ANALYSIS OF COLD TOLERANCE IN SORGHUM [*Sorghum bicolor* (L.) Moench]

by

FRANK MAULANA

B.S., Bunda College of Agriculture, University of Malawi, 2004

A THESIS

Submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2011

Approved by:

Dr. Tesfaye Tesso
Major Professor

ABSTRACT

Cold temperature stress is an important abiotic constraint to grain sorghum production in temperate regions. In the United States, low temperature in late spring and early fall has limited sorghum production to a narrow growing period. Deployment of cold tolerance traits may widen this window and hence contribute to increased production. The objectives of this study were (1) to determine the effect of early and mid-season cold temperature stress on growth, phenology and yield components of sorghum, and identify key traits that are most sensitive to cold stress at seedling and flowering stages, and (2) to identify new sources of cold tolerance for use in breeding programs. Series of controlled environment (greenhouse/growth chamber) and field experiments were carried out. Three sorghum genotypes of variable response, Shan Qui Red (tolerant), SRN39 (susceptible) and Pioneer 84G62 (unknown) were subjected to cold (15/13ºC day/night) and normal (25/23ºC day/night) temperature at seedling (Experiment I) and flowering (Experiment II) stages. The genotypes were planted in a greenhouse using a 5L polytainer pots. Each pot consisted of a single plant and each plot was represented by three pots. A split-plot design with three replications was used in both experiments with temperature regimes as main plots and genotypes as sub-plots. Three days after emergence, experiment I plants were moved to the growth chamber and subjected to the designated temperature treatments. For experiment II, the treatments were assigned at heading stage immediately before anthesis had begun. The treatments lasted 10 d in both experiments. Data were collected on seedling characteristics and leaf chlorophyll content in experiment I, days to flowering, maturity, and yield components in both experiments, and anthesis duration in experiment II. For the field experiment, 150 sorghum germplasm collections of potential cold tolerance along with tolerant and susceptible checks were evaluated for emergence and seedling traits under early planting (April 13) at soil

temperature of 20.1/13.4 ºC max/min. The normal temperature treatment was applied by planting at regular season (May 26) at soil temperature of 30.0/20.4ºC max/min. Twenty-four genotypes selected based on field emergence and seedling vigor were further screened under controlled environment. Early-season stress significantly reduced leaf chlorophyll content, all seedling traits (height, vigor and dry weight), and also delayed flowering and maturity. But it had no effect on final leaf number, plant height and yield components. Genotypic response to early stress was significant for all traits with the susceptible checks having the lowest score for all seedling traits. Mid-season cold stress prolonged anthesis duration, delayed maturity and highly reduced all yield components. Several genotypes among the 150 had higher seedling vigor and emergence than the tolerant check, Shan Qui Red. In conclusion, reduced seedling vigor as a result of early stress had no effect on final yield provided that stand establishment was not compromised while mid season stress is damaging to yield. The wide genetic variation for the traits indicates the potential for improvement of cold tolerance in sorghum.

TABLE OF CONTENTS

LIST OF FIGURES vi
LIST OF TABLES vii
ACKNOWLEDGEMENTS viii
INTRODUCTION 1
Chapter 1 - LITERATURE REVIEW 3
Germination and emergence 4
Seedling growth and vigor 6
Photosynthetic activity 8
Effect of cold temperature stress on phenology and reproductive growth 9
Chapter 2 - EFFECT OF EARLY AND MID-SEASON COLD TEMPERATURE STRESS ON GROWTH, PHENOLOGY AND YIELD COMPONENTS OF SORGHUM 11
INTRODUCTION 12
MATERIALS AND METHODS 14
Genetic materials 15
Temperature regimes 16
Experimental design and layout 16
RESULTS 19
Effect of cold temperature stress on seedling growth and phenology 19
Effect of cold temperature stress on yield components 21
DISCUSSION 29
CONCLUSION 33
Chapter 3 - SCREENING OF SORGHUM GERMPLASM FOR TOLERANCE TO EARLY-SEASON COLD TEMPERATURE STRESS 34
INTRODUCTION 35
MATERIALS AND METHODS 37
Field experiment 37
Controlled environment experiment 38
Growth chamber experiment-seedling vigor test 39

