based on their *per se* performance. The performance of a cross can deviate from the average general combining ability of two parents due to genetic

effects that are specific to that cross and this deviation is referred to as specific combining ability (SCA) (Bernardo, 2014). Combining ability studies provide useful information regarding the selection of suitable parents for effective hybridization programs and indicate the nature and magnitude of various types of gene action involved in the expression of quantitative characters (Bernardo, 2014).

Earlier studies on chilling tolerance established positive associations between grain yield and the maturity period in sorghum. These findings would help to breed early and extra-early maturing non-tannin hybrids that can compromise yield potential under chilling stress (Franks et al., 2006; Maulana and Tesso, 2013). However, earlier studies in sorghum were mainly focused on inbred lines and association or mapping panels and resulted in identifying several quantitative trait loci (QTL) for early-stage chilling tolerance (Knoll et al., 2008; Burow et al., 2011; Fiedler et al. 2012; Bekele et al., 2014; Marla et al., 2019). Yu and Tuinstra, (2001) and Tiryaki and Andrews, (2001) confirmed heterosis and combining ability for chilling tolerance on seedling traits and provided the first valuable insights regarding their inheritance. The study suggested for developing vigorous pollinators that contribute to a heterotic seedling growth under low temperature chilling stress. Windpassinger et al., (2017) studied the *per se*, heterosis and combining ability for sorghum seedling traits, like emergence, early shoot development, and root development, in chilling conditions. Schaffasz et al., (2019) focused on later stage reproductive chilling tolerance in sorghum and concluded that robust and efficient enhancement of reproductive chilling tolerance is feasible via hybrid breeding. Very limited combining ability studies in corn were reported earlier on the hybrids under chilling stress environments (Revilla et al., 2003; Wijewardana et al., 2015). These limited classical studies on heterosis and combining ability warrants more detailed research to focus on seedling and agronomic traits improvement for chilling tolerance in sorghum.

Most importantly, hybrid varieties are representing the main source of sorghum acreage and production in the Great Plains of the United States (Daly et al., 2012). Therefore, it is important to understand the inheritance of traits to select the most appropriate parents for heterotic vigor to develop high yielding hybrids under chilling stress environments. This critical research would be beneficial for sorghum cultivation in Kansas and its extension into northern regions of the United States. Our hypothesis is that with the development of hybrids, early season chilling stress will decrease the emergence and seedling vigor but can help overcome the stress quicker than their inbred parents do. Therefore, the objectives of this study are i) to assess the combining ability of parents and hybrids, ii) to determine the nature and magnitude of gene actions, and iii) to estimate heterosis for seedling, agronomic, yield, and yield component traits for chilling tolerance in sorghum hybrids.

Materials and Methods

Genetic materials

Twelve inbred sorghum lines consisting of eight cytoplasmic male sterile (CMS) seed parental female (A-lines) lines and four advanced breeding restorer male (R-lines) tester parents were used to develop 27 new F_1 hybrids. The eight female lines were ARCH11070A (L1), ARCH11192A (L2), ARCH11201A (L3), ATx645 (L4), KS116A (L5), KS133A (L6), KS136A (L7), and Redbine58A (L8).The four male testers are ARCH10747-1R (T1), ARCH10747-2R (T2), ARCH12012R (T3), and KS115R (T4). Shan Qui Red, a potential chilling tolerant source, was included as check line. The parental ARCH (Agricultural Research Center, Hays) lines used in this study were developed for chilling tolerance over many years following population improvement and pedigree breeding methods. The original parents involved in the development of these ARCH lines are SC35 and 803B, PI574599, PI574560, PI 574562, DLO357B and one genetic male-sterile (GMS) line KP8B (Chiluwal et al., 2018). All the developed advanced ARCH lines were with desirable agronomic traits (short, early, semi-compact with complete panicle exertion). The developed ARCH line sources were all tannin-free except for ARCH10747-2R. ATx645 is a released line from Texas possessing pre-flowering drought tolerance and a slight degree of stay-green with lodging resistance that combines well with many pollinators (personal communication from Dr. William Rooney). KS116A is an early, white-seeded, tan plant type with resistance to biotype I Greenbug (Kofoid and Harvey, 2005). KS133A and KS136A are early, white seeded, tan plant and stay green (Perumal et al., 2015). Including Redbine58A, all seed parental lines combine well with many pollinators and are involved in the ongoing breeding program for test hybrids evaluation at the Agricultural Research Center, Hays Kansas. KS115R

pollinator is a durra-caudatum type released from Kansas State University and known for its large seeded and yellow-endosperm seed qualities (Tuinstra et al., 2001).

Controlled environment experiment

The 27 hybrids and 13 parents, including SQR, were evaluated in controlled environment chambers (Conviron Model CMP6050, Manitoba, Canada) at the Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas, USA. The chambers were automated to control temperature, light, and relative humidity following the diurnal changes in temperature similar to field conditions. The experiment was conducted using two different temperature treatments (20/10°C [chilling stress] and 30/20°C [optimal control] day/night temperatures). The two treatments were provided with the same 12 h photoperiod (06:00 to 18:00 h), around 600 µmol $m^{-2}s^{-1}$ light intensity and 60% relative humidity. The microclimatic conditions (air temperatures) were recorded at 15-minute intervals using HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA) in each chamber. The genotypes were sown in plastic trays (51 cm in length, 36 cm in width and 8 cm in depth) filled with a 2:1 proportion of sand and farm soil, respectively, to closely match the field soil texture. The genotypes were grown under the two temperature conditions mentioned above, with two independent chambers for the chilling stress treatment to account for chamber effect and one chamber for the control. Each genotype was planted in a single row consisting of 25 seeds at a 3.5 cm depth. The genotypes were randomized in a Split Plot Design.

Hybrid development

All eight cytoplasmic male-sterile lines (L1 to L8) were used as females and crossed to each of the four restorer male-fertile testers (T1 to T4) to produce 27 F_1 hybrids. Sufficient hybrid seeds in five crosses (L3/T2, L4/T1, L4/T2, L4/T3 and L8/T4) could not be obtained due to poor

seed set. Hybrid seeds of 27 hybrids and all parents were developed at Puerto Vallarta, Mexico in the 2017 winter nursery program.

Experimental layout

A total of 40 genotypes (27 hybrids, 12 parents and SQR) were evaluated in field trials in 2018 and 2019 growing season at the Kansas State University, Agricultural Research Center, Hays, Kansas, USA (lat. 38.9798N, long. 99.3268W; 611 m in elevation). The genotypes were planted on two different dates; April 16, which is considered as early planting, and May 27, as regular planting based on current sorghum cultivation practices in Kansas. The early planting was conducted to impose early-stage chilling stress, while the regular planting was to have a comparative response with the same set of genotypes grown under optimal conditions. The same set of genotypes were grown in 2019, similar to 2018, following two planting dates (April 17 and June 5) and management strategies. Each genotype was planted in a four-row plot with two replications for each planting. Each row was 3.6 m long and accommodated 48 seeds with an inter-row spacing of 0.75 m. The seeding depth was ~3.8 cm with a seed spacing of 7.6 cm. Each experiment followed a randomized complete block design. The maximum/minimum soil temperature at 5 cm depth for each planting date was recorded at 15-minute intervals using HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA). The air temperature at 15-minute intervals was obtained from the weather station located on the Agricultural Research Center station (https://mesonet.k-state.edu/).

Observations

Seedling traits, including (i) seedling emergence percentage (ii) emergence index, were recorded from the chamber and field experiments. Whereas, traits including (iii) seedling vigor (shoot dry weight), (iv) growing degree units (GDU) to 50% flowering, (v) plant height, (vi) two-

hundred kernel weight, and (vii) plot yield were all observed in the field experiments. It should be noted that *per se* is the mean phenotypic performance of a given genotype.

Seedling traits

Number of seedlings that emerged were counted temporally on a Monday-Wednesday-Friday schedule in the field and on a daily basis in the controlled environment chambers, and were used to measure the seedling emergence index. The final emergence counts were used to determine the emergence percentage. In the chamber experiment, the emergence was documented on a daily basis from planting until no further change was recorded. In the field experiments, emergence was documented on a Monday-Wednesday-Friday schedule, starting from planting until no further change was obtained. Emergence percentage and emergence index were calculated using the following equations described by Yu et al., (2004):

$$Emergence\ Percent\ (EP) = \left(\frac{TSE}{TSP}\right)100$$

$$Emergence\ Index\ (EI)\ = \sum \frac{(n_i\ \times DAP_i)}{TSE}$$

Where total seedlings emerged (*TSE*), total seeds planted (*TSP*), and number of emerged seedlings on *i*th day after planting (n_i).

In the field experiments, the seedling biomass was harvested on average 30 days after 50% emergence had occurred. The above ground shoot dry weight was recorded after oven drying at 60°C and constant weight was achieved.

Agronomic traits

In field experiments, growing degree units with 50% flowering was measured on a plot average basis. Plant height (cm) was estimated at time of physiological maturity by measuring from the ground to the tip of the panicle. In the 2018 field experiment, after 100% flowering had

occurred, twenty main tiller panicles in the middle two rows from each plot in each replication were covered with a mesh bag to prevent any bird damage. In the 2019 field experiment, a bird deterrent system (Guardian 2 Single Rotary Propane Cannon) was used to prevent bird damage. At physiological maturity, twenty panicles were harvested to estimate yield. To account for a large range in the plant stand under chilling conditions, the obtained twenty-panicle grain weight was normalized by multiplying the yield with plant stand, using the following formula:

$$Yield = \frac{(\frac{TGW}{H_n})TSE)}{PA}$$

Where, *TGW*; total grain weight (g), H_n; number of heads harvested, *TSE*; total seedlings emerged, *PA*; plot area (m^2).

Statistical analysis

Statistical analyses were performed using the RStudio statistical software version 3.6.1 (https://rstudio.com/). The genetic analysis was performed, using line × tester model for female × male hybrid (Singh and Chaudhary, 1979), to obtain values of general combining ability (GCA) for both male and female parents and the specific combining ability (SCA) effects for their corresponding hybrids. Variance component attributed to general combining ability of male (σ^2GCA (M)), female (σ^2GCA (F)), and specific combining ability of hybrid (σ^2SCA (F×M)) were estimated following (Ortis and Golmirzaie, 2002) and implemented in the R package agricolae (De Mendiburu, 2017). Significance test for GCA and SCA effects were performed using t-test. The magnitude of heterosis of each hybrid was computed using the average mean values of combined analysis of each planting dates. The better-parent heterosis (BPH) estimate was calculated for each hybrid as according to the formula described by Falconer and Mackay, (1996) as follow:

$$BPH\ (\%) = \left(\frac{(F_1 - BP)}{BP}\right)100$$

In which F_1 denoted to the mean performance of hybrid and *BP* refers to the mean performance of the better parent. The significance of heterosis was carried out by adopting t-test as suggested by Nadarajan and Gunasekaran, (2005). Pearson's correlation coefficients (r) among the traits was calculated (R Core Team 2017). To compare the phenology differences between genotypes, growing degree units (GDU) was calculated using the following formula:

$$GDU = \left(\frac{Daily\ maximum\ air\ temperature + \ Daily\ minimum\ air\ temperature}{2}\right) - 10$$

Results

Climate

Soil and air temperatures were significantly different with field experiments in years 2018 and 2019 from planting until biomass sampling (Table 3.1). Although attempting to replicate the chilling stress conditions between years with similar planting dates, the two years of temperatures deviated dramatically. In 2018, the average maximum and minimum soil temperatures were 25.4 and 23.0°C, respectively, while the average minimum soil temperatures followed similar patterns (Table 3.1). The average maximum air temperatures in 2018 and 2019 were 26.1 and 22.9°C, respectively, while the average minimum temperatures were 9.5 and 8.8°C, respectively (Table 3.1). The controlled environment chamber experiment stress treatment (20/10°C day/night) was not as stressful to that of both early planting field trials where the average temperature from planting to sampling was 17.8 and 15.9°C in 2018 and 2019, respectively..

Analysis of variance

The year wise coefficients of variation were more than 15% for all the traits studied due to significant differences in soil and air temperatures in both years. In this study, individual year wise analysis was carried out separately for each experiment and discussed due to these significant environmental differences (data not shown). There were significant differences among all genotypes within the study in regards to seedling traits (emergence percent, emergence index and seedling biomass) across the stress/early planting treatments in the controlled environment chambers and both years in the field (Table 3.2). Significant differences appeared among all genotypes in the control and regular planting treatments for all seedling traits except seedling biomass in both field experiments (Table 3.4). There were significant differences among parents in both stress/early and control/regular treatments for emergence percent. Emergence index only

showed significant differences among parents in the 2018 and 2019 early planting treatment as well as the control chamber treatment. A significant difference between parents and crosses was recorded for emergence percent, emergence index and seedling biomass in 2019 early and regular planting treatments (Table 3.2; 3.4). Emergence percent showed a significant difference between parents and crosses in the control/regular planting of the chamber and 2018 field experiments (Table 3.4). Emergence index was significant for parent vs. crosses in the stress and control treatment in the chamber experiment were it was only significant in the 2018 regular planting treatment (Table 3.2; 3.4). Differences of parents and crosses were also significant for seedling biomass in the 2018 early planting field treatment (Table 3.2). Crosses had significant difference for emergence percent in all stress/early and control/regular treatments except for the 2019 early planting. Emergence index showed significant difference among crosses for all control/regular treatments as well as the early planting in 2019 (Table 3.2; 3.4). In the 2018 early planting, crosses were significantly different as well (Table 3.2). A significant difference among female parents were reported for both stress/early and control/regular treatments in the chamber and 2019 field experiments as well as in the 2018 early planting (Table 3.2, 3.4). Female parents also showed a significant difference for emergence index in the 2019 early planting (Table 3.2). The only report of a significant difference between male and female parents were that of emergence percent and emergence index in the control chamber treatment as well as for emergence index in the 2018 early planting.

Significant difference among all genotypes were reported for all agronomic traits (days to flower, plant height, 200-kernel weight and plot yield) in all early and regular planting treatments in the field experiments except for plot yield in 2019 regular planting. There were significant differences among all parents for all agronomic traits in the 2018 as well as for plant height 2019

early and regular planting(Table 3.3; 3.5). Kernel weight was significantly different among parents in the 2019 regular planting (Table 3.5). Time to flower, plant height and kernel weight was significantly different between parents and crosses in both early and regular plantings of 2018 and 2019 (Table 3.2; 3.5). Plot yield only showed a significant different between parents and crosses in the early planting of 2018 (Table 3.3). Crosses were significantly different for all agronomic traits in both early and regular planting of both years besides regular planting of 2019 (Table 3.2; 3.5). Time to flower had a significant difference among female parents in the 2018 regular planting (Table 3.5) and the 2019 early planting (Table 3.3). Plant height, kernel weight and plot yield was significantly different among female parents in both the early and regular plantings of 2018 (Table 3.3; 3.5). In 2019, plant height was only significant among female parents in the regular planting treatment (Table 3.5). Male parents were significantly different amongst themselves for time to flower and plant height in both years of early and regular plantings. Kernel weight was significant among male parents in both early and regular plantings of 2018 (Table 3.3; 3.5). The only report of yield being significantly different among male parents was in the regular planting of 2018 (Table 3.5). A significant different between male and female parents was reported for time to flower in the early and regular plantings of 2018. Plant height was significantly different between male and females lines in the early planting of 2018 and the regular planting of 2019 (Table 3.3; 3.4). Only one treatment displayed a significant different between male and female parents for kernel weight, which was in the 2019 regular planting (Table 3.5).

Per se and general combing ability (GCA) of parents

Emergence percentage ranged from 7 to 94% among all stress/early planting treatments among parents. Redbine58B and ARCH10747-2R had the highest *per se* emergence percentage in the stress treatment and 2108 early planting of the female and males parents, respectively.

ARCH10747-2R and BTx645 showed the highest emergence percentage *per se* in 2019 early planting (Table 3.6). ARCH11201B and KS116B had a significant GCA for emergence percent in the stress chamber treatment and the 2018 early planting treatment, respectively. Emergence index ranged from 15 to 23 days across all stress/early planting treatments among parents. BTx645 and ARCH12012R had the lowest emergence index *per se* in stress chamber treatments of female and male parents, respectively. In 2018, ARCH11201B and ARCH10717-1R took the shortest amount of time to emerge in 2018, while KS116B and ARHC10747-1R were the shortest in 2019. In regards to GCA, KS133B had a significant negative emergence index in the stress chamber treatment (Table 3.6). ARCH11201B, KS136B and Redbine58B all had significantly negative GCA for emergence index in the 2018 and 2019 regular plantings. Seedling biomass ranged from 0.06 to 4.3 g across the two early plantings among parents. KS115R had the greatest amount of seedling biomass *per se* in both 2018 and 2019 early plantings among the male parents. While ARCH11170B had the highest *per se* in 2018 and BTx645B had the highest in 2019 among the female parents. Seedling biomass GCA in was found significant in the 2018 early planting of BTx645. Of both years of regular planting, BTx645 was shown to be significant in 2019 (Table 3.18).

Time from planting to 50% flowering ranged from 1632 to 2217 GDU among the early plantings of 2018 and 2019 within parents. Among the female parents, Redbine58B, and among the male parents, ARCH10747-2R required the least amount of GDU *per se* to flower in both years of early plantings (Table 3.7). There was no negative GCA significance found for time to flower in both plantings and years. Among parents, plant height ranged from 89 to 116 cm between the two early planting treatments. In 2018 and 2019 early plantings, KS115R was the tallest parent among all, while in 2018, ARCH11201B and KS116B were the tallest among the female parents

in 2018 and 2019, respectively (Table 3.7). GCA of ARCH11201B was found significant for plant height in both 2018 early and regular plantings as well as the 2019 regular planting (Table 3.7; 3.9). KS115R was significant for plant height GCA in 2019 early planting (Table 3.7). Kernel weight ranged from 3.6 to 7.6 g among parents in the early plantings. KS115R and BTx645B had the highest kernel weight *per se* in both years among male and female parents, respectively (Table 3.7). KS113A was found to be significant for kernel weight in both early and regular plantings of 2018 (Table 3.7; 3.9). ARCH11192B and KS115B were significant for kernel weight GCA in early planting of 2019. ARCH10747-2R produced the highest plot yield *per se* in both years of early planting among male parents. Plot yield ranged from 85 to 772 g m-2 within the early planting treatments among parents. Among the female parents, ARCH11192B had the highest yield *per se* in 2019 and KS136B had the highest yield *per se* in 2019 early plantings. The only findings of significant yield GCA were in the early planting treatments including BTx645 (Table 3.7).

