Screening maize and sorghum for chilling tolerance at seedling stage

by

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Abstract

Low temperature is one of the most limiting stresses to crops that are adapted to tropical and subtropical regions, such as maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench], when introduced into temperate regions. However, no studies have compared the chilling tolerance of maize and sorghum grown together. Therefore, the objective of this research was to screen maize hybrids and sorghum genotypes for chilling tolerance at the germination and seedling stages. With the hypothesis that grain composition of maize and sorghum could lead to varying chilling tolerance, the seeds were analyzed for concentrations of protein, starch, and amylose. Five commercial hybrids of maize and 18 genotypes of sorghum were maintained in growth chambers for 31 days at two temperatures: a control temperature (25/20 ^{o}C, day/night) and at chilling temperatures (11/8 ^{o}C for 14 days; 12.5/9.5 ^{o}C for 14 days, and 14/11 ^{o}C for 3 days). Emergence and seedling height were measured during the experiment. At the end of the experiment, shoot dry weight, root dry weight, and leaf area were determined.

Emergence of sorghum under the chilling temperature regime was low (18%). Average height of the emerged sorghum seedlings in the cold temperatures at the end of the experiment was 1.4 cm compared to 55.5 cm in the control treatment. All maize hybrids emerged, but emergence and growth were slowed by the cold temperatures, and average height at the end of the experiment was 4.6 cm compared to 96.1 cm in the control treatment. Shoot dry weight, root dry weight, and leaf area of the sorghum under the chilling temperatures were too small to measure, and, for maize, they were greatly reduced. The results showed that, for sorghum, temperatures should be above 14 ^{o}C for emergence, while maize could emerge at lower temperatures.

The analyses of the sorghum seeds showed that Redbine 60 and RTx430 had the highest protein concentrations (15.71% and 15.35%, respectively), and Segaolane had the lowest protein concentration (9.83%). Segaolane had the highest starch concentration (72.71%), and RTx430 had the lowest starch concentration (65.31%). There was an inverse relationship between protein and starch concentrations in the sorghum seeds ($R^2 = 0.69$). Amylose concentrations did not vary significantly among the sorghum seeds. The analyses of the maize seeds showed that Dekalb 51-20 and Pioneer 1151 had the highest protein concentrations (10.98% and 10.95%, respectively), and Pioneer 1105 had the lowest protein concentration (9.26%). Starch and amylose concentrations did not vary significantly among the maize seeds.

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Dedication

This thesis work is dedicated to my husband, Roshan Jerome, who has been a constant source of support and encouragement during the challenges of graduate school and life. I am truly thankful for having you in my life. This work is also dedicated to my parents, M.T.Antony and Sali Antony, who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve and to Dr. Kirkham for her unwavering support throughout my life at K-State.

And lastly, I dedicate this thesis to the Almighty God, thank you for the guidance, strength, power of mind, protection and skills and for giving us a healthy life.

All of these, we offer to you.

Chapter 1 - Literature Review

Because plants are immobile, abiotic stresses such as drought, cold, and heat are often unavoidable and can cause serious yield losses in crops worldwide, threatening global food security (Sreenivasulu et al., 2007; Ahmad and Prasad, 2012). Low temperature is one of the most harmful abiotic stresses affecting temperate plants (Janská et al., 2010; Thakur et al., 2010) and is especially damaging to crops that originate from tropical and sub-tropical regions, such as maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench], when introduced into the higher latitudes of temperate regions (Knoll et al., 2008; Burow et al., 2011; Salas Fernandez et al., 2015). Maize and sorghum are susceptible to damage when temperatures fall below 15 ^{o}C (Ruelland et al., 2009). Both maize and sorghum are C_4 plants, and C_4 plants photosynthesize at higher temperatures than C_3 plants (Hatch, 1992). When grown in temperate regions, cold temperatures limit their productivity. In the central regions of U.S.A., early planting and use of minimum tillage require the development of maize and sorghum genotypes with early season cold tolerance (Yu et al. 2004). Therefore, a goal of breeders is to improve early season cold tolerance (Crosbie et al., 1980).

Chilling stress has been defined as damage caused by low but above-freezing temperatures (Ercoli et al., 2004), and, conversely, chilling tolerance describes beneficial reactions of plants to low temperatures (Farooq et al., 2009). This review will consider the chilling tolerances of maize and sorghum and then compare them.

Chilling tolerance of maize

Many studies have been done concerning the chilling tolerance of maize at the seedling stage (Assefa et al., 2014). A review of the historical literature shows the importance that researchers in the maize-growing states of the U.S.A. put on early planting to increase grain yields. They

recognized that early dates of planting in the U.S. Corn Belt (e.g., mid-April) necessitate emergence and growth under conditions of cold soil and low air temperatures (Mosely et al., 1984), and therefore, a need for cold-tolerant maize for use in locations where early planting subjects the crop to low temperatures during germination and early seedling growth (Mock and Bakri, 1976).

In the spring of 1974 and 1976 in Iowa, Mock and McNeill (1979) evaluated cold tolerance in the field of 34 maize inbred lines adapted to various latitudes in North America. Their objective was to assess genetic variability and breeding potential for improvement of cold tolerance within maize germplasm adapted to North America. They found that seedling dry weight was significantly correlated with grain yield, indicating good seedling vigor was related to high grain yield. Cold tolerance was generally not related to geographical location of adaptation, and three of the best cold-tolerant inbreds were adapted to the central latitudes of the U.S. Corn Belt. Two originated from Iowa and one was from Missouri.

Cutforth et al. (1986) studied root growth between germination and emergence of three maize hybrids grown under soil temperatures of 15, 19, 25, and 30.5 ^{o}C. Decreases in soil temperature decreased root growth rate, and all hybrids responded to soil temperature in the same way. The root growth rate at 15 ^{o}C was about 1/10 that at 30.5 ^{o}C.

Minimum temperatures for growth of maize have been studied. Mock and McNeill (1979), whose work is cited previously, calculated growing degree days for the 34 maize inbred lines and maximum temperature and minimum temperature were restricted to those below 30 ^{o}C and above 10 ^{o}C, respectively. In a study to quantify the temperature responses of the rates of development of five maize hybrids from emergence to tassel initiation, Birch et al. (1998) used base, optimum, and maximum temperatures of 8, 34, and 40 ^{o}C, respectively. To determine

differences in chilling tolerance, Haskell (1952) germinated inbred sweet corn, dent maize, and their reciprocal hybrids in unsterilized field soil at a constant 10 ^{o}C for 10 days. All plant lines germinated with percentage germinations ranging from 2.5 to 98%. Aidun et al. (1991) also used a temperature of 10 ^{o}C for 5 weeks to study cold tolerance of inbreds and their hybrids grown in sand. Plants were germinated in plastic Petri dishes and grown for 8 days at 20 ^{o}C before the cold temperature began. It was reported that some plants died, but the number is unknown because no growth data were presented.

Landi and Crosbie (1982) studied response of four inbred maize lines grown in soil (fine loam) exposed for 72 hours to temperatures of 8/3, 10/5, and 12/7 ^{o}C day/night for three different periods during vegetative growth (when the plants had 2-3 leaves; when they had 3-4 leaves; and when they had 4-5 leaves). Control plants grew under 16/10 ^{o}C. All inbred lines grew when given the three 72-hour cold treatments, but the seedling growth of one inbred was reduced by about 50% at both 8/3 ^{o}C and 10/5 ^{o}C. Eagles (1988) studied emergence at two low temperatures of four lines of maize and crosses between them. Plants were grown in a potting mixture of fine gravel, peat, and vermiculite (70:15:15, v/v). The first temperature treatment was 15 ^{o}C for 12 h each day and 10 ^{o}C for 8 h each day with changeover periods of 2 h. The second temperature treatment was 15 ^{o}C for 12 h and 5 ^{o}C for 4 h with changeover periods of 4 h. After 28 days at the 15/10 ^{o}C temperatures and 35 days at the 15/5 ^{o}C temperatures, any seeds that were going to germinate had germinated and emergence had ceased. All seeds germinated at 15/10 ^{o}C, and emergence varied from 84.4 to 100%. Except for one parent, which had 0% emergence, all seeds emerged at 15/5 ^{o}C, and emergence ranged from 84.4 to 100%. For the maize seeds that germinated, most of the plants could survive up to 4 hours per day at 5 ^{o}C for up to 35 days. Stewart et al. (1990) studied growth and respiration of four maize inbreds at cold temperatures.

Seedlings were germinated and grown in a vermiculite-peat mixture in darkness at 30 ^{o}C (constant), 14 ^{o}C (constant), or 15/8 ^{o}C in a 16 h/8 h day/night cycle for up to 21 days. All inbreds grew at the cold temperatures, but two inbreds were more cold tolerant than the other two inbreds. These studies indicate that maize can germinate and grow for up to 10 days at 10 ^{o}C (Haskell, 1952), and, up to 35 days for short periods of cold (5 ^{o}C for 4 hours per day) (Eagles, 1988).

More recent studies indicate that the chilling tolerance of maize may have improved since these early studies. Farooq et al. (2009) reviewed the literature and reported that the optimum temperature for growth of maize is 25 to 28 ^{o}C. Temperatures below 12 to 15 ^{o}C induced chilling stress. A minimum temperature threshold for maize germination was between 8 and 12 ^{o}C, and autotrophic growth occurs between 13 to 15 ^{o}C. Maize root growth can occur at a temperature as low as ~9 ^{o}C (Farooq et al., 2009). As there has been no study directly comparing the chilling tolerance of maize cultivars released at different time periods, it is difficult to make comparisons between maize grown 40 years ago and maize grown today. However, the data cited by Farooq et al. (2009) indicates that chilling tolerance of maize may have increased over the decades.

Chilling tolerance of sorghum

Fewer studies have been done studying the chilling tolerance of sorghum compared to maize at the seedling stage. Yu et al. (2004) examined the cold tolerance of 30 commercial hybrid seed lots grown under growth chamber conditions to evaluate the consistency of controlled-condition test results with data from the field, where seeds were planted at least 30 days earlier than normal production to ensure low-temperature stress. The plants were grown in a commercial horticultural medium (Terra-Lite® Redi-Earth, Scotts-Sierra Horticulture Products Company,

Marysville, OH) under 15/10, 13/10, 11/8, and 25/20 ºC day/night temperatures in a 13/11 h cycle in a growth chamber for 30 days. Combined analysis of the three low temperatures showed that the temperature effect was significant for emergence, shoot dry weight, and seedling height. Significant genotypic differences were detected for all traits. While the temperatures in the field could not be controlled, the data from the growth-chamber experiment were positively correlated with data from the field. Even though Yu et al. (2004) gave no supplementary information for the individual responses of the 30 genotypes under each temperature regime, they noted that entry by temperature interactions were not significant for any of the traits measured, indicating that the relative performances of entries were constant across the three cold-temperature regimes. Emergence of the 30 hybrids under the cold temperatures, was reported to range from 58.36% to 83.06%.

Ercoli et al. (2004) grew one hybrid sorghum (Venturoli Aralba) in soil (sandy loam) in a growth chamber at a constant 27 ºC, which was the optimal temperature for this hybrid, with a 14-h day and 10-h night period until the plants reached the third-leaf stage, which was about 10 days after emergence or stage 1 (scale of Vanderlip and Reeves, 1972). Plants were then transferred to one of three temperatures (2, 5, or 8 ºC) and exposed to three different lengths of time (1, 4, or 8 days). Next, plants were then transferred back to the 27 ºC temperature for 10 days. All plants exposed to the chilling treatments survived with no visual symptoms of injury. Ercoli et al. (2004) concluded that this hybrid could be held for 8 days at the lowest temperature (2 ºC) without apparent damage. However, generally growth chambers, even the new ones, do not go as low as 2 ºC. Growth chambers can be set at 2 ºC, but the temperature level achieved is generally higher than 2 ºC (Dr. Krishna Jagadish, personal communication, 9 May 2018). Data loggers need to be put in the chambers to document the temperatures. Therefore, one needs to

exercise caution in interpreting data from growth chambers set a 2 ^{o}C. Ercoli et al. (2004) did not determine if sorghum seeds could germinate and emerge at 2, 5, or 8 ^{o}C.

Maulana and Tesso (2013) investigated the impact of cold temperature at two growth stages of sorghum to identify genetic sources of cold tolerance for use in breeding programs. In a growth-chamber experiment, three genotypes of sorghum, Shan Qui Red (tolerant), SRN39 (susceptible), and Pioneer 84G62 (a commonly grown commercial hybrid in the U.S.A.) were subjected to two temperature regimes: cold temperature [15 ^{o}C (day) and 13 ^{o}C (night)] and a control temperature [25 ^{o}C (day) and 23 ^{o}C (night)] for 10 days at seedling and flowering stages. The plants grew in 50:50 mixture of soil and Metro-Mix 360 growing medium (Sun Gro Horticulture, Agawam, MA). Early season cold reduced seedling dry weight, but all three genotypes grew under the cold temperatures. Under the cold temperatures, the seedling dry weights of Shan Qui Red, SRN39, and Pioneer 84G62 at the end of the 10-day experiment were 0.47, 0.10, and 0.41 g; under the control temperatures they were 1.52, 1.21, and 1.29 g, respectively. The data confirmed that Shan Qui Red was cold tolerant. Early season cold stress delayed flowering by 8 days, but had no effect on yield components. Maulana and Tesso (2013) concluded that cold stress at the seedling stage may not be critical for yield provided that emergence is not affected.

Razmi et al. (2013) examined seed germination and seedling growth of three sorghum genotypes (KFS1; KFS1; Speedfeed) grown under four temperature regimes: 25/20, 15/10, 13/10, and 11/8 ^{o}C, day/night temperatures, for up to 15 days. Seeds were germinated in Petri plates with wetted filter paper. Germination percentage was reduced from 60 to 90% at 25/20 ^{o}C to 10 and 40% at 11/8 ^{o}C. Even though Razmi et al. (2013) state that some seeds did not germinate at the cold temperatures, apparently some seeds of all genotypes did germinate under

the cold temperatures. The differential germination of genotypes is a major issue that needs to be resolved in sorghum. Speedfeed was most susceptible and KFS2 was the most tolerant. At the end of the experiment at 11/8 ^{o}C, Speedfeed had a shoot length of 1 mm and KFS2 had a shoot length of 8 mm. Plants grown at 25/20 ^{o}C germinated about as well as those at 15/10 ^{o}C. Razmi et al. (2013) concluded that temperatures below 15/10 ^{o}C could be considered as cold stress for sorghum.

Tiryaki and Andrews (2001a, b) developed a screening test to identify cold tolerant sorghums. During a 13-day experiment using Petri plates, they screened 36 genotypes for cold tolerance at germination using control temperatures of 25-30 ^{o}C (day) and 20-25 ^{o}C (night) in a greenhouse and at constant cold temperature in a growth chamber of 15 ^{o}C. Germination rate in cold conditions was found to give good relative separation among genotypes in cold tolerance.

Salas Fernandez et al. (2015) screened 56 accessions (38 kaoliangs, described in the next paragraph, and 18 non-kaoliangs) in the laboratory using a 7-d and a 14-d cold test at 10 ^{o}C with soil. The accessions were also evaluated under early planting conditions in the field at two locations in the Midwest of the USA and it was found that the 7-d cold test was the best predictor of seed emergence in the field.

Although most of the available sorghum germplasm is of tropical origin, some sorghums have evolved in isolation in temperate regions of China. These sorghums, referred to as "kaoliangs," tend to exhibit higher seedling emergence under cool temperatures than most other sorghums (Yu et al., 2004; Knoll and Ejeta, 2008; Knoll et al., 2008; Burow et al., 2011; Salas Fernandez et al., 2014). Shan Qui Red is one of these kaoliangs, and several studies have shown that it has a high level of cold tolerance (Tiryaki and Andrews, 2001a; Knoll et al., 2008). In Chinese, "kao" means "high" and "liang" means "grain" or "food staple" (George L. Liang, retired

sorghum breeder, Kansas State University; personal communication 2 April 2018). Farmers use the stalks of the kaoling types for fences or even as roofing material. A kaoliang sorghum has a special feature. When the weather is hot, even iron wires on the stalk will not burn the plant; the stalk serves as an insulation layer. The kaoliangs were used for human consumption in the old days, including during World War II, when food was scarce in China (Dr. Liang, personal communication, 2 April 2018). Though it has a bad taste for humans, kaoliangs are used for livestock or chicken feed.

Mold sensitivity is of concern during seed germination (Rami et al., 1998). Chilling stresses occur when planting coincides with periods of cool, wet weather (Kapanigowda et al., 2013). The cold temperatures not only affect germination and seedling establishment, but also increase susceptibility to a number of seedling pathogens, including *Pythium* spp., predominantly *P. aphanidermatum*, *P. ultimum*, and *P. arrhenomanes* (Kapanigowda et al., 2013). Seed physiologists have developed laboratory tests to assess seed vigor. One of them is the cold test that imitates the emergence of seedlings under realistic field conditions. This stress test relies on exposing non-treated seeds to a cold temperature (10 ^{o}C), excess moisture, and non-sterilized soil to introduce soil-borne pathogens (Salas Fernandez et al., 2014). Such a test is widely used in the maize seed industry to determine the fitness of a maize seed lot for emergence and survival under stressful conditions in the field. In several of the studies investigating cold tolerance of sorghum, temperatures greater than 10 ^{o}C have been used as the minimum temperature (e.g., 15 ^{o}C; see Tiryaki and Andrews, 2001a, b; Maulana and Tesso, 2013), and at temperatures warmer than 10 ^{o}C, the seedling has a growth competitive advantage over the soil pathogens and could escape infection. Therefore, it is important to use lower temperatures (10 ^{o}C) and soil in the cold test to slow seedling growth and promote seed-pathogen interaction (Salas Fernandez et al.,

2014). On average over the long term (30 years), the temperature of soil in the field increases on average by 0.5 ^{o}C every 3 days (Mary C. Knapp, Associate Agronomist, Weather Data Library, Kansas State University, Manhattan, KS; personal communication, 10 May 2018). However, this average is highly variable depending on the soil type, soil cover, soil moisture, and solar radiation. When air temperature increases, there is more of a lag of increase in temperature of a wet soil than in a dry soil. Therefore, a wet soil warms up more slowly than a dry soil. In spring, air temperature is usually warmer during the day than soil temperature. But if a cold front moves in, then the soil is warmer than the air temperature. Solar radiation on a bare soil will warm it up faster than solar radiation on a soil covered with residue. Therefore, there are many factors affecting the temperature of the soil and the pathogen-plant interaction.

Minimum temperatures for growth of sorghum have been studied. The mean optimum temperature range for sorghum is 21 to 35 ^{o}C for seed germination, 26 to 34 ^{o}C for vegetative growth and development, and 25 to 28 ^{o}C for reproductive growth (Prasad et al., 2008). Stand establishment and early-season vigor of sorghum were adversely affected by air and soil temperatures below 15 ^{o}C during germination, emergence, and early seedling growth (Burow et al., 2011; Kapanigowda et al., 2013). Tari et al. (2013) stated that sorghum cannot germinate below a soil temperature of 10 ^{o}C. Sorghum is normally grown where minimum mean temperatures during the growing season generally are above 18 ^{o}C (Singh, 1985). Cold-tolerant sorghums have been shown to have 6-10 ^{o}C lower optimum temperature requirements than cold-susceptible sorghums (Singh, 1985).