Statistical analysis 40
RESULTS 40
Field experiment 40
Controlled environment 42
Association among cold tolerance traits 44
DISCUSSION 53
CONCLUSION 56
REFERENCES 57
Appendix 1. Mean number of leaves and plant height of sorghum genotypes as affected by early season cold temperature stress. 71
Appendix 2. List of sorghum genotypes with their respective country of origin used in this study. 72
Appendix 3. The daily maximum and minimum soil temperatures during early planting in the field experiment at Manhattan, 2010. 78
Appendix 4. The daily maximum and minimum soil temperatures during normal planting in the field experiment at Manhattan, 2010. 80
Appendix 5. Mean germination percentages of 26 sorghum genotypes at three time intervals evaluated under controlled cold conditions. 82
Appendix 6. Mean cold tolerance related traits of sorghum genotypes evaluated under early planting in the field conditions at Manhattan, 2010. 83

LIST OF FIGURES

Figure 2.1. Effect of early-season cold temperature stress on seedling vigor. 27
Figure 2.2. Effect of mid-season cold temperature stress on seed setting and grain development: (a) tolerant genotype exposed to normal (left) and cold (right), and (b) susceptible genotype exposed to normal (left) and cold (right) temperature treatments. 28

LIST OF TABLES

Table 2.1. Combined analysis of variance for reaction of sorghum genotypes to early and mid-season cold temperature stress evaluated under controlled environment condition. 23
Table 2.2. Phenology and seedling growth parameters as affected by early-season cold temperature stress..... 24
Table 2.3. Mean yield components of sorghum genotypes as affected by early and mid-season cold temperature stress..... 25
Table 2.4. Phenology and yield components of sorghum genotypes subjected to cold temperature stress at seedling and flowering 26
Table 3.1.Combined analysis of variance for cold tolerance traits under growth chamber and field conditions evaluated at Manhattan 46
Table 3.2.Mean performance of cold tolerance traits and the number of genotypes significantly higher than Shan Qui red under early planting in field conditions 47
Table 3.3.Mean cold tolerance related traits among the top 24 accessions evaluated under cold (early planting) and normal (regular) field planting at Manhattan, 2010. 48
Table 3.4.Mean cold tolerance related traits among the top 24 accessions evaluated under cold (15/13°C day/night) and normal (25/23°C day night) temperature under growth chamber condition. 49
Table 3.5.Relative ranking of genotypes based on germination percentage and seedling vigor under controlled environment and field conditions. 50
Table 3.6.Pearson correlation coefficients among cold tolerance traits of sorghum germplasm accessions evaluated under growth chamber and field conditions. 51
Table 3.7. Pearson correlation coefficients between cold tolerance traits scored under growth chamber and field conditions. 52

ACKNOWLEDGEMENTS

First of all, I would like to thank God Almighty for His mercy and blessing on me and for giving me strength to carry out this work. I would like also to express my sincere appreciation to my major advisor, Dr. Tesfaye Tesso, for his constructive guidance towards my study from the beginning to the end. My sincere thank goes to my committee members, Dr. Jianming Yu and Dr. Vara Prasad for their advice and valuable suggestions towards my study that ultimately helped to improve this work. I would like also to thank my fellow graduate students, Adedayo Adeyanju and Jayfred V. Godoy, and Leah Miller, research assistant in the Sorghum breeding, for their unwavering support during field operations and data collection.

I am also thankful to all my family members who have been with me all the way in both good and bad times. Special appreciation goes to my mother and father, Abbey Kapangaza, and Reginald Maulana, respectively, who tried their best to send me to school so that I can grow up to become a productive person in the society. My sincere gratitude and appreciation go to my wife, Enellece, for her patience, continued support and encouragement during my study.