***Per se* and specific combining ability (SCA) of hybrids**

Emergence percentage ranged from 56 to 96% in the stress chamber treatment while ranging from 18 to 63% in the early planting field experiments among hybrids. L5/T4 had the highest *per se* of emergence percentage in the stress chamber treatment, while L5/T2 had the highest *per se* in the early planting treatments (Table 3.10). L1/T3 had significant SCA in both years of early and regular plantings. L2/T3 and L5/T2 had significant SCA for emergence percent in both early and regular planting of 2018 (Table 3.10; 3.14). In the 2018 early planting, L5/T1 was found significant. In the 2019 early planting, L3/T1, L6/T4, L7/T3 and L8/T1 showed significant emergence percent SCA (Table 3.10). In the control treatment of the chamber experiment, L1/T1, L3/T3, L5/T2, L5/T3, L5/T4 and L7/T3 all had significant emergence percent SCA. Across both years of regular planting L1/T2 showed significant negative SCA. In the 2018

regular planting, L3/T1, L5/T4, L6/T1 and L7/T1 had significant SCA. In the same planting and year, L4/T3, L6/T3, L6/T4 and L8/T1 showed SCA of negative significance. L2/T4, L8/T1 and L8/T2 had a significant emergence percent SCA, while L1/T1 and L3/T4 were negatively significant (Table 3.14).

Emergence index ranged from 11 to 26 d in the early plantings across both years among hybrids. L6/T4 took the shortest amount of days to emerge in the stress chamber treatment as well as the early planting of 2019. L5/T2 was found to have the lowest *per se* of the 2018 early planting. Within the early plantings of both years, L4/T3 and L7/T3 were negatively significant for SCA. Within the 2018 early planting, emergence index SCA was negatively significant for hybrids L1/T3, L2/T1, L3/T4, L5/T3, L6/T1 and L6/T4. Among the hybrids in the 2019 early planting L1/T1, L1/T2, L2/T2, L6/T4 and L8/T1 were significantly negative (Table 6a). While L2/T3, L5/T2 and L8/T2 were negatively significant for emergence index in the control chamber treatment as well as the regular planting or 2018. In the control chamber treatment, hybrids L4/T3, L5/T3, L5/T4 and L7/T3 were negatively significant. L1/T3, L2/T4, L3/T3, L6/T1 and L7/T2 were found to be negatively significant for emergence index. (Table 7a). Seedling biomass ranged from 2 to 5 g in 2018 early planting, while ranging from 0.2 to 0.4 in 2019. L5/T2 and L6/T4 had the highest *per se* of 2018 and 2019, respectively (Table 3.11). L3/T1 showed a significant seedling biomass SCA in both early and regular planting of both years (Table 3.11; 3.15). Within the early planting treatment, hybrids L1/T4, L5/T1 and L8/T3 had significant biomass SCA across both years. In the 2018 early planting, L4/T3, L5/T3, L6/T4 and L7/T2 showed a significant biomass SCA. In the 2019 early planting, L1/T3, L5/T2, L6/T2, L7/T3, L7/T4, and L8/T2 reported SCA of significance (Table 3.11). Among they hybrids in the regular planting across both years, L4/T3, L6/T3 and L7/T4 were significant for seedling biomass SCA. Within the 2018 regular planting, hybrids

L2/T1, L5/T1, L5/T2 and L7/T3 had a significant biomass SCA. Among the hybrids in the regular planting of 2019, L2/T4, L5/T4 and L8/T3 were significant for biomass SCA (Table 3.15).

Among the hybrids, time until flowering ranged from 1572 to 1951 GDU. While L7/T2 and L2/T2 required the least amount of GDU to flowering in 2018 and 2019 early plantings, respectively. Hybrids L3/T4 and L4/T3 showed negative significance across both years of early planting. Within the early planting of 2018, crosses L1/T3, L2/T4, L3/T3 and L7/T2 were negatively significant. In regards to the early planting of 2019, L3/T1, L6/T3 and L8/T3 had a negative SCA significance (Table 3.12). Among regular plantings across both years, hybrids L1/T3 and L3/T1 were negatively significant for time to flower SCA. Within the regular planting of 2018, L3/T3, L4/T3, L5/T4, L6/T2 and L8/T2 were negatively significant. As of the regular planting in 2019, L1/T2, L2/T1, L2/T3, L3/T4, L5/T2, L6/T3 and L7/T1 had significantly negative SCA (Table 3.16).

Plant height ranged from 89 to 216 cm tall in the early plantings among hybrids. L5/T3 recorded the lowest *per se*, while L5/T4 recorded the highest (Table 3.12). L4/T3, L8/T1 and L8/T2 all showed significant SCA for plant height across both early and regular plantings in both years. L3/T3 and L5/T4 each were significantly negative for plant height SCA in the 2018 early planting. While L5/T4 showed significant SCA for plant height in the early planting of 2019 (Table 3.12). Within the regular planting, L8/T3 had significant SCA of plant height in 2018 (Table 3.16).

Kernel weight ranged from 3.4 to 8.1 g among all hybrids in the early plantings of both years. L7/T4 showed the highest *per se* of all (Table 3.13) L8/T2 was had significant SCA for kernel weight across all plantings and years of experiments (Table 3.13; 3.17). Among the early plantings, hybrid L3/T4 was significant for kernel weight SCA. L8/T1 was significant in 2018, while L2/T4, L5/T1, L5/T2, L6/T2 and L7/T3 were significant in the 2019 (Table 3.13). Of the

regular planting in 2018, hybrids L4/T3, L7/T4, L8/T2 and L8/T3 showed a significant SCA of kernel weight. In the 2019 regular planting, L2/T1, L2/T4, L5/T3, L5/T4, L6/T2 and L7/T2 had SCA significance for negative kernel weight SCA (Table 3.17). Plot yield ranged from 68 to 113 g m-2 in the early plantings (Table 3.17). Hybrid L2/T2 showed a significant SCA for plot yield across both early and regular plantings in both years (Table 3.13; 3.17). Among the early plantings of both years, cross L6/T4 had a significant SCA. Within the 2018 early planting, L2/T3, L7/T1 and L7/T3 were significant. In regards to the plot yield SCA of 2019, L1/T4, L2/T2, L2/T4, L3/T4, L5/T1, L5/T3, L7/T2 and L8/T2 were significant in early planting (Table 3.13). In the regular plantings of 2018 and 2019, L7/T3 was found to have significant SCA for plot yield. Within the regular planting of 2018, hybrids L1/T4, L2/T3, L3/T3 and L6/T1 had significant SCA. Within the 2019 regular planting cross L1/T1, L2/T4, L3/T1, L5/T4 and L6/T2 showed a SCA significance (Table 3.17).

Better-parent heterosis pattern of hybrids

Within the stress/early planting treatments, hybrids L3/T3 and L3/T4 showed significant heterosis of emergence (Table 3.18). In the control/regular planting treatments hybrids L4/T3 and L7/T4 had a negative heterosis of emergence index (Table 3.20). Hybrid L2/T3 in the stress chamber treatment showed a negative significance (Table 3.18). There was no significant heterosis recorded in regards to seedling biomass across both plantings in both years. Hybrids L2/T4, L3/T1, L5/T2, L5/T4 and L6/T1 were significantly negative across stress/early and control/regular treatments throughout both years of the study (Table 3.19; 3.21). Within the early plantings of both years L2/T1, L3/T4, L5/T1, L6/T4 and L7/T4 had a negative significance for time to flower heterosis (Table 3.19). Within the regular plantings of 2018 and 2019, hybrids L3/T3, L4/T3, L6/T3, L8/T1 and L8/T2 were negatively significant for time to flower heterosis (Table 3.21).

Between each planting in both years, hybrids L1/T1, L1/T2, L1/T4, L2/T4, L3/T4, L5/T4, L6/T4, L7/T3 and L7/T4 were all significant for plant height heterosis (Table 3.19; 3.21). Within the regular plantings of both years, L1/T3, L5/T3, L6/T3, L7/T1 and L8/T2 showed significant heterosis while L7/T2 showed significantly negative heterosis for plant height (Table 3.21). No significant heterosis was found for kernel weight across all experiments and treatments. Hybrids L5/T1 and L5/T3 reported a significant heterosis for plot yield (Table 3.19; 3.21). From the early planting treatments from 2018 and 2019, hybrid L5/T2 showed a significant heterosis (Table 3.19). Within the regular plantings of 2018 and 2019 L1/T1, L1/T2, L4/T3 and L7/T4 had significant heterosis (Table 3.21).

Interrelationship between traits

Emergence percentage was correlated significantly negative with emergence index and days to 50% flowering across both years of early planting. Seedling biomass was also correlated significantly negative to time to flower while it was correlated positively significant to plant height in both years of early planting. Plant height also had a positively significant correlation with kernel weight (Table 3.22). Like the early planting, seedling biomass had significantly negative correlation to time to flower, and plant height had a significantly positive correlation to kernel weight across both years of regular plantings (Table 3.23). For traits such as emergence percentage, emergence index, seedling biomass, days to flower and plant height the early and regular plantings were significantly correlated across both years. Whereas kernel weight had a negative correlation between early and regular plantings in 2018. Plot yield correlated significantly between the two plantings in 2018 (Table 3.12).

Discussion

Early-stage chilling tolerance in sorghum can be defined as the ability of sorghum genotypes to tolerate chilling conditions (<15°C) early in the growth stage; germinate, emerge and grow and recover once optimal conditions have been met. The early-stage chilling conditions pose serious challenges for the expansion and earlier planting of sorghum in the Great Plains of United States. Many efforts have been made and fortunately new parental lines have been identified as sources for chilling tolerance (Franks et al., 2006; Knoll and Ejeta, 2008; Kapanigowda et al., 2013; Chiluwal et al., 2018). Hybrids that can be planted earlier while not affecting the germination, emergence or seedling vigor would allow producers to benefit from early spring moisture, earlier canopy closure and possibly a reduction in canopy transpiration during later stages of crop growth. In this study, emphasis on assessing the combining ability and heterosis of hybrids derived from eight seed parents as lines and four pollinators as testers. The variation among the different studied seedling and agronomic traits across planting dates and years can be attributed to the variation of the inter- and intra-annual environments.

Data were analyzed for combining ability and heterosis and discussed separately over four field experiments due to significant variation recorded in all the traits studied. Within this study, a regular planting was included as a comparison to optimal conditions. That being said, the focus is on the performance in the early planting. Kapanigowda et al., (2013) reported that early planting took longer time to complete emergence compared to regular planting time, meaning that chilling stress reduced the seedling biomass tremendously. While Yu et al., (2004) reported that chilling stress reduced the emergence by 37% and increased the time to emerge by twice that of regular planting, which are the results of our study confirm. Generally, a significant delay of time to flower was reported when sorghum was exposed to chilling stress (Kapanigowda et al., 2013; Maulana

and Tesso 2013; Windpassinger et al., 2015). Across all genotypes in this study, the time and thermal units it took to flower was lengthened by the earlier planting in both years, which would be a result in the delayed time to emerge and accumulate seedling vigor. Where these delays show no real impact on the overall plant height, kernel weight and yield.

Per se and general combining ability effects

In the current study, the *per se* of seedling traits in early planting impacted inconsistent performance due to chilling stress and significant temperature differences across years. The impact was comparatively less in all agronomic traits in the later growing stages under early regular plantings in both years. It clearly indicates that a parent good in *per se* performance may not necessarily produce better hybrids when used in hybridization (Shukla and Pandey, 2008). It also indicated that one parent of the worst combination could make the best combination if the other parent was selected rationally (Bao et al., 2009).

Hybrid breeding programs should focus on GCA to select genetically diverse parents to maximize heterosis rather than on selection among lines based on their *per se* performance (Windpassinger et al., 2017). In the current study, the lines ARCH11201B and KS116B showed significant GCA for stress/early planting treatments for emergence percentage in the chamber experiment and 2018 early planting, respectively, while line ARCH11201B also had significant GCA for plant height in 2018. Line BTx645 had a significant GCA for seedling biomass in 2018 and for yield in 2019 of early plantings. Whereas, the tester KS113B reported a significant GCA value for shorter emergence index in the stress chamber treatment as well as larger kernel weight in 2018 early planting. The tester KS115R had significant GCA for plant height in both years as well as kernel weight in 2019 early planting (Table 3.6; 3.7). Significant GCA recorded on the previously mentioned parents in different traits indicates potential evidence of desirable gene flow

of additive genes from parents to offspring at high intensity (Franco et al., 2001). It also indicates higher heritability with less environmental effects and gene interactions and higher achievement in selection with high adaptability to chilling stress environments (Topal et al., 2004; Tyagi and Lal, 2005; Chigeza et al., 2014).

Per se and specific combining ability effects

The estimates of SCA provide important information about the hybrid performance to its parents, showing the importance of non-additive interaction due to large or minor gene effects in particular hybrid combination (Kenga et al., 2004; Fasahat et al., 2016). High SCA effects results from crosses where both parents are good general combiners (i.e., good GCA × good GCA) are due to additive × additive gene action (Dey et al., 2014; Verma and Srivastava, 2004). Hybrids with additive × additive gene interaction results fixable nature with limited environmental influence of the trait. Due to the impact of significant temperature fluctuations across years, none of the parental combinations for the traits in the current study showed consistently high GCA effects.

As stated above, two poor performing parents could produce a top performing hybrid. In this study, multiple hybrids were able to display significant SCA for emergence percentage. In regards to emergence index, KS133A/KS115R and KS136A/ARCH12012R had a significantly negative SCA in both 2018 and 2019 early plantings. KS133B had a significantly low GCA value for emergence percent while KS115R was not significant. A hybrid derived from one parent with a significant GCA and one without may be attributed from favorable additive effects of the good general combiner parent and epistatic effects from the poor general combiner, which fulfils the favorable plant emergence index (Milić et al., 2011).

Four hybrids (ARCH11170A/KS115R, ARCH11201A/ARCH10747-1R, KS116A/ARCH10747-1R and Redbine58A/ARCH12012R) had a significant SCA across both 2018 and 2019 early plantings for seedling biomass. Among these four hybrids, none of the parents showed significant GCA effects, meaning the SCA effect may be due to a dominance × dominance of non-allelic gene interaction which leads to over dominance. Predominance of non-additive effect has been reported for inheritance of one trait and related traits in which there were cross combinations with high SCA effect arising from parents with high and low GCA (Azad et al., 2014). ARCH11201A/KS115R and ATx645/ARCH12012R each showed significant reduction in time to flower in regards to SCA across both years of early planting. While ATx645/ARCH12012R, KS116A/KS115R, Redbine58A/ARCH10747-1R and Redbine58A/ARCH10747-2R had significant plant height SCA in both years as well. These hybrids display the dominance × dominance of non-allelic gene interaction (Wassimi et al., 1986).

KS116A/KS115R showed additive effects of the good general combiner parent and epistatic effects from the poor general combiner. Significant SCA for kernel weight was recorded in both years for ARCH11201A/KS115R and Redbine58A/ARCH10747-2R. The hybrid Redbine58A/ARCH10747-2R could have a dominance × dominance of non-allelic gene interaction, while ARCH11201A/KS115R could have an additive effects of the good general combiner parent and epistatic effects from the poor general combiner interaction for kernel weight. Hybrid crosses of ARCH11201A/ARCH10747-1R and KS133A/KS115R showed significant SCA for plot yield in 2018 and 2019. None of the parents of these hybrids shows a significant GCA for yield, meaning their SCA comes from in interaction of the dominance × dominance gene action (Fasahat et al, 2016) (Table 3.15; 3.17).

Heterosis

The success of hybrid breeding depends also on the magnitude of heterosis for the traits related to chilling tolerance. Earlier studies confirmed heterosis for chilling tolerance traits and provided valuable insights regarding their inheritance (Yu and Tuinstra, 2001; Tiryaki and Andrews, 2001; Windpassinger et al., 2015). Significant heterosis was expressed in all traits besides seedling biomass and kernel weight, which confirms the findings of the previous studies. Hybrids ARCH11201A/ARCH12012R and ARCH11201A/KS115R showed a significant heterosis for emergence percentage from 15 to 150% when planted in all stress/early conditions. In the 2019 early planting, hybrid ARCH11170A/ARCH10747-2R had a significantly negative heterosis for emergence index of -25%, indicating that the hybrid was able to emerge 25% earlier than the performance of the better parent. Ten hybrids were able to consistently outperform their better parents in regards to time to flower. These hybrids were able to flower with 2 to 10% less thermal units required in early planting compared to their better parents. Hybrids KS116A/ARCH10747-1R, KS116A/ARCH10747-2R and KS116A/ARCH12012R significantly outperformed their better parents for plot yield in early planting across both years. The wide range (24 to 103%) of heterosis for plot yield clearly indicates a great scope for improving the yield performance in hybrids under chilling stress conditions (Table 3.18; 3.19).

Inter relationship between traits

The interrelationships of traits included in this study showed interesting results. Where emergence percentage was significantly correlated with emergence index in both years of early planting. This shows that a genotype that has the ability to emerge quicker can emerge a greater number of plants which, as stated in the introduction, is a key component of chilling tolerance. This emergence timing also correlates significantly with seedling biomass in both years. This

correlation is excepted in that when a seedling can emerge sooner, it has the capability of accumulating more biomass. This also leads to the significant correlation of the biomass and plant height, where if the genotype can emerge sooner and accumulate more biomass, it will be taller at maturity (Table 3.22). The correlation among plantings within a year are significant for emergence percent, emergence index, seedling biomass, time to flower and plant height for both 2018 and 2019. This shows that when selecting for chilling tolerant genotypes, the top performing ones in an optimal condition will most likely carry over to the chilling stress conditions. Although, this correlation is not 100%, and some genotypes could perform at a higher level in the chilling conditions than in optimal (Table 3.12).