Comparison of chilling tolerance of maize and sorghum

Summarizing results for minimum temperatures, older studies indicate that maize can germinate and grow for up to 10 days at 10 ^{o}C (Haskell, 1952), and, up to 35 days for short

periods of cold (5 ^{o}C for 4 hours per day) (Eagles, 1988). More recently, Farooq et al. (2009) state that maize root growth can occur at a temperature as low as ~9 ^{o}C. Hybrids of sorghum can emerge and grow at 11/8 ^{o}C day/night for up to 30 days (Yu et al., 2004).

No study could be found in which the chilling tolerance of maize and sorghum has been directly compared. The biochemical properties of the maize and sorghum seeds have been compared (Rooney and Pflugfelder, 1986; Chandrashekar and Mazhar, 1999), but temperature was not a factor in the comparison. Therefore, the objective of this research was to screen and compare maize hybrids and sorghum genotypes, grown under the same controlled-environmental conditions, for chilling tolerance at the germination and seedling stages. The hypothesis was that maize hybrids and sorghum genotypes vary in chilling tolerance. Because apparently no information exists concerning the grain quality of maize and sorghum of varying cold tolerance, the seeds were analyzed for concentrations of protein, starch, and amylose.

Chapter 2 - Screening Maize and Sorghum for Chilling Tolerance at Seedling Stage

Abstract

Low temperature is one of the most limiting stresses to crops that are adapted to tropical and subtropical regions, such as maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench], when introduced into temperate regions. However, no studies have compared the chilling tolerance of maize and sorghum grown together. Therefore, the objective of this research was to screen maize hybrids and sorghum genotypes for chilling tolerance at the germination and seedling stages. With the hypothesis that grain composition of maize and sorghum could lead to varying chilling tolerance, the seeds were analyzed for concentrations of protein, starch, and amylose. Five commercial hybrids of maize and 18 genotypes of sorghum were maintained in growth chambers for 31 days at two temperatures: a control temperature (25/20 ^{o}C, day/night) and at chilling temperatures (11/8 ^{o}C for 14 days; 12.5/9.5 ^{o}C for 14 days, and 14/11 ^{o}C for 3 days). Emergence and seedling height were measured during the experiment. At the end of the experiment, shoot dry weight, root dry weight, and leaf area were determined.

Emergence of sorghum under the chilling temperature regime was low (18%). Average height of the emerged sorghum seedlings in the cold temperatures at the end of the experiment was 1.4 cm compared to 55.5 cm in the control treatment. All maize hybrids emerged, but emergence and growth were slowed by the cold temperatures, and average height at the end of the experiment was 4.6 cm compared to 96.1 cm in the control treatment. Shoot dry weight, root dry weight, and leaf area of the sorghum under the chilling temperatures were too small to measure, and, for maize, they were greatly reduced. The results showed that, for sorghum,

temperatures should be above 14 ^{o}C for emergence, while maize could emerge at lower temperatures.

The analyses of the sorghum seeds showed that Redbine 60 and RTx430 had the highest protein concentrations (15.71% and 15.35%, respectively), and Segaolane had the lowest protein concentration (9.83%). Segaolane had the highest starch concentration (72.71%), and RTx430 had the lowest starch concentration (65.31%). There was an inverse relationship between protein and starch concentrations in the sorghum seeds ($R^2 = 0.69$). Amylose concentrations did not vary significantly among the sorghum seeds. The analyses of the maize seeds showed that Dekalb 51-20 and Pioneer 1151 had the highest protein concentrations (10.98% and 10.95%, respectively), and Pioneer 1105 had the lowest protein concentration (9.26%). Starch and amylose concentrations did not vary significantly among the maize seeds.

Introduction

Maize and sorghum are two tropical crops now grown in temperate latitudes, where they are susceptible to chilling temperatures in the spring. In Kansas, maize is usually planted between April 1 to May 20, and sorghum is usually planted a month later, from May 1 to June 20 (Shroyer et al., 1996). There is a push for earlier planting, because of the potential to increase yields. In addition, early plantings may spread the risk of drought, avoid damage from early fall frost, eliminate grain moisture dockage at harvest, increase profitability associated with better harvest season prices, spread field work load, and facilitate rotation to wheat or cover crops in the fall (Claassen et al., 1999).

Because of the economic importance of maize, much more research has been done concerning the chilling tolerance of it compared to sorghum. Farooq et al. (2009) reviewed the voluminous literature concerning the chilling tolerance of maize. For maize, temperatures below 12-15 ^{o}C induce chilling stress, but plants may be able to grow at temperatures as low as 9 ^{o}C. Less research has been done with sorghum. A key paper is the comprehensive study by Yu et al. (2004), who found that 30 commercial hybrids of sorghum emerged and grew at temperatures of 15/10, 13/10, and 11/8 ^{o}C, day/night.

Limited information exists on the chilling tolerance of extremely diverse germplasm of sorghum. Sunoj et al. (2017) studied the impact of heat stress on pollen germination and other post-flowering physiological processes of 24 diverse sorghum genotypes, which were nested association mapping (NAM) founder sorghum genotypes. The NAM design, which uses multiple recombinant inbred line families connected by a single common parent, has been successful for complex trait dissection (Bouchet et al., 2017). The populations developed using these 24 sorghum genotypes as founders provided a rich genetic resource for heat tolerance

(Sunoj et al., 2017). In the current study on chilling tolerance, eleven of the 24 sorghum genotypes studied by Sunoj et al. (2017) were used. In addition, five advanced breeding lines developed at the Agricultural Research Center at Hays (ARCH), Kansas, also were used, along with two, commercial hybrids. It was hypothesized that these 18 diverse sorghum genotypes would provide substantial variation in chilling tolerance.

It is generally thought that maize has greater chilling tolerance than sorghum. However, apparently no study has compared the chilling tolerance maize and sorghum. Therefore, the objective of this research was to screen and compare maize hybrids and sorghum genotypes, grown under the same controlled-environmental conditions, for chilling tolerance at the germination and seedling stages. The hypothesis was that maize hybrids and sorghum genotypes vary in chilling tolerance. In addition, it was desired to see if, indeed, maize was more chilling tolerant than sorghum. Because apparently no information exists concerning the grain composition of maize and sorghum of varying cold tolerance, the seeds were analyzed for concentrations of protein, starch, and amylose.

Materials and Methods

The 31-day experiment was carried out in August and September 2017 in four growth chambers (Conviron Model No. CMP 6050, Conviron, Winnipeg, Manitoba, Canada) at Kansas State University in Manhattan, Kansas. For all growth chambers, the photoperiod was 13 hours light (05:00 to 18:00 h) and 11 hours dark (18:00 to 5:00 h), and the relative humidity was set at 60%. Two growth chambers were kept at control temperature, which were 25 ^{o}C day and 20 ^{o}C night, and two other growth chambers were kept at cold temperature. The temperatures in the cold chambers were increased 1.5 ^{o}C every 14 days to simulate what might occur under field conditions, as the temperature warms up during the spring. At the beginning of the experiment,

the temperatures were set at 11/8 ^{o}C (day/night), for 14 days; then the temperatures were increased to 12.5/9.5 ^{o}C for the next 14 days; and finally the temperatures were increased to 14/11 ^{o}C for the last 3 days (total of 31 days). The low temperature regimes were selected based on the study of Yu et al. (2004), who found that hybrid sorghum germinated at 11/8, 13/10, and 15/10 ^{o}C.

Genetic materials

Eighteen genotypes of sorghum and five hybrids of maize were studied in the experiment. The 18 genotypes of sorghum were as follows:

Two commercial ones:

Dekalb 41-50

Pioneer 8500

Five from the breeding program at the Agricultural Research Center in Hays, KS (ARCH):

ARCH 10747-1

ARCH 10747-2

ARCH 12002

ARCH 12012

ARCH 12045

Eleven from the sorghum Nested Associated Mapping (NAM) program (Casa et al., 2008; see their Supplement 1; Sunoj et al., 2017):

Ajabsido

Macia

P898012

Redbine 60

RTx430

SC35

SC283

SC971

SC1345

Segaolane

Shan Qui Red

Table 2.1 gives a description of the NAM lines (Casa et al., 2008, Supplement 4; Bouchet et al., 2017; Sunoj et al., 2017).

The five hybrids of maize were commercial hybrids:

Dekalb 51-20

Dekalb 64-69

Pioneer 1105

Pioneer 1151

Pioneer 8387

Experimental design and handling

In one control and one cold chamber, the seeds were planted on 23 August 2017 (called the "first set" of growth chambers) and in the second control chamber and the second cold chamber, the seeds were planted on 25 August 2017 (called the "second set" of growth chambers). The two-day difference in planting was necessary to plant all the seeds in the four growth chambers. Seeds were planted in cone-shaped plastic pots (6.4 cm inside diameter; 25 cm in length; 4 drainage holes) with 182 grams of a commercial growth medium (Metro Mix 360, Sun Gro Horticulture, Agawam, MA) in each pot. There were four pots for each genotype in each

chamber for a total of 72 containers with sorghum (4 containers; 18 genotypes) and 20 containers of maize (4 containers; 5 hybrids). At planting, 300 mL tap water were added to each container, and water drained from the drainage holes at the bottom of the containers. The soil in the cold growth chambers remained wet, so no more water was added to the pots in the cold growth chambers. The water content of the growth medium of pots in the control chambers was monitored using a soil moisture meter (HoldAll Moisture Meter; Panacea Products Corp., Columbus, OH; Product Code 0 70686 26002 9). It uses a bimetallic tip to generate electricity in the presence of moisture. The electricity causes a pointer to move across a scale indicating the degree of soil wetness (Faber et al., 1994). The probe, inserted into the soil at the 5 cm depth, was small (4 mm in diameter) and had essentially no effect on root growth. Using the probe it was determined how much water was needed to keep the soil moist. In general, this meant that the maize in the control pots received about 200 mL weekly and the sorghum in the control pots received about 200 mL every two weeks. Therefore, the plants were kept well-watered during the experiment.

For the control temperature, 2 seeds were planted in each pot of maize and sorghum, and plants were thinned to 1 plant per pot on 29 August 2017 for the first set of growth chambers and on 1 Sept. 2017 for the second set of growth chambers. For the maize under the cold treatment, 2 seeds were planted in each pot and plants were thinned to one plant on 19 Sept. 2017 for the seeds planted on 23 Aug. 2017 and on 21 Sept. 2017 for the seeds planted on 25 Aug. 2017. For the sorghum under the cold treatment, 4 seeds of sorghum were planted in each pot and were not thinned because of low emergence.

In each growth chamber, the sorghum grew on one side of the chamber and the maize grew on the other side of the chamber (although the sides were switched, as noted below). Trays

containing maize or sorghum were rotated in an anticlockwise direction about every three days. In addition to the rotations, the position of the maize and sorghum trays within a chamber were interchanged every 7 days. That is, every week the maize pots and sorghum pots were switched from one side of a chamber to the other side of a chamber. A split-plot design was used with "chamber" the experimental unit for temperature and "pot" the experiment unit for genotype (4 pots per genotype in each chamber).

HOBO data loggers (Onset Computer Corp., Bourne, MA) were used to measure the air and soil temperature and relative humidity inside the chambers. Soil temperature was measured at the 5 cm depth. In Appendix A, Figure A.1 gives air and soil temperatures in Chamber 19 (control temperature) and Chamber 15 (cold temperature), the first set of growth chambers with planting on 23 Aug. 2017, and Figure A.2 gives the air and soil temperatures in Chamber 17 (control temperature) and Chamber 11 (cold temperature), the second set of growth chambers with planting on 25 Aug. 2017.

Data collection

Emergence was recorded and emergence percentage was calculated as follows:

Emergence percentage = (n/N) x 100

where n is the number of seeds emerged and N is the number of seeds planted.

Height was measured using a ruler every three days during the experiment. Height was measured from the surface of the growth medium to the tip of the most recently expanded leaf, which was extended to make the measurement. At harvest on 23 Sept. 2017 for the first set of growth chambers and 25 Sept. 2017 for the second set of growth chambers, shoots were cut at the surface of the growth medium. Total leaf area of each plant was measured with a leaf-area meter (Model No. 3100 Area Meter, Li-Cor, Inc., Lincoln, NE). Roots were washed from the

growth medium and root dry weight was determined. Roots and shoots were dried for a week at 60 ^{o}C and dry weights were measured.

The seeds of all maize and sorghum entries were analyzed for protein, starch, and amylose. Three separate lots of seeds were analyzed. The ARCH and NAM seeds and seeds of the commercial sorghum hybrid, Pioneer 8500, were provided by Dr. Ramasamy Perumal, Associate Professor, Sorghum Breeding, Agricultural Research Center, Kansas State University, Hays, KS. The Dekalb 41-50 hybrid sorghum seeds were provided by Michael Lenz, Innovative Seed Solutions LLC, Mt. Hope, KS. The maize seeds were provided by Dr. Ignacio Ciampitti, Associate Professor, Crop Production, Department of Agronomy, Kansas State University.

Protein concentration was determined by the total combustion method. A LECO TruSpec CN Carbon/Nitrogen combustion analyzer (Model No. FP628) was used, which reports total levels (inorganic and organic) of C and N on a weight percent basis, according to the TruSpec CN instrument method "Carbon, Hydrogen, and Nitrogen in Flour and Plant Tissue," published by LECO Corporation, St. Joseph, MI, in 2005. Protein concentration was determined by multiplying nitrogen by 6.25 (Buxton et al., 1996).

Starch is a mixture of two types of compounds that are separable from each other: amylose and amylopectin. Amylose is the component that is a long, unbranched chain, and amylopectin has been shown to be a branched-chain polysaccharide (White et al., 1964). Starch and amylose were determined, and amylopectin then can be calculated as the difference between starch concentration and amylose concentration.

Total starch was analyzed using the Megazyme International Ireland (2015) total starch assay procedure. This is an official AOAC (Association of Official Analytical Chemists) method and an AACC (American Association of Cereal Chemists) method. The principle of the analysis is

as follows. Thermostable α-amylase hydrolyzes starch into soluble branched and unbranched maltodextrins. Amylogucosidase quantitatively hydrolyzes maltodextrins to D-glucose. D-Glucose is oxidized to D-gluconate with the release of one mole of hydrogen peroxide, which is quantitatively measured in a colorimetric reaction employing peroxidase and the production of a quinoneimine dye.

Two procedures were used to analyze for starch: 1) seed samples were not washed; 2) samples were washed with 80% ethanol. Samples containing high levels of D-glucose and maltodextrins are washed with aqueous ethanol (80%) before analysis (Megazyme International Ireland, 2015, p. 2). However, if one does not know beforehand if samples contain high levels of D-glucose and maltodextrins, one would have to analyze seeds to determine their concentrations. The two procedures were used, as suggested by the USDA Center for Grain and Animal Health Research, where the analyses were done.

Procedure 1: With washing with 80% ethanol

The seeds were milled to pass a 0.5 mm screen and then 25 mg of the ground sample was weighed into a glass centrifuge tube and 750 μL of 80% ethanol was added and the mixture was incubated for 5 minutes at 85 ^{o}C. Another 750 μL of 80% ethanol was added and the mixture was vortexed and then centrifuged for 10 minutes. The supernatant was collected and put into glass centrifuge tubes. To the sample, 1.5 mL 80% ethanol was added, vortexed, and centrifuged again, and the supernatant was collected. This step was repeated once more. In the next step, the enzyme (α-amylase) was added and the steps described for Procedure 2 (see below) were carried out.

Procedure 2: Without washing

The seeds were milled to pass a 0.5 mm screen and then 25 mg of the ground sample was weighed and put in a glass test tube and 50 µL of 80% ethanol was added followed by adding 750 µL of thermostable α-amylase in a sodium acetate buffer (600 µL α-amylase and 17.4 µL buffer). The mixture was incubated for 12 minutes on a 100 ^{o}C block heater with shaking at 4, 8, and 12 minutes (every 4 minutes). The tubes were then placed in a 50 ^{o}C water bath for 5 minutes and then 25 µL amyloglucosidase was added and incubated for 30 minutes at 50 ^{o}C. The entire content of the test tube was then transferred to a 25 mL volumetric flask and was adjusted to the volume of the flask. An aliquot of 10 mL was centrifuged and 6 µL of the sample was then transferred to a 96-well plate. Then 180 µL of GOPOD (glucose oxidase/peroxidase) reagent was added using a plate-pipetting technique. The plates were then placed in a UV plate reader, and the absorbance was measured at 510 nm. Regular maize starch with 93% starch (dry weight basis) was used as the reference, and a D-glucose (1.0 mg/mL in 0.2 benzoic acid) standard was used as a control.

The difference between procedure 1 and procedure 2 was that the ethanol extract from procedure 1 was removed and the residue was used for the starch analysis. In procedure 2 the ethanol was not removed.

For determination of amylose, starch was isolated from samples according to the procedure of Park et al. (2006). Then the method of Kaufman et al. (2015) was used to determine amylose content. The method is as follows. From the starch, 5 mg of a sample or a standard in 1 mL 90% dimethyl sulfoxide (DMSO) was transferred to a 2 mL centrifuge tube. It was heated at 90 ^{o}C for 1 hour and vortexed at 0, 5, 15, 30, 45, and 60 minutes. The blank used only DMSO. Then a 100 µL aliquot of the sample was transferred to a 96-well plate. Then 100 µL of 3.04 g/L

iodine in 90% DMSO was added to each sample, followed by stirring the sample for 2 minutes. Then 20 μL of the mixture was transferred to a new optical 96-well plate and 180 μL deionized water was added to each well. The sample was stirred for 1 minute, and the absorbance was read at 620 nm and 510 nm. A regression equation was determined for the standard curve on each plate analyzed that combines the absorbance values at 620 nm and 510 nm, and dual wavelength amylose was calculated. The standard curve can be obtained from the USDA Center for Grain and Animal Health Research, where the analyses were done.

Grain characteristics of the sorghum seeds also were determined using a single kernel characterization system (SKCS) (Model SKCS 4100, Perten Instruments, Stockholm, Sweden). The system gives an average weight (mg), diameter (mm), and hardness index for 75 kernels fed into the system for each analysis. The hardness index values are based on algorithms from instrumental data that describe the force required to crush individual kernels (Gaines et al., 1996). For reference, hard wheats have a hardness index of about 75 and soft wheats have a hardness index of about 25. The SKCS instrument can analyze seeds of wheat (*Triticum aestivum* L.; *Triticum durum* Desf.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), and sorghum, but it cannot analyze seeds of maize.

Statistical analysis

The four growth chambers allowed two replications for each temperature treatment. The two-day difference in planting between the first set of growth chambers and the second set of growth chambers was small enough so that the two chambers could be considered two true replications. Statistical analyses were performed using SAS (Version 9.4, Statistical Analysis System, 2013) PROC GLIMMIX with a Gaussian distribution. A mixed model analysis, with chamber as a random effect, was conducted for all variables and model fit was evaluated based on the

residuals. Genotype means were separated based on the Tukey-Kramer adjustment with alpha = 0.05.