Lastly, I would like to thank the Kansas State Center for Sorghum Improvement and ARET for financial support towards my study, and the Kansas State University, Department of Agronomy for provision of required facilities used to conduct my research. I am also grateful to Dr. Anita Dille for sharing growth chamber space needed to carry out the experiments

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is an important grain crop worldwide. In the United States, it ranks third in total production behind maize and wheat (FAO, 2004). Sorghum is believed to have evolved under warm dry conditions in the tropical Africa (Doggett, 1988), and therefore, it is well adapted to drought and high temperature conditions than other cereals. Given this, sorghum is more sensitive to low temperature compared to most monocot species (Doggett, 1970). Previous studies determined temperature range of 21 to 35ºC as optimal for germination, 26 to 34ºC for vegetative growth and development, and 25 to 28ºC for reproductive growth (Maiti, 1996; Prasad et al., 2008). Although its gradual expansion to higher altitude areas within Africa has resulted in emergence of ideotypes well suited to the cooler climate of the highlands, its introduction to other parts of the world resulted in further development of not only cold tolerant variants but also early-maturing and photoperiod insensitive types.

Unlike the tropical environment where it has extended warm growing period of up to 8 months, sorghum in the United States is grown in a narrow window period between June and September. All hybrids grown in the United States are bred to fit to this short growing window of 3-4 months certainly at the expense of higher yield. Low soil temperature during early-growing season (April and May), and low night temperatures during mid-growth stage (August through October) in some locations pose serious challenge to expansion of sorghum. It is often planted 3 to 5 weeks after the maize crop because it is sensitive to early-season cold stress. As a result, it does not fully benefit from the late spring sunshine and early season moisture. Cold tolerant hybrids with enhanced early-season seedling vigor may withstand low soil temperature during early planting. Thus deployment of cold tolerance traits may allow the use of high yielding full

season cultivars in the current sorghum areas or help expand sorghum production to areas currently considered too cold for the crop.

Cold temperature stress has been reported to have adverse effects on many other crops as well including the major world cereals such as rice (Nishiyama, 1995; Ali et al., 2006; Basnayake et al., 2003; Shimono et al., 2004, 2007) and maize (Rymen et al., 2007). When encountered early in the season, the stress can result in reduced germination and emergence, poor seedling growth and reduced vigor in sorghum (Yu and Tuinstra, 2001; Knoll et al., 2008; Tiryaki and Andrews, 2001). Likewise, cold stress has been shown to reduce photosynthetic activity in maize primarily due to impaired chloroplast function (Allen and Ort, 2001; Gomez et al., 2004) ultimately leading to reduced seedling growth and increased cell death. Perhaps due to its impact on growth rate, cold stress often delays phenological development (flowering and maturity) (Quinby et al., 1973; Zinn et al., 2010) and causes spikelet sterility, flower abortion, and reduction in the number of pollen grains intercepted by the stigma in several crop species resulting in poor seed-set and ultimately low grain yield (Khan et al., 1986; Singh, 1977; Farrell et al., 2001; Lee, 2001; Gunawardena et al., 2003a, 2003b; Oliver et al., 2005; Thakur et al., 2010). Moreover, tremendous yield losses due to cold temperature stress on major grain crops have also been reported in sub-tropical and temperate regions (Thakur et al., 2010).

This study has two components. The first part focuses on investigating the effect of both early and mid-season cold temperature stress on growth, phenology and yield components of sorghum, and to identify important plant characteristics that are more sensitive to low temperature episode at two growth stages. The second part of the study focuses on screening of sorghum germplasm accessions for tolerance to early-season cold temperature stress under controlled and field conditions to identify sources of cold tolerance for use in breeding programs.