Summary

Most of the hybrids average *per se* performance is higher than their parents for all the traits both in early and regular plantings across two years. Parental choice only based on SCA effect has limited value in breeding programs. Therefore, SCA effect should be used in combination with a high *per se* performance of hybrid, favorable SCA and heterosis estimates, and involving at least one parent with high GCA (Franco et al., 2001; Makanda et al., 2010; Umakanth et al., 2012). Hybrid ARCH11201A/ARCH10747-1R proved to have high *per se* performance, favorable SCA and heterosis estimates as well has at least one parent with high GCA in multiple traits crucial for chilling tolerance.

Table 3.1 Planting, emergence and sampling dates, daily average soil and air temperature and precipitation from the field experiments in 2018 and 2019.

Planting	Planting	Emergence	Sampling	Soil temperature (°C)		Air temperature (°C)		Precipitation
				Max	Min	Max	Min	(mm)
2018								544.6
Early	16-Apr-18	6-May-18	4-Jun-18	25.4 ± 6.9	15.4 ± 5.0	26.1 ± 6.7	9.5 ± 6.3	
Regular	26-May-18	30-May-18	2-Jul-18	32.8 ± 3.1	22.1 ± 2.5	33.2 ± 3.8	16.9 ± 3.4	
2019								602.5
Early	17-Apr-19	1-May-19	7-Jun-19	23.0 ± 5.6	14.4 ± 4.2	22.9 ± 6.3	8.8 ± 5.0	
Regular	5-Jun-19	12-Jun-19	9-Jul-19	31.1 ± 3.2	21.7 ± 3.1	30.4 ± 4.3	15.8 ± 3.5	

Temperature data presented is the average daily maximum and minimum temperatures (°C ± standard deviation) during early seedling growth (from planting until sampling). Precipitation is the cumulative rainfall from April through August.

Table 3.2 Mean squares of the seedling traits of the parental lines and hybrids of chilling stress/early planning in the controlled environment chambers and field experiments of 2018 and 2019.

Source of variance	Df	Seedling traits							
		Chamber		2018			2019		
		EP	EI	EP	EI	SB	EP	EI	SB
Replications	1	0.8	0.1	193.7	0.6	0.1	5.0	3.9	0.1**
Genotypes	38	209.8**	0.4*	304.6**	5.3**	1.8**	331.8**	14.1**	0.0*
Parents	11	172.3**	0.1	541.9**	5.5*	1.4	442.6**	30.5**	0.0
Parents vs. Crosses	1	50.7	4.7**	23.6	2.2	13.0**	2001.7**	34.7**	0.3**
Crosses	26	232.6**	0.3	215.0**	5.4**	1.5*	220.7	6.3*	0.0
Lines (L)	7	612.0**	0.3	691.8**	4.1	1.1	561.3**	10.3*	0.0
Testers (T)	3	152.7	0.4	44.5	13.5	2.9	137.2	11.0	0.0
Lines × Tester	16	71.6	0.3	38.3	4.4*	1.4	87.3	3.7	0.0
Error	38	56.6	0.2	91.0	2.2	0.7	150.9	3.3	0.0
σ^2GCA (L)		67.5	0.0	81.7	0.0	0.0	59.3	0.8	0.0
σ^2GCA (t)		5.1	0.0	0.4	0.6	0.1	3.1	0.5	0.0
σ^2SCA (L×T)		111.1	0.1	83.6	2.6	0.5	55.5	2.5	0.0

*,** Significant at 5 and 1% probability levels respectively. σ^2GCA - variance attributed to general combining ability; σ^2SCA - variance attributed to specific combining ability. EP - emergence (%); EI - emergence index (d); SB - seedling biomass (g).

Table 3.3 Mean squares of the agronomic traits of the parental lines and hybrids of chilling stress/early planning in the controlled environment chambers and field experiments of 2018 and 2019.

Source of variance	Df	Agronomic Traits							
		2018				2019			
		DF	PH	KW	PY	DF	PH	KW	PY
Replications	1	195272.1**	125.7	0.0	3023.5	226659.2**	2288.5	0.4*	242387.4*
Genotypes	38	31325.1**	1987.9**	3.1**	18135.6**	41032.8**	1665.8**	0.3**	66272.2*
Parents	11	48951.2**	272.4**	2.5**	29939.5**	57163.2**	256.7	0.2	44131.7
Parents vs. Crosses	1	204925.2**	10340.4**	8.5**	54113.5**	569538.5**	7992.8**	1.4**	6295.2
Crosses	26	17191.0**	2392.4**	3.1**	11757.9*	13881.3**	2018.5**	0.3**	77946.1*
Lines (L)	7	9838.1	1109.1**	2.8**	25577.1**	17330.2**	294.1	0.3	72234.7
Testers (T)	3	94752.6**	17440.3**	20.1**	11931.7	59784.0**	14067.4**	0.5	186857.6
Lines × Tester	16	5865.0**	132.4**	0.1	5679.4	3765.7	513.8	0.2	60023.9
Error	38	2208.9	40.5	0.1	5849.3	3554.8	751.3	0.1	37191.3
σ^2GCA (L)		496.6	2.2	0.3	2487.2	1695.6	-27.5	0.0	1526.3
σ^2GCA (T)		5555.5	1.6	1.2	390.8	3501.1	847.1	0.0	7927.1
σ^2SCA (L×T)		17304.8	3.2	3.8	4273.4	11702.6	2103.6	0.1	34590.4

*,** Significant at 5 and 1% probability levels respectively. σ^2GCA - variance attributed to general combining ability; σ^2SCA - variance attributed to specific combining ability. DF - days to 50% flowering (GDU); PH - plant height (cm); KW - 200 kernel weight (g); PY - plot yield (g m^{-2}).

Table 3.4 Mean squares of the seedling traits of the parental lines and hybrids of control/regular planting in the controlled environment chambers and field experiments of 2018 and 2019.

Source of variance	Df	Seedling traits							
		Chamber		2018			2019		
		EP	EI	EP	EI	SB	EP	EI	SB
Replications	1	0.0	0.0	25.7	0.6	8.4	10.1	0.4*	3.7
Genotypes	38	177.6**	0.2**	221.1**	0.4**	6.3	830.2**	0.3**	3.7
Parents	11	204.4**	0.1**	499.6**	0.2	6.5	901.1**	0.2	2.7
Parents vs. Crosses	1	133.0**	0.6**	655.1**	2.2**	19.4	5010.8**	1.4**	40.1*
Crosses	26	167.6**	0.2**	86.6*	0.3*	5.7	639.4*	0.3**	2.7
Lines (L)	7	343.4*	0.2	78.5	0.4	7.8	1583.1*	0.3	1.5
Testers (T)	3	103.1	0.3	178.6	0.6	6.6	464.0	0.5	6.7
Lines × Tester	16	98.5**	0.1**	72.9	0.2	4.6	259.4	0.2	2.5
Error	38	0.0	0.0	47.6	0.2	5.6	211.2	0.1	3.1
σ^2GCA (L)		30.6	0.0	0.7	0.0	0.4	165.5	0.0	-0.1
σ^2GCA (T)		0.3	0.0	6.6	0.0	0.1	12.8	0.0	0.3
σ^2SCA (L×T)		90.8	0.1	31.2	0.1	0.3	278.8	0.1	0.2

*,** Significant at 5 and 1% probability levels respectively. σ2GCA - variance attributed to general combining ability; σ2SCA - variance attributed to specific combining ability. EP – emergence (%); EI – emergence index (d); SB – seedling biomass (g).

Table 3.5 Mean squares of the agronomic traits of the parental lines and hybrids of control/regular planting in the controlled environment chambers and field experiments of 2018 and 2019.

Source of variance	Df	Agronomic Traits							
		2018				2019			
		DF	PH	KW	PY	DF	PH	KW	PY
Replications	1	27497.7**	159.4	0.1	15180.2	89999.4**	644.2*	0.0	126914.4
Genotypes	38	17369.1**	3243.3**	3.2**	23223.1**	18261.7**	1573.0**	2.3**	92511.6
Parents	11	26161.5**	435.1**	3.0**	42707.0**	20275.2**	125.0	3.1**	103966.1
Parents vs. Crosses	1	126831.7**	14630.2**	6.8**	11014.7	244024.8**	8849.8**	13.2**	124151.6
Crosses	26	9439.2**	3993.4**	3.1**	15449.4**	8726.6*	1905.8**	1.6**	86448.6
Lines (L)	7	10112.3*	1551.9**	1.2*	18952.0*	10327.5	1203.4*	2.2	85148.3
Testers (T)	3	44385.1**	30747.3**	21.9**	57568.4**	19789.2*	11821.5**	1.4	101975.5
Lines × Tester	16	2592.3**	45.3	0.4	6019.7	5952.0	353.9*	1.3**	84106.1
Error	38	736.0	57.4	0.4	4096.8	3202.1	130.3	0.1	83244.7
σ^2GCA (L)		940.0	188.3	0.1	1616.5	546.9	106.2	0.1	130.3
σ^2GCA (T)		2612.1	1918.9	1.3	3221.8	864.8	716.7	0.0	1116.8
σ^2SCA (L×T)		9147.0	5362.1	3.7	11708.3	4410.4	2164.6	0.8	3582.7

*,** Significant at 5 and 1% probability levels respectively. σ^2GCA - variance attributed to general combining ability; σ^2SCA - variance attributed to specific combining ability. DF - days to 50% flowering (GDU); PH - plant height (cm); KW - 200 kernel weight (g); PY - plot yield (g m^{-2}).

Table 3.6 *Per se* and general combining ability effects (GCA) of parental lines and testers for seedling traits in stress/early planting treatment from controlled environment chamber and 2018 and 2019 field experiments.

Parents	Seedling traits															
	EP						EI						SB			
	Chamber		2018		2019		Chamber		2018		2019		2018		2019	
	per se	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA
Lines																
L1 (ARCH11170B)	*70.0*	-20.59*	*47.9*	-8.14	*15.1*	-15.71*	*16.3*	0.44	*18.6*	0.28	*19.2*	1.49	*2.68*	0.01	*0.06*	-0.04
L2 (ARCH11192B)	*80.0*	5.58	*52.6*	6.84	*46.9*	7.73	*15.9*	-0.10	*17.7*	0.36	*10.8*	-0.18	*2.50*	0.43	*0.28*	0.01
L3 (ARCH11201B)	*76.0*	9.41*	*31.3*	7.27	*24.0*	4.13	*16.2*	-0.03	*18.5*	-0.20	*15.7*	1.71	*2.12*	-0.65*	*0.08*	0.05
L4 (BTx645)	*74.0*	-13.92*	*48.4*	-4.36	*55.7*	-6.98	*15.5*	0.09	*20.1*	-0.21	*13.7*	1.52	*2.62*	0.63*	*0.41*	-0.04
L5 (KS116B)	*92.0*	8.08	*11.5*	15.95*	*25.0*	8.25	*16.2*	0.06	*23.4*	-1.18	*10.6*	-1.25	*1.25*	0.05	*0.16*	0.05
L6 (KS133B)	*88.0*	-2.42	*26.6*	-4.75	*41.1*	-6.98	*15.6*	-0.31*	*22.3*	1.30	*14.6*	-0.16	*1.56*	0.02	*0.14*	-0.02
L7 (KS136B)	*94.0*	0.08	*24.0*	-3.32	*50.5*	6.43	*16.1*	0.04	*20.5*	-0.46	*14.9*	-1.54	*1.71*	0.27	*0.14*	-0.01
L8 (Redbine58B)	*94.0*	0.74	*59.4*	-14.60*	*44.3*	-1.43	*15.9*	-0.02	*20.4*	-0.12	*14.0*	-0.04	*2.36*	-0.60*	*0.28*	-0.03
Testers																
T1 (ARCH10747-1R)	*68.0*	-3.64	*24.0*	-1.76	*32.8*	-1.48	*16.1*	0.04	*20.0*	-0.08	*24.5*	0.42	*1.60*	-0.02	*0.21*	-0.01
T2 (ARCH10747-2R)	*90.0*	2.74	*37.0*	-1.76	*25.5*	2.91	*16.2*	0.07	*21.8*	-1.21	*14.0*	-0.54	*3.13*	0.41	*0.25*	0.01
T3 (ARCH12012R)	*78.0*	0.08	*25.0*	1.57	*35.1*	-3.40	*15.9*	0.09	*21.4*	1.04	*19.6*	0.96	*2.82*	-0.54	*0.12*	-0.02
T4 (KS115R)	*82.0*	1.41	*6.8*	1.72	*44.8*	3.35	*16.3*	-0.22	*20.0*	-0.08	*15.7*	-1.23	*4.32*	0.33	*0.20*	0.03
Check																
SQR	*56.0*		*44.8*		*45.3*		*16.3*		*20.4*		*11.9*		*3.28*		*0.17*	
SE± (GCAi) F	*3.65*		*6.32*		*5.51*		*0.11*		*0.74*		*1.04*		*0.20*		*0.05*	
SE± (GCAj) M	*5.28*		*7.18*		*9.99*		*0.10*		*0.55*		*2.69*		*0.65*		*0.03*	

* Significant at 5% probability level; EP - emergence (%); EI - emergence index (d); SB - seedling biomass (g).

Table 3.7 *Per se* and general combining ability effects (GCA) of parental lines and testers for agronomic traits in stress/early planting treatment from controlled environment chamber and 2018 and 2019 field experiments.

Parents	Agronomic traits															
	DF				PH				KW				PY			
	2018		2019		2018		2019		2018		2019		2018		2019	
	per se	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*Per se*	GCA	*Per se*	GCA
Lines																
L1 (ARCH11170B)	*1762.0*	33.5	*2160.2*	30.3	*89.5*	-6.2	*85.4*	0.5	*3.57*	-0.66	*3.85*	-0.30*	*326.2*	-35.3	*259.4*	60.7
L2 (ARCH11192B)	*1937.7*	-7.9	*1979.9*	-62.6	*117.5*	9.9	*108.3*	1.5	*4.22*	-0.33	*3.58*	0.29*	*494.7*	82.4	*667.9*	37.3
L3 (ARCH11201B)	*1815.7*	-23.7	*2113.9*	-25.5	*123.8*	17.2*	*115.4*	0.5	*4.93*	0.56	*4.18*	-0.09	*281.7*	1.3	*592.7*	-81.0
L4 (BTx645)	*1937.7*	39.9	*2010.8*	140.3*	*116.0*	-13.1*	*113.3*	-14.5*	*6.18*	0.44	*4.20*	0.06	*370.6*	-46.3	*387.7*	185.9*
L5 (KS116B)	*2057.5*	-27.1	*2131.5*	-20.5	*89.0*	-10.0	*116.3*	5.7	*3.87*	-0.16	*3.37*	0.16	*105.8*	42.7	*431.7*	130.3
L6 (KS133B)	*2028.9*	37.7	*2188.3*	2.0	*105.0*	5.6	*115.4*	-0.5	*4.55*	0.81*	*3.75*	0.03	*242.5*	-6.0	*586.1*	-159.5*
L7 (KS136B)	*2116.2*	25.1	*2216.8*	60.3	*104.3*	7.8	*102.1*	6.1	*5.10*	0.51	*3.92*	-0.02	*243.9*	19.9	*771.6*	-17.0
L8 (Redbine58B)	*1659.1*	-71.4	*1821.3*	-34.0	*104.0*	-22.3*	*101.7*	-13.3*	*4.72*	-0.94*	*3.57*	-0.14	*442.2*	-124.1*	*316.0*	-50.1
Testers																
T1 (ARCH10747-1R)	*1834.5*	-27.3	*1815.1*	-1.7	*92.3*	-14.3*	*88.8*	-9.5	*4.60*	-0.40	*4.0*	-0.18	*227.7*	-27.6	*404.1*	26.8
T2 (ARCH10747-2R)	*1632.1*	-115.4	*1949.0*	-100.7	*91.0*	-20.6*	*100.4*	-14.7*	*4.03*	-0.84	*4.2*	-0.08	*261.0*	-29.1	*409.0*	105.5
T3 (ARCH12012R)	*1834.5*	66.1	*1698.0*	67.8	*97.0*	-17.5*	*96.3*	-20.1*	*4.17*	-0.54	*3.9*	-0.18	*188.2*	7.8	*474.9*	-130.7*
T4 (KS115R)	*2025.5*	59.1	*1874.8*	12.2	*99.5*	60.6*	*87.9*	52.6*	*7.63*	2.03	*3.7*	0.51*	*85.0*	50.9	*445.2*	37.6
Check																
SQR	*1949.0*		*1949*		*162.5*		*92.5*		*5.20*		*4.3*		*444.3*		*1083.2*	
SE± (GCAi) F	*59.52*		*49.97*		*4.77*		*4.01*		*0.31*		*0.11*		*46.70*		*68.06*	
SE± (GCAj) M	*92.74*		*61.23*		*2.30*		*3.48*		*0.98*		*0.12*		*44.09*		*19.18*	

* Significant at 5% probability level; DF - days to 50% flowering (GDU); PH - plant height (cm); KW - 200 kernel weight (g); PY - plot yield (g m^{-2}).

Table 3.8 *Per se* and general combining ability effects (GCA) of parental lines and testers for seedling traits in control/regular planting treatment from controlled environment chamber and 2018 and 2019 field experiments.