Results

Emergence

At the end of the experiment, all plants emerged in both replications of the five maize genotypes under the cold temperature, and, therefore, emergence percentage was 100%. Table 2.2 gives the emergence percentage of the sorghum genotypes under the cold temperature at the end of the experiment. As noted, 4 seeds of each genotype were planted in the pots with sorghum in the cold treatment, while 2 seeds were planted in the other treatments. Despite doubling the number of seeds in each pot, emergence percentage was low. Out of 576 total sorghum seeds planted under the cold temperatures (18 genotypes x 4 pots per genotype in each chamber x 4 seeds in each pot x 2 chambers), only 104 emerged (18.0%). SC283 had the highest percentage emergence (50.0%), followed by SC971 and Segaolane, each with 40.6%, and the two hybrids (Dekalb 41-50 and Pioneer 8500) and Shan Qui Red, each with 25.0%. ARCH 10747-1 and RTx430 had 0% emergence under the cold treatment. The average height of the emerged sorghum seedlings in the cold temperatures at the end of the experiment was 1.4 cm. For all genotypes, the length of each seedling 31 days after planting ranged from 0.2 cm to 3.5 cm; Ajabsido and Dekalb 41-50 each had a seedling that reached 3.5 cm tall.

Emergence percentage for maize under the control treatment was 100%. For sorghum under the control treatment, it was 100% for all genotypes except for ARCH 10747-1, ARCH 12002, Macia, SC35, and Segaolane, where it was 87.5% for each; ARCH 12012 where it was 75.0%; and RTx430 where it was 50%. Kapanigowda et al. (2013) noted that ARCH 10747 (no number 1 or 2 given after it) planted early (2 May 2011) in the field at two locations in Kansas (Hays and

Colby) had an emergence of 39.45% and was not identified as being a potential source of cold tolerance.

Height, dry weight, and leaf area

Appendix Figure A.3 shows the height of the maize genotypes under the control and cold temperatures. The Dekalb hybrids are shown on the left-hand side of the figure and the Pioneer hybrids are shown on the right-hand side of the figure. Under the control temperature and at the end of the experiment 30 days after planting, the three Pioneer hybrids were the tallest, with Pioneer 1151 being the tallest at 102.5 cm. The shortest hybrid was Dekalb 64-69 with a height of 91.5 cm. At the end of the experiment under the control temperature, the Pioneer hybrids were about 10 cm taller than the Dekalb hybrids.

Under the cold temperature, maize did not start to emerge until 21 to 24 days after planting, while under the control temperature, it emerged by six days after planting. Under the cold temperature, final heights varied from 7.3 cm (Pioneer 8387) to 5.7 cm (Dekalb 64-69). At the end of the experiment, height of the maize genotypes under the cold treatment was 1/15 that of the height of the maize genotypes under the control treatment, because the average heights of the maize genotypes under the cold and control temperatures were 6.5 and 96.1 cm, respectively.

Appendix Figures A.4, A.5, and A.6 show the height of the sorghum genotypes under the control temperature for the hybrids, the ARCH populations, and the NAM populations, respectively. Heights of the sorghum genotypes under the cold temperature were not analyzed statistically because of the many missing values (no plants emerged). On the final measurement day, 30 days after planting, the order of heights, from tallest to shortest, was as follows with the heights in parentheses: SC35 (68.4 cm); Segaolone (65.1 cm); SC971 (64.4 cm); SC1345 (60.3 cm); ARCH 10747-2 (60.3 cm); Shan Qui Red (60.1 cm); ARCH 12045 (60.1 cm); P898012

(56.2 cm); RTx430 (56.2 cm); Redbine 60 (54.2 cm); Dekalb 41-50 (53.7 cm); Pioneer 8500 (52.9 cm) Macia (52.3 cm); ARCH 10747-1 (49.1 cm); ARCH 12002 (48.7 cm); SC283 (47.5 cm); ARCH 12012 (45.2 cm), and Ajabsido (44.2 cm). The average height of the sorghum genotypes under the control temperature was 55.5 cm. As noted, under the cold temperatures, the average height was 1.4 cm. The cold treatment reduced the height by 40 times.

As noted under emergence, RTx430 had the poorest emergence under the control conditions (50%) which was due to fungal infection. It was known that RTx430 had fungal infection, because in the incubator experiments (see Appendix B) fungal infection was observed on the seeds. RTx430 was the shortest genotype at the end of the experiment (Appendix A, Figure A.6).

Under the cold temperature treatment, both maize and sorghum grew too poorly to carry out a statistical analysis of the growth. Therefore, only the maize and sorghum under the control temperature were analyzed statistically. Tables 2.3 and 2.4 give the shoot dry weight, root dry weight, and total leaf area of maize and sorghum, respectively, at harvest, 31 days after planting under the control temperature. Among the five maize hybrids, Pioneer 8387 had significantly highest shoot dry weight and leaf area (Table 2.3). Root dry weight did not differ among the hybrids. Among the sorghum genotypes, SC35, Shan Qui Red, SC1345, and SC971 had the highest shoot dry weights (Table 2.4). Shan Qui Red had the highest root dry weight. RTx430 had the lowest shoot and root dry weights, as well as the lowest leaf area. As noted, the poor growth of RTx430 was due to fungal infection. The shoot dry weights of the 18 sorghum genotypes averaged 1.32 g, and the root dry weights averaged 1.31 g (Table 2.4). The shoot dry weights of the maize averaged 4.17 g and the root dry weights averaged 1.81 g (Table 2.3). The

shoot and root dry weights of sorghum were similar, while the shoot dry weights of maize were 2.3 times greater than the root dry weights.

The statistical model used to generate the data for dry weights and leaf area in Tables 2.3 and 2.4 gives estimated values. Appendix A gives the actual values of the shoot and root dry weights and leaf areas of maize under the cold and control temperatures (Table A.1 in the Appendix) and the shoot and root dry weights and leaf area of sorghum under the control temperature (Table A.2 in the Appendix). (Shoot and root dry weights and leaf area for sorghum under the cold temperatures were not taken, because the plants were too small to measure.) The data show that shoot dry weight and leaf area of maize were reduced at least 100 fold by the cold and root dry weight was reduced on average by about 70 fold.

Protein, starch, and amylose

For the maize seeds, protein varied among the hybrids, but starch and amylose did not vary (Table 2.5). Dekalb 51-20 and Pioneer 1151 had the highest concentrations of protein with values of 10.98% and 10.95%, respectively. Pioneer 1105 had the lowest concentration of protein (9.26%).

For the sorghum seeds, both protein and starch varied among the genotypes, but amylose did not vary (Table 2.6). Redbine 60 and RTx430 had the highest concentrations of protein with values of 15.71% and 15.35%, respectively. Segaolane had the lowest concentration of protein (9.83%). Segaolane had the highest concentration of starch (72.71%), and RTx430 and ARCH 12002 had the lowest concentrations of starch with values of 65.31% and 66.11%, respectively.

The procedure to analyze starch was highly significant (Table 2.5 and 2.6). For maize, when all hybrids were averaged together, Procedure 1 gave an estimate of 66.62% starch, and Procedure 2 gave an estimate of 69.31% starch. For sorghum, when all genotypes were averaged

together, Procedure 1 gave an estimate of 67.84% starch, and Procedure 2 gave an estimate of 69.79% starch. Therefore, Procedure 2, in which the seeds were not washed with 80% ethanol, gave higher estimates of starch than Procedure 1, in which the seeds were washed with ethanol.

The sorghum seeds had a wider range in concentration of protein than did the maize seeds. This perhaps reflects that fact that diverse, unadapted accessions of sorghum were used in the study, while the maize seeds were hybrids. The range for protein in the sorghum seeds varied from 9.83% (Segaolane) to 15.71% (Redbine 60), with a difference of 5.88%. The range for protein in the maize seeds varied from 9.26% (Pioneer 1105) to 10.98% (Dekalb 51-20), with a difference of 1.72%. The wider variability in protein in the sorghum seeds allows for more diversity in a breeding program, if one wants to select for seeds either with high or low protein. The average value for protein in the maize hybrids (all 5 averaged together) was 10.25%, and the average value for protein in the sorghum genotypes (18 averaged together) was 13.12%. On average, the sorghum seeds had 2.87% more protein in them than did the maize seeds.

Different types of starch have different proportions of amylose and amylopectin. Waxy starch contains almost exclusively amylopectin and little amylose. Normal starch contains 15-30% amylose. High amylose starch usually has more than 50% amylose (Yangcheng, 2012). Both maize and sorghum in this experiment had seeds with normal starch. The concentration of amylose in the maize seeds varied from 23.36% to 26.90% (Table 2.5), and the concentration of amylose in the sorghum seeds varied from 22.82% to 27.67% (Table 2.6).

Weight, diameter, and hardness index differed significantly among the sorghum genotypes (Table 2.7). Ajabsido had the heaviest seed (46.39 mg), the largest diameter (3.18 mm), and the lowest hardness index (33.24). P898012 also had the lowest hardness index, and its value (33.23) did not differ significantly from that of Ajabsido. SC971 had the lightest seed (21.17

mg). ARCH 10747-1 had the smallest diameter (2.16 mm). SC283 had the highest hardness index. But its hardness index was suspect because of an outlier in the data. The three values that were averaged for hardness index for SC283 were 78.49, 56.43, and 59.69. The value 78.49 was 16.19 points higher than any other value determined for hardness index. If the value 78.49 is not considered valid, then the average value for the hardness index for SC283 was 58.06. If 58.06 is a more reasonable value for SC283, then Shan Qui Red had the highest hardness index (59.67) followed closely by the hardness index of ARCH 12002 (59.51).

Discussion

The cold temperatures used in this 31-day experiment [11 ^{o}C/8 ^{o}C (day/night) for 14 days; 12.5 ^{o}C/9.5 ^{o}C for 14 days; 14 ^{o}C/11 ^{o}C for 3 days] resulted in poor emergence of the 18 genotypes of sorghum studied. Out of 576 total sorghum seeds planted, 104 emerged (18.0%) under the cold treatment with an average height at the end of the experiment of 1.4 cm compared to an average height of 55.5 cm for the control plants. These results agree with the statement of Tari et al. (2013), who said that generally stand establishment and early-season vigor of sorghum are adversely affected by temperatures below 15 ^{o}C.

At the temperatures used in the experiment, 100% of the maize seeds emerged, but the plants were chlorotic and grew poorly. Compared to the control temperature, height of maize was reduced 93 %, and shoot dry weight was reduced at least 99 %, by the cold temperatures. The results of this experiment showed that, for the 18 genotypes of sorghum, the day temperature should be above 14 ^{o}C for sorghum to emerge. Maize could tolerate the temperatures for 31 days, but the day temperature should be greater than 14 ^{o}C for survival after 31 days. The data show that the maize hybrids used in this study were more cold tolerant than the sorghum genotypes; however, all plants were severely damaged by the cold.

In the study by Yu et al. (2004), sorghum hybrids grew at the temperatures used in this experiment. The hybrids in this experiment (Dekalb 41-50 and Pioneer 8500) were not cold tolerant like those used by Yu et al. (2004). Even though the emergence of the hybrids was only 25.0%, one of the two tallest seedlings under the cold conditions was a hybrid (Dekalb 41-50), and it reached a height of 3.5 cm by the end of the experiment. Generally, hybrids are more vigorous than inbred parent lines (Yu and Tuinstra, 2001), so one would expect the hybrids to perform better under the cold temperatures; however, in this experiment they did not. It is not known why the sorghum hybrids in this experiment were not cold tolerant like the ones used by Yu et al. (2004). The answer is probably in the methodology as well as in the different hybrids used. Yu et al. (2009) studied hybrids that had been evaluated for cold tolerance under field conditions in Kansas by Claassen et al. (1999), who stated that they evaluated early-planted grain sorghum hybrids. Therefore, the hybrids that Yu et al. (2009) studied already had been selected to grow well under early spring (April) temperatures. Sorghum normally is planted in June at the location in Kansas where Claassen et al. (1999) did their research. The two hybrids used in this study (Dekalb 41-50 and Pioneer 8500) had not been previously evaluated and were of unknown cold tolerance.

The sorghum inbred line RTx430 had 0% emergence under the cold temperatures. This was due to fungal infection. The results for RTx430 confirm what others have found for this genotype, i.e., it is cold sensitive (Burow et al., 2011). This may be due in part to its susceptibility to pathogens. As noted in the literature review, in order for a seed to be cold tolerant, it must be able to withstand attack from microorganisms, because chilling stress occurs at planting time in the spring when soil is cool and wet. RTx430 was developed at the Texas Agricultural Experiment Station and released 12 Feb. 1976 (Miller, 1984). It was bred to

withstand head smut and downy mildew. It is an important and widely adapted pollinator/restorer line of sorghum, but it has been designated as cold sensitive (Burow et al., 2016). One reason it is cold sensitive is because its seeds are susceptible to grain mold. Of 19 lines investigated at Corpus Christi, Texas, and College Station, Texas, it had the highest ranking for susceptibility to grain mold (Peterson et al., 2009). Studies of the interaction/association between grain mold and chilling susceptibility need further investigation.

Emergence is strongly influenced by seed quality (Yu and Tuinstra, 2001). But, because the seeds grew poorly under the cold temperatures, no conclusion could be made about seed quality and cold tolerance. However, among the 18 genotypes of sorghum, RTx430, which is cold sensitive due to fungal infection, did have the highest concentration of protein (15.35%, along with Redbine 60, which had 15.71% protein), and the lowest concentration of starch (65.31%). The chemical composition of its seed may be related to its cold sensitivity or its mold sensitivity. Segaolane, which had the lowest protein concentration among the 18 sorghum genotypes at 9.83%, but the highest starch concentration at 72.71%, also had one of the highest emergence percentages (40.6%) under the cold temperatures.

Hardness affects cereal grain from resistance to fungal infection (Chandrasheka and Mazhar, 1999). However, RTx430, the genotype susceptible to mold, did not have a low hardness index (Table 2.7). Its hardness index did not differ significantly from the hardness index of the genotype with the highest hardness index (SC283).

Two sorghum genotypes did not emerge under the cold temperatures, and they were RTx430, discussed in the two previous paragraphs, and ARCH 10747-1. ARCH 10747-1 had the smallest seed diameter at 2.16 mm (Table 2.7). When sorghum seed lots were separated into three size groups, significantly fewer of the smallest seeds germinated in tests or in the field (Vanderlip et

al., 1973). A larger seed, therefore, may be desirable under cold temperatures to ensure emergence.

The biochemical data for sorghum (Table 2.6) indicated a negative relationship between protein concentration and starch concentration, and Figure 2.1 shows a plot of the data. The regression coefficient was 0.69. These results agree with those of Hicks et al (2002), who found significant negative correlations between protein and starch when sorghum lines and their hybrids were grown under dryland conditions in Kansas.

In order to try and determine a relationship between grain composition and chilling stress, the protein concentration, starch concentration, grain size, and hardness index of the sorghum genotypes that had emergence of 25 percent or greater were considered (Table 2.8). The data have been extracted from Table 2.2 for emergence, Table 2.6 for the protein and starch concentrations, and Table 2.7 for the grain size and hardness index. Amylose concentration is not considered, because its concentration did not vary among the genotypes (see Table 2.6). All the genotypes with the highest emergence (25% or greater) had a high starch concentration. However, the starch concentration was not a good measure to segregate sorghum seeds, because 16 of the 18 genotypes had the same starch concentration (see Table 2.6). RTx430 and ARCH 12002 were the two genotypes with starch concentrations significantly lower than the other 16 genotypes (less than 67%). Except for San Qui Red and SC971, the genotypes with the highest emergence had the largest diameters. And, except for P898012 and Pioneer 8500, the genotypes with the highest emergence had the highest hardness index. So, in general, the seeds that had 25% emergence or more had the common characteristics of having values of starch that were greater than 67%, a large diameter, and a high hardness index.

In summary, in this 31-day experiment, the temperatures [11/8 ^{o}C (day/night) for 14 days; 12.5/9.5 ^{o}C for 14 days; 14/11 ^{o}C for 3 days] were too cold for the emergence of most of the genotypes of sorghum. Of 576 sorghum seeds planted under the cold temperatures, 104 plants emerged (18.0%). The average height of the emerged sorghum seedlings at the end of the experiment was 1.4 cm compared to 55.5 cm for the control plants. The maize hybrids emerged, but growth was poor, and their average height at the end of the experiment was 4.6 cm compared to 96.1 cm for the control plants. Both the sorghum and maize seedlings were chlorotic and would have died had the experiment been continued. Because of the limited growth of the maize and sorghum genotypes under the cold temperatures, they could not be compared for cold tolerance. The results suggest that, for both maize and sorghum, temperatures should be above 14 ^{o}C for emergence and growth.

Future Research

The 18 sorghum genotypes emerged poorly under the cold temperatures used in this experiment (11/8 ^{o}C for 14 days; 12.5/9.5 ^{o}C for 14 days; 14/11 ^{o}C for 3 days). Therefore, chilling tolerance could not be compared among the genotypes. Future research should maintain seedlings at higher temperatures of 14/11 ^{o}C for equal duration or for longer periods, so that the genotypes emerge and chilling tolerance can be determined.

From plants grown under chilling temperatures, their tissues should be analyzed for lipid composition. One of the most extensively studied aspects in membrane biology is the relationship between lipid composition and the ability of organisms to adjust to temperature changes (Somerville et al., 2000). Studies with *Arabidopsis* mutants have shown that diminished unsaturation resulted in plants that grew well at 22 ^{o}C, and they were less robust than the wild type when grown at 2 ^{o}C to 5 ^{o}C. (*Arabidopsis* is a chilling-resistant plant unlike many tropical and subtropical plants, as studied in this thesis). Thus, reduction in unsaturation appears to lead to chilling sensitivity. If the maize hybrids and sorghum genotypes could grow at chilling temperatures, then their lipid composition should be compared among hybrids and genotypes. Do chilling tolerant hybrids or genotypes have more unsaturated fatty acids than chilling sensitive hybrids or genotypes?. If maize is, indeed, more chilling tolerant than sorghum, does it have more unsaturated fatty acids than sorghum?

In addition to the lipid composition, the amino acid composition should be studied. Does amino acid composition change under chilling temperatures? These analyses should include measurements of the amino acid proline. It is known that, under drought, osmotic adjustment occurs. It is a biochemical mechanism that helps plants adapt to dry soil by increasing the solute particles in plant cells. Proline is an osmolyte that is accumulated in plants in response to stress.

Do plants exposed to chilling temperatures accumulate more proline than plants grown under control temperatures?

It was noted that the maize hybrids grown under the chilling temperatures had longer roots than the sorghum genotypes grown under the chilling temperatures. For example, in the second growth chamber experiment reported in the Appendix, the roots of maize under the chilling temperatures were longer than the shoots. However, the roots of the sorghum genotypes were too small to measure (less than 1 mm), even though the shoot lengths were measurable (ranging in height from about 2 to 6 cm). Why are roots of sorghum shorter under cold temperatures than roots of maize? Plant growth and development are under hormonal control, and the difference in root growth probably relates to different hormonal compositions. The root-forming activity of the hormone auxin has been recognized for decades. The concentration of auxins in maize and sorghum exposed to chilling temperatures should be compared. Brassinosteroids also have been shown to provide protection to plants during chilling stress (Clouse et al., 1998). Therefore, the concentration of brassinosteroids in maize hybrids and sorghum genotypes varying in chilling tolerance should be measured.