Chapter 1 - LITERATURE REVIEW

Growth is the result of series of biochemical reactions that involves complex of enzymatic activities performing different functions. These processes require optimal temperature for normal functions and deviation from optimum conditions may disrupt the normal physiological processes and thus affect growth and development. Previous studies indicate that all components of crop growth and development are affected by low temperature stress though there are remarkable variability among species, genotypes and the nature of the specific functions. A number of methods have been utilized to screen genotypes for cold tolerance in several species including maize (Gardner et al., 1987; Rodriquez et al., 2007), sorghum (Tiryaki and Andrews, 2001), and rice (Satya and Saha, 2010). Both field based cold nursery and controlled laboratory and growth chamber experiments have been widely applied for characterizing genotypes for early season cold tolerance. A range of traits related to seedling growth and establishment under cold temperature have been characterized (Tiryaki and Andrews, 2001; Gardner et al., 1987; Satya and Saha, 2010). In sorghum, improved early-season cold tolerance is believed to help increase production by allowing expansion of the crop in time and space. Apart from the increased growth duration, early planting of sorghum may help the crop escape latent diseases such as stalk rot that appear during later growth stages, late season drought stress and associated pests and diseases.

Germination and emergence

Cold temperature stress is one of the abiotic factors that affects sorghum production in temperate regions including the United States. The crop is sensitive to cold stress at all stages of growth than any other cereal. In the United States, for instance, sorghum is planted 3-5 weeks after maize just to wait for soil temperature to warm up because it is very sensitive to early-season cold stress (De La Soujeole and Miller, 1984).

The most visible effect of cold temperature early in the season seems to be on the establishment of the crop. Reports from earlier studies show that germination, emergence and seedling vigor are highly compromised when the crop is subjected to the stress early in the season (Pinthus and Rosenblum, 1961; Singh, 1985; Harris et al., 1987; Anda and Pinter, 1994). These reports are substantiated by more recent studies where the effect of early-season cold stress has been noted to reduce plant population with the effect being variable between genotypes (Yu et al., 2002; Tiryaki et al., 2001; Franks et al., 2006). Besides directly affecting germination and seedling growth, early-season cold stress was noted to increase the frequency of seed decay and vulnerability of emerging seedlings to soil-borne diseases such as *Pythium* and *Fusarium* spp. contributing to low plant populations (Forbes et al., 1987).

Even in other cereals that are supposedly less sensitive to cold temperature such as maize, early-season cold stress has been identified as one of the environmental challenges (Sezegen and Carena, 2009). Though there is little or no quantitative information on yield losses due to cold stress under early planting, significant reduction in potential maize yield has been reported in areas with colder spring season. Similar to sorghum, planting maize early in the season is expected to improve grain yield, not only through allowing the use of spring sunlight or affording longer growing period but also through helping the crop escape the blistering heat at

the time of pollination that often undermines fertilization and seed-set (Schoper et al., 1987). As it is the case with sorghum, development of cold tolerant maize cultivars has been a priority for many years in order to increase yields in areas with short growing seasons (Revilla et al., 1999, 2000; Rodriquez et al., 2007). The main focus for maize has been on breeding for improved germination and crop establishment under cold conditions because seedling stage is more sensitive to cold temperatures than mature stages (Greaves, 1996). So far, a number of studies have been conducted to identify cold-tolerant maize genotypes both in the laboratory (Lee et al., 2002; Revilla et al., 2003) and in field conditions (Verheul et al., 1996). But there seems to be little or no consistency in genotypic response to cold temperature stress under controlled environment and field condition. Menkir and Larter (1985) observed no significant correlation among emergence-related traits between controlled cold and field conditions. In recent study, Rodriquez et al. (2007) also observed no significant correlation in crop performance between controlled cold and field conditions. However, they observed a positive and negative correlation for seedling vigor under both controlled environment and field conditions. Maize scientists suggest that screening for cold tolerance sources should be conducted under both controlled environment and field conditions (Revilla et al., 2000). This is because controlled condition provides more accurate response of genotypes as it allows monitoring of the growing environments,and the need to have information on the reaction of genotypes before they are exposed to the field conditions where many other factors may come into play (Revilla et al., 2005).