Parents	Seedling traits															
	EP						EI						SB			
	Chamber		2018		2019		Chamber		2018		2019		2018		2019	
	per se	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA
Lines																
L1 (ARCH11170B)	*88.0*	-15.85*	*64.1*	-0.36	*49.2*	-27.67*	*3.4*	0.38*	*7.3*	0.01	*7.7*	0.02	*8.16*	0.91	*6.02*	-0.45
L2 (ARCH11192B)	*96.0*	3.15	*66.7*	-1.14	*29.7*	-4.61	*3.0*	-0.08	*7.2*	0.10*	*7.4*	0.06	*6.95*	0.98	*4.90*	0.12
L3 (ARCH11201B)	*96.0*	6.82	*73.4*	4.46	*44.3*	11.84	*3.2*	-0.02	*7.3*	-0.26*	*7.4*	-0.27*	*10.79*	-1.27	*3.02*	-0.50
L4 (BTx645)	*100.0*	-7.85	*61.5*	-1.79	*77.0*	-2.13	*2.6*	0.42*	*7.2*	0.43*	*7.2*	0.44*	*9.00*	-0.77	*5.03*	1.50*
L5 (KS116B)	*72.0*	5.15	*50.5*	5.11	*37.0*	15.25	*3.2*	0.03	*7.4*	0.26*	*7.4*	0.24*	*5.06*	-1.40	*4.32*	0.30
L6 (KS133B)	*100.0*	-3.85	*64.6*	-4.01	*64.3*	3.60	*2.9*	-0.10	*7.4*	0.18*	*7.2*	0.08	*10.07*	-0.66	*4.62*	0.25
L7 (KS136B)	*92.0*	2.15	*49.5*	0.16	*37.6*	12.86	*3.1*	-0.14	*7.4*	-0.34*	*7.7*	-0.23*	*9.05*	1.12	*4.42*	0.12
L8 (Redbine58B)	*88.0*	2.82	*77.6*	-3.53	*71.4*	-10.38	*3.0*	-0.11	*7.5*	-0.17*	*7.6*	-0.11*	*6.35*	0.25	*3.40*	-0.45
Testers																
T1 (ARCH10747-1R)	*92.0*	0.15	*50.5*	-1.79	*16.7*	-4.77	*3.0*	-0.19	*7.4*	0.15	*7.4*	0.08	*6.42*	0.49	*2.82*	0.16
T2 (ARCH10747-2R)	*68.0*	2.82	*59.9*	1.24	*52.1*	-2.60	*3.2*	0.04	*8.2*	-0.08	*8.2*	-0.12	*10.49*	0.79	*4.80*	0.21
T3 (ARCH12012R)	*84.0*	0.15	*49.5*	3.28	*63.5*	-1.94	*2.8*	0.13	*8.2*	0.15	*8.1*	0.21	*7.65*	-0.91	*2.22*	-0.75
T4 (KS115R)	*92.0*	-3.18	*16.7*	-3.53	*8.9*	10.75	*2.8*	0.03	*7.3*	-0.29	*7.2*	-0.25	*7.21*	-0.15	*2.62*	0.60
Check																
SQR	*84.0*		*50.5*		*8.1*		*3.8*		*8.2*		*7.8*		*8.3*		*4.0*	
SE± (GCAi) F	*3.47*		*3.72*		*6.62*		*0.09*		*0.04*		*0.07*		*0.73*		*0.36*	
SE± (GCAj) M	*6.53*		*10.92*		*15.37*		*0.12*		*0.29*		*0.28*		*1.02*		*0.67*	

* Significant at 5% probability level; DF- days to 50% flowering (GDU); PH - plant height (cm); KW - 200-kernel weight (g); PY - plot yield (g m^{-2}).

Table 3.9 *Per se* and general combining ability effects (GCA) of parental lines and testers for agronomic traits in control/regular planting treatment from controlled environment chamber and 2018 and 2019 field experiments.

Parents	Agronomic traits															
	DF				PH				KW				PY			
	2018		2019		2018		2019		2018		2019		2018		2019	
	per se	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA	*per se*	GCA
Lines																
L1 (ARCH11170B)	*1795.0*	11.4	*1693.8*	71.2*	*104.5*	-2.7	*94.6*	-8.4*	*4.02*	-0.39	*5.85*	0.23	*341.7*	-41.1	*449.5*	-83.5
L2 (ARCH11192B)	*1900.1*	-26.8	*1747.1*	-13.0	*136.0*	12.7	*105.4*	5.2	*3.98*	-0.12	*3.95*	-0.69	*448.6*	-38.7	*1208.1*	24.6
L3 (ARCH11201B)	*1795.0*	-17.2	*1803.6*	-2.8	*141.0*	18.0*	*113.3*	29.2*	*5.05*	0.36	*6.10*	0.44	*571.1*	-50.8	*476.5*	183.1
L4 (BTx645)	*1974.5*	24.0	*1836.4*	64.9	*128.3*	-25.4*	*101.3*	-13.6*	*5.83*	-0.40	*5.63*	0.36	*463.4*	84.8	*609.9*	72.6
L5 (KS116B)	*2057.5*	38.0	*1779.0*	-35.4	*98.0*	-7.6	*94.2*	-3.5	*3.77*	-0.28	*2.70*	-0.93	*231.3*	-5.6	*615.7*	-50.7
L6 (KS133B)	*1857.5*	-29.5	*1695.0*	-14.2	*116.3*	1.1	*109.6*	-3.8	*5.55*	0.72*	*5.93*	0.34	*603.6*	12.0	*670.2*	130.2
L7 (KS136B)	*2031.9*	55.2	*1912.3*	7.7	*126.8*	10.7	*117.9*	4.8	*4.93*	0.18	*5.80*	0.22	*358.0*	97.1	*593.8*	-137.7
L8 (Redbine58B)	*1772.0*	-55.0	*1695.7*	-40.6	*107.5*	-28.5*	*105.4*	-17.2*	*4.72*	-0.37	*6.52*	0.54	*494.8*	-9.2	*537.7*	-51.1
Testers																
T1 (ARCH10747-1R)	*1795.0*	-10.5	*1808.9*	-5.7	*107.0*	-16.8	*100.4*	-8.6	*4.22*	-0.56	*4.30*	0.07	*286.7*	-1.1	*742.4*	78.4
T2 (ARCH10747-2R)	*1665.0*	-82.0	*1523.0*	-44.9	*104.8*	-26.3*	*97.5*	-19.1	*4.73*	-0.38	*3.60*	-0.42	*351.2*	50.9	*574.5*	45.2
T3 (ARCH12012R)	*1819.1*	47.4	*1833.5*	-3.5	*99.5*	-25.8*	*101.3*	-15.8	*4.77*	-0.78	*3.72*	-0.01	*313.8*	39.7	*467.3*	-43.3
T4 (KS115R)	*1837.5*	31.1	*1695.7*	56.2	*125.8*	80.3*	*115.0*	50.3	*8.37*	2.06	*5.68*	0.36	*87.5*	-102.6	*258.8*	-78.9
Check																
SQR	*1720.2*		*1808.9*		*178.5*		*135.8*		*4.70*		*4.1*		*363.5*		*646.5*	
SE± (GCAi) F	*42.37*		*29.74*		*5.90*		*3.19*		*0.29*		*0.49*		*46.84*		*90.40*	
SE± (GCAj) M	*45.08*		*81.70*		*6.60*		*4.51*		*1.10*		*0.55*		*68.06*		*116.96*	

* Significant at 5% probability level; DF- days to 50% flowering (GDU); PH - plant height (cm); KW - 200-kernel weight (g); PY - plot yield (g m^{-2}).

Table 3.10 *Per se* and specific combining ability effects (SCA) for emergence (%) from stress/early planting treatment from controlled environmental chamber and 2018 and 2019 field experiments.

Hybrids	Emergence %					
	Chamber		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*56.0*	-3.70	*24.0*	-0.20	*34.4*	1.3
L1/T2 (ARCH11170A/ARCH10747-2R)	*72.0*	5.92	*22.4*	-1.76	*27.6*	-9.8*
L1/T3 (ARCH11170A/ARCH12012R)	-	-	*32.3*	4.82*	*38.5*	7.4*
L1/T4 (ARCH11170A/KS115R)	*62.0*	-2.74	*25.0*	-2.63	*37.5*	-0.4
L2/T1 (ARCH11192A/ARCH10747-1R)	*88.0*	2.14	*35.9*	-3.19	*47.4*	-9.1*
L2/T2 (ARCH11192A/ARCH10747-2R)	*90.0*	-2.24	*38.0*	-1.11	*73.4*	12.6*
L2/T3 (ARCH11192A/ARCH12012R)	*94.0*	4.42	*52.1*	9.63*	*51.6*	-3.0
L2/T4 (ARCH11192A/KS115R)	*86.0*	-4.91	*37.5*	-5.10*	*59.4*	-1.9
L3/T1 (ARCH11201A/ARCH10747-1R)	*92.0*	2.30	*35.9*	-3.63	*58.9*	6.0*
L3/T3 (ARCH11201A/ARCH12012R)	*94.0*	0.59	*42.7*	-0.18	*47.9*	-3.0
L3/T4 (ARCH11201A/KS115R)	*94.0*	-0.74	*45.3*	2.28	*56.3*	-1.4
L4/T3 (ATx645/ARCH12012R)	*70.0*	-0.08	*29.7*	-1.57	*43.2*	3.4
L5/T1 (KS116A/ARCH10747-1R)	*84.0*	-4.36	*53.1*	4.88*	*57.3*	0.3
L5/T2 (KS116A/ARCH10747-2R)	*94.0*	-0.74	*54.2*	5.92*	*62.5*	1.1
L5/T3 (KS116A/ARCH12012R)	*94.0*	1.92	*49.0*	-2.61	*52.1*	-3.0
L5/T4 (KS116A/KS115R)	*96.0*	2.59	*43.8*	-7.97*	*62.0*	0.2
L6/T1 (KS133A/ARCH10747-1R)	*80.0*	2.14	*28.6*	1.10	*35.9*	-5.8*
L6/T2 (KS133A/ARCH10747-2R)	*86.0*	1.76	*28.1*	0.58	*40.6*	-5.5*
L6/T3 (KS133A/ARCH12012R)	*66.0*	-15.58	*27.1*	-3.78	*43.2*	3.4
L6/T4 (KS133A/KS115R)	*94.0*	11.09	*33.3*	2.32	*53.1*	6.5*
L7/T1 (KS136A/ARCH10747-1R)	*80.0*	-0.36	*30.7*	1.76	*55.7*	0.6
L7/T2 (KS136A/ARCH10747-2R)	*90.0*	3.26	*30.2*	1.24	*59.9*	0.3
L7/T3 (KS136A/ARCH12012R)	*86.0*	1.92	*32.3*	0.00	*57.8*	4.6*
L7/T4 (KS136A/KS115R)	*80.0*	-5.41	*29.7*	-2.76	*53.1*	-6.9*
L8/T1 (Redbine58A/ARCH10747-1R)	*82.0*	0.97	*17.7*	0.02	*51.6*	4.3*
L8/T2 (Redbine58A/ARCH10747-2R)	*88.0*	0.59	*20.8*	3.14	*54.7*	3.0
L8/T3 (Redbine58A/ARCH12012R)	*84.0*	-0.74	*19.8*	-1.22	*40.1*	-5.3*
SE±(SCAij)	*2.12*		*2.03*		*2.06*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.11 *Per se* and specific combining ability effects (SCA) for emergence index (d) and seedling biomass (g) from stress/early planting treatment from controlled environmental chamber and 2018 and 2019 field experiments.

Hybrids	Emergence index						Seedling biomass			
	Chamber		2018		2019		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*16.1*	0.09	*23.4*	2.44*	*15.0*	-1.06*	*2.66*	-0.61*	*0.28*	0.01
L1/T2 (ARCH11170A/ARCH10747-2R)	*15.9*	-0.06	*19.6*	-0.22	*14.3*	-0.78*	*3.44*	-0.25	*0.17*	-0.12*
L1/T3 (ARCH11170A/ARCH12012R)	-	-	*19.9*	-2.15*	*16.9*	0.31	*2.49*	-0.26	*0.30*	0.04*
L1/T4 (ARCH11170A/KS115R)	*15.8*	0.08	*21.2*	0.26	*16.3*	1.91*	*4.54*	0.93*	*0.37*	0.06*
L2/T1 (ARCH11192A/ARCH10747-1R)	*15.6*	0.16	*19.2*	-1.84*	*15.8*	1.39*	*3.66*	-0.03	*0.34*	0.02
L2/T2 (ARCH11192A/ARCH10747-2R)	*15.2*	-0.30	*19.7*	-0.16	*11.9*	-1.58*	*4.21*	0.10	*0.33*	0.00
L2/T3 (ARCH11192A/ARCH12012R)	*15.3*	-0.17	*23.3*	1.16*	*15.8*	0.85*	*2.91*	-0.25	*0.28*	-0.03*
L2/T4 (ARCH11192A/KS115R)	*15.5*	0.34	*22.2*	1.17*	*12.5*	-0.27	*4.03*	0.00	*0.36*	0.01
L3/T1 (ARCH11201A/ARCH10747-1R)	*15.7*	0.19	*20.0*	-0.47	*16.7*	0.37	*3.45*	0.84*	*0.42*	0.07*
L3/T3 (ARCH11201A/ARCH12012R)	*15.8*	0.27	*22.0*	0.39	*16.3*	-0.49	*2.02*	-0.06	*0.29*	-0.05*
L3/T4 (ARCH11201A/KS115R)	*14.9*	-0.36	*19.7*	-0.80*	*14.6*	-0.04	*2.40*	-0.55*	*0.37*	-0.02
L4/T3 (ATx645/ARCH12012R)	*15.6*	-0.09	*20.5*	-1.04*	*15.7*	-0.96*	*3.90*	0.54*	*0.28*	0.02
L5/T1 (KS116A/ARCH10747-1R)	*15.7*	0.14	*19.2*	-0.29	*12.7*	-0.58	*4.31*	1.00*	*0.43*	0.07*
L5/T2 (KS116A/ARCH10747-2R)	*16.0*	0.38	*18.7*	0.33	*13.1*	0.72*	*2.96*	-0.77*	*0.44*	0.07*
L5/T3 (KS116A/ARCH12012R)	*15.7*	0.09	*19.8*	-0.86*	*14.3*	0.38	*3.27*	0.48*	*0.31*	-0.04*
L5/T4 (KS116A/KS115R)	*14.7*	-0.59	*20.6*	1.16*	*11.6*	-0.14	*2.76*	-0.89*	*0.30*	-0.10*
L6/T1 (KS133A/ARCH10747-1R)	*14.9*	-0.34	*21.2*	-0.78*	*14.1*	-0.32	*2.56*	-0.72*	*0.27*	-0.02
L6/T2 (KS133A/ARCH10747-2R)	*15.6*	0.35	*20.5*	-0.33	*15.0*	1.52*	*3.96*	0.26	*0.42*	0.12*
L6/T3 (KS133A/ARCH12012R)	*15.6*	0.29	*26.1*	3.05*	*15.9*	0.96*	*1.69*	-1.07*	*0.25*	-0.03*
L6/T4 (KS133A/KS115R)	*14.7*	-0.27	*20.4*	-1.61*	*11.0*	-1.78*	*4.97*	1.35*	*0.25*	-0.07*
L7/T1 (KS136A/ARCH10747-1R)	*15.3*	-0.28	*21.3*	1.05*	*14.7*	1.64*	*3.74*	0.21	*0.22*	-0.08*
L7/T2 (KS136A/ARCH10747-2R)	*15.3*	-0.32	*19.7*	0.61	*13.0*	0.88*	*4.84*	0.89*	*0.25*	-0.07*
L7/T3 (KS136A/ARCH12012R)	*15.5*	-0.08	*20.3*	-1.06*	*11.2*	-2.36*	*2.70*	-0.32	*0.34*	0.06*
L7/T4 (KS136A/KS115R)	*16.0*	0.70	*19.9*	-0.27	*11.6*	0.23	*2.92*	-0.96*	*0.41*	0.08*
L8/T1 (Redbine58A/ARCH10747-1R)	*15.5*	-0.03	*20.5*	-0.10	*13.1*	-1.49*	*2.44*	-0.22	*0.20*	-0.08*
L8/T2 (Redbine58A/ARCH10747-2R)	*15.4*	-0.15	*19.0*	-0.39	*14.5*	0.92*	*2.67*	-0.41*	*0.32*	0.03*
L8/T3 (Redbine58A/ARCH12012R)	*15.5*	-0.02	*22.4*	0.74*	*14.8*	-0.27	*2.91*	0.78*	*0.33*	0.06*
SE±(SCAij)	*0.07*		*0.32*		*0.35*		*0.17*		*0.01*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.12 *Per se* and specific combining ability effects (SCA) for days to 50% flowering (GDU) and plant height (cm) from early planting treatment from 2018 and 2019 field experiments.