It has to be noted that in a more robust testing of the above hypotheses, sorghum hybrids and not just sorghum genotypes need to be included in the comparisons to account for the heterotic contribution, which could have a strong confounding influence.

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Legend to Figure

Figure 2.1. Protein concentration versus starch concentration of 18 genotypes of sorghum. The regression coefficient was 0.69.

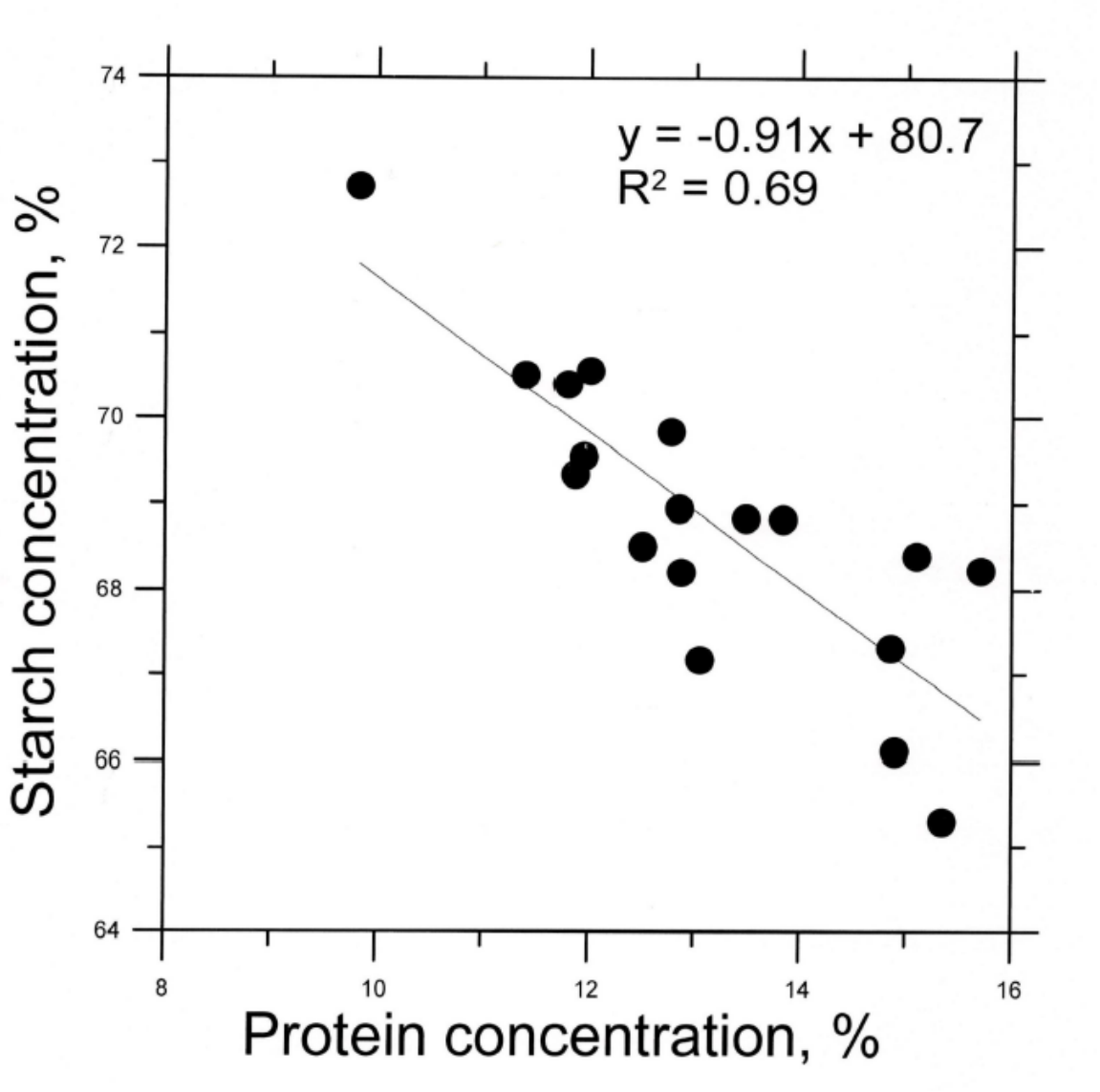

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Table 2.1. Description of the NAM parent lines (from Bouchet et al., 2017; Sunoj et al., 2017)

Genotype	PI[†]	Origin[‡]	Type	Botanical race	Genetic structure[§]
Ajabsido	656015	Sudan	Selected landrace	Caudatum (Feterita)	C=0.50; D=0.29; G=0.21
Macia	565121	ICRISAT, India	Global variety	Caudatum (Zerazera)	C=0.99
P898012	656057	Purdue	…[¶]	…	C=0.95
Redbine 60[#]					
RTx430	655996	Texas A&M	Pollinator parent	…	G=0.65; C=0.23; D=0.08
SC35[$]	534133	Ethiopia	Converted landrace	Durra	D=0.97
SC283	533869	Tanzania	Converted landrace	Guinea (Conspicuum)	K=0.74; G=0.26
SC971	656111	Puerto Rico	Converted landrace	Durra-Kafir	K=0.42; G=0.4
SC1345	597980	Mali	Converted landrace	Caudatum-Bicolor	C=0.85
Segaolane	656023	Botswana	Selected landrace	Kafir	K=0.98
ShanQuiRed[#]		China	Landrace		

[†] Plant Introduction number

[‡] Geographic origin for landrace-derived accessions or breeding program origin for improved lines

[§] Estimated admixture coefficients (G = Guinea, K = Kafir, D = Durra, C = Caudatum)

[¶] Data not available

[#] Bouchet et al., 2017 do not list Redbine 60 or Shan Qui Red. See text for discussion of Shan Qui Red. According to Duncan et al. (1991; see their Table 5-5), Redbine 60 was released in 1950 by Texas. Redbine (Tx3042) is used as an old standard cultivar (Balota et al, 2010; see their Table 1).

[$] Lines with an "SC" designation are partially or fully converted exotic lines from the sorghum conversion program (Peterson et al., 2009).

Table 2.2. Emergence percentage of 18 genotypes of sorghum 31 days after planting and subjected to 11/8 ºC for 14 days, 12.5/9.5 ºC for 14 days and 14/11 ºC for 3 days. Each emergence percentage represents the average of two replications; in each replication there were 4 pots with 4 seeds planted per pot. Therefore, a total of 16 seeds were planted in each replication per genotype. Mean and standard deviation of the two replications are shown. In the right-hand column, the number of seedlings that emerged is given.

Genotype	Emergence, %	Number of seedlings
Ajabsido	18.8±17.7	6
ARCH 10747-1	0+0	0
ARCH 10747-2	3.1±4.4	1
ARCH 12002	9.4±4.4	3
ARCH 12012	3.1±4.4	1
ARCH 12045	9.4±4.4	3
Dekalb 41-50	25.0±0	8
Macia	9.4±4.4	3
P898012	28.1±22.1	9
Pioneer 8500	25.0±8.8	8
Redbine 60	6.3±8.8	2
RTx430	0±0	0
SC35	15.6±4.4	5
SC283	50.0±26.5	16
SC971	40.6±4.4	13
SC1345	15.6±4.4	5
Segaolane	40.6±13.3	13
ShanQuiRed	25.0±8.8	8

Table 2.3. Shoot dry weight, root dry weight, and total leaf area at harvest (31 days after planting) of 5 hybrids of maize grown under the control temperature. Each value is the mean of eight plants (four pots in two growth chambers). Analysis of variance for each parameter is given at the bottom of the table.

Genotype	Shoot dry weight, g/plant	Root dry weight, g/plant	Leaf area, cm^2/plant
Pioneer 8387	4.71A[†]	1.87A	896.3A
Pioneer 1105	4.15AB	1.75A	776.7B
Pioneer 1151	4.08B	1.71A	777.9B
Dekalb 51-20	4.07B	1.96A	786.7B
Dekalb 64-69	3.85B	1.76A	814.4AB
Analysis of variance			
Effect	Numerator DF	Denominator DF	Pr>F
Shoot dry weight			
Genotype	4	34	0.0074
Root dry weight			
Genotype	4	34	0.1176
Leaf area			
Genotype	4	34	0.0061

[†] Within each column, values (least square means; alpha = 0.05) with the same letter are not significantly different using Tukey-Kramer grouping for genotype.

Table 2.4. Shoot dry weight, root dry weight, and total leaf area at harvest (31 days after planting) of 18 genotypes of sorghum grown under the control temperature. Each value is the mean of eight plants (four pots in two growth chambers). Analysis of variance for each parameter is given at the bottom of the table.

Genotype	Shoot dry weight, g/plant	Root dry weight, g/plant	Leaf area, cm^2/plant
SC35	1.94A[†]	1.68AB	320.3ABC
ShanQuiRed	1.94A	2.03A	400.2AB
SC1345	1.89A	1.57AB	410.6A
SC971	1.76A	1.44ABC	436.6A
ARCH 10747-2	1.68AB	1.61AB	344.1ABC
Segaolane	1.58AB	1.62AB	322.1ABC
Pioneer 8500	1.53AB	1.60AB	364.5ABC
ARCH 12045	1.45AB	1.64AB	327.0ABC
Dekalb 41-50	1.41ABC	1.14ABCD	298.9ABCD
P898012	1.38ABC	1.38ABC	311.8ABCD
Ajabsido	1.27ABCD	1.20ABCD	322.5ABC
Macia	1.27ABCD	1.44ABC	248.2ABCDE
SC283	1.12ABCD	1.28ABCD	302.7ABCD
Redbine 60	1.08ABCD	1.04BCD	275.8ABCDE
ARCH 12002	0.83BCD	0.88BCD	210.0BCDE
ARCH 10747-1	0.83BCD	1.05BCD	186.1CDE
ARCH 12012	0.52CD	0.55CD	129.0DE
RTx430	0.46D	0.39D	106.4E
Analysis of variance			
Effect	Numerator DF	Denominator DF	Pr>F
Shoot dry weight			
Genotype	17	125	<0.0001
Root dry weight			
Genotype	17	124	<0.0001
Leaf area			
Genotype	17	125	<0.0001

[†] Within each column, values (least square means; alpha = 0.05) with the same letter are not significantly different using Tukey-Kramer grouping for genotype.

Table 2.5. Protein, starch, and amylose concentrations in seeds of five hybrids of maize. Starch was analyzed using two different methods. See text for description of methods. Each value for protein and amylose is the mean of six values (two measurements taken on each of three replications). The values obtained using the two methods to analyze for starch have been averaged together, and each value for starch is the mean of 12 values.

Genotype	Protein, %	Starch, %	Amylose, %
Dekalb 51-20	10.98A[†]	65.67A	25.59A
Pioneer 1151	10.95A	68.08A	25.44A
Pioneer 8387	10.47AB	68.15A	23.36A
Dekalb 64-69	9.61AB	68.44A	26.90A
Pioneer 1105	9.26B	69.50A	25.55A
Analysis of variance			
Effect	Numerator DF	Denominator DF	Pr > F
Protein			
Genotype	4	10	0.0196
Starch			
Genotype	4	10	0.1553
Procedure	1	10	0.0109
Genotype*Procedure	4	10	0.6311
Amylose			
Genotype	4	10	0.3910

[†]Within each column values (least square means; alpha = 0.05) with the same letter are not significantly different using Tukey-Kramer grouping for genotype.

Table 2.6. Protein, starch, and amylose concentrations in seeds of 18 genotypes of sorghum. Starch was analyzed using two different methods. See text for description of methods. Each value for protein and amylose is the mean of six values (two measurements taken on each of three replications). The values obtained using the two methods to analyze for starch have been averaged together, and each value for starch is the mean of 12 values.

Genotype	Protein, %	Starch, %	Amylose,%
Redbine 60	15.71A[†]	68.23AB	23.77A
RTx430	15.35A	65.31B	26.94A
Dekalb 41-50	15.10AB	68.40AB	24.60A
ARCH 12002	14.90ABC	66.11B	24.27A
Pioneer 8500	14.86ABC	67.31AB	25.80A
Ajabsido	13.84ABCD	68.82AB	23.08A
SC35	13.49ABCD	68.83AB	24.57A
ShanQuiRed	13.06ABCD	67.17AB	27.64A
SC1345	12.88ABCDE	68.21AB	26.57A
ARCH 10747-2	12.86ABCDE	68.94AB	22.82A
SC283	12.78ABCDE	69.83AB	23.99A
ARCH 12012	12.51BCDE	68.51AB	24.74A
ARCH 10747-1	12.01BCDE	70.56AB	24.30A
P898012	11.95BCDE	69.54AB	25.60A
Macia	11.87CDE	69.33AB	23.27A
ARCH 12045	11.80CDE	70.40AB	24.24A
SC971	11.41ED	70.51AB	25.65A
Segaolane	9.83E	72.71A	27.67A
Analysis of variance			
Effect	Numerator DF	Denominator DF	Pr > F
Protein			
Genotype	17	36	<0.0001
Starch			
Genotype	17	36	0.0434
Procedure	1	36	<0.0001
Genotype*Procedure	17	36	0.7350
Amylose			
Genotype	17	36	0.6836

[†]Within each column values (least square means; alpha = 0.05) with the same letter are not significantly different using Tukey-Kramer grouping for genotype.

Table 2.7. Weight, diameter, and hardness index of seeds of 18 genotypes of sorghum. See text for number of seeds analyzed in each procedure.

Genotype	Weight, mg	Diameter, mm	Hardness index
Ajabsido	46.39A	3.18A	33.24C
P898012	42.94AB	2.88AB	33.23C
RTx430	40.70ABC	2.88AB	52.45ABC
SC1345	37.90ABDC	2.70ABCDE	49.51BC
SC35	35.93ABCDE	2.63ABCDE	58.94AB
Dekalb 41-50	35.41BCDE	2.77ABCD	51.58ABC
Pioneer 8500	34.96BCDE	2.81ABC	43.47BC
Redbine 60	34.69BCDE	2.57BCDE	50.17ABC
Segaolane	32.66BCDEF	2.75ABCDE	58.14AB
SC283	32.36CDEF	2.63ABCDE	64.87A‡
ShanQuiRed	30.47CDEFG	2.49BCDE	59.67AB
ARCH 12045	30.34DEFG	2.62ABCDE	55.89AB
Macia	29.13DEFG	2.21DE	55.27AB
ARCH 10747-2	29.00DEFG	2.38BCDE	40.75BC
ARCH 12002	27.15EFG	2.33BCDE	59.51AB
ARCH 12012	25.04EFG	2.29CDE	41.76BC
ARCH 10747-1	23.54FG	2.16E	43.45BC
SC971	21.17G	2.24CDE	53.84AB
Analysis of variance			
Effect	Numerator DF	Denominator DF	Pr > F
Weight			
Genotype	17	36	<0.0001
Diameter			
Genotype	17	36	<0.0001
Hardness index			
Genotype	17	36	<0.0001

†Within each column, values (least square means; alpha = 0.05) with the same letter are not significantly different using Tukey-Kramer grouping for genotype.

‡ This high value is suspect due to one, high questionable measurement. See text for explanation.

Table 2.8. Concentration of protein, concentration of starch, seed weight, seed diameter, and hardness index of the seven genotypes of sorghum that had an emergence of 25 percent or greater under the cold temperature.

Genotype	Germ.,%	Protein, %	Star., %	Wt., mg	Diam., mm	Hardness
SC283	50±26.5	12.78ABCDE	69.83AB	32.36CDEF	2.63ABCDE	…[†]
SC971	40.6±4.4	11.41ED	70.51AB	21.17G	2.24CDE	53.84AB
Segaolane	40.6±13.3	9.83E	72.71A	32.66BCDEF	2.75ABCDE	58.14AB
P898012	28.1±22.1	11.95BCDE	69.54AB	42.94AB	2.88AB	33.23C
Dklb. 41-50	25.0±0	15.10AB	68.40AB	35.41BCDE	2.77ABCD	51.58ABC
Pion. 8500	25.0±8.8	14.86ABC	67.31AB	34.96BCDE	2.81ABC	43.47BC
ShanQuiRed	25.0±8.8	13.06ABCD	67.17AB	30.47CDEFG	2.49BCDE	59.67AB

[†] Value was suspect due to one, high questionable measurement.

Appendix A - Supplementary Information for Chapter 2

Table A.1. Shoot dry weight, root dry weight, and total leaf area at harvest (31 days after planting) of five commercial hybrids of maize grown under control and cold temperatures. Each value is the mean and standard error of eight plants (four pots in two growth chambers). For treatments, see the text.

Genotype	Control temperature	Cold temperature
	Shoot dry weight, g/plant	
Dekalb 51-20	4.06±0.20	0.04±0.01
Dekalb 64-69	3.85±0.19	0.02±0.01
Pioneer 1105	4.14±0.20	0.03±0.01
Pioneer 1151	4.07±0.20	0.02±0.01
Pioneer 8387	4.70±0.23	0.05±0.01
	Root dry weight, g/plant	
Dekalb 51-20	1.96±0.06	0.03±0.01
Dekalb 64-69	1.76±0.06	0.02±0.01
Pioneer 1105	1.75±0.06	0.03±0.01
Pioneer 1151	1.71±0.06	0.02±0.01
Pioneer 8387	1.87±0.06	0.05±0.01
	Total leaf area, cm^2/plant	
Dekalb 51-20	781.7±99.7	7.60±1.91
Dekalb 64-69	809.3±103.1	6.86±1.79
Pioneer 1105	771.8±98.4	7.04±1.82
Pioneer 1151	773.0±98.6	5.16±1.51
Pioneer 8387	890.7±113.3	10.7±2.4

Table A.2. Shoot dry weight, root dry weight, and total leaf area at harvest (31 days after planting) of two commercial hybrids of sorghum, five sorghum genotypes from the Agricultural Research Center breeding program in Hays, Kansas (ARCH), and 11 sorghum genotypes from the Nested Associated Mapping (NAM) program, all grown under the control temperature. Each value is the mean and standard error of eight plants (four pots in two growth chambers). Data for the cold temperature are not shown, because all the values were zero.

Genotype	Control temperature		
	Shoot dry wt., g/plant	Root dry wt., g/plant	Total leaf area, cm^2/plant
	Commercial genotypes		
Dekalb 51-50	1.41±0.15	1.14±0.38	298.9±26.7
Pioneer 8500	1.53±0.15	1.60±0.38	354.5±26.7
	Agricultural Research Center Hays, Kansas (ARCH) genotypes		
ARCH 10747-1	0.83±0.15	1.05±0.38	186.1±26.7
ARCH 10747-2	1.68±0.15	1.61±0.38	344.1±26.7
ARCH 12002	0.82±0.15	0.88±0.38	210.0±26.7
ARCH 12012	0.52±0.15	0.55±0.38	129.0±26.7
ARCH 12045	1.45±0.15	1.64±0.38	327.0±26.7
	Nested Associated Mapping (NAM) genotypes		
Ajabsido	1.27±0.15	1.20±0.38	322.5±26.7
Macia	1.27±0.15	1.44±0.38	248.2±26.7
P898012	1.38±0.15	1.38±0.38	311.8±26.7
Redbine 60	1.08±0.15	1.04±0.38	275.8±26.7
RTx430	0.46±0.15	0.39±0.38	106.4±26.7
SC35	1.94±0.15	1.68±0.38	320.3±26.7
SC283	1.12±0.15	1.28±0.38	302.7±26.7
SC971	1.76±0.15	1.44±0.38	436.6±26.7
SC1345	1.89±0.15	1.57±0.38	410.6±26.7
Segaolane	1.58±0.15	1.62±0.38	322.1±26.7
ShanQuiRed	1.94±0.15	2.03±0.38	400.2±26.7

Figure A.1. Air and soil temperatures in Chamber 19 (control temperature) and Chamber 15 (cold temperature), the first set of growth chambers with planting on 23 Aug. 2017.