Like sorghum and maize, cold stress in rice delays germination and emergence; soil temperature of below 10°C can result in complete failure of germination (Yoshida, 1981b). Screening for cold tolerance based on germination and seedling growth have been attempted in

rice as well (Nilanjaya et al., 2003; Cruz et al., 2006) and there was marked genetic variability for the traits (Satya and Saha, 2010). Yoshida (1981b) studied the effect of cold stress at three phases: germination, imbibition, activation and post-germination growth. The effect of cold stress was more pronounced at imbibition phase and this was regarded as the most sensitive phase. The exposure of seeds to cold stress during this phase has resulted in increased escape of solutes from the seeds. This has been attributed to the incomplete plasma membrane of the dry seed and to the disturbance caused on its reconstruction (Renata Pereira da Cruz et al., 2004). Cold stress at this stage has been reported to target the cellular membrane and thus is the primary cause of other metabolic disorders usually observed within the cells (Lyons, 1973).

Seedling growth and vigor

The early vegetative growth stage of the plant life cycle is vulnerable to cold stress (Nishiyama, 1995). Marked injuries due to cold stress have been observed on rice seedlings planted in early spring in temperate and subtropical environments (Andaya et al., 2003). However, the degree of injury due to cold stress varies with duration of exposure, crop species and stage of development. Both root and shoot development has been shown to be very sensitive to cold stress at seedling stage (Stone et al., 1999; Engel 1994; Stamp 1984). In poorly developed root system, absorption and translocation of nutrients and water is hindered affecting shoot development (Engels and Marschner, 1990). Moreover, cold stress has been shown to arrest leaf growth by extending the duration of meristematic cycles (Rymen et al., 2007). It reduces root hydraulic conductance resulting in low leaf water and turgor potential and ultimately reduces growth (Fennell and Markhart, 1998; Aroca et al., 2001) that once this becomes irreversible, it ends up with cell death. Nevertheless, brief exposure of seedlings to short-duration of chilling

temperatures may reduce leaf number and plant height, and once the stress is over, the plants quickly recover and resume normal growth (Majora, 1981).

Seedling vigor is one of the most important components of crop growth in all environments (Ludlow and Muchow, 1990; Cisse et al., 2003). In arid areas, crops with high seedling vigor and good stand establishment are capable of using the little available soil water, and ultimately result in higher biomass accumulation and increased grain yield (Cisse and Ejeta, 2003). The effect of seedling vigor can be directly reflected on grain yield since it is associated with vegetative growth processes that ultimately affect production. A crop with good vegetative cover can help reduce excessive evaporative water loss from the soil surface thereby helping maintain more water for transpiration and growth (Condon et al., 1987; Cooper et al., 1987; Gregory et al., 1992). In addition, a vigorous crop with larger leaf area can help increase carbon assimilation when vapor pressure deficit is low per unit transpirational water loss compared to if growth occurs when the temperature is very high (Tanner and Sinclair, 1983). Further, a vigorous crop can help suppress weed growth and minimize the use of chemicals as a weed control strategy ultimately reducing the risk of herbicide resistance in weed species (Lopez-Castaneda et al., 1995). The ability of a crop to accumulate biomass may be directly influenced by seedling vigor. Crops with delayed emergence may have poor seedling vigor that can consequently impact yield at harvest (Tekrony et al., 1991). Increased biomass accumulation prior to anthesis may help to increase yield. Brar (1994) suggested selection of sorghum genotypes for high and uniform germination under different temperature regimes as an important step for identifying genotypes with improved seedling establishment in the field. Thus seedling vigor under all environments is an important indicator of a successful crop. The major seedling vigor traits include seedling height, dry weight and growth rate. Seedling vigor is often scored

using numeric scale of 1 through 5 at pre-determined dates usually 7, 14 and 21days after emergence (Maiti et al., 1981). This scoring system takes into consideration the height of the plant, spread of leaf canopy, and/or the length and width of individual leaves. Plant population density is also influenced by germination and emergence percentages.