Hybrids	Days to 50% flowering				Plant height			
	2018		2019		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*1829.7*	47.7*	*1821.3*	-18.8	*112.0*	5.2	*118.3*	2.9
L1/T2 (ARCH11170A/ARCH10747-2R)	*1733.8*	39.9*	*1732.3*	-8.9	*105.5*	5.0	*119.2*	8.8
L1/T3 (ARCH11170A/ARCH12012R)	*1829.7*	-45.6*	*1892.7*	-16.9	*98.0*	-5.7	*98.7*	-6.1
L1/T4 (ARCH11170A/KS115R)	*1843.8*	-24.6	*1920.8*	66.9	*169.0*	-12.7	*163.8*	-13.9*
L2/T1 (ARCH11192A/ARCH10747-1R)	*1762.0*	21.4	*1732.3*	-15.0	*119.3*	-3.7	*118.3*	1.8
L2/T2 (ARCH11192A/ARCH10747-2R)	*1685.8*	33.4	*1663.9*	15.6	*118.5*	1.8	*118.3*	7.0
L2/T3 (ARCH11192A/ARCH12012R)	*1863.5*	29.7	*1846.7*	29.9	*116.3*	-3.5	*111.3*	5.3
L2/T4 (ARCH11192A/KS115R)	*1759.9*	-67.0*	*1752.9*	-8.2	*195.0*	-2.9	*156.3*	-22.4*
L3/T1 (ARCH11201A/ARCH10747-1R)	*1720.2*	-4.6	*1718.6*	-65.7*	*120.0*	-10.2	*110.0*	-5.5
L3/T3 (ARCH11201A/ARCH12012R)	*1773.9*	-44.2*	*1895.5*	41.6*	*107.0*	-20.1*	*114.6*	9.7
L3/T4 (ARCH11201A/KS115R)	*1762.0*	-49.1*	*1744.0*	-54*	*206.8*	1.6	*150.4*	-27.2*
L4/T3 (ATx645/ARCH12012R)	*1815.7*	-66.1*	*1951.8*	-67.8*	*114.3*	17.5*	*110.0*	20.1*
L5/T1 (KS116A/ARCH10747-1R)	*1685.3*	-36.1	*1815.1*	25.8	*108.0*	5.0	*118.3*	-2.3
L5/T2 (KS116A/ARCH10747-2R)	*1645.2*	11.9	*1663.9*	-26.5	*96.0*	-0.7	*96.7*	-18.9*
L5/T3 (KS116A/ARCH12012R)	*1834.5*	19.7	*1895.5*	36.6*	*104.5*	4.7	*89.2*	-20.9*
L5/T4 (KS116A/KS115R)	*1829.7*	22.0	*1789.7*	-13.5	*160.8*	-17.2*	*216.7*	33.8*
L6/T1 (KS133A/ARCH10747-1R)	*1759.9*	-26.2	*1846.7*	34.9*	*110.5*	-8.1	*114.6*	0.1
L6/T2 (KS133A/ARCH10747-2R)	*1680.1*	-17.9	*1698.0*	-14.9	*108.3*	-4.1	*97.9*	-11.5
L6/T3 (KS133A/ARCH12012R)	*1905.3*	25.9	*1842.0*	-39.4*	*117.5*	2.0	*102.5*	-1.5
L6/T4 (KS133A/KS115R)	*1908.2*	35.7	*1867.3*	41.7*	*195.5*	1.9	*181.3*	4.6
L7/T1 (KS136A/ARCH10747-1R)	*1762.0*	-11.6	*1892.5*	22.5	*113.8*	-7.1	*109.2*	-11.9
L7/T2 (KS136A/ARCH10747-2R)	*1572.1*	-113.4*	*1806.4*	35.3*	*105.5*	-9.1	*106.7*	-9.3
L7/T3 (KS136A/ARCH12012R)	*1964.0*	97.1*	*1920.8*	-18.8	*120.8*	3.0	*112.1*	1.6
L7/T4 (KS136A/KS115R)	*1905.3*	45.4*	*1867.3*	-16.6	*200.8*	4.9	*194.6*	11.3
L8/T1 (Redbine58A/ARCH10747-1R)	*1720.2*	43.1*	*1842.0*	66.2*	*107.5*	16.8*	*116.3*	14.5*
L8/T2 (Redbine58A/ARCH10747-2R)	*1645.2*	56.2*	*1700.7*	23.9	*106.8*	22.3*	*120.4*	23.8*
L8/T3 (Redbine58A/ARCH12012R)	*1747.7*	-22.7	*1789.7*	-55.6*	*100.8*	13.2	*97.1*	5.9
SE±(SCAij)	*18.18*		*16.34*		*6.78*		*6.23*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.13 *Per se* and specific combining ability effects (SCA) for 200-kernel weight (g) and plot yield (g m^{-2}) from early planting treatment from 2018 and 2019 field experiments.

Hybrids	200-kernel weight				Plot yield			
	2018		2019		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*4.75*	0.30	*3.63*	0.07	*272.2*	5.6	*491.6*	-55.4
L1/T2 (ARCH11170A/ARCH10747-2R)	*4.22*	0.19	*3.52*	-0.15	*295.3*	30.1	*575.7*	-50.0
L1/T3 (ARCH11170A/ARCH12012R)	*4.02*	-0.31	*3.37*	-0.19*	*266.0*	-36.0*	*240.3*	-149.1*
L1/T4 (ARCH11170A/KS115R)	*6.45*	-0.43	*4.43*	0.18	*343.3*	-1.8	*773.0*	215.3*
L2/T1 (ARCH11192A/ARCH10747-1R)	*4.57*	-0.18	*3.93*	-0.23*	*278.0*	-106.3*	*473.3*	-50.1
L2/T2 (ARCH11192A/ARCH10747-2R)	*4.60*	0.26	*4.15*	-0.11	*385.3*	2.4	*786.2*	184.1*
L2/T3 (ARCH11192A/ARCH12012R)	*4.27*	-0.33	*3.77*	-0.38*	*518.5*	98.8*	*101.2*	-264.8*
L2/T4 (ARCH11192A/KS115R)	*7.22*	0.00	*5.48*	0.63*	*465.9*	3.1	*625.9*	91.7*
L3/T1 (ARCH11201A/ARCH10747-1R)	*4.92*	-0.72*	*3.53*	-0.25*	*371.2*	68.0*	*535.9*	130.7*
L3/T3 (ARCH11201A/ARCH12012R)	*5.52*	-0.02	*3.82*	0.05	*291.7*	-46.9*	*239.0*	-8.7
L3/T4 (ARCH11201A/KS115R)	*5.92*	0.54*	*4.10*	0.19*	*283.2*	-7.8	*645.3*	130.7*
L4/T3 (ATx645/ARCH12012R)	*7.77*	-0.34	*4.50*	0.03	*329.6*	-52.1*	*360.3*	-55.6
L5/T1 (KS116A/ARCH10747-1R)	*5.08*	0.15	*4.33*	0.30*	*329.3*	-15.4	*844.4*	228.0*
L5/T2 (KS116A/ARCH10747-2R)	*4.32*	-0.16	*4.38*	0.26*	*354.8*	11.6	*534.4*	-160.8*
L5/T3 (KS116A/ARCH12012R)	*4.65*	-0.16	*4.17*	0.15	*381.1*	1.1	*630.5*	171.6*
L5/T4 (KS116A/KS115R)	*7.32*	-0.08	*3.92*	-0.80*	*423.7*	0.6	*349.3*	-277.9*
L6/T1 (KS133A/ARCH10747-1R)	*5.88*	-0.01	*3.88*	0.04	*301.6*	5.7	*369.7*	43.0
L6/T2 (KS133A/ARCH10747-2R)	*5.23*	-0.22	*4.25*	0.31*	*259.0*	-35.4*	*232.1*	-173.4*
L6/T3 (KS133A/ARCH12012R)	*5.75*	-0.02	*3.63*	-0.19*	*325.8*	-5.5	*134.3*	-34.9
L6/T4 (KS133A/KS115R)	*8.32*	0.01	*4.28*	-0.25*	*407.5*	33.2*	*463.7*	126.2*
L7/T1 (KS136A/ARCH10747-1R)	*5.47*	-0.11	*3.85*	0.00	*356.0*	34.2*	*362.0*	-107.1*
L7/T2 (KS136A/ARCH10747-2R)	*5.20*	0.03	*3.40*	-0.54*	*315.9*	-4.4	*653.9*	106.0*
L7/T3 (KS136A/ARCH12012R)	*5.20*	-0.27	*4.13*	0.30*	*413.4*	56.2*	*344.0*	32.4
L7/T4 (KS136A/KS115R)	*8.13*	0.11	*4.68*	0.15	*312.2*	-88.0*	*409.5*	-70.4
L8/T1 (Redbine58A/ARCH10747-1R)	*4.93*	0.78*	*3.87*	0.14	*205.2*	27.3	*326.2*	-109.9*
L8/T2 (Redbine58A/ARCH10747-2R)	*4.42*	0.67*	*4.05*	0.22*	*192.5*	16.1	*607.2*	92.3*
L8/T3 (Redbine58A/ARCH12012R)	*4.37*	0.33	*3.78*	0.07	*218.6*	5.4	*294.7*	16.0
SE±(SCAij)	*0.25*		*0.09*		*15.04*		*38.72*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.14 *Per se* and specific combining ability effects (SCA) for emergence (%) from control/regular planting treatment from controlled environmental chamber and 2018 and 2019 field experiments.

Hybrids	Emergence %					
	Chamber		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*80.0*	3.85*	*46.4*	-2.24	*16.1*	-14.7*
L1/T2 (ARCH11170A/ARCH10747-2R)	*80.0*	1.18	*44.3*	-7.36*	*24.2*	-8.8*
L1/T3 (ARCH11170A/ARCH12012R)	-	-	*63.0*	9.35*	*50.0*	16.3*
L1/T4 (ARCH11170A/KS115R)	*88.0*	-1.15	*47.9*	1.06	*52.3*	5.9
L2/T1 (ARCH11192A/ARCH10747-1R)	*92.0*	-4.82*	*43.8*	-4.07*	*43.2*	-10.7*
L2/T2 (ARCH11192A/ARCH10747-2R)	*100.0*	-7.15*	*47.9*	-2.94*	*51.3*	-4.8
L2/T3 (ARCH11192A/ARCH12012R)	*100.0*	-5.82*	*58.9*	5.96*	*63.0*	6.2
L2/T4 (ARCH11192A/KS115R)	*100.0*	0.18	*47.9*	1.84	*77.3*	7.9*
L3/T1 (ARCH11201A/ARCH10747-1R)	*100.0*	4.85	*57.3*	3.88*	*76.3*	5.9
L3/T3 (ARCH11201A/ARCH12012R)	*100.0*	8.18*	*57.8*	-0.68	*73.2*	-0.1
L3/T4 (ARCH11201A/KS115R)	*84.0*	-1.15	*50.5*	-1.16	*76.0*	-9.9*
L4/T3 (ATx645/ARCH12012R)	*84.0*	-0.15	*49.0*	-3.28*	*61.2*	1.9
L5/T1 (KS116A/ARCH10747-1R)	*68.0*	2.18	*53.1*	-0.94	*74.2*	0.4
L5/T2 (KS116A/ARCH10747-2R)	*100.0*	3.85*	*62.5*	5.40*	*77.3*	1.4
L5/T3 (KS116A/ARCH12012R)	*96.0*	9.18*	*51.0*	-8.10*	*75.5*	-1.1
L5/T4 (KS116A/KS115R)	*96.0*	5.18*	*56.8*	4.44*	*87.2*	-2.1
L6/T1 (KS133A/ARCH10747-1R)	*100.0*	-0.15	*51.0*	6.09*	*67.7*	5.6
L6/T2 (KS133A/ARCH10747-2R)	*96.0*	-12.82*	*50.0*	2.01	*63.5*	-0.8
L6/T3 (KS133A/ARCH12012R)	*92.0*	-6.15*	*46.9*	-3.15*	*61.5*	-3.5
L6/T4 (KS133A/KS115R)	*100.0*	2.51	*39.1*	-4.15*	*75.0*	-2.7
L7/T1 (KS136A/ARCH10747-1R)	*88.0*	-0.82	*51.0*	5.05*	*72.7*	1.2
L7/T2 (KS136A/ARCH10747-2R)	*88.0*	-2.15	*50.0*	-0.07	*78.2*	4.6
L7/T3 (KS136A/ARCH12012R)	*96.0*	9.18*	*46.9*	2.05	*77.3*	3.1
L7/T4 (KS136A/KS115R)	*92.0*	-10.82*	*39.1*	-6.24*	*76.6*	-10.4*
L8/T1 (Redbine58A/ARCH10747-1R)	*96.0*	1.18	*37.0*	-8.45*	*59.6*	11.5*
L8/T2 (Redbine58A/ARCH10747-2R)	*100.0*	1.18	*55.2*	6.74*	*69.8*	19.4*
L8/T3 (Redbine58A/ARCH12012R)	*72.0*	0.51	*49.5*	-1.03	*29.4*	-21.6*
SE±(SCAij)	*1.83*		*1.32*		*3.55*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.15 *Per se* and specific combining ability effects (SCA) for emergence index (d) and seedling biomass (g) from control/regular planting treatment from controlled environmental chamber and 2018 and 2019 field experiments.

Hybrids	Emergence index						Seedling biomass			
	Chamber		2018		2019		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*3.2*	0.19*	*8.2*	0.20*	*8.2*	0.25	*10.29*	-0.30	*5.20*	-0.08
L1/T2 (ARCH11170A/ARCH10747-2R)	*3.2*	-0.08	*8.1*	0.29*	*7.9*	0.15	*11.18*	0.31	*5.65*	0.33
L1/T3 (ARCH11170A/ARCH12012R)	-	-	*7.5*	-0.48*	*7.8*	-0.28	*8.84*	-0.34	*4.30*	-0.07
L1/T4 (ARCH11170A/KS115R)	*2.8*	-0.07	*7.6*	0.07	*7.6*	-0.03	*10.05*	0.11	*5.32*	-0.40
L2/T1 (ARCH11192A/ARCH10747-1R)	*2.6*	0.01	*8.5*	0.37*	*8.2*	0.26	*11.33*	0.68*	*5.07*	-0.79*
L2/T2 (ARCH11192A/ARCH10747-2R)	*2.8*	0.27*	*8.0*	0.10	*8.0*	0.21	*11.38*	0.44	*5.37*	-0.53*
L2/T3 (ARCH11192A/ARCH12012R)	*2.6*	-0.16*	*7.9*	-0.18*	*8.0*	-0.12	*8.89*	-0.36	*4.47*	-0.47*
L2/T4 (ARCH11192A/KS115R)	*2.7*	0.20*	*7.4*	-0.22*	*7.4*	-0.27	*9.04*	-0.98*	*7.85*	1.56*
L3/T1 (ARCH11201A/ARCH10747-1R)	*2.6*	-0.06	*7.8*	0.09	*7.6*	-0.01	*10.46*	2.05*	*7.62*	2.39*
L3/T3 (ARCH11201A/ARCH12012R)	*2.7*	-0.05	*7.4*	-0.33*	*7.5*	-0.29	*6.65*	-0.35	*2.80*	-1.52*
L3/T4 (ARCH11201A/KS115R)	*2.8*	0.07	*7.5*	0.23*	*7.6*	0.26	*6.63*	-1.13*	*4.78*	-0.89*
L4/T3 (ATx645/ARCH12012R)	*3.2*	-0.13*	*8.3*	-0.15	*8.3*	-0.21	*8.41*	0.91*	*7.07*	0.75*
L5/T1 (KS116A/ARCH10747-1R)	*2.6*	0.08	*8.2*	0.34*	*8.2*	0.35	*8.86*	1.22*	*5.88*	-0.59*
L5/T2 (KS116A/ARCH10747-2R)	*3.2*	-0.21*	*7.9*	-0.39*	*7.7*	-0.48	*9.65*	1.37*	*5.45*	-0.58*
L5/T3 (KS116A/ARCH12012R)	*2.7*	-0.45*	*8.1*	0.07	*8.1*	0.12	*6.46*	-2.11*	*6.47*	0.40
L5/T4 (KS116A/KS115R)	*3.1*	-0.15*	*8.3*	0.06	*8.4*	0.09	*6.17*	-0.71*	*5.67*	0.55*
L6/T1 (KS133A/ARCH10747-1R)	*2.6*	-0.03	*7.3*	-0.45*	*7.4*	-0.24	*8.16*	-0.22	*5.38*	-1.03*
L6/T2 (KS133A/ARCH10747-2R)	*3.1*	0.34*	*8.0*	-0.14	*7.8*	-0.22	*7.32*	-1.70*	*6.40*	0.42
L6/T3 (KS133A/ARCH12012R)	*3.0*	0.25*	*8.0*	0.04	*7.7*	-0.07	*11.47*	2.17*	*6.97*	0.95*
L6/T4 (KS133A/KS115R)	*2.1*	0.00	*8.8*	0.62*	*8.7*	0.61	*7.13*	-0.48	*4.50*	-0.56*
L7/T1 (KS136A/ARCH10747-1R)	*3.2*	0.24*	*8.0*	-0.13	*7.8*	0.10	*7.32*	-2.42*	*5.03*	-0.82*
L7/T2 (KS136A/ARCH10747-2R)	*2.5*	0.06	*8.0*	-0.21*	*7.3*	-0.20	*11.47*	-0.87*	*5.17*	-0.73*
L7/T3 (KS136A/ARCH12012R)	*3.0*	-0.32*	*8.8*	0.34*	*8.0*	0.17	*7.13*	1.76*	*4.77*	-0.18
L7/T4 (KS136A/KS115R)	*2.9*	-0.03	*7.3*	0.07	*7.4*	0.02	*8.16*	1.31*	*7.80*	1.51*
L8/T1 (Redbine58A/ARCH10747-1R)	*2.7*	0.02	*8.0*	0.22*	*8.1*	0.32	*10.30*	0.37	*5.35*	0.07
L8/T2 (Redbine58A/ARCH10747-2R)	*2.4*	-0.25*	*7.3*	-0.33*	*7.3*	-0.28	*9.08*	-1.14*	*5.02*	-0.31
L8/T3 (Redbine58A/ARCH12012R)	*3.0*	0.25*	*7.7*	-0.10	*7.7*	-0.21	*8.92*	0.40	*4.98*	0.62*
SE±(SCAij)	*0.06*		*0.08*		*0.07*		*0.34*		*0.23*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.16 *Per se* and specific combining ability effects (SCA) for days to 50% flowering (GDU) and plant height (cm) from regular early planting treatment from 2018 and 2019 field experiments.