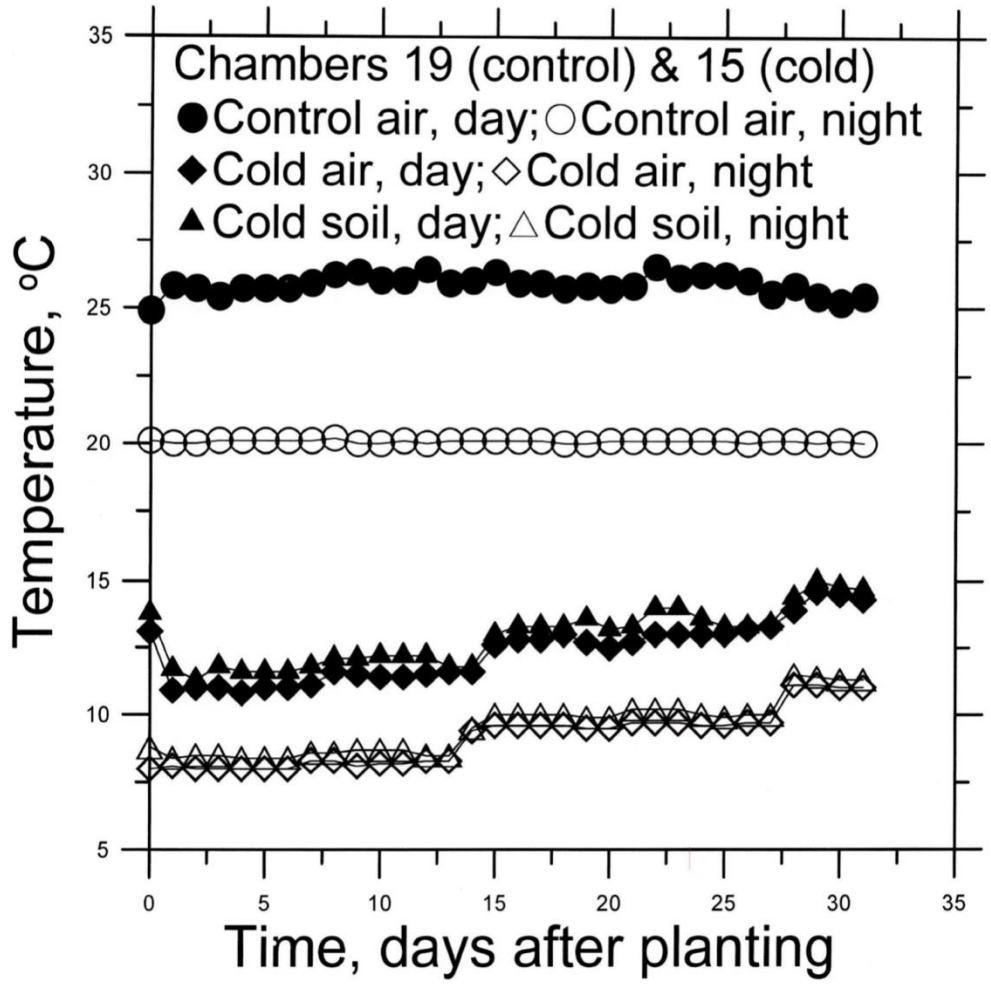


Figure A.2. Air and soil temperatures in Chamber 17 (control temperature) and Chamber 11 (cold temperature), the second set of growth chambers with planting on 25 Aug. 2017.

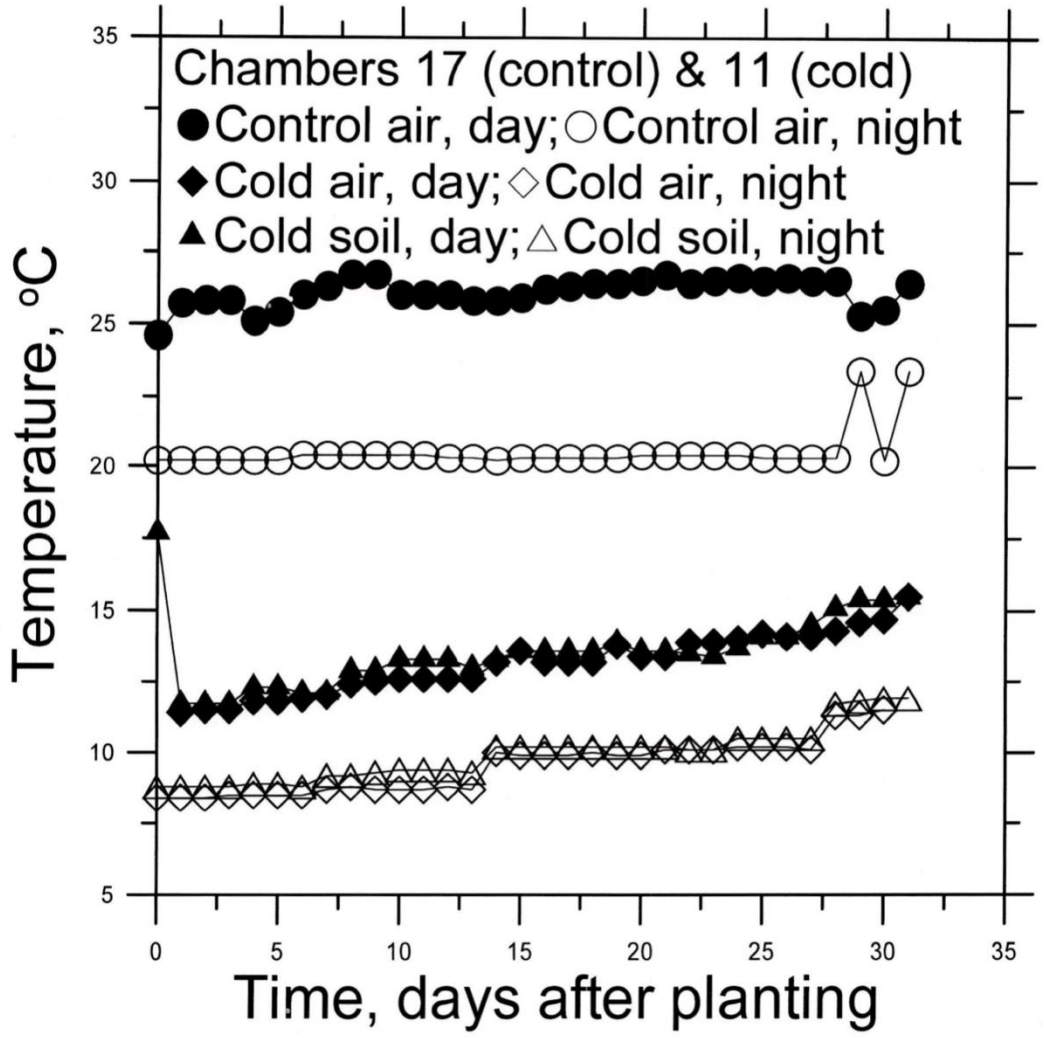


Figure A.3. Height of five commercial hybrids of maize grown under control or cold temperatures for 31 days. Left: Dekalb 51-20 and Dekalb 64-69. Right: Pioneer 1105, Pioneer 1151, and Pioneer 8387. Mean and standard error are shown. If the standard error bar fell within a symbol, it does not show. Each point is the average of eight values (four plants in two growth chambers). See text for details of the treatments.

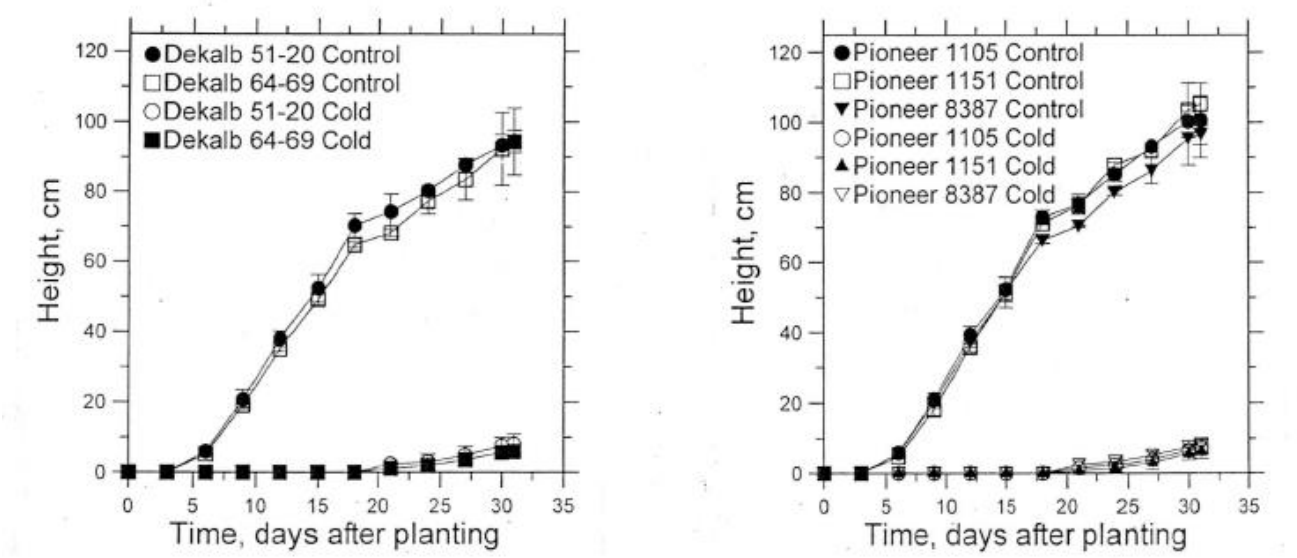


Figure A.4. Height of two commercial genotypes of sorghum grown under the control temperature for 30 days. Mean and standard error are shown. If the standard error bar fell within a symbol, it does not show. Each point is the average of eight values (four plants in two growth chambers).

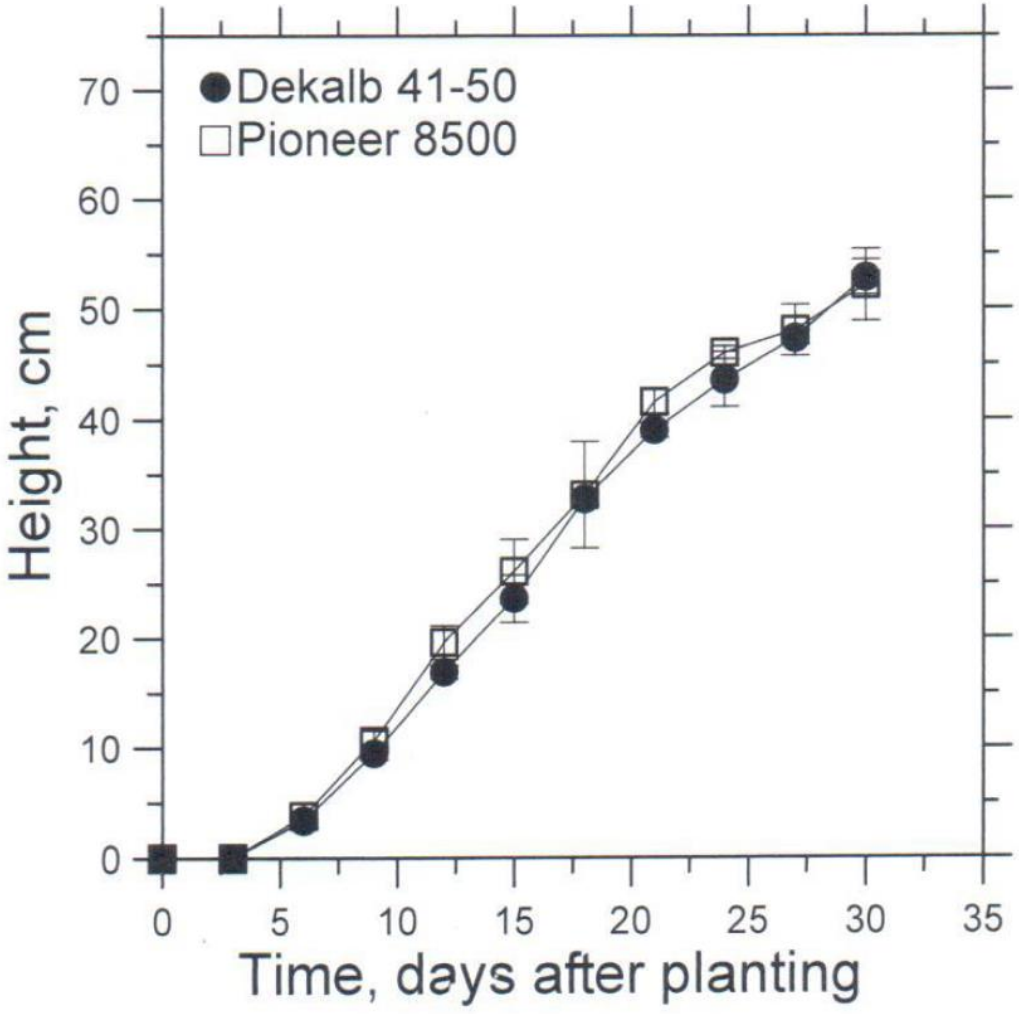


Figure A.5. Height of five genotypes of sorghum from the breeding program at the Agricultural Research Center in Hays, Kansas (ARCH), grown under the control temperature for 30 days. Left: ARCH 20747-1 and ARCH 20747-2. Right: ARCH 12002, ARCH 12012, and ARCH 12045. Mean and standard error are shown. If the standard error bar fell within a symbol, it does not show. Each point is the average of eight values (four plants in two growth chambers). The five genotypes are shown in two graphs so each line is visible.

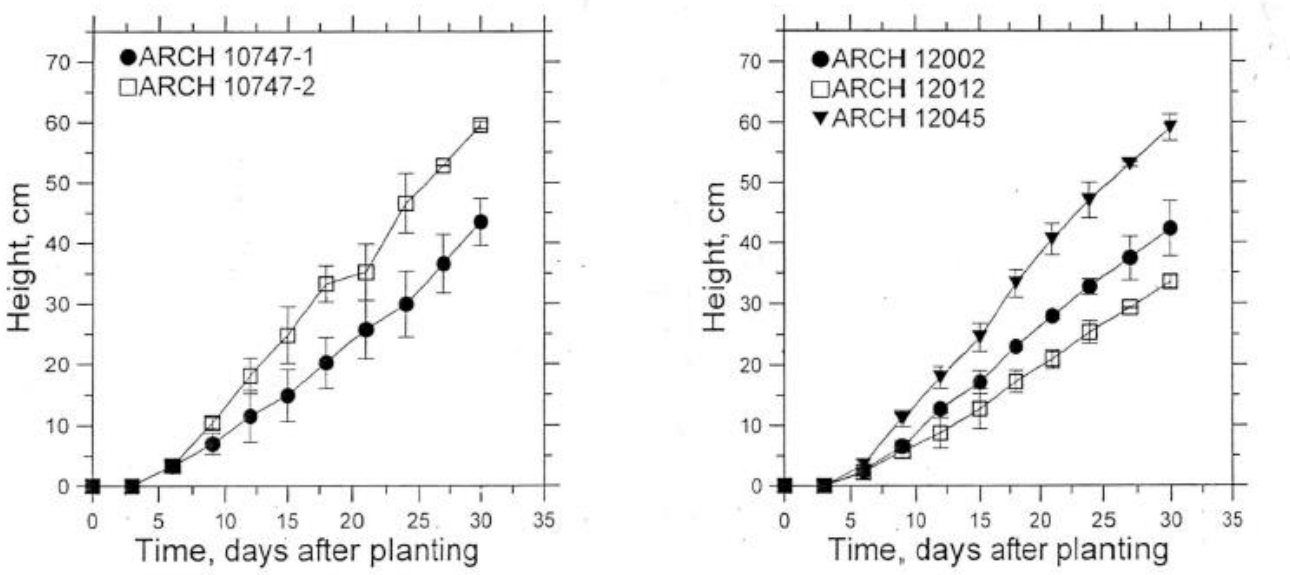
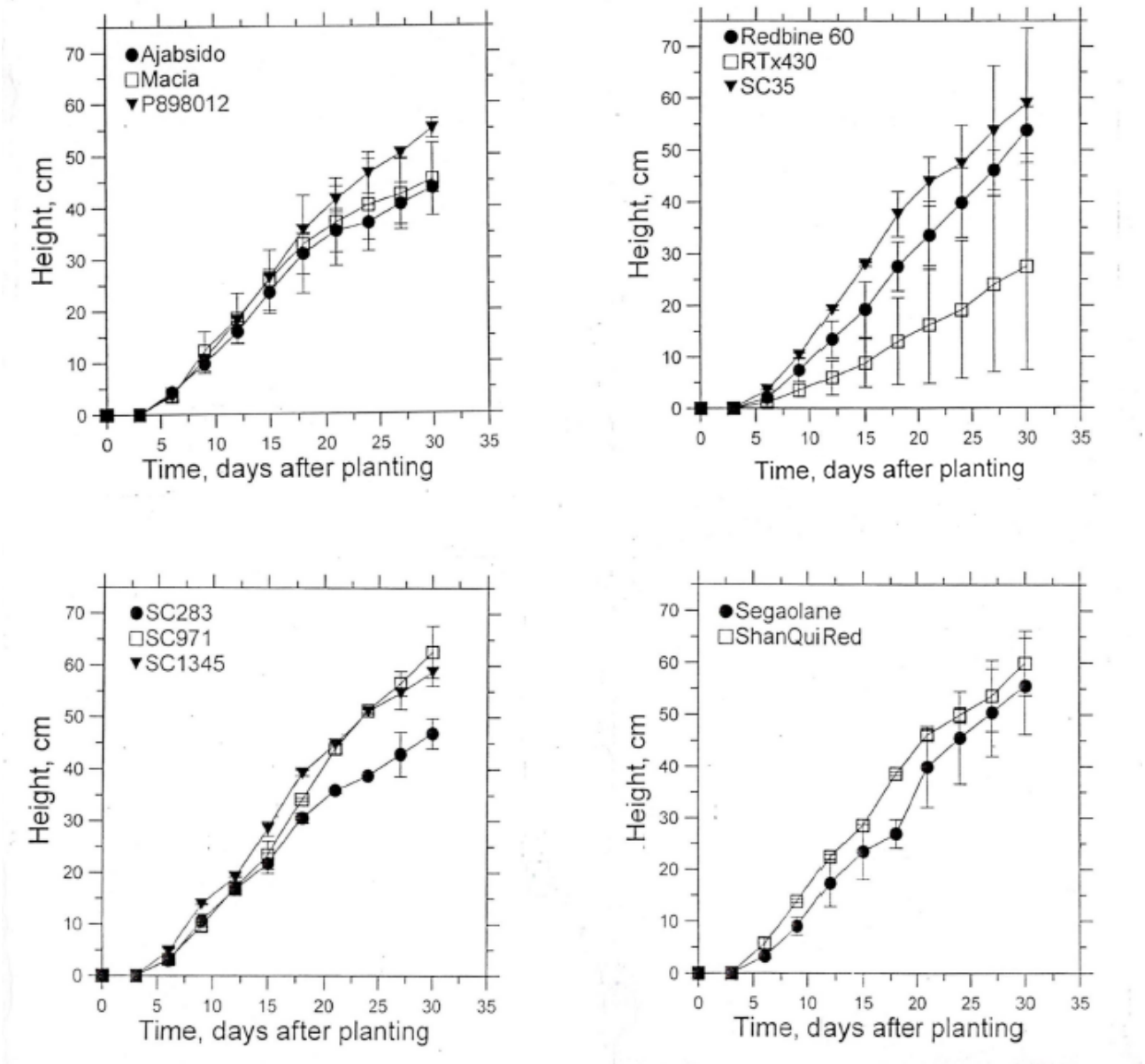


Figure A.6. Height of 11 genotypes of sorghum from the Nested Associated Mapping (NAM) program grown under the control temperature for 30 days. Upper left: Ajabsido, Macia, and P898012; upper right: Redbine 60, RTx430, and SC35; lower left: SC283, SC971, and SC1345; lower right: Segaolane and ShanQuiRed. Mean and standard error are shown. If the standard error bar fell within a symbol, it does not show. Each point is the average of eight values (four plants in two growth chambers). The 11 genotypes are shown alphabetically in four graphs so each line is visible.