Photosynthetic activity

Cold stress has been shown to affect photosynthetic activity of the seedlings (Fryer et al., 1998; Stirling et al., 1991). In maize, temperatures as low as 15°C or below reduce photosynthetic activity of the leaves (Nie et al., 1992; Haldimann et al., 1996; Fracheboud et al., 1999), alter composition of leaf pigment (Haldimann et al., 1995; Haldimann, 1998; Fracheboud et al., 1999) and affect chloroplast development (Robertson et al., 1993). Recent reports on the negative effect of cold temperature stress on photosynthesis, carbon exchange rates, and quantum efficiency of Photosystem-II substantiate the previous findings (Ying et al., 2002; Yan et al., 2006). This may be the result of the effect of the stress on development and function of chloroplasts reported earlier (Robertson et al., 1993; Fracheboud et al., 1999; Allen and Ort, 2001).

Moreover, temperature range of 10 to 15°C has been reported to significantly reduce chlorophyll content, and below 10°C, the chlorophyll content reduction is more pronounced due to the fact that membrane-bound chlorophyll is destroyed by the free radicals of oxygen despite the protective action of carotenoids (Bradbury and Baker, 1983; Wise and Naylor, 1987; Smillie et al., 1987). This reduction in chlorophyll content has been implicated to metabolic blocks in the porphyrin pathway that leads to chlorophyll synthesis (Hodgins and Van Huystee, 1986). Low temperature stress also affects enzymes that carry out photosynthetic processes in the plant.

Because enzymes that are routinely involved in photosynthesis have little energy, the rate of photosynthesis is slowed down under low temperature (Pramod and Vinay, 2007).

Effect of cold temperature stress on phenology and reproductive growth

Cold stress has been shown to affect both phenology and grain filling period. Early season stress has been demonstrated to prolong flowering duration and grain filling period in wheat (Subedi et al., 1998) and delay panicle emergence and heading in sorghum (Majora et al., 1981). In rice, these processes have been reported to be delayed under low temperature often by up to 40 days highly reducing the time between harvesting of one crop and planting of the next (Gunawardena et al., 2003b; Sipaseuth et al., 2007).

Exposure of plants to cold stress during reproductive stage has tremendous effects on yield components. Generally, it appears that reproductive stage is more sensitive to cold stress than any other stage. Significant reduction in spikelet fertility has been reported in rice plants subjected to cold stress (Pereira da Cruz et al., 2006; Gunawardena et al., 2003). As a result, significant yield reduction of 30-40% has been common under low temperature environments in the temperate region (Andaya and Mackill, 2003; Clarke and Siddique, 2004).

Satake and Hayase (1970) observed higher cytological and histological disorders in the anthers of cold-stressed rice plants as compared to non-stressed plants. Cold temperature stress at reproductive stage has also been reported to cause flower abscission, pollen sterility, pollen tube distortion, ovule abortion and poor seed-set (Thakur et al., 2010). Structural and functional abnormalities in the reproductive organs of cold-stressed plants, and failed fertilization or premature abortion of the embryo have been observed. Brooking (1979) reported varying degrees of sterility in a sensitive sorghum line after exposure to night temperatures between 5

and 14^{o}C. Even crops inherently tolerant to cold temperature do suffer from cold stress during flowering. In both field and growth chamber experiment, chickpea plants exposed to cold temperature at flowering had highly reduced pollen germination and pollen tube growth (Savithri et al., 1980; Srinivasan et al., 1999) and also suffered significant flower abortion (Lawlor et al., 1997; Siddique and Sedgley, 1986) leading to reduced yield despite their adaptation to cooler climate. These events are similar in both cereals and legumes and ultimately result in reduced yield and quality of grains (Yang and Zhang, 2006). However, the specific duration and time when the floret development is more sensitive to low temperature is unknown.