Hybrids	Days to 50% flowering				Plant height			
	2018		2019		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*1795.0*	23.1	*1808.9*	112.6*	*123.5*	-3.0	*119.2*	8.4
L1/T2 (ARCH11170A/ARCH10747-2R)	*1744.6*	44.2*	*1608.3*	-48.8*	*115.8*	-1.2	*108.3*	8.1
L1/T3 (ARCH11170A/ARCH12012R)	*1795.0*	-34.8*	*1639.2*	-59.3*	*109.5*	-7.9	*105.8*	2.3
L1/T4 (ARCH11170A/KS115R)	*1795.0*	-18.5	*1751.5*	-6.6	*224.3*	0.7	*144.2*	-25.5*
L2/T1 (ARCH11192A/ARCH10747-1R)	*1721.6*	-12.0	*1579.9*	-32.3*	*132.3*	-9.7	*128.3*	4.0
L2/T2 (ARCH11192A/ARCH10747-2R)	*1665.0*	2.9	*1669.8*	96.9*	*132.8*	0.4	*117.9*	4.1
L2/T3 (ARCH11192A/ARCH12012R)	*1795.0*	3.4	*1551.7*	-62.6*	*130.5*	-2.4	*121.7*	4.6
L2/T4 (ARCH11192A/KS115R)	*1795.0*	19.7	*1669.8*	-4.1	*239.3*	0.3	*163.8*	-19.4*
L3/T1 (ARCH11201A/ARCH10747-1R)	*1694.2*	-49.0*	*1579.9*	-42.5*	*137.8*	-9.4	*130.8*	-17.5*
L3/T3 (ARCH11201A/ARCH12012R)	*1772.0*	-29.1*	*1665.1*	40.6*	*127.3*	-10.8	*114.6*	-26.6*
L3/T4 (ARCH11201A/KS115R)	*1795.0*	10.1	*1639.2*	-45.0*	*226.8*	-17.5	*225.4*	18.2*
L4/T3 (ATx645/ARCH12012R)	*1795.0*	-47.4*	*1695.7*	3.5	*120.5*	25.8*	*114.2*	15.8*
L5/T1 (KS116A/ARCH10747-1R)	*1796.1*	-2.3	*1608.5*	18.8	*120.8*	-0.9	*100.8*	-14.8*
L5/T2 (KS116A/ARCH10747-2R)	*1744.6*	17.7	*1494.9*	-55.7*	*107.8*	-4.3	*102.9*	-2.2
L5/T3 (KS116A/ARCH12012R)	*1900.1*	43.7*	*1608.5*	16.6	*113.5*	0.9	*108.3*	-0.1
L5/T4 (KS116A/KS115R)	*1795.0*	-45.1*	*1669.8*	18.2	*211.5*	-7.2	*185.0*	10.5
L6/T1 (KS133A/ARCH10747-1R)	*1772.0*	41.1*	*1608.5*	-2.4	*130.8*	0.5	*112.5*	-2.8
L6/T2 (KS133A/ARCH10747-2R)	*1603.7*	-55.7*	*1582.4*	10.6	*122.5*	1.8	*100.8*	-4.0
L6/T3 (KS133A/ARCH12012R)	*1795.0*	6.1	*1579.9*	-33.3*	*118.5*	-2.7	*115.8*	7.7
L6/T4 (KS133A/KS115R)	*1795.0*	22.5	*1695.7*	23.0	*216.3*	-11.1	*166.7*	-7.5
L7/T1 (KS136A/ARCH10747-1R)	*1795.0*	-20.6	*1579.9*	-53.0*	*138.5*	-1.4	*124.2*	0.3
L7/T2 (KS136A/ARCH10747-2R)	*1772.0*	27.9*	*1608.5*	14.9	*121.5*	-8.9	*109.2*	-4.2
L7/T3 (KS136A/ARCH12012R)	*1900.1*	26.5	*1669.8*	34.8*	*127.3*	-3.6	*113.8*	-2.9
L7/T4 (KS136A/KS115R)	*1837.5*	-19.7	*1695.7*	1.1	*239.5*	2.5	*182.9*	0.2
L8/T1 (Redbine58A/ARCH10747-1R)	*1749.1*	43.7*	*1610.5*	25.9	*120.8*	20.1*	*117.9*	16.0*
L8/T2 (Redbine58A/ARCH10747-2R)	*1603.7*	-30.2*	*1551.7*	6.3	*117.5*	26.4*	*112.5*	21.1*
L8/T3 (Redbine58A/ARCH12012R)	*1795.0*	31.7*	*1608.5*	21.8	*114.0*	22.4*	*101.3*	6.5
SE±(SCAij)	*13.71*		*12.30*		*8.37*		*6.14*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.17 *Per se* and specific combining ability effects (SCA) for 200-kernel weight (g) and plot yield (g m^{-2}) from regular planting treatment from 2018 and 2019 field experiments.

Hybrids	200-kernel weight				Plot yield			
	2018		2019		2018		2019	
	per se	SCA	*per se*	SCA	*per se*	SCA	*per se*	SCA
L1/T1 (ARCH11170A/ARCH10747-1R)	*4.83*	0.14	*6.20*	0.03	*395.7*	32.8	*889.9*	208.3*
L1/T2 (ARCH11170A/ARCH10747-2R)	*4.95*	0.08	*5.78*	0.10	*400.1*	-14.7	*654.4*	5.9
L1/T3 (ARCH11170A/ARCH12012R)	*4.40*	-0.07	*6.20*	0.11	*312.8*	-90.9*	*454.9*	-105.1*
L1/T4 (ARCH11170A/KS115R)	*6.80*	-0.51*	*6.23*	-0.24	*347.3*	85.9*	*413.9*	-110.5*
L2/T1 (ARCH11192A/ARCH10747-1R)	*5.30*	0.34	*6.42*	1.16*	*297.7*	-67.6*	*692.1*	-97.8*
L2/T2 (ARCH11192A/ARCH10747-2R)	*5.22*	0.07	*4.08*	-0.68*	*470.7*	53.5*	*851.7*	95.0*
L2/T3 (ARCH11192A/ARCH12012R)	*4.25*	-0.49	*3.95*	-1.22*	*448.7*	42.6*	*469.7*	-198.4*
L2/T4 (ARCH11192A/KS115R)	*7.32*	-0.27	*6.30*	0.75*	*248.4*	-15.4	*832.4*	199.8*
L3/T1 (ARCH11201A/ARCH10747-1R)	*5.55*	0.11	*6.32*	-0.06	*380.4*	27.2	*1160.7*	212.4*
L3/T3 (ARCH11201A/ARCH12012R)	*4.93*	-0.28	*6.22*	-0.08	*431.0*	37.0*	*661.7*	-164.9*
L3/T4 (ARCH11201A/KS115R)	*7.50*	-0.56*	*6.40*	-0.28	*251.4*	-0.3	*787.3*	-3.8
L4/T3 (ATx645/ARCH12012R)	*5.23*	0.78*	*6.23*	0.01	*489.9*	-39.7*	*759.4*	43.3
L5/T1 (KS116A/ARCH10747-1R)	*4.95*	0.15	*3.77*	-1.24*	*395.8*	-2.6	*602.9*	-111.5*
L5/T2 (KS116A/ARCH10747-2R)	*4.93*	-0.05	*3.63*	-0.89*	*453.5*	3.1	*611.9*	-69.3
L5/T3 (KS116A/ARCH12012R)	*4.18*	-0.39	*6.00*	1.07*	*436.1*	-3.1	*630.3*	37.5
L5/T4 (KS116A/KS115R)	*7.35*	-0.07	*6.37*	1.06*	*312.6*	15.7	*699.2*	142.0*
L6/T1 (KS133A/ARCH10747-1R)	*4.80*	-1.00*	*6.27*	-0.01	*513.8*	97.7*	*665.1*	-230.3*
L6/T2 (KS133A/ARCH10747-2R)	*5.97*	-0.02	*6.23*	0.44*	*444.3*	-23.7	*1112.7*	250.4*
L6/T3 (KS133A/ARCH12012R)	*5.88*	0.30	*6.02*	-0.18	*450.0*	-6.9	*712.3*	-61.5
L6/T4 (KS133A/KS115R)	*8.78*	0.36	*6.33*	-0.24	*260.4*	-54.1*	*778.2*	40.0
L7/T1 (KS136A/ARCH10747-1R)	*5.03*	-0.22	*6.40*	0.24	*519.7*	18.6	*699.9*	72.3
L7/T2 (KS136A/ARCH10747-2R)	*5.03*	-0.40	*6.12*	0.45*	*506.9*	-46.1*	*466.5*	-127.8*
L7/T3 (KS136A/ARCH12012R)	*4.75*	-0.28	*6.07*	-0.01	*587.3*	45.4*	*446.7*	387.7*
L7/T4 (KS136A/KS115R)	*8.43*	0.56*	*5.78*	-0.67*	*394.7*	-4.8	*68.3*	-333.6*
L8/T1 (Redbine58A/ARCH10747-1R)	*5.10*	0.39	*6.20*	-0.28	*325.0*	-69.8*	*645.8*	-68.3
L8/T2 (Redbine58A/ARCH10747-2R)	*5.47*	0.58*	*6.85*	0.86*	*460.0*	13.3	*694.8*	14.0
L8/T3 (Redbine58A/ARCH12012R)	*5.23*	0.75*	*6.18*	-0.21	*402.6*	-33.0	*566.4*	-26.0
SE±(SCAij)	*0.24*		*0.18*		*17.43*		*41.91*	

* Significant at 5% probability level; L – line, T – tester.

Table 3.18 Better-parent heterosis (d_{ii}) of seedling traits from stress/early planting treatment from controlled environment chambers and 2018 and 2019 field experiments.

Hybrids	Seedling traits							
	EP			EI			SB	
	Chamber	2018	2019	Chamber	2018	2019	2018	2019
L1/T1 (ARCH11170A/ARCH10747-1R)	-20.0*	-50.0*	-23.3*	-0.5	25.8*	-4.5	-0.6	43.1
L1/T2 (ARCH11170A/ARCH10747-2R)	-20.0*	-53.3*	-57.6*	-1.7	5.4	-25.3*	10.0	44.8
L1/T3 (ARCH11170A/ARCH12012R)	-	-32.6*	17.5*	-	7.2	-11.8	-11.7	44.3
L1/T4 (ARCH11170A/KS115R)	-24.4*	-47.8*	46.9*	-3.7*	14.0	16.4	5.0	46.8
L2/T1 (ARCH11192A/ARCH10747-1R)	10.0*	-31.7*	1.1	-2.0	8.8	46.6*	46.5	22.5
L2/T2 (ARCH11192A/ARCH10747-2R)	0.0	-27.7*	12.8*	-4.7*	11.8	10.1	34.6	19.7
L2/T3 (ARCH11192A/ARCH12012R)	17.5*	-1.0	10.0	-3.8*	32.1*	46.6*	3.3	0.4
L2/T4 (ARCH11192A/KS115R)	4.9	-28.7*	26.7*	-2.5*	25.7	15.9	-6.8	31.0
L3/T1 (ARCH11201A/ARCH10747-1R)	21.1*	15.0	31.4*	-2.8*	8.0	5.9	62.6	114.0
L3/T3 (ARCH11201A/ARCH12012R)	20.5*	36.7*	46.0*	-0.7	18.6	3.8	-28.3	40.1
L3/T4 (ARCH11201A/KS115R)	14.6*	45.0*	120.4*	-8.0*	6.1	4.1	-44.4	48.0
L4/T3 (ATx645/ARCH12012R)	-12.8	-38.7*	-22.4*	-2.9*	2.5	14.9	38.4	-31.9
L5/T1 (KS116A/ARCH10747-1R)	-8.7*	121.7*	27.9*	-2.5*	-3.8	20.6	169.0	118.7
L5/T2 (KS116A/ARCH10747-2R)	2.2	116.7*	90.5*	0.4	-12.9	23.9	4.9	112.0
L5/T3 (KS116A/ARCH12012R)	2.2	95.8*	58.7*	-1.3	-7.9	34.9	15.8	49.9
L5/T4 (KS116A/KS115R)	4.3	281.8*	142.9*	-8.9*	3.1	9.3	-36.1	17.9
L6/T1 (KS133A/ARCH10747-1R)	-9.1*	7.8	-19.8*	-4.7*	6.1	-3.1	59.8	37.4
L6/T2 (KS133A/ARCH10747-2R)	-4.4	-23.9*	-37.6*	-0.1	-6.0	3.0	26.7	207.0
L6/T3 (KS133A/ARCH12012R)	-25.0*	2.0	5.1	-0.4	21.9*	9.5	-39.9	17.7
L6/T4 (KS133A/KS115R)	-9.1*	25.5*	29.1*	2.5	1.6	-21.6	15.1	-0.7
L7/T1 (KS136A/ARCH10747-1R)	-14.9*	28.3*	10.3*	-5.0*	6.5	-1.6	118.5	12.8
L7/T2 (KS136A/ARCH10747-2R)	-4.3	-18.3*	-8.0*	-5.2*	-4.1	-13.1	54.7	77.8
L7/T3 (KS136A/ARCH12012R)	-8.5*	29.2*	14.4*	-2.5*	-1.2	-24.8	-4.4	65.2
L7/T4 (KS136A/KS115R)	0.0	23.9*	5.2	-8.9*	-0.5	-17.2	-32.5	64.2
L8/T1 (Redbine58A/ARCH10747-1R)	-6.4	-70.2*	15.1*	-3.5*	2.4	-6.4	3.4	-29.3
L8/T2 (Redbine58A/ARCH10747-2R)	-10.6	-64.9*	-16.0*	-2.5*	-6.5	4.0	-14.7	14.5
L8/T3 (Redbine58A/ARCH12012R)	-10.3	-66.7*	-9.4	0.8	10.1	6.2	3.4	15.9

* Significant at 5% probability level; L – line, T – tester.

Table 3.19 Better-parent heterosis (d_{ii}) of seedling traits from early planting treatment from 2018 and 2019 field experiments.

Hybrids	Agronomic traits							
	DF		PH		KW		PY	
	2018	2019	2018	2019	2018	2019	2018	2019
L1/T1 (ARCH11170A/ARCH10747-1R)	3.8*	-2.9*	21.4*	34.6*	3.3	-5.6	-16.5*	10.4*
L1/T2 (ARCH11170A/ARCH10747-2R)	6.2*	2.0*	15.9*	23.8*	4.5	-8.7	-9.5*	21.2*
L1/T3 (ARCH11170A/ARCH12012R)	3.8*	4.3*	1.0	11.3*	-3.6	-16.5	-18.4*	-40.5*
L1/T4 (ARCH11170A/KS115R)	4.6*	-1.4*	69.8*	63.1*	-15.5	5.1	5.2*	89.0*
L2/T1 (ARCH11192A/ARCH10747-1R)	-4.0*	-7.6*	1.5	9.2*	-0.7	5.4	-43.8*	-29.1*
L2/T2 (ARCH11192A/ARCH10747-2R)	3.3*	-2.0*	0.9	9.2*	9.1	7.8	-22.1*	17.7*
L2/T3 (ARCH11192A/ARCH12012R)	1.6*	1.7*	-1.1	2.7*	1.2	-6.6	4.8*	-84.9*
L2/T4 (ARCH11192A/KS115R)	-9.2*	-10.1*	66.0*	44.2*	-5.5	30.0	-5.8*	-6.3*
L3/T1 (ARCH11201A/ARCH10747-1R)	-5.3*	-8.3*	-3.0	-4.7*	-0.3	-15.5	31.8*	-9.6*
L3/T3 (ARCH11201A/ARCH12012R)	-2.3*	4.4*	-13.5*	-0.7	11.8	-8.8	3.6*	-59.7*
L3/T4 (ARCH11201A/KS115R)	-3.0*	-10.5*	67.1*	30.3*	1.7	6.7	17.0*	-39.2*
L4/T3 (ATx645/ARCH12012R)	-1.0*	7.5*	-1.5	-2.9*	-4.3	-2.4	-23.6*	59.7*
L5/T1 (KS116A/ARCH10747-1R)	-8.1*	-3.2*	17.1*	1.8	10.5	16.1	44.6*	89.7*
L5/T2 (KS116A/ARCH10747-2R)	-10.3*	-8.3*	-1.0	-16.8*	3.6	8.7	88.5*	23.8*
L5/T3 (KS116A/ARCH12012R)	0.0	4.4*	7.7*	-23.3*	11.6	3.3	102.5*	46.1*
L5/T4 (KS116A/KS115R)	-9.7*	-8.2*	61.6*	86.4*	-4.1	-7.1	300.7*	-19.1*
L6/T1 (KS133A/ARCH10747-1R)	-4.1*	-1.5*	5.2*	-0.7	27.9	3.6	24.4*	-36.9*
L6/T2 (KS133A/ARCH10747-2R)	2.9*	0.0	3.1	-15.2*	15.0	10.4	-0.8	-60.4*
L6/T3 (KS133A/ARCH12012R)	3.9*	1.5*	11.9*	-11.2*	26.4	-9.9	34.4*	-77.1*
L6/T4 (KS133A/KS115R)	-5.8*	-4.2*	86.2*	57.0*	9.0	1.6	68.1*	-20.9*
L7/T1 (KS136A/ARCH10747-1R)	-4.0*	0.9*	9.1*	6.9*	7.2	-1.7	45.9*	-53.1*
L7/T2 (KS136A/ARCH10747-2R)	-3.7*	6.4*	1.2	4.5*	2.0	-13.2	21.0*	-15.3*
L7/T3 (KS136A/ARCH12012R)	7.1*	5.8*	15.8*	9.8*	2.0	2.5	69.5*	-55.4*
L7/T4 (KS136A/KS115R)	-5.9*	-4.2*	92.6*	90.6*	6.6	11.1	28.0*	-46.9*
L8/T1 (Redbine58A/ARCH10747-1R)	3.7*	1.1*	3.4	14.3*	4.6	3.6	-53.6*	-26.7*
L8/T2 (Redbine58A/ARCH10747-2R)	0.8*	0.2	2.6	18.4*	-6.4	5.2	-56.5*	27.9*
L8/T3 (Redbine58A/ARCH12012R)	5.3*	-1.4*	-3.1	-4.5*	-7.4	-6.2	-50.6*	-27.1*

* Significant at 5% probability level; L – line, T – tester.

Table 3.20 Better-parent heterosis (d_{ii}) of seedling traits from control/regular planting treatment from controlled environment chambers and 2018 and 2019 field experiments.