Appendix B - Incubator Experiments

This appendix summarizes the eight incubator experiments done before the experiment reported in the body of the thesis.

First incubator experiment (control temperature; seeds soaked overnight)

The same genotypes used in the experiment reported in the body of the thesis were used (5 genotypes of maize and 18 genotypes of sorghum).

For maize, the experiment ran from 14 Nov. 2016 to 23 Nov. 2016 (9 days) and, for sorghum, the experiment ran from 23 Nov. 2016 to 2 Dec. 2016 (9 days).

Seeds were soaked overnight at room temperature in distilled water before being sterilized and placed on Petri plates. Seeds were surface sterilized with commercial bleach (sodium hypochlorite at 5.25%). The bleach was diluted (50 mL bleach in 1000 mL distilled water). The seeds were washed with bleach for 2 minutes. After sterilization, the seeds were washed 4 to 5 times with distilled water. Five seeds of maize and 10 seeds of sorghum were put in each Petri plate, which had germination paper cut to the size of the Petri plate. The paper was wetted with distilled water. Germination papers were wetted with distilled water whenever necessary. There were 8 Petri plates for each genotype of maize (8 replications) and 4 Petri plates for each genotype of sorghum (4 replications).

The temperature of the incubator was 25/20 ^{o}C, day/night. For the first 5 days of the experiment, seeds were in dark. For the last 4 days of the experiment, lights were switched on to provide a 13 h photoperiod (11 h dark). Germination was recorded, and root and shoot lengths were measured on the sixth and ninth (last) day of the experiment. After the root and shoot measurements on the sixth day of the experiment, 3 seedlings of corn and 5 seedlings of sorghum were discarded to avoid congestion and allow the remaining seedlings to grow. Therefore, on

the final measurement day (ninth day of experiment), 2 seedlings of corn and 5 seedlings of sorghum were measured.

Results

Germination percentage of maize on 20 Nov. 2016 and root and shoot length of maize on 23 Nov. 2016 (ninth and last day of experiment) grown at 25/20 ^{o}C. The germination percentages are the averages of 5 seeds per Petri plate and 8 Petri plates (40 seeds). The root and shoot lengths are the averages and standard deviations of 16 seedlings (2 seedlings per Petri plate x 8 Petri plates).

Genotype	Germination, %	Root length, cm	Shoot length, cm
Dekalb 51-20	97.5	11.5±1.7	3.0±0.6
Dekalb 64-69	100	10.1±1.4	2.5±0.9
Pioneer 1105	90.0	9.9±1.8	2.7±0.7
Pioneer 1151	97.4	7.4±0.8	3.1±0.6
Pioneer 8387	100	8.9±2.0	2.8±0.9

Germination percentage of sorghum on 29 Nov. 2016 and root and shoot lengths of sorghum on 2 Dec. 2016 (ninth and last day of experiment) grown at 25/20 ^{o}C. The germination percentages are the averages of 10 seeds per Petri plate and 4 Petri plates (40 seeds). The root and shoot lengths are the averages and standard deviations of 20 seedlings (5 seedlings per Petri plate x 4 Petri plates).

Genotype	Germination, %	Root length, cm	Shoot length, cm
Dekalb 41-50	95.0	6.7±1.9	4.8±1.3
Pioneer 8500	97.5	7.7±2.0	5.5±1.4
ARCH 10747-1	85.0	3.8±2.3	4.3±2.2
ARCH 10747-2	100	7.1±2.3	5.4±2.1
ARCH 12002	100	8.1±1.9	5.1±1.6
ARCH 12012†	37.5	1.5±2.0	2.1±2.6
ARCH 12045	90.0	6.5±1.7	5.6±1.3
Ajabsido	100	9.0±3.1	4.5±1.8
Macia	100	7.8±2.2	4.1±1.3
P898012	72.5	7.6±4.0	3.8±2.0
Redbine 60	97.5	6.5±3.4	5.8±3.5
RTx430	72.5	7.2±3.3	5.4±2.3
SC35	87.5	4.3±3.3	3.1±2.2
SC283	97.5	4.5±1.6	6.4±2.0
SC971	95.0	8.4±2.6	5.6±1.2
SC1345	100	5.8±1.6	5.7±2.3
Segaolane	82.5	6.0±1.8	5.2±2.0
Shan Qui Red†	37.5	2.3±2.6	2.8±2.7

† ARCH 12012 and Shan Qui Red showed fungal infections even after sterilizing with bleach.

Second incubator experiment (seeds soaked overnight; cold temperature, 11/8 ºC)

The preparation of the seeds was the same as for the first incubator experiment. That is, the seeds were soaked in distilled water overnight and sterilized with bleach. For both maize and sorghum, the experiment ran from 23 Dec. 2016 to 1 Feb. 2017 (9 days). There were 30 seeds for each genotype of maize and sorghum. Five seeds of maize and 10 seeds of sorghum were put in each Petri plate. There were 6 Petri plates for each genotype of maize (6 replications) and 3 Petri plates for each genotype of sorghum (3 replications) (30 seeds total for each genotype). Grain weight of the 30 seeds was measured.

The temperature of the incubator was 11/8 ºC, day/night. For the first 4 days of the experiment, the seeds were in the dark. On the fifth day of the experiment (28 Dec. 2017), lights were switched on to provide a 13 h photoperiod (11 h dark). Germination was recorded, and root and shoot lengths were measured on 1 Feb. 2017, which was the ninth (last) day of the experiment.

Results

Grain weight of 30 seeds of maize, germination percentage of 30 seeds of maize, and root and shoot lengths of maize on 1 Feb. 2017 (ninth and last day of experiment) grown at 11/8 ºC. The root and shoot lengths are the averages and standard deviations of 5 seeds in each of 6 Petri plates (30 seeds). Even though many seeds germinated, root and shoot lengths often were too small to measure. They were measured if they were at least 0.1 cm long. Many values were zero.

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm
Dekalb 51-20	8.2	96.7	1.1±0.6	0.2±0.1
Dekalb 64-69	7.2	100	0.6±0.3	0
Pioneer 1105	6.6	96.7	0.3±0.5	…[†]
Pioneer 1151	6.7	100	0.2±0.3	0
Pioneer 8387	9.3	100	0.7±0.6	0.1±0.1

[†] Of the 30 seeds of Pioneer 1105 germinated, one seed (in Replication 3) produced a shoot that was 0.2 cm long.

Grain weight of 30 seeds of sorghum, germination percentage of 30 seeds of sorghum, and root and shoot lengths of maize on 1 Feb. 2017 (ninth and last day of experiment) grown at 11/8 ºC. The root and shoot lengths are the averages and standard deviations of 10 seeds in each of 3 Petri plates (30 seeds). Even though many seeds germinated, root and shoot lengths often were too small to measure. They were measured if they were at least 0.1 cm long. Many values were zero.

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm
Dekalb 41-50	1.0	86.7	0.20±0.21	0
Pioneer 8500	1.0	93.3	0.13±0.14	0.01±0.04
ARCH 10747-1	0.9	76.7	0.22±0.20	0.05±0.05
ARCH 10747-2	0.9	96.7	0.20±0.25	0.03±0.05
ARCH 12002	0.9	90.0	0.23±0.19	0.06±0.07
ARCH 12012	0.6	26.7	0	0
ARCH 12045	0.8	100	0.11±0.17	0
Ajabsido	1.4	100	0.41±0.25	0.13±0.09
Macia	0.8	76.7	0.07±0.14	0
P898012	1.2	93.3	0.48±0.34	0.01±0.03
Redbine 60	0.9	93.3	0.16±0.20	0
RTx430	1.1	83.3	0.11±0.15	0
SC35	0.6	90.0	0.05±0.13	0
SC283	0.6	86.7	0.17±0.20	0
SC971	0.5	100	0.16±0.17	0
SC1345	0.9	90.0	0.25±0.30	0
Segaolane	0.9	70.0	0.07±0.16	0
Shan Qui Red	0.7	76.7	0.07±0.15	0

Third incubator experiment (repeat of second incubator temperature; seeds soaked overnight; cold temperature, 11/8 ºC)

The experimental procedure was the same as for the second incubator experiment. For both maize and sorghum, the experiment ran from 27 Jan. 2017 to 5 Feb. 2017 (9 days). There were 30 seeds for each genotype of maize and sorghum. Five seeds of maize and 10 seeds of sorghum were put in each Petri plate. There were 6 Petri plates for each genotype of maize (6 replications) and 3 Petri plates for each genotype of sorghum (3 replications) (30 seeds total for each genotype). Grain weight of the 30 seeds was measured.

The temperature of the incubator was 11/8 ºC, day/night. For the first 4 days of the experiment, the seeds were in the dark. On the fifth day of the experiment (1 Feb. 2017), lights were switched on to provide a 13 h photoperiod (11 h dark). Germination was recorded, and root and shoot lengths were measured on 5 Feb. 2017, which was the ninth (last) day of the experiment.

Results

Grain weight of 30 seeds of maize, germination percentage of 30 seeds of maize, and root and shoot lengths of maize on 5 Feb. 2017 (ninth and last day of experiment) grown at 11/8 ºC. The root and shoot lengths are the averages of 5 seeds in each of 6 Petri plates. Even though many seeds germinated, root and shoot lengths were too small to measure. They were measured if they were at least 0.1 cm long.

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm[†]
Dekalb 51-20	7.6	93.3	…[‡]	0
Dekalb 64-69	7.3	100	0	0
Pioneer 1105	7.0	73.3	…[§]	0
Pioneer 1151	6.7	56.7	0	0
Pioneer 8387	9.7	66.7	…[¶]	0

[†] No genotype produced a measurable shoot.

[‡] Of the 30 seeds of Dekalb 51-20 germinated, 15 seeds produced roots of measurable lengths. The lengths in cm were as follows: Replication 1: 0.5, 0.5; Replication 3: 0.5, 0.5,0.2; Replication 4: 0.9, 0.4, 0.3, 0.3; Replication 5: 0.5, 0.5, 0.4; Replication 6: 0.6, 0.3, 0.2.

[§] Of the 30 seeds of Pioneer 1105 germinated, one seed (in Replication 4) produced a root that was 0.4 cm long.

[¶] Of the 30 seeds of Pioneer 8387 germinated, 4 seeds produced roots of measurable lengths. The lengths in cm were as follows: Replication 3: 0.5, 0.7; Replication 5: 0.4; Replication 6: 0.4.

Grain weight of 30 seeds of sorghum, germination percentage of 30 seeds of sorghum, and root and shoot lengths of maize on 5 Feb. 2017 (ninth and last day of experiment) grown at 11/8 ºC. The root and shoot lengths are the averages of 10 seeds in each of 3 Petri plates. Even though many seeds germinated, root and shoot lengths were too small to measure. They were measured if they were at least 0.1 cm long.

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm[†]
Dekalb 41-50	1.0	80.0	0	0
Pioneer 8500	1.0	80.0	0	0
ARCH 10747-1	0.8	50.0	0	0
ARCH 10747-2	0.8	43.3	0	0
ARCH 12002	0.8	80.0	0	0
ARCH 12012	0.7	23.3	0	0
ARCH 12045	1.0	83.3	0	0
Ajabsido	1.5	83.3	…[‡]	0
Macia	1.0	83.3	0	0
P898012	1.2	93.3	…[§]	0
Redbine 60	1.0	56.7	0	0
RTx430	1.5	16.7	0	0
SC35	1.1	33.3	0	0
SC283	0.7	76.7	0	0
SC971	0.6	23.3	0	0
SC1345	1.0	93.3	0	0
Segaolane	1.0	26.7	0	0
Shan Qui Red	0.7	13.3	0	0

[†] No genotype produced a measurable shoot.

[‡] Of the 30 seeds of Ajabsido germinated, two seeds, one in Replication 2 and one in Replication 3, each produced a root that was 0.4 cm long.

[§] Of the 30 seeds of P898012 germinated, 17 seeds produced roots of measurable lengths. The lengths in cm were as follows: Replication 1: 0.4, 0.5, 0.3; Replication 2: 0.7, 0.5, 0.3, 0.3, 0.6, 0.3; Replication 3: 1.2, 0.7, 0.8, 0.7, 0.6, 0.8., 0.7, 0.5.

Fourth incubator experiment (seeds soaked overnight; cold temperature; 12.5/10.5 ºC)

The experimental procedure was the same as for the second incubator experiment, except for the temperatures used. For both maize and sorghum, the experiment ran from 10 Jan. 2017 to 19 Jan. 2017 (9 days). There were 30 seeds for each genotype of maize and sorghum. Five seeds of maize and 10 seeds of sorghum were put in each Petri plate. There were 6 Petri plates for each genotype of maize (6 replications) and 3 Petri plates for each genotype of sorghum (3 replications) (30 seeds total for each genotype). Grain weight of the 30 seeds was measured.

The temperature of the incubator was 12.5/10.5 ºC, day/night. For the first 5 days of the experiment, the seeds were in the dark. On the sixth day of the experiment (16 Jan. 2017), lights were switched on to provide a 13 h photoperiod (11 h dark). Germination was recorded, and root and shoot lengths were measured on 19 Jan. 2017, which was the ninth (last) day of the experiment.

Results

Grain weight of 30 seeds of maize, germination percentage of 30 seeds of maize, and root and shoot lengths of maize on 19 Jan. 2017 (ninth and last day of experiment) grown at 12.5/10.5 ºC. The root and shoot lengths are the averages and standard deviations of 5 seeds in each of 6 Petri plates (30 seeds). Even though many seeds germinated, root and shoot lengths often were too small to measure. They were measured if they were at least 0.1 cm long.

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm
Dekalb 51-20	7.7	100	2.3±1.3	0.4±0.2
Dekalb 64-69	7.1	100	1.0±2.0	0.1±0.1
Pioneer 1105	6.9	100	1.4±0.9	0.2±0.2
Pioneer 1151	6.6	86.7	0.8±0.9	0.2±0.2
Pioneer 8387	9.6	96.7	1.5±1.3	0.4±0.3

Grain weight of 30 seeds of sorghum, germination percentage of 30 seeds of sorghum, and root and shoot lengths of maize on 19 Jan. 2017 (ninth and last day of experiment) grown at 12.5/10.5 ºC. The root and shoot lengths are the averages and standard deviations of 10 seeds in each of 3 Petri plates (30 seeds). Even though many seeds germinated, root and shoot lengths often were too small to measure. They were measured if they were at least 0.1 cm long. Many values were zero.

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm†
Dekalb 41-50	1.1	90.0	0.82±0.78	0.09±0.13
Pioneer 8500	1.0	86.7	0.56±0.48	0.12±0.13
ARCH 10747-1	0.7	80.0	0.77±0.70	0.12±0.16
ARCH 10747-2	0.7	86.7	0.40±0.51	0.04±0.09
ARCH 12002	0.7	90.0	0.54±0.69	0.09±0.16
ARCH 12012	0.7	26.7	0.07±0.26	0.01±0.05
ARCH 12045	0.9	76.7	0.44±0.46	0
Ajabsido	1.3	96.7	1.47±0.89	0.29±0.22
Macia	1.0	96.7	0.99±0.51	0.07±0.11
P898012	1.1	96.7	1.48±1.29	0.23±0.21
Redbine 60	1.0	70.0	0.13±0.23	0
RTx430	1.3	20.0	0.13±0.42	0.02±0.07
SC35	1.1	76.7	0.29±0.53	0.03±0.09
SC283	0.8	86.7	0.40±0.40	0.05±0.10
SC971	0.6	63.3	0.37±0.59	0.03±0.09
SC1345	1.0	100	1.23±0.48	0.08±0.11
Segaolane	1.0	76.7	0.14±0.33	0
Shan Qui Red	0.7	80.0	0.52±0.53	0

The fifth through eight incubator experiments were similar to the first through fourth incubator experiments, except the seeds were not soaked overnight.

Fifth incubator experiment (seeds not soaked overnight; cold temperature, 11/8 ºC)

The same procedure was used as in the second and third incubator experiments, except the seeds were not soaked overnight. The temperature was 11/8 ºC, day/night. The starting date of the experiment was 2 Feb. 2017, and the ending date (ninth day) was 11 Feb. 2017. On the fifth day of the experiment (7 Feb. 2017), lights were switched on to provide a 13 h photoperiod (11 h dark). Root and shoot lengths were measured on 11 Feb. 2017, the ninth and last day of the experiment. However, no measurable roots or shoots were produced (all measurements less than 0.1 cm). For maize, there were 5 seeds per Petri dish and 6 Petri dishes were used (6 replications) for each genotype. For sorghum, there were 10 seeds per Petri dish and 3 Petri dishes were used (3 replications) for each genotype. Grain weight of 30 seeds of each genotype was measured.

Results

Grain weight of 30 seeds of maize and germination percentage with standard deviation of 30 seeds of maize germinated at 11/8 ºC and without soaking seeds before incubation.†

Genotype	Grain wt. 30 seeds, g	Germination, %
Dekalb 51-20	8.1	40.0±25.3
Dekalb 64-69	7.2	73.3±30.1
Pioneer 1105	6.6	43.3±8.2
Pioneer 1151	6.7	6.7±10.3
Pioneer 8387	9.3	93.3±10.3

† Root and shoot lengths were not measured, because no measurable roots or shoots were produced.