Chapter 2 - EFFECT OF EARLY AND MID-SEASON COLD TEMPERATURE STRESS ON GROWTH, PHENOLOGY AND YIELD COMPONENTS OF SORGHUM

INTRODUCTION

Sorghum is one of the most important food and feed grains in the world. Originated in the semi-arid sub-Saharan Africa, the crop is adapted to hot and dry conditions commonly considered marginal for other cereals. However, the introduction and continued exposure of the crop into conditions different from its native environment has gradually led to evolution of new variants adapted to the new areas. For example, the expansion of the crop from its setting in the hot and dry environments in sub-Saharan Africa to the temperate regions has resulted in development of early-maturing and photoperiod insensitive types that makes the basis of sorghum production in these areas (Sally et al., 2007; Stephens et al., 1967). This and similar movement from the hot dry lowlands to high altitude areas within Africa also lead to the evolution of sorghum variants adapted to cooler weather. Nevertheless, many of these stresses, drought, low soil fertility, cold temperature, etc, continue to be among the major challenges to sorghum production in all areas where the crop is grown.

In the United States, low temperature in late spring and early fall has limited sorghum production to a narrow window period between May and October. All of the cultivated sorghums in the United States are bred to fit to this short time window, certainly at the expense of higher yield. Reduced seedling vigor under early-season cold stress and low night temperature during later growth stages in parts of the sorghum belt has been a serious limitation to expanding sorghum production both in time and space.

Though sorghum appears to be more sensitive, many other cereals do suffer from cold stress at different stages of growth. In rice, early season low temperature stress has been reported to reduce germination (Basnayake et al., 2003; Ali et al., 2006) and stand establishment

(Shimono et al., 2004; Shimono et al., 2007). It is also reported to delay phenological development and increase spikelet sterility resulting in low yield when encountered during flowering (Farrell et al., 2001; Lee, 2001; Gunawardena et al., 2003a, b). Similar effects of cold stress have been reported in maize. Low temperature of 15ºC or below has been noted to seriously reduce photosynthetic activity (Nie et al., 1992; Haldimann et al., 1996), alter composition of leaf pigments (Haldimann et al., 1995; Haldimann, 1998) as well as affect chloroplast development (Robertson et al., 1993). Recent studies agree with earlier findings that chloroplast function and photosynthetic capacity are seriously reduced under cold temperature and in extreme cases can result in cell death (Allen and Ort, 2001; Gomez et al., 2004; Rymen et al., 2007).

Cold temperature late in the season has been shown to affect reproductive development and hence directly reduce grain yield. In rice, low temperature after panicle initiation stage results in spikelet sterility (Nahar et al., 2009). Besides its direct effect on vital plant process, low temperature during flowering stage may also predispose crops to other yield limiting factors. In sorghum, low night temperature during flowering increases the incidence of ergot disease (Stack, 2000).

Given that it is naturally adapted to warmer conditions, the impact of low temperature stress on sorghum may be even more critical. Previous studies indicate that low soil temperature during early-growing season can severely reduce germination, emergence and seedling growth (Yu and Tuinstra, 2001; Franks et al., 2006). Incidence of certain types of soil-borne diseases such as *Pythium* and *Fusarium* appear to increase under cold temperature contributing to increased frequency of failed germination and seedling death resulting in poor stand establishment (Forbes et al., 1987). As a result sorghum in the United States is often planted 3-5

weeks after maize and other summer crops have been planted to avoid poor stand establishment due to low soil temperature in late spring. Though the impact has not been studied yet, there is a growing concern that low night temperature during grain filling stage in parts of sorghum growing areas in the United States may have adverse effect on yield. Analysis of weather data for some of the major sorghum growing counties in Kansas indicate that night temperature regularly drops below 20ºC starting from around pollination time which is below the range of 25-28ºC reported as optimal for reproductive growth (Maiti, 1996).

However, opportunities exist to deploy cold tolerant traits and thereby enhance sorghum production. Variants of sorghum that have improved germination and seedling vigor under cold temperature have been reported (Franks et al., 2006). Interest to deploy cold tolerance traits has increased in recent years. Sorghum hybrids with improved cold tolerance will not only result in increased yield but also lead to expansion of sorghum into areas traditionally considered too cold for the crop, and also enhance yield by allowing early planting in current production areas. However, assessment of the ultimate effects of both early and mid-season cold temperature stress is important to justify investment for improvement of the trait. Therefore, the objectives of this study are to determine the effect of early and mid-season cold temperature stress on crop establishment, growth, and yield components of sorghum, and to identify key plant characteristics that are most sensitive to low temperature episode at seedling and flowering stages**.**