Hybrids	Seedling traits							
	EP			EI			SB	
	Chamber	2018	2019	Chamber	2018	2019	2018	2019
L1/T1 (ARCH11170A/ARCH10747-1R)	-13.0*	-27.6*	-67.2*	-4.9	12.3	12.3	26.2	-13.6
L1/T2 (ARCH11170A/ARCH10747-2R)	-9.1*	-30.9*	-53.5*	-6.4	10.4	10.4	6.6	-6.1
L1/T3 (ARCH11170A/ARCH12012R)	-	-1.6	-21.3*	-	3.1	3.1	8.4	-28.5
L1/T4 (ARCH11170A/KS115R)	-26.1*	-25.2*	100.7*	2.2	5.1	6.9	23.3	26.3
L2/T1 (ARCH11192A/ARCH10747-1R)	-4.3	-34.4*	6.2	-16.2	17.1	5.1	63.0	-11.6
L2/T2 (ARCH11192A/ARCH10747-2R)	-4.2	-28.1*	45.6*	-13.0	10.1	17.1	8.5	3.4
L2/T3 (ARCH11192A/ARCH12012R)	4.2	-11.7*	-1.5	-13.5	9.4	10.1	16.1	9.5
L2/T4 (ARCH11192A/KS115R)	19.0*	-28.1*	21.7*	-14.1	2.7	9.9	25.4	49.8
L3/T1 (ARCH11201A/ARCH10747-1R)	4.2	-22.0*	-0.8	-12.0	7.4	9.4	-3.1	-8.8
L3/T3 (ARCH11201A/ARCH12012R)	4.2	-21.3*	160.5*	-10.7	1.6	2.7	-38.4	60.2
L3/T4 (ARCH11201A/KS115R)	14.3*	-31.2*	18.9*	-2.6	3.7	12.9	-38.5	31.3
L4/T3 (ATx645/ARCH12012R)	-16.0*	-20.3*	-20.5*	15.3	14.6	14.6	-6.5	40.4
L5/T1 (KS116A/ARCH10747-1R)	4.3	5.2	135.9*	-18.4	6.9	12.3	50.3	36.3
L5/T2 (KS116A/ARCH10747-2R)	0.0	23.7*	5.3	2.7	9.9	9.1	-15.6	38.6
L5/T3 (KS116A/ARCH12012R)	-4.0	1.0	-1.2	-6.0	12.9	8.5	-19.4	45.1
L5/T4 (KS116A/KS115R)	4.3	12.4*	-16.4*	-11.1	12.3	8.1	22.9	57.4
L6/T1 (KS133A/ARCH10747-1R)	-8.0*	-21.0*	-4.5	-28.5	9.1	19.5	-27.4	-2.5
L6/T2 (KS133A/ARCH10747-2R)	0.0	-22.6*	16.6*	-11.0	8.5	0.4	9.3	16.6
L6/T3 (KS133A/ARCH12012R)	-4.3	-27.4*	93.3*	3.1	19.5	2.2	-29.2	14.0
L6/T4 (KS133A/KS115R)	13.6*	-39.5*	-2.2	-25.8	0.4	-3.7	-19.0	4.5
L7/T1 (KS136A/ARCH10747-1R)	-4.3	1.0	50.2*	-22.7	8.9	-2.2	-19.2	7.6
L7/T2 (KS136A/ARCH10747-2R)	4.3	-16.5*	21.7*	-4.2	8.3	8.4	9.3	7.9
L7/T3 (KS136A/ARCH12012R)	0.0	-5.3	103.7*	-7.0	19.3	0.3	-21.2	76.6
L7/T4 (KS136A/KS115R)	-18.2*	-21.1*	-58.8*	1.5	0.4	2.5	-9.9	46.6
L8/T1 (Redbine58A/ARCH10747-1R)	4.2	-52.3*	72.4*	-17.9	8.1	7.4	60.4	152.5
L8/T2 (Redbine58A/ARCH10747-2R)	4.2	-28.9*	15.2*	-14.1	-3.7	1.6	-13.4	-7.2
L8/T3 (Redbine58A/ARCH12012R)	-12.5*	-36.2*	71.8*	-11.3	2.5	3.7	16.6	58.6

* Significant at 5% probability level; L – line, T – tester.

Table 3.21 Better-parent heterosis (d_{ii}) of seedling traits from regular planting treatment from the 2018 and 2019 field experiments.

Hybrids	Agronomic traits							
	DF		PH		KW		PY	
	2018	2019	2018	2019	2018	2019	2018	2019
L1/T1 (ARCH11170A/ARCH10747-1R)	0.0	6.8*	15.4*	18.7*	14.6	6.0	15.8*	19.9*
L1/T2 (ARCH11170A/ARCH10747-2R)	4.8*	5.6*	10.5*	11.1*	4.6	-1.1	13.9*	13.9*
L1/T3 (ARCH11170A/ARCH12012R)	0.0	-3.2*	4.8*	4.5*	-7.7	6.0	-8.4*	-2.7*
L1/T4 (ARCH11170A/KS115R)	0.0	-9.6*	78.3*	25.4*	-18.7	6.6	1.6	-18.8*
L2/T1 (ARCH11192A/ARCH10747-1R)	-4.1*	3.4*	-2.8*	21.7*	25.7	49.2	-33.6*	-7.9*
L2/T2 (ARCH11192A/ARCH10747-2R)	0.0	-9.6*	-2.4*	11.9*	10.2	3.4	4.9*	-42.7*
L2/T3 (ARCH11192A/ARCH12012R)	-1.3*	9.6*	-4.0*	15.4*	-10.8	0.0	0.0	-29.5*
L2/T4 (ARCH11192A/KS115R)	-2.3*	-16.0*	75.9*	42.4*	-12.5	10.9	-44.6*	-0.6
L3/T1 (ARCH11201A/ARCH10747-1R)	-5.6*	-11.2*	-2.3*	15.4*	9.9	3.6	-33.4*	-61.1*
L3/T3 (ARCH11201A/ARCH12012R)	-1.3*	-1.5*	-9.8*	1.1	-2.3	1.9	-24.5*	-31.1*
L3/T4 (ARCH11201A/KS115R)	0.0	-9.6*	60.8*	96.0*	-10.4	4.9	-56.0*	2.4*
L4/T3 (ATx645/ARCH12012R)	-1.3*	-7.5*	-6.0*	12.8*	-10.3	10.7	5.7*	24.5*
L5/T1 (KS116A/ARCH10747-1R)	0.1	-1.5*	12.9*	0.4	17.4	-12.4	38.1*	13.6*
L5/T2 (KS116A/ARCH10747-2R)	-4.1*	-5.1*	8.3*	1.6	3.5	-2.2	44.5*	-10.4*
L5/T3 (KS116A/ARCH12012R)	4.5*	3.9*	14.1*	7.0*	-12.2	61.4	39.0*	66.0*
L5/T4 (KS116A/KS115R)	-2.3*	-5.0*	68.2*	60.9*	-12.2	12.0	35.1*	-13.0*
L6/T1 (KS133A/ARCH10747-1R)	-1.3*	-6.8*	12.5*	2.7	-13.5	5.6	-14.9*	6.3*
L6/T2 (KS133A/ARCH10747-2R)	-3.7*	0.0	5.4*	-8.0*	7.5	5.1	-26.4*	16.1*
L6/T3 (KS133A/ARCH12012R)	-1.3*	-12.7*	1.9*	5.7*	6.0	1.4	-25.5*	-5.7*
L6/T4 (KS133A/KS115R)	-2.3*	1.9*	72.0*	44.9*	5.0	6.7	-56.9*	21.0*
L7/T1 (KS136A/ARCH10747-1R)	0.0	5.6*	9.3*	5.3*	2.0	10.3	45.2*	-21.4*
L7/T2 (KS136A/ARCH10747-2R)	6.4*	-8.9*	-4.1*	-7.4*	2.0	5.5	41.6*	-24.8*
L7/T3 (KS136A/ARCH12012R)	4.5*	0.0	0.4*	-3.5*	-3.7	4.6	64.1*	-88.5*
L7/T4 (KS136A/KS115R)	0.0	-5.1*	89.0*	55.1*	0.8	-0.3	10.3*	5.3*
L8/T1 (Redbine58A/ARCH10747-1R)	-1.3*	-12.4*	12.3*	11.9*	8.1	-4.9	-34.3*	56.3*
L8/T2 (Redbine58A/ARCH10747-2R)	-3.7*	-7.7*	9.3*	6.7*	15.5	5.1	-7.0*	38.9*
L8/T3 (Redbine58A/ARCH12012R)	1.3*	-3.3*	6.0*	-4.0	9.8	-5.1	-18.6*	65.2*

* Significant at 5% probability level; L – line, T – tester.

Table 3.22 Correlation coefficient between traits in early planting field experiments.

		EI	SB	DF	PH	KW	PY
EP	2018	-0.34*	0.12	-0.33*	0.17	-0.13	0.78**
	2019	-0.34*	0.53**	-0.55*	0.30*	0.11	0.21
EI	2018		-0.30*	0.29*	-0.04	0.08	-0.14
	2019		-0.29*	0.02	-0.43*	0.10	-0.27*
SB	2018			-0.42*	0.31*	0.26	0.25
	2019			-0.60**	0.32*	0.21	-0.01
DF	2018				0.08	0.27*	-0.15
	2019				-0.18	0.07	0.05
PH	2018					0.74**	0.45**
	2019					0.26*	0.03
KW	2018						0.11
	2019						0.19

*,** Significant at 5 and 1% probability levels respectively; EP - emergence (%); EI - emergence index (d); SB - seedling biomass (g); DF - days to 50% flowering (GDU); PH - plant height (cm); KW - 200-kernel weight (g); and PY - plot yield (g m^{-2}).

Table 3.23 Correlation coefficients between traits in regular planting field experiments.

		EI	SB	DF	PH	KW	PY
EP	2018	-0.19	0.00	-0.05	-0.23	-0.48**	0.54**
	2019	0.04	0.42**	-0.42**	0.31*	0.29*	0.01
EI	2018		0.07	-0.19	-0.21	-0.25	0.23
	2019		0.10	-0.26	-0.07	-0.07	0.07
SB	2018			-0.56**	0.07	0.13	0.18
	2019			-0.46**	0.29*	0.28*	0.32*
DF	2018				-0.05	-0.14	-0.12
	2019				-0.01	-0.19	-0.17
PH	2018					0.76**	-0.27
	2019					0.34*	0.00
KW	2018						-0.38*
	2019						0.05

*,** Significant at 5 and 1% probability levels respectively; EP - emergence (%); EI - emergence index (d); SB - seedling biomass (g); DF - days to 50% flowering (GDU); PH - plant height (cm); KW - 200-kernel weight (g); and PY - plot yield (g m^{-2}).

Table 3.24 Correlation coefficients for two years between early and regular planting field experiments.

Traits	Year	
	2018	2019
EP	0.62**	0.55**
EI	0.30*	0.26*
SB	0.47**	0.50**
DF	0.79**	0.70**
PH	0.97**	0.80**
KW	-0.33*	-0.02
PY	0.32*	0.14

*,** Significant at 5 and 1% probability levels, respectively; EP - emergence (%); EI - emergence index (d); SB - seedling biomass (g); DF - days to 50% flowering (GDU); PH - plant height (cm); KW - 200-kernel weight (g); and PY - plot yield (g m^{-2}).

Chapter 4 - General discussion

Enhancing chilling tolerance in sorghum is gaining importance due to the increased challenges of heat and drought stresses across the world. Chilling tolerance improvement at various growth stages is recognized as a key factor in allowing the expansion of sorghum growing regions in the United States. Additionally, chilling tolerance has the potential to allow domestic growers to capitalize on early season moisture and provide for fuller season hybrids with higher yield potential. With enhanced chilling tolerance, sorghum can be planted early and later developmental stages including anthesis and grain filling may have the opportunity to avoid detrimental production stresses.

Some major key points from these studies are as follows:

- A new phenotyping approach was systematically implemented, tested, and recommended for further chilling tolerance experiment in controlled environmental chambers. This approach better relates the dynamically changing temperatures in the field to the chambers (Figure 2.1) and carries over to key chilling traits such as shoot, root, and total biomass.
- For the first time, new hybrids utilizing promising chilling tolerant inbred lines previously identified by Chiluwal et al., (2018) were developed. These hybrids were tested under controlled environmental chambers and two years under field conditions.
- These studies showed that the grain-filling period could vary within a single genotype due to environmental factors such as early planting. The increase in the grain-filling period could useful to increase sorghum productivity, which is the end goal of early-stage chilling tolerance. Previous reports state that the grain filling duration is relatively fixed with very few opportunities to extend. These conclusions were drawn by using diverse genetic

material but were all planted in the regular planting window, and the environmental factor was not studied.

- Seedlings that survive after exposure to severe chilling stress have a significantly higher recovery rate and attain comparable levels of growth with regular planting. A functional aspect of the chilling tolerance response in hybrids may be due to heterotic vigor but can partly be due to the physiological priming that enhances the robustness of the plant. Priming due to early-stage chilling stress can possibly enhance the plants ability to address subsequent stresses during the life cycle of a crop.
- A tannin-free early-stage chilling tolerant grain sorghum hybrid (ARCH11192A/ ARCH12012R) was developed. This hybrid took longer to reach flowering compared to the hybrids in regular but exhibited a significantly longer grain-filling period.
- The general combing ability of 12 parents were analyzed under controlled environment conditions and two years of field conditions. Three parental lines (ARCH11201B, KS116B, and KS133B) demonstrated high combining ability concerning chilling tolerance traits.
- Gene actions within the chilling tolerant traits were brought to light under early-stage chilling tolerance stress. Most traits displayed dominance × dominance of non-allelic gene interactions. Other traits may be attributed from favorable additive effects of the high general combiner parent and epistatic effects from the low general combiner.
- The hybrids tested in these studies outperformed their best performing parents by a wide range. This wide range of heterosis indicates there is a great scope for improving the performance of the hybrids under chilling stress conditions.

- In these studies, we were successful in discovering that when selecting for chilling tolerant genotypes, the top performing genotypes in an optimal condition will most likely be top performing genotypes within chilling stress conditions. Although, this correlation is not 100%, and some genotypes could perform at a higher level in the chilling conditions than in optimal.
- The specific combining ability of 27 newly developed hybrids concerning chilling tolerance traits across the same environmental conditions was assessed. As a result, a specific hybrid combination for chilling tolerant traits (ARCH11201A/ARCH10747-1R) was selected. This hybrid performed highly among most chilling tolerant traits. The hybrid proved to have high *per se* performance, favorable SCA and heterosis estimates as well has at least one parent with high GCA in multiple traits crucial for chilling tolerance.

The research of early-stage chilling tolerance has many great challenges. Unpredictable weather fluctuations complicate field screening of complex chilling tolerance traits. This fluctuating climate changes inter- and intra-annually, making research on chilling tolerance even more difficult. Not only does the climate bring along challenges in field-testing, other factors like seed quality for planting and pest control of weeds, birds, and insects have their own. It is imperative to try to reduce the amount of outside factors being integrated into the data from field-based screening. Numerous studies have been performed concerning the development of chilling tolerant sorghum. The current and most tolerant genotypes remain sensitive to chilling stress in April and early May. However, continuous research efforts with available advanced breeding lines, diversity panel, bi-parental, and nested association mapping (NAM) populations from different breeding programs are being made to enhance chilling tolerance with adapted traits including

germplasm with non-tannin backgrounds. The collaborative research needs to focus on understanding the mechanism of complex quantitative traits related to chilling tolerance. In addition to sustained classical breeding approaches, efforts through molecular markers such as quantitative trait loci (QTL) integration and prediction models to identify the optimal parent combinations are required to accelerate the breeding process and develop high yielding hybrids with enhanced chilling tolerance for early planting.

Chapter 5 - References

Almodares, A. and Mostafafi Darany, S.M. (2006). Effects of planting date and time of nitrogen application of yield and sugar content of sweet sorghum. *Journal of Environmental Biology*. 27(3), 601-605.

Anda, A. and Pinter, L. (1994). Sorghum germination and development as influenced by soil temperature and water content. *Agronomy Journal*. 86(4), 621-624.

Assefa, Y., Staggenborg, S.A. and Prasad, V.P. (2010). Grain sorghum water requirement and responses to drought stress: A review. *Crop Management*. 9(1), 1-11.

Azad, M.A.K., Shah-E-Alam, M. and Hamid, M.A. (2014). Combining ability of pod yield and related traits of groundnut (*Arachis hypogaea* L.) under salinity stress. *Science World Journal*. 2014, 1–7.

Bahuguna, R.N., Jha, J., Pal, M., Shah, D., Lawas, L.M., Khetarpal, S. and Jagadish, K.S.V. (2015). Physiological and biochemical characterization of NERICA-L-44: a novel source of heat tolerance at the vegetative and reproductive stages in rice. *Physiologia Plantarum.* 154(4), 543-559.

Bao, Y., Wang, S., Wang, X., Wang, Y., Li, X., Wang, L. and Wang, H. (2009). Heterosis and combining ability for major yield traits of a new wheat germplasm Shannong 0095 derived from *Thinopyrum intermedium*. *Agricultural Sciences in China.* 8(6), 753-760.

Bean, S.R., Chung, O.K., Tuinstra, M.R., Pedersen, J.F. and Erpelding, J. (2006). Evaluation of the single kernel characterization system (SKCS) for measurement of sorghum grain attributes. *Cereal Chemistry*. 83(1), 108-113.

Bekele, W.A., Fiedler, K., Shiringani, A., Schnaubelt, D., Windpassinger, S., Uptmoor, R., Friedt, W. and Snowdon, R.J. (2014). Unravelling the genetic complexity of sorghum seedling development under low-temperature conditions. *Plant, Cell & Environ*. 37(3), 707-723.

Bernardo, R. (2014). *Essentials of plant breeding*. Stemma Press Woodbury, MN.

Bhosale, S.U., Rymen, B., Beemster, G.T.S., Melchinger, A.E. and Reif, J.C. (2007). Chilling tolerance of Central European maize lines and their factorial crosses. *Annals of Botany*. 100(6), 1315-1321.

Blum, A. (1970). Effect of plant density and growth duration on grain sorghum yield under limited water supply[1]. *Agronomy Journal*. 62(3), 333-336.

Blum, A. (2004). Sorghum physiology. In '*Physiology and biotechnology integration for plant breeding*'. [Nguyen H.T. and Blum, A. (eds)] Marcel Dekker, New York (p. 141-223).

Branolini, A., Landi, P., Monfredini, G. and Tano, F. (2000). Variation among Andean raves of maize for cold tolerance during heterotrophic and early autotrophic growth. *Euphytica*. 111, 33-41.