Grain weight of 30 seeds of sorghum and germination percentage and standard deviation of 30 seeds of sorghum grown at 11/8 ºC and without soaking seeds before incubation.†

Genotype	Grain wt. 30 seeds, g	Germ., %
Dekalb 41-50	1.0	86.7±11.6
Pioneer 8500	0.9	90.0±10.0
ARCH 10747-1	0.7	23.3±5.8
ARCH 10747-2	0.7	3.3±5.8
ARCH 12002	0.7	46.7±15.3
ARCH 12012	0.6	0
ARCH 12045	0.9	40.0±36.1
Ajabsido	1.4	66.7±11.6
Macia	0.9	53.3±11.6
P898012	1.1	96.7±5.8
Redbine 60	0.9	26.7±15.3
RTx430	1.3	6.7±11.6
SC35	1.1	16.7±11.6
SC283	0.8	60.0±10.0
SC971	0.5	10.0±10.0
SC1345	1.0	50.0±34.6
Segaolane	0.9	6.7±11.6
Shan Qui Red	0.7	6.7±5.8

† Root and shoot lengths were not measured, because no measurable roots or shoots were produced.

Sixth incubator experiment (seeds not soaked overnight; cold temperature, 12.5/9.5 ºC)

The same procedure was used as in the fifth incubator experiments, except the seeds were germinated at 12.5/9.5 ºC, day/night. The starting date of the experiment was 3 March 2017, and the ending date (ninth day) was 12 March 2017. On the fifth day of the experiment (9 March 2017), lights were switched on to provide a 13 h photoperiod (11 h dark). For maize, there were 5 seeds per Petri dish and 6 Petri dishes were used (6 replications) for each genotype. For sorghum, there were 10 seeds per Petri dish and 3 Petri dishes were used (3 replications) for each genotype. Grain weight of 30 seeds of each genotype was measured. Germination was recorded, and root and shoot lengths were measured on 12 March 2017, which was the ninth (last) day of the experiment.

Results

Grain weight of 30 seeds of maize and germination percentage with standard deviation of 30 seeds of maize germinated at 12.5/9.5 ºC and without soaking seeds before incubation. The root and shoot lengths, taken on 12 March 2017 (ninth and last day of experiment) are the averages and standard deviations of 5 seeds in each of 6 Petri plates (30 seeds). Even though many seeds germinated, root and shoot lengths often were too small to measure. They were measured if they were at least 0.1 cm long.†

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm
Dekalb 51-20	7.7	96.7±8.2	1.13±0.83
Dekalb 64-69	7.1	100±0	0.41±0.40
Pioneer 1105	6.9	96.7±8.2	0.74±0.45
Pioneer 1151	6.3	73.3±30.1	0.01±0.07
Pioneer 8387	9.6	100±0	0.97±0.81

† Shoot length was not measured, because no genotype produced a measurable shoot.

Grain weight of 30 seeds of sorghum and germination percentage and standard deviation of 30 seeds of sorghum grown at 12.5/9.5 ºC and without soaking seeds before incubation. Root and shoot lengths were measured on 12 March 2017 (ninth and last day of experiment), but all but one genotype had no measurable roots or shoots.

Genotype	Grain wt. 30 seeds, g	Germ., %	Root length, cm†
Dekalb 41-50	1.1	90.0±10.0	
Pioneer 8500	1.0	100±0	
ARCH 10747-1	0.7	43.3±15.3	
ARCH 10747-2	0.8	36.7±15.3	
ARCH 12002	0.7	73.3±5.8	
ARCH 12012	0.7	6.7±5.8	
ARCH 12045	0.9	63.3±11.6	
Ajabsido	1.3	86.7±5.8	
Macia	1.0	90.0±10.0	
P898012	1.1	100±0	0.47±0.44
Redbine 60	1.0	53.3±5.8	
RTx430	1.3	13.3±11.6	
SC35	1.1	56.7±15.3	
SC283	0.8	83.3±11.6	
SC971	0.6	70.0±17.3	
SC1345	1.0	90.0±10.0	
Segaolane	1.0	43.3±15.3	
Shan Qui Red	0.7	66.7±5.8	

† P898012 was the only genotype to produce measurable root growth. Roots on the other genotypes were too small to measure or the seeds did not germinate. Shoot length was not measured, because no genotype produced measurable shoot growth.

Seventh incubator experiment (seeds not soaked overnight; cold temperature, 14/11 ºC)

The same procedure was used as in the fifth incubator experiments, except the seeds were germinated at 14/11 ºC, day/night. The starting date of the experiment was 9 April 2017, and the ending date (ninth day) was 17 April 2017. On the fifth day of the experiment (13 April 2017), lights were switched on to provide a 13 h photoperiod (11 h dark). For maize, there were 5 seeds per Petri dish and 6 Petri dishes were used (6 replications) for each genotype. For sorghum, there were 10 seeds per Petri dish and 3 Petri dishes were used (3 replications) for each genotype. Grain weight of 30 seeds of each genotype was measured. Germination was recorded, and root and shoot lengths were measured on 17 April 2017, which was the ninth (last) day of the experiment.

Results

Grain weight of 30 seeds of maize and germination percentage with standard deviation of 30 seeds of maize germinated at 14/11 ºC and without soaking seeds before incubation. The root and shoot lengths, taken on 17 April 2017 (ninth and last day of experiment), are the averages and standard deviations of 5 seeds in each of 6 Petri plates (30 seeds). Even though many seeds germinated, root and shoot lengths often were too small to measure. They were measured if they were at least 0.1 cm long. Many values were zero.

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm
Dekalb 51-20	7.4	93.3±10.3	1.9±0.9	0.3±0.2
Dekalb 64-69	7.2	96.7±8.2	1.1±0.7	0.1±0.1
Pioneer 1105	6.8	96.7±8.2	1.6±0.7	0.1±0.1
Pioneer 1151	6.5	90.0±10.9	0.4±0.5	0.1±0.1
Pioneer 8387	9.4	100±0.0	2.5±0.9	0.4±0.1

Grain weight of 30 seeds of sorghum and germination percentage and standard deviation of 30 seeds of sorghum grown at 14/11 ºC and without soaking seeds before incubation.†

Genotype	Grain wt. 30 seeds, g	Germ., %
Dekalb 41-50	1.0	90.0±10.0
Pioneer 8500	1.0	100±0
ARCH 10747-1	0.7	46.7±28.9
ARCH 10747-2	0.7	50.0±20.0
ARCH 12002	0.7	66.7±15.3
ARCH 12012	0.6	26.7±15.3
ARCH 12045	0.9	90.0±17.3
Ajabsido	1.3	80.0±10.0
Macia	1.0	90.0±0.0
P898012	1.1	100±0
Redbine 60	0.9	43.3±28.9
RTx430	1.3	16.7±11.6
SC35	1.0	53.3±5.8
SC283	0.8	80.0±20.0
SC971	0.6	56.7±25.2
SC1345	1.0	96.7±5.8
Segaolane	1.0	50.0±10.0
Shan Qui Red	0.7	73.3±5.8

† Root and shoot lengths were not measured, because no genotype produced a measurable root or shoot.

Eighth incubator experiment (seeds not soaked overnight; control temperature)

The same procedure was used as in the fifth incubator experiment, except the seeds were soaked for three hours in distilled water (not overnight) and the seeds were germinated at 25/20 ^{o}C, day/night. The starting date of the experiment was 6 June 2017, and the ending date (ninth day) was 28 June 2017. On the fifth day of the experiment (25 June 2017), lights were switched on to provide a 13 h photoperiod (11 h dark). For maize, there were 5 seeds per Petri dish and 6 Petri dishes were used (6 replications) for each genotype. For sorghum, there were 10 seeds per Petri dish and 3 Petri dishes were used (3 replications) for each genotype. Grain weight of 30 seeds of each genotype was measured. Germination was recorded, and root and shoot lengths were measured on 25 June 2017, which was the ninth (last) day of the experiment.

Results

Grain weight of 30 seeds of maize and germination percentage with standard deviation of 30 seeds of maize germinated at 25/20 ^{o}C and with three hours of soaking seeds before incubation. The root and shoot lengths are the averages and standard deviations of 5 seeds in each of 6 Petri plates (30 seeds) taken on 28 June 2017 (ninth and last day of experiment).

Genotype	Grain wt. 30 seeds, g	Germination, %	Root length, cm	Shoot length, cm
Dekalb 51-20	7.6	100±0	10.5±5.4	5.2±2.2
Dekalb 64-69	6.7	100±0	9.2±4.0	7.5±2.2
Pioneer 1105	7.3	93.3±10.3	11.3±3.6	6.7±1.7
Pioneer 1151	9.7	93.3±10.3	7.7±3.6	6.5±2.0
Pioneer 8387	7.0	100±0	6.8±2.0	4.1+1.2

Grain weight of 30 seeds of sorghum and germination percentage and standard deviation of 30 seeds of sorghum grown at 25/20 ^{o}C and with three hours of soaking seeds before incubation. The root and shoot lengths are the averages and standard deviations of 10 seeds in each of 3 Petri plates (30 seeds) taken on 28 June 2017 (ninth and last day of experiment).

Genotype	Grain wt. 30 seeds, g	Germ., %	Root length, cm	Shoot length, cm
Dekalb 41-50	1.0	90.0±10.0	4.1±2.7	2.8±0.8
Pioneer 8500	1.2	80.0±10.0	10.2±2.1	4.8±0.9
ARCH 10747-1	0.8	80.0±17.3	4.6±0.9	4.7±0.4
ARCH 10747-2	0.8	63.3±5.8	6.1±3.8	4.5±2.8
ARCH 12002	0.8	93.3±5.8	8.5±2.1	4.8±1.4
ARCH 12012	0.7	30.0±10.0	2.8±1.4	3.3±1.7
ARCH 12045	1.0	90.0±10.0	6.3±2.1	5.0±0.5
Ajabsido	1.5	83.3±5.8	9.8±2.5	4.1±1.9
Macia	1.0	96.7±5.8	7.3±1.5	3.4±0.5
P898012	1.0	93.3±11.6	6.6±3.3	4.0±0.3
Redbine 60	1.0	76.7±5.8	5.0±1.0	3.7±0.9
RTx430	1.5	36.7±11.6	2.7±1.6	1.3±0.9
SC35	1.1	73.3±5.8	3.7±2.1	3.5±1.2
SC283	1.0	90.0±10.0	4.5±1.6	4.7±0.4
SC971	0.7	100±0	5.4±1.5	3.3±0.6
SC1345	0.6	93.3±5.8	6.0±0.5	4.1±0.6
Segaolane	1.0	70.0±0.0	6.3±1.1	3.8±1.7
Shan Qui Red	0.7	93.3±5.8	7.6±1.3	5.4±1.0

Appendix C - Growth Chamber Experiments

This appendix summarizes three experiments done in growth chambers before the experiment reported in the body of the thesis.

First growth chamber experiment (seeds soaked overnight; control temperature and cold temperature of 11/8 ºC)

The same genotypes used in the experiment reported in the body of the thesis were used (5 genotypes of maize and 18 genotypes of sorghum).

The experiment ran for 31 days. The seeds were soaked overnight at room temperature before planting. The maize seeds were planted on 3 Nov. 2016, and the plants were harvested on 5 Dec. 2016. The sorghum seeds were planted on 8 Nov. 2016, and the plants were harvested on 9 Dec. 2016. The seeds were planted in cone-shaped containers (6.4 cm inside diameter; 25 cm in length; 4 drainage holes) with 182 grams in each container of a commercial growth medium (Metro Mix 360, Sun Gro Horticulture, Agawam, MA). The day before planting, each container was fertilized with two fertilizers: Osmocote (Scotts Miracle-Gro Co., Marysville, OH) at a rate of 1.34 g per container and Micromix (Micromix Plant Health Ltd., Langar, Nottinghamshire, UK) at a rate of 0.144 g per container. Fertilizer was added on the surface of the soil and watered until the water drained out of the container from drainage holes at the bottom of each container. Two growth chambers were used: a control growth chamber kept at 25/20 ^{o}C, day/night, and a cold growth chamber kept at 11/8 ^{o}C, day/night. For both growth chambers, the photoperiod was 13 hours light (05:00 to 18:00 hr) and 11 hours dark (18:00 to 5:00 hr), and the relative humidity was set at 60%. For maize, two seeds were planted in each container that was in the control and cold chambers. For sorghum, two seeds were planted in each container that was in the control chamber, but four seeds were planted in each container that was in the cold

chamber. On 23 Nov. 2016, sorghum plants in the control chamber were thinned to two plants per container. There were four replications per genotype, and a completely randomized experimental design for each species was used. This meant that for both the control and cold growth chambers, on one side of each growth chamber there were 20 containers with maize (randomly arranged), and on the other side of the growth chamber, there were 72 containers with sorghum (randomly arranged). Every week, the maize containers and sorghum containers were switched from one side of a chamber to the other side of a chamber. Emergence was recorded during the experiment. On the last day of the experiment, number of leaves and plant height were measured. Plant height was measured only if an emerged seedling was at least 0.1 cm long. Plants were cut at the soil surface, and shoots were dried and shoot dry weight was measured. Roots were not extracted, and so there are no root dry weight data reported for this experiment.

Results

Germination, height, number of leaves, and shoot dry weight of 5 genotypes of maize grown for 31 days at 25/20 ^{o}C.

Genotype	Germination, %	Height, cm	Leaves, no.	Shoot dry wt., g
Dekalb 51-20	87.5	76.8±17.1	7.0±0.6	2.8±1.7
Dekalb 64-69	100	70.4±6.4	6.8±0.4	1.5±0.2
Pioneer 1105	100	87.2±10.4	7.0±0.5	3.7±1.1
Pioneer 1151	87.5	76.5±18.7	7.0±0.9	2.3±0.8
Pioneer 8387	100	81.1±6.8	7.8±0.7	4.5±1.8

Germination, height, number of leaves, and shoot dry weight of 5 genotypes of maize grown for 31 days at 11/8 ºC.†

Genotype	Germination, %	Height, cm	Shoot dry wt., g
Dekalb 51-20	100	3.8±1.5	0.018±0.005
Dekalb 64-69	87.5	2.5±1.0	0.011±0.005
Pioneer 1105	100	3.5±1.9	0.023±0.012
Pioneer 1151	50	2.5±0.9	0.007±0.001
Pioneer 8387	100	5.9+1.3	0.053±0.008

† Plants too small to measure number of leaves; only the first leaf began to emerge

Germination, height, number of leaves, and shoot dry weight of 18 genotypes of sorghum grown for 31 days at 25/20 ºC.

Genotype	Germination, %	Height, cm	Leaves, no.	Shoot dry wt., g
Dekalb 41-50	87.5	54.9±3.7	8.4±0.5	1.8±0.5
Pioneer 8500	100	57.1±1.5	8.1±0.4	2.2±0.2
ARCH 10747-1	68.8	61.5±7.1	7.9±0.4	2.2±0.5
ARCH 10747-2	93.8	66.1±17.9	7.8±0.5	2.7±0.7
ARCH 12002	93.8	56.7±6.5	9.0±0.9	1.9±0.9
ARCH 12012	18.8	27.0±16.3	6.5±0.7	0.1±0.1
ARCH 12045	100	55.7±2.9	7.9±0.4	1.6±0.5
Ajabsido	93.8	51.4±4.5	9.8±1.4	1.5±0.4
Macia	87.5	53.6±4.0	7.5±0.5	1.7±0.2
P898012	68.8	51.1±10.6	6.8±0.9	1.2±1.0
Redbine 60	93.8	51.6±3.8	8.0±0.8	1.3±0.2
RTx430	62.5	53.4±4.5	8.0±1.1	1.3±0.4
SC35	56.3	51.2±17.3	7.3±1.5	1.4±0.7
SC283	87.5	49.8±2.0	9.1±2.2	1.2±0.2
SC971	87.5	55.4±2.7	8.8±2.1	1.3±0.3
SC1345	93.8	56.1±2.2	6.9±0.4	1.7±0.3
Segaolane	50.0	59.0±5.7	7.3±0.5	1.0±0.3
Shan Qui Red	31.3	61.8±4.7	7.0±0.7	1.3±0.7

Germination, height, number of leaves, and shoot dry weight of 18 genotypes of sorghum grown for 31 days at 11/8 ºC.†

Genotype	Germination, %
Dekalb 41-50	25.0
Pioneer 8500	18.8
ARCH 10747-1	0
ARCH 10747-2	6.3
ARCH 12002	25.0
ARCH 12012	0
ARCH 12045	0
Ajabsido	6.3
Macia	0
P898012	6.3
Redbine 60	37.5
RTx430	6.3
SC35	12.5
SC283	6.3
SC971	12.5
SC1345	0
Segaolane	12.5
Shan Qui Red	0

† Height, number of leaves, and shoot dry weight were not measured, because no genotype produced measurable growth. No seeding that emerged reached 0.1 cm.

Second growth chamber experiment (seeds soaked overnight; control temperature and cold temperatures of 11/8 ºC; 12.5/9.5 ºC; 14/11 ºC)

The experiment ran for 31 days. The same procedures, as reported for the first growth chamber experiment, were used, except for number of seeds per container and the cold temperatures used. Because sorghum did not grow at 11/8 ºC, the temperature regime as reported in the body of the thesis was used. That is, the temperatures in the cold chambers were increased 1.5 ºC every 14 days. For the first 14 days, the temperatures were 11/8 ºC; for the second 14 days the temperatures were 12.5/9.5 ºC; and for the last three days the temperatures were 14/11 ºC. Both maize and sorghum were planted on 21 Dec. 2016 and harvested on 21 Jan. 2017. Fertilizers were added on 20 Dec. 2016. For maize, two seeds per container were planted for each container that was in the control and cold chambers. For sorghum, four seeds per container were planted for each container that was in the control and cold chambers. On 29 Dec. 2016, maize and sorghum were thinned to one plant per container, except for sorghum in the cold chamber, which was not thinned because of essentially no emergence. Emergence was recorded during the experiment. On the last day of the experiment, number of leaves, total leaf area, and plant height were measured. Plant height was measured only if an emerged seedling was at least 0.1 cm long. Plants were cut at the soil surface. Leaf area was measured using a leaf-area meter (Model No. 3100 Area Meter, LiCor, Inc., Lincoln, NE). Shoots were dried and shoot dry weight was measured. Roots were extracted from the growth medium and washed. Maximum root length was measured. The maize roots then were scanned using a plant image analysis system (WinRHIZO, Regent Instruments, Inc., Quebec City, Canada). Sorghum roots in both the control and cold temperatures were too small to determine root dry weights or to scan.

Results

Germination, height, number of leaves, total leaf area, shoot dry weight, maximum root length, and root dry weight of 5 genotypes of maize grown for 31 days at 25/20 ºC. Mean and standard deviation are given.

Genotype	Gr., %	Ht., cm	Lvs.	Area, cm^2	Sht. wt., g	Rt. ln., cm	Rt. wt., g
Dekalb51-20	100	75.7±3.9	7.0±0	345.0±62.1	2.47±0.62	36.4±8.3	1.02±0.21
Dekalb64-69	100	75.9±6.0	7.0±0	436.6±68.4	2.47±0.43	43.6±12.3	0.87±0.11
Pioneer 1105	100	81.3±4.1	7.0±0	387.1±61.8	2.53±0.89	39.4±5.6	0.81±0.23
Pioneer 1151	100	86.5±14.2	7.3±0.5	496.5±252.0	3.03±2.10	33.4±2.2	0.98±0.45
Pioneer 8387	100	81.7±5.8	7.3±0.5	573.0±83.8	3.84±0.82	35.2±1.1	1.19±0.17

Germination, height, number of leaves, total leaf area, shoot dry weight, maximum root length, and root dry weight of 5 genotypes of maize grown for 31 days at the cold temperatures. See text for temperatures. Mean and standard deviation are given.