MATERIALS AND METHODS

Two sets of experiments (Experiment I and II) were conducted using controlled environment facilities (greenhouse and growth chamber) at Kansas State University Department

of Agronomy. In experiment I, the cold temperature stress was imposed during the early days of seedling establishment starting on the third day after seedling emergence; whereas in experiment II, the temperature stress was imposed at heading stage immediately before anthesis had begun. In both experiments, the seedlings were raised in the greenhouse and were moved to the growth chambers for applying the temperature treatments. The treatment in both experiments lasted for 10 d. At the end of treatment application, the plants were returned to normal temperature in the greenhouse. The experiments were repeated three times.

Genetic materials

Three sorghum genotypes of contrasting response to early-season cold temperature (Shan Qui Red, SRN39 and Pioneer 84G62) were included in this study. Shan Qui Red (SQR), a Chinese kaoliang, exhibits higher germination and excellent seedling vigor when planted in cold temperature condition (Yu and Tuinstra, 2001; Yu et al., 2004; Knoll et al., 2008) and hence is considered tolerant to early-season cold stress. It is a red-seeded early-maturing genotype with high tannin concentration in the grain. SRN39 is an African caudatum often having difficulty establishing under cold temperature condition and as a result is considered susceptible to early-season cold temperature stress (Knoll et al., 2008; Cisse and Ejeta, 2003). Pioneer 84G62 is a popular high yielding commercial hybrid widely grown in the United States. Its reaction to cold temperature is unknown, but it has an overall excellent stand establishment and very good early-season vigor under normal conditions compared to most experimental hybrids. The experimental seeds for SQR and SRN39 were produced at Manhattan, KS during the 2008 season. Upon harvest the seeds were cleaned and surface sterilized using standard sorghum seed treatment (a mixture of Maxim 4FS, Apron XL, Concep III, Colorant and water). The 2007 batch of Pioneer 84G62 was acquired from the company. This source came with the standard seed treatment and

additional surface sterilization was not necessary. All the seed sources were stored in cold storage facility until needed.

Temperature regimes

The genotypes for the controlled experiments were evaluated under two temperature regimes, (25/23ºC) day/night, representing normal temperature condition, and (15/13ºC) day/night selected as cold temperature stress. The temperature transition time between day and night was thirty minutes. The temperature treatments were imposed by calibrating growth chamber temperature to the required settings. The specific temperature regimes were selected based on previous published information (Mann et al., 1985; Tiryaki and Andrews, 2001), a standard room temperature designation as control and an arbitrarily selected 5/3ºC units above base temperature (10ºC) for sorghum. A relative humidity of 60% was maintained throughout the treatment application period. The growth chambers were set to 10 hours of dark and 14 hours of light using incandescent bulb.

Experimental design and layout

A total of fifty-four 5L Poly-Tainer pots filled with a 1:1 soil: Metro-Mix 360 growing medium (Sun Gro, Bellevue, WA) were used. The pots were arranged in split-plot design with three replications. Three pots represented one plot. Temperature treatments were assigned to the whole-plot unit and genotypes to the sub-plot unit in both experiments. Experimental plants were initiated under normal temperature in the greenhouse. Three seeds were sown into each pot, and after emergence, the seedlings were thinned to one plant per pot. The pots were watered regularly. A slow release fertilizer (polycoate, Hummert International, Earth City, MO) was applied at seedling and mid-growth stages. Insect pests were controlled by spraying the plants

with Talstar P (FMC Corporation, Agricultural Products Group, Philadelphia, PA) or Floramite (Crompton Manufacturing Company, Inc. Middlebury CT) at the labeled rates.

On the third day after seedling emergence, experiment I plants were moved to the designated temperature treatments in the growth chambers and remained there for the duration of treatment period. Upon the end of treatment application, the plants were returned to normal temperature in the greenhouse and managed through maturity. Similarly, temperature treatments for experiment II were imposed by moving the plants to the growth chamber at panicle