Brar, G.S. and Stewart, B.A. (1994). Germination under controlled temperature and field emergence of 13 sorghum cultivars. *Crop Science*. 34(5), 1336-1340.

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

Chigeza, G., Mashingaidze, K. and Shanahan, P. (2014). Advanced cycle pedigree breeding in sunflower. II: combining ability for oil yield and its components. *Euphytica*. 195(2), 183-195.

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

Chopra, R., Burow, G., Burke, J.J., Gladman, N. and Xin, Z. (2017). Genome-wide association analysis of seedling traits in diverse sorghum germplasm under thermal stress. *BMC Plant Biology*. 17(12).

Cisse, N. and Ejeta, G. (2003). Genetic variation and relationships among seedling vigor traits in sorghum. *Crop Science*. 43(3), 824-828.

Craine, J.M. and Dybzinski, R. (2013). Mechanisms of plant competition for nutrients, water and light. *Functional Ecology*. 27(4), 833-840.

Doggett, H. (1988). *Sorghum, 2nd Ed*. Wiley, NY.

Dykes L. (2019). *Tannin analysis in sorghum grains*. [Zhao, Z.Y. and Dahlberg J. (eds)] Sorghum, Methods in Molecular Biology, Humana Press, NY, (vol. 1931).

Falconer, D.S. (1989). *Introduction to quantitative genetics, 3rd Ed.* Longman Sci. Tech., NY.

Fasahat, P., Rajabi, A., Rad, J.M. and Derera, J. (2016). Principles and utilization of combining ability in plant breeding. *Biometrics & Biostatistics International Journal*. 4(1), 1–22.

Fernandez, M.G.S., Schoenbaum, G.R. and Goggi, A.S., (2015). Novel germplasm and screening methods for early cold tolerance in sorghum. *Crop Science*. 54(6), 2631-2638.

Franco, M.C., Cassini, S.T. and Oliveira, V.R. (2001). Combining ability for nodulation in common bean (*Phaseolus vulgaris* L.) genotypes from Andean and Middle American gene pools. *Euphytica*. 118(3), 265– 270.

Franks, C.D., Burow, G.B. and Burke, J.J. (2006). A comparison of U.S. and Chinese sorghum germplasm for early season cold tolerance. *Crop Science*. 46(3), 1371-1376.

Gesch, R.W. and Archer, D.W. (2005). Influence of sowing date on emergence characteristics of maize seed coated with a temperature-activated polymer. *Agronomy Journal*. 97(6), 1543-1550.

Hariprasanna, K. and Rakshit, S. (2016). Economic importance of sorghum. [Rakshit, S. and Wang, Y., (eds)] *The Sorghum genome*. Springer, (p. 1-25).

Harris, D., Handi, Q.A. and Oda, A.C.T. (1987). Germination and emergence of Sorghum bicolor. Genotypic and environmentally induced variation in the response to temperature and depth of sowing. *Plant, Cell & Environment*. 10(6), 501-508.

Kapanigowda, M.H., Perumal, R., Aiken, R.M., Herald, T.J., Bean, S.R. and Little, C.R. (2013). Analyses of sorghum (*Sorghum bicolor* (L.) Moench) lines and hybrids in response to early-season planting and cool conditions. *Canadian Journal of Plant Science*. 93(5), 773-784.

Kenga, R., Alabib, S.O. and Gupta, S.C. (2004). Combining ability studies in tropical sorghum (*Sorghum bicolor* (L.) Moench). *Field Crops Research*. 88(2), 251–260.

Kerchev, P., van der Meer, T., Sujeeth, N., Verlee, A., Stevens, C.V., Van Breusegem, F. and Gechev, T. (2019). Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. *Biotechnology Advances*. 107503.

Kheir, E.A., Sheshshayee, M.S., Prasad, T.G. and Udayakumar, M., (2012). Temperature induction response as a screening technique for selecting high temperature-tolerant cotton lines. *Journal of Cotton Science*. 16(3), 190-199.

Knoll, J. and Ejeta, G. (2008). Marker-assisted selection for early-season cold tolerance in sorghum: QTL validation across populations and environments. *Theoretical and Applied Genetics*. 116(4), 541-553.

Knoll, J., Gunaratna, N. and Ejeta, G. (2008). QTL analysis of early-season cold tolerance in sorghum. *Theoretical and Applied Genetics*. 116, 577-587.

Kofoid, K.D. and Harvey, T.L. (2005). Registration of greenbug resistant sorghum germplasm lines KS 116 A/B through KS 120 A/B. Crop Sci., 45(2), 802-803.

Kumar, V., Rani, A., Pandey, V., Mande, P. and Chauhan, G.S. (2006). Compositional traits of soybean seeds as influenced by planting date in India. *Experimental Agriculture*. 42(1), 19–28.

Larkindale, J. and Vierling, E. (2008). Core genome responses involved in acclimation to high temperature. *Plant Physiology*. 146, 748-761.

Lawas, L.M.F., Erban, A., Kopka, J., Jagadish, S.K., Zuther, E. and Hincha, D.K. (2019). Metabolic responses of rice source and sink organs during recovery from combined drought and heat stress in the field. *GigaScience*, 8(8), 102.

Leff, B., Ramankutty, N. and Foley, J.A. (2004). Geographic distribution of major crops across the world. *Global Biogeochemical Cycles*. 18(1), GB1009.

Leon-Velasco, H., Mendoza-Onofre, L.E., Castillo-Gonzalez, F., Cervantes-Santana, T. and Martinez-Garza, A. (2009). Evaluation of two generations of cold tolerant sorghum hybrids and parental lines. I: Genetic variability and adaptability. *Agrociencia*. 43(5), 483-496.

Lu, Q. and Dahlberg, J. (2001). Chinese sorghum genetic resources. *Economic Botany*. 55, 401–425.

Ludlow, M. and Muchow, R. (1990). A critical evaluation of traits for improving crop yields and water-limited environments. *Advances in Agronomy*. 43, 107-153.

Majora, D.J., Hammana, W.M. and Rooda, S.B. (1982). Effects of short-duration chilling temperature exposure on growth and development of sorghum. *Field Crops Research*. 5, 129-136.

Makanda, I., Tongoona, P., Derera, J., Sibiya, J. and Fato, P. (2010). Combining ability and cultivar superiority of sorghum germplasm for grain yield across tropical low and mid-altitude environments. *Field Crops Research*. 116(1), 75–85.

Maulana, F. and Tesso, T.T. (2013). Cold temperature episode at seedling and flowering stages reduces growth and yield components in sorghum. *Crop Science*. 53(2), 564-574.

McMaster, G.S., Edmunds, D.A., Jones, S.M., Johnson, J.J. and Vigil, M.F. (2016). Simulating the probability of grain sorghum maturity before the first frost in Northeastern Colorado. *Agronomy*, 6(4), 44.

Mendoza-Onefre, L., Eastern, J.D., Sullivan, C.Y. and Ross, W.M. (1979). Relations between laboratory and field observations of sorghum seedlings grown under cold

temperature. p. 137-145. Development of improved high-yielding sorghum cultivars. Ann. Rep. No. 9. Univ. of Nebraska, ARS, USDA, Lincoln, NE.

Milić, D., Katić, S., Karagić, Đ., Gvozdanović–Varga, J., Petrović, S. and Jan Boćanski, J. (2011). Genetic control of agronomic traits in alfalfa (*M sativa*ssp. *sativa*L.). *Euphytica.* 182(1), 25-33.

Moghimi, N., Desai, J.S., Bheemanahalli, R., Impa, S.M., Vennapusa, A.R., Sebela, D., Perumal, R., Doherty, C.J. and Jagadish, S.V.K. (2019). New candidate loci and marker genes on chromosome 7 for improved chilling tolerance in sorghum. *Journal of Experimental Botany*. 70(12), 3357-3371.

Nagaraj, N., Basavaraj, G., Rao, P.P., Bantilan, C. and Haldar, S. (2013). Sorghum and pearl millet economy of India: Future outlook and options. *Economic & Political Weekly*. 48(52) 74-81.

Nordquist, P.T. (1971). Use of earliness and cold tolerance in breeding sorghums. *Proc. Ann. Corn and Sorghum Research Conference*. 26, 33–41.

Ortiz, D., Hu, J. and Salas Fernandez, M.G. (2017). Genetic architecture of photosynthesis in *Sorghum bicolor* under non-stress and cold stress conditions. *Journal of Experimental Botany*. 68(16), 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. (p. 143-159).

Peiris, K.H.S., Bean, S.R., Chiluwal, A., Perumal, R. and Jagadish, S.V.K. (2019). Moisture effects on robustness of sorghum grain protein near-infrared spectroscopy calibration. *Cereal Chemistry*. 96(4), 678-688.

Pennisi, E. (2009). How sorghum withstands heat and drought. *Science*. 323(5914), 573-573.

Perumal, R., Tesso, T., Kofoid, K.D., Prasad, P.V.V., Aiken, R.M., Bean, S.R., Wilson, J.D., Herald, T.J. and Little, C.R. (2015). Registration of nine grain sorghum seed parent (A/B) lines. *Journal of Plant Registrations*. 9(2), 244-248.

Pinthus, M.J. and Rosenblum, J. (1961). Germination and seedling emergence of sorghum at low temperatures. *Crop Science*. 1(4), 293-296.

Poorter, H., Fiorani, F., Pieruschka, R., Wojciechowski, T., van der Putten, W.H., Kleyer, M., Schurr, U. and Postma, J. (2016). Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. *New Phytologist*. 212(4), 838-855.

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

Proietti, I., Frazzoli, C. and Mantovani, A. (2015). Exploiting nutritional value of staple foods in the world's semi-arid areas: risks, benefits, challenges and opportunities of sorghum. In *Healthcare* (Vol. 3, No. 2, pp. 172-193). Multidisciplinary Digital Publishing Institute.

Quinby, J.R. (1972). Grain filling period of sorghum parents and hybrids[1]. *Crop Science*. 12(5), 690-691.

Revilla, P., Hotchkiss, J.R. and Tracy. W.F. (2003). Chilling tolerance evaluation in a diallel among open-pollinated sweet corn cultivars. *Horticulture Science.* 38(1), 88–91.

Rooney, L., McDonough, C. and Dykes, L. (2004). Myths about sorghum tannins. Retrieved from http://crsps.net/wp-content/downloads/INTSORMIL/Inventoried%209.6/3-0000-8-680.pdf.

Rooney, W.L. (2004). Sorghum improvement-integrating traditional and new technology to produce improved genotypes. *Advances in Agronomy*. 83, 37-109.

Saballos, A. (2008). Development and utilization of sorghum as a bioenergy crop. In *Genetic improvement of bioenergy crops* (pp. 211-248). Springer, New York, NY.

Schaffasz, A., Windpassinger, S., Snowdon, R. and Wittkop, B. (2019). Reproductive cold stress tolerance in sorghum F_1 hybrids is a heterotic trait. *Agronomy*. 9(9), 508.

Senthil-Kumar, M., Srikanthbabu, V., Mohan Raju, B., Shivaprakash, N. and Udayakumar, M. (2003.) Screening of inbred lines to develop a thermotolerant sunflower hybrid using the temperature induction response (TIR) technique: a novel approach by exploiting residual variability. *Journal of Experimental Botany*. 54(392), 2569-2578.

Shi, W., Lawas, L.M.F., Raju, B.R. and Jagadish, S.V.K. (2016). Acquired thermo-tolerance and trans-generational heat stress response at flowering in rice. *Journal of Agronomy and Crop Science*. 202(4), 309-319.

Shukla, S.K. and Pandey, M.P. (2008). Combining ability and heterosis over environments for yield and yield components in two-line hybrids involving thermosensitive genic male sterile lines in rice (*Oryza sativa* L.). *Plant Breeding*. 127(1):28-32.

Shull, G. (1948). What is "Heterosis"?. *Genetics*. 33(5): 439–446.

Singh, S.P. (1985). Sources of cold tolerance in grain sorghum. *Canadian Journal of Plant Science*. 65(2), 251–257.

Smith, C.W. and Frederiksen, R.A. (2000). History of cultivar development in the United States: From "Memoirs of A.B. Maunder - Sorghum Breeder'. [Smith, C.W. and Frederiksen, R.A. (eds.)] Sorghum: Origin, History, Technology, and Production. John Wiley & Sons, New York, NY, (p.191–223).

Soujeole, A.A. and Miller. F.R. (1984). Cold tolerance of sorghum during early developmental stages. *Proc. Ann. Corn and Sorghum Research Conference*. 39, 18–32.

Srikanthbabu, V., Krishnaprasad, B.T., Gopalakrishna, R., Savitha, M. and Udayakumar, M. (2002). Identification of pea genotypes with enhanced thermotolerance using temperature induction response technique (TIR). *Journal of Plant Physiol*. 159(5), 535-545.

Stoffer, R.V. and Riper, G.E.V. (1963). Effect of soil temperature and soil moisture on the physiology of sorghum. *Agronomy Journal*. 55(5), 447-450.

Tack, J., Lingenfelser, J. and Jagadish, S.V.K. (2017). Disaggregating sorghum yield reductions under warming scenarios exposes narrow genetic diversity in US breeding programs. *Proceedings of the National Academy of Sciences. U.S.A.* 114 (35), 9296-9301.

Taylor, A.O. and Rowley, J.A. (1971). Plants under climatic stress: I. low temperature, high light effects on photosynthesis. *Plant Physiology*. 47(5), 713-718.

Tiryaki, I. and Andrews, D.J. (2001). Germination and seedling cold tolerance in sorghum: I. Evaluation of rapid screening methods. *Agronomy Journal*. 93(6), 1386-1391.

Topal, A., Aydın, C., Akgun, N. and Babaoglu, M. (2004). Diallel cross analysis in durum wheat (*Triticum durum* Desf.): identification of best parents for some kernel physical features. *Field Crops Research*. 87(1), 1-12.

Tremblay, G.J., Beausoleil, J.M., Filion, P. and Saulnier, M. (2006). Response of three soybean cultivars to seeding date. *Canadian Journal of Plant Science*. 86(4), 1071–1078.

Tuinstra, M.R., Liang, G.L., Hicks, C., Kofoid, K.D. and Vanderlip, R.L. (2001). Registration of KS 115 Sorghum. *Crop Science*. 41(3), 932-93.

Tyagi, A.P. and Lal, P. (2005). Line × tester analysis in sugar cane (*Saccharum officinarum*). *South Pacific Journal of Natural Science*. 23(1), 30-36.

Umakanth, A.V., Patil, J.V., Rani, C.H., Gadakh, S.R., Siva Kumar, S., Rao, S.S. and Kotasthane, T.V. (2012). Combining ability and heterosis over environments for stalk

and sugar related traits in sweet sorghum (*Sorghum bicolor* (L.) Moench.). *Sugar Tech.* l14(3), 237–246.

Upadhyay, HD, Wang, Y., Sastry, D., Dwivedi, SL, Prasad, P.V., Burrell, A., Klein, R., Morris, G. and Klein, P., 2016. Association mapping of germinability and seedling vigor in sorghum under controlled low-temperature conditions. *Genome*. 59(2), 137–145.

Upadhyaya, H., Pundir, R., Dwivedi, S., Gowda, C., Reddy, V. and Singh S. (2009). Developing a mini core collection of sorghum for diversified utilization of germplasm. *Crop Science*. 49(5), 1769–1780.

USDA-NASS., 2019. Agricultural Statistics Board. Received online at https://downloads.usda.library.cornell.edu/usda-esmis/files/j098zb09z/0k225n39n/jw827p632/acrg0619.pdf

Villalobos, F.J., Sadras, V.O. and Fereres, E. (2016). Plant density and competition. In *Principles of Agronomy for Sustainable Agriculture* (pp. 159-168). Springer, Cham.

Wang, X., Cai, J., Liu, F., Jin, M., Yu, H., Jiang, D., Wollenweber, B., Dai, T. and Cao, W. (2012). Pre-anthesis high-temperature acclimation alleviates the negative effects of post-anthesis heat stress on stem stored carbohydrates remobilization and grain starch accumulation in wheat. *Journal of Cereal Science*. 55(3), 331-336.

Warrington, I.J. and E.T. Kanemasu. (1983). Corn growth response to temperature and photoperiod I. seedling emergence, tassel initiation, and anthesis. *Agronomy Journal*. 75(5), 749-754.

Wassimi, N.N., Isleib, T.G. and Hosfield, G.L. (1986). Fixed effect genetic analysis of a diallel cross in dry beans (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*. 72(4), 449-454.

Wijewardana, C., Hock, M., Henry, B. and Raja Reddy, K. (2015). Screening corn hybrids for cold tolerance using morphological traits for early-season seedling. *Crop Science*. 55(2), 851-867.

Windpassinger, S., Friedt, W., Deppé, I., Werner, C., Snowdon, R. and Wittkop, B. (2017). Towards enhancement of early-stage chilling tolerance and root development in sorghum F_1 hybrids. *Journal of Agronomy and Crop Science*. 203(2), 146-160.

Wu, Y., Li, X., Xiang, W., Zhu, C., Lin, Z., Wu, Y., Li, J., Pandravada, S., Ridder, D.D., Bai, G. and Wang, M.L. (2012). Presence of tannins in sorghum grains is conditioned by different natural alleles of Tannin1. *Proceedings of the National Academy of Sciences, U.S.A.* 109(26), 10281-10286.

Yu, J. Tuinstra, M.R. (2001). Genetic analysis of seedling growth under cold temperature stress in grain sorghum. *Crop Science*. 41(5), 1438-1443.

Yu, J., Tuinstra, M.R., Claassen, M.M., Gordon, W.B. and Witt, M.D. (2004). Analysis of cold tolerance in sorghum under controlled environment conditions. *Field Crops Research*. 85(1), 21-30.

Zegada-Lizarazu, W., Fernando Luna, D. and Monti, A. (2016). Differential characteristics of photochemical acclimation to cold in two contrasting sweet sorghum hybrids. *Physiologia Plantarum*. 157(4), 479-489.