Genotype	Gr., %	Ht., cm	Lvs.	Area, cm^2	Sht. wt., g	Rt. ln., cm	Rt. wt., g
Dekalb51-20	100	7.1±0.8	2.5±05	4.1±1.5	0.03±0.004	10.6±2.0	0.02±0.01
Dekalb64-69	100	6.3±1.6	2.4±0.7	4.5±2.2	0.02±0.001	10.5±4.0	0.01±0.01
Pioneer 1105	100	6.8±1.0	2.8±0.5	4.3±1.5	0.03±0.01	11.5±2.1	0.01±0.004
Pioneer 1151	100	6.4±0.7	2.4±0.5	4.0±1.6	0.02±0.01	12.3±3.7	0.01±0.004
Pioneer 8387	100	7.3±1.2	3.0±0.0	7.5±1.7	0.04±0.01	9.8±1.8	0.02±0.01

Root length, projected area, surface area, average diameter, length per volume, and root volume of 5 genotypes of maize grown for 31 days at 25/20 ºC. Data obtained from WinRHIZO. Mean and standard deviation are given.

Genotype	Len., cm	Proj. ar., cm^2	Sur. ar., cm^2	Diam., mm	L/V, cm/cm^3	Vol., cm^3
Dkl. 51-20	2414±171	70.0±6.7	219.9±21.1	0.293±0.012	2414±171	1.62±0.22
Dkl. 64-69	1473±284	44.3±8.9	139.2±27.9	0.320±0.014	1473±284	1.07±0.23
Pion. 1105	2467±279	73.5±8.5	231.0±26.7	0.299±0.002	2467±279	1.74±0.20
Pion. 1151	3343±277	109.3±10.5	343.4±32.9	0.328±0.007	3343±277	2.82±0.31
Pion. 8387	3756±236	128.5±12.1	403.7±38.1	0.341±0.014	3756±236	3.48±0.45

Root length, projected area, surface area, average diameter, length per volume, and root volume of 5 genotypes of maize grown for 31 days at the cold temperatures. See text for temperatures. Data obtained from WinRHIZO. Mean and standard deviation are given.

Genotype	Len., cm	Proj. ar., cm^2	Sur. ar., cm^2	Diam., mm	L/V, cm/cm^3	Vol., cm^3
Dkl. 51-20	37.7±8.2	2.9±0.5	9.1±1.5	0.783±0.079	37.7±8.2	0.18±0.03
Dkl. 64-69	27.4±6.1	2.2±0.6	7.0±1.9	0.778±0.070	27.3±6.1	0.15±0.05
Pion. 1105	49.6±3.8	3.9±0.5	12.4±1.4	0.791±0.031	49.6±3.8	0.25±0.04
Pion. 1151	37.5±10.2	2.9±0.9	9.2±2.7	0.774±0.022	37.5±10.2	0.18±0.06
Pion. 8387	48.2±4.3	4.0±0.6	12.4±1.7	0.808±0.042	48.2±4.3	0.26±0.05

Germination, height, number of leaves, total leaf area, shoot dry weight, and maximum root length of 18 genotypes of sorghum grown for 31 days at 25/20 ºC. Root dry weight was too small to measure. Mean and standard deviation are given.

Genotype	Gr., %	Ht., cm	Lvs..	Area, cm^2	Sht. wt., g	Root ln., cm
Dekalb 41-50	94	47.3±4.0	8.5±0.6	152.5±39.8	1.13±0.41	41.2±4.9
Pioneer 8500	100	51.9±4.0	10.3±3.2	218.2±70.9	1.62±0.42	41.3±3.4
ARCH 10747-1	75	55.7±3.2	9.0±2.2	247.0±73.2	1.86±0.54	42.5±4.0
ARCH 10747-2	100	53.9±3.6	8.0±0	235.8±44.0	1.82±0.29	40.8±5.4
ARCH 12002	75	47.3±2.5	12.0±2.9	247.6±34.8	1.60±0.27	47.4±5.0
ARCH 12012	81	48.9±5.1	8.0±0	136.2±42.6	0.86±0.25	40.5±4.9
ARCH 12045	88	46.4±7.5	8.5±1.0	186.7±87.0	1.22±0.52	42.3±3.2
Ajabsido	94	40.8±4.5	13.3±3.9	286.3±199.4	1.19±0.30	45.6±7.5
Macia	100	46.0±4.2	8.5±1.7	186.1±88.7	1.40±0.74	37.5±1.8
P898012	88	54.8±3.3	13.0±3.7	313.1±114.0	2.04±0.72	39.3±2.3
Redbine 60	94	46.3±4.5	12.8±4.9	190.5±67.1	1.31±0.39	45.6±6.8
RTx430	81	48.9±6.2	10.0±3.5	150.9±59.8	1.19±0.43	38.9±3.5
SC35	50	52.3±0.9	7.5±0.6	167.1±57.1	1.27±0.34	40.8±7.0
SC283	81	44.4±2.8	15.3±3.9	206.6±67.0	1.36±0.57	37.5±3.6
SC971	94	53.4±3.3	16.3±2.2	324.6±48.3	2.01±0.43	34.2±5.5
SC1345	88	51.5±3.9	8.8±2.7	224.0±46.0	1.67±0.57	36.6±5.0
Segaolane	75	56.6±3.0	8.0±0	229.0±68.1	1.57±0.60	43.1±3.4
Shan Qui Red	94	54.7±4.6	8.3±0.5	302.8±65.0	2.40±0.53	40.8±4.7

Germination, height, number of leaves, and shoot dry weight of 18 genotypes of sorghum grown for 31 days at the cold temperatures. See text for the temperatures. Plants were too small to measure leaf area, root length, or root dry weight. Mean and standard deviation are given.†

Genotype	Gr., %	Ht., cm	Lvs.	Sht. wt., g†
Dekalb 41-50	62.5	5.4±1.3	2.0±0	0.71±0.0005
Pioneer 8500	81.3	5.8±0.7	2.0±0	0.77±0.0007
ARCH 10747-1	0	… ‡	…	…
ARCH 10747-2	37.5	3.0±1.1	1.6±0.7	0.34±0.0003
ARCH 12002	12.5	3.6±0.9	2.0±0	0.44±0.0001
ARCH 12012	0	…	…	…
ARCH 12045	25.0	2.0±0.5	1.5±0.7	0.23±0.0006
Ajabsido	43.8	3.1±0.6	1.9±0.4	0.44±0.0005
Macia	6.3	3.2±0	2.0±0	0.87±0.0000
P898012	87.5	5.2±1.1	2.7±0.5	1.12±0.0005
Redbine 60	31.3	4.8±1.2	2.0±0	0.49±0.0006
RTx430	6.3	2.8±0	2.0±0	0.44±0.0000
SC35	0	…	…	…
SC283	12.5	2.4±1.6	1.0±0	0.13±0.0001
SC971	37.5	3.7±0.8	1.6±0.6	0.31±0.0005
SC1345	12.5	2.4±0.9	1.5±0.7	0.23±0.0006
Segaolane	18.8	3.9±1.0	2.0±0	0.32±0.0005
Shan Qui Red	37.5	3.3±1.6	1.8±0.5	0.38±0.0003

† Plants too small to measure leaf area or root length

‡ No germination

Third growth chamber experiment (seeds not soaked overnight; no control temperature; cold temperatures: 11/8 ºC; 12.5/9.5 ºC; 14/11 ºC)

The third growth chamber experiment was like the second growth chamber experiment, except the seeds were not soaked overnight and there was no growth chamber with a control temperature. There was only a growth chamber for the cold temperatures. Both maize and sorghum were planted on 15 Feb. 2017 and harvested on 18 March 2017. Fertilizers were added on 14 Feb. 2017. For maize, two seeds per container were planted for each container, and, for sorghum, four seeds per container were planted. The same measurements that were taken in the second growth chamber experiment were taken in the third growth chamber experiment, except no WinRHIZO data were collected.

Results

Germination, height, number of leaves, total leaf area, shoot dry weight, and maximum root length, and root dry weight of 5 genotypes of maize grown for 31 days at the cold temperatures. See text for temperatures. Mean and standard deviation are given. Plants were too small to measure root dry weight.

Genotype	Gr., %	Ht., cm	Lvs.	Area, cm^2	Sht. wt., g	Rt. ln., cm
Dekalb51-20	100	7.6±0.8	3.0±0.0	5.5±1.0	0.031±0.007	12.2±2.4
Dekalb64-69	100	6.4±1.2	2.9±0.4	6.7±2.2	0.028±0.006	12.1±2.5
Pioneer 1105	90	6.6±1.7	2.7±0.5	5.0±2.7	0.030±0.011	13.5±3.6
Pioneer 1151	90	6.3±1.0	2.6±0.5	4.7±1.7	0.029±0.004	13.2±3.9
Pioneer 8387	90	9.3±0.5	3.3±0.5	10.8±1.5	0.059±0.011	14.3±1.8

Germination, height, number of leaves, total leaf area, shoot dry weight, and maximum root length of 18 genotypes of sorghum grown for 31 days at the cold temperatures. See text for the temperatures. Plants were too small to measure root dry weight. Mean and standard deviation are given.

Genotype	Gr., %	Ht., cm	Lvs.	Area, cm^2	Sht. wt., g	Root ln., cm
Dekalb 41-50	80	5.5±1.7	2.1±0.3	1.0±0.2	0.0†	6.7±1.3
Pioneer 8500	72	4.5±1.4	2.0‡	0.7±0.2	0.0†	4.9±1.4
ARCH 10747-1	0					
ARCH 10747-2	25	2.7±1.9	1.5±0.7	0.3±0.2	0.002±0.003	3.0±1.3
ARCH 12002	25	1.7‡	…§	…§	0.001‡	1.2‡
ARCH 12012	0					
ARCH 12045	0					
Ajabsido	30	2.8±1.2	1.8±0.5	0.6±0.2	0.0†	4.2±1.0
Macia	32	3.6±0.8	2.0‡	0.8±0.4	0.007±0.002	4.0±0.7
P898012	43	3.9±2.0	2.5±0.6	1.0±0.6	0.006±0.003	5.3±2.7
Redbine 60	0					
RTx430	0					
SC35	25	6.6±1.0	2.0‡	0.8±0.2	0.009±0.003	7.0±1.0
SC283	60	5.0±0.6	1.3±0.6	0.3±0.2	0.006±0.001	5.9±0.4
SC971	50	4.6±1.0	2.0‡	0.5±0.1	0.005±0.002	6.2±2.2
SC1345	25	2.5‡	2.0‡	0.4‡	0.004‡	4.2‡
Segaolane	25	4.8±1.7	2.0‡	0.6±0.2	0.006±0.004	6.0±1.5
Shan Qui Red	25	5.5‡	2.0‡	0.4‡	0.005‡	10.0‡

† Plants too small to measure shoot weight; weight less than 0.0001 g

‡ Standard deviation not calculated

§ Not measured

Appendix D - Grain Biochemical and Size Data

This appendix summarizes biochemical and grain size data not reported in the body of the thesis. Except for the SKCS (Single Kernel Characterization System), which was used to get grain characteristics of sorghum, all data were obtained in the Department of Agronomy at Kansas State University. The SKCS data were obtained at the USDA-ARS Center for Grain and Animal Health Research (CGAHR) in Manhattan, Kansas. For grain weight, 30 seeds of the maize and sorghum were measured to provide a mean and standard deviation. The SKCS is described in the main body of the thesis. Area, length, and width of the maize seeds were determined by scanning 100 seeds on an Epson (Suwa, Nagano, Japan) scanner. Then Image-J software (a public domain, Java-based image processing program developed at the National Institutes of Health) was used to determine the area, length, and width of each seed. The 100 analyses were averaged together by the software to give a mean and standard deviation. Total N and total C were determined by the Soil Testing Laboratory at Kansas State University using a LECOTruSpec CN Carbon/Nitrogen combustion analyzer, which reports total levels (inorganic and organic) of C and N on a weight percent basis, according to the TruSpec CN instrument method "Carbon, Hydrogen, and Nitrogen in Flour and Plant Tissue," LECO Corporation, St. Joseph, MI, 2005. Total protein was determined by multiplying the concentration of nitrogen by 6.25. Only the total protein is reported. Three seed samples were analyzed to get a mean and standard deviation for the total protein.

Starch was analyzed as follows. A powdered sample (50 mg) was used for the analysis. Samples were washed with 1.5 mL, 80% ethanol and kept in a water bath at 80 to 85 ^{o}C for 30 minutes. The extraction mixture was centrifuged and decanted into a 25 mL glass tube and the supernatant was collected. The extraction was repeated three more times. An alcohol extract

was used for sugar analysis and the residue dried in an oven for starch analysis. Distilled water (0.75 mL) was added to the residue, and, while stirring, 0.75 mL 9.6 N perchloric acid was added (digestion). The mixture was stirred continuously for 5 minutes (and then occasionally for the next 15 minutes) and then centrifuged. The supernatant liquid was poured into a 50 mL volumetric flask. The digestion procedure was repeated with 4.2 N perchloric acid. The combined extract was diluted to 50 mL with distilled water. Exactly 0.1 mL of the diluted solution was pipetted into a Pyrex tube. It was then made to 1 mL with distilled water, and 4 mL anthrone reagent was added. After the anthrone was added to all of a series of sample tubes, the tubes were cooled in water and each one was mixed thoroughly. All tubes were heated together for 7.5 minutes in a boiling water bath. The tubes were cooled rapidly in ice, and color intensities were determined using light of a wavelength of 630 nm. Similar test tubes were used for all samples and standards, because their size and thickness influence the color development.

Sugar was analyzed as follows. An alcohol extract was evaporated to remove the alcohol. Five mL distilled water was added to the tubes and vortexed, followed by addition of 5 mL of chloroform. The mixture was homogenized by vortexing and kept undisturbed until a bilayer was formed. Then 0.3 mL of the sample was pipetted into a Pyrex tube and diluted to l mL with distilled water. Then 1 mL phenol reagent, followed by 5 mL concentrated sulfuric acid, were added. The sulfuric acid reagent was added rapidly to the test tube and vortexed to obtain a good mixing. The tubes were allowed to stand for 30 min at room temperature and vortexed again before reading absorbance at 400 nm.

Joaquin De Leon, Biological Laboratory Technician, USDA, ARS, Grain Quality and Structure Research, Manhattan, Kansas, tried to do total sugar analyses on the maize and sorghum seeds. The dinitrosalicylic acid (DNSA), necessary for the analyses, had been stored too

long and deteriorated. The results done by him were not valid. The only results for sugar are those I did in the Department of Agronomy, and they are reported below.

Results

Weight of seeds, and starch, sugar, and protein concentrations of seeds, of 5 genotypes of maize. Mean and standard deviation are given.

Genotype	Weight of 30 seeds, g	Starch, mg/g	Sugar, mg/g	Protein, %
Dekalb 51-20	7.73±0.31	622.8±6.0	15.2±0.5	9.62±0.10
Dekalb 64-69	7.06±0.24	626.3±9.6	20.8±0.4	9.56±0.06
Pioneer 1105	6.89±0.31	613.9±9.1	17.9±0.5	8.86±0.12
Pioneer 1151	7.39±1.54	704.2±12.9	18.2±0.4	11.21±0.02
Pioneer 8387	8.82±1.22	630.1±8.1	27.2±0.7	10.65±0.08

Weight of seeds, and starch, sugar, and protein concentrations of seeds, of 18 genotypes of sorghum. Mean and standard deviation are given.

Genotype	Weight of 30 seeds, g	Starch, mg/g	Sugar, mg/g	Protein %
Dekalb 41-50	1.01±0.03	...†	…†	…†
Pioneer 8500	1.03±0.12	…†	…†	…†
ARCH 10747-1	0.72±0.06	483.4±38.5	8.7±1.1	11.95±0.40
ARCH 10747-2	0.76±0.04	436.5±49.5	8.7±1.2	9.96±0.19
ARCH 12002	0.74±0.05	389.2±11.3	11.6±0.4	13.77±0.26
ARCH 12012	0.65±0.04	419.6±29.5	13.2±1.1	12.73±0.39
ARCH 12045	0.91±0.06	616.0±34.4	13.2±1.6	10.36±0.14
Ajabsido	1.38±0.08	470.6±13.2	8.8±1.0	13.32±0.07
Macia	0.98±0.03	544.3±21.9	16.9±3.4	9.55±0.06
P898012	1.07±0.06	574.2±68.3	13.8±1.5	10.36±0.14
Redbine 60	0.93±0.07	626.9±40.0	11.8±0.9	12.16±0.16
RTx430	1.34±0.11	499.1±58.9	9.7±0.7	12.50±0.13
SC35	1.08±0.04	513.7±37.1	9.2±1.5	11.66±0.18
SC283	0.85±0.10	490.1±24.2	12.9±2.1	13.44±0.12
SC971	0.61±0.07	490.7±54.7	11.0±2.8	10.58±0.08
SC1345	0.90±0.20	514.5±45.7	9.1±1.9	13.57±0.26
Segaolane	0.97±0.03	492.3±96.5	8.1±1.5	9.60±0.19
Shan Qui Red	0.70±0.03	565.5±23.3	11.8±0.9	9.72±0.11

† Dekalb 41-50 and Pioneer 8500 are not included in the starch, sugar, and protein analyses, because they were added later to the experiment.

Area, length, and width of seeds of 5 genotypes of maize. Mean and standard deviation are given.

Genotype	Area, mm^2	Length, mm	Width, mm
Dekalb 51-20	58.5±6.6	10.17±0.69	7.31±0.44
Dekalb 64-69	57.9±4.1	9.21±0.49	8.00±0.32
Pioneer 1105	59.3±6.5	10.14±0.82	7.43±0.42
Pioneer 1151	46.3±3.6	8.38±0.54	7.04±0.33
Pioneer 8387	71.9±6.1	10.55±0.83	8.68±0.38

Mean hardness index, mean kernel diameter, and mean kernel weight of 18 genotypes of sorghum as determined using the Single Kernel Characterization System (SKCS).

Genotype	Hardness index	Diameter, mm	Weight, mg
Dekalb 41-50	73.0	2.9	33.5
Pioneer 8500	69.7	2.9	31.6
ARCH 10747-1	49.7	2.1	21.7
ARCH 10747-2	58.1	2.1	21.3
ARCH 12002	79.1	2.3	24.6
ARCH 12012	59.1	2.2	18.8
ARCH 12045	77.7	2.6	28.7
Ajabsido	54.5	3.1	43.9
Macia	73.3	2.6	31.1
P898012	49.6	2.8	36.3
Redbine 60	71.9	2.4	29.0
RTx430	67.8	3.0	40.3
SC35	77.3	2.7	35.1
SC283	97.7	2.2	25.2
SC971	72.6	2.3	19.0
SC1345	51.2	2.5	35.1
Segaolane	73.0	2.9	30.1
Shan Qui Red	80.6	2.2	23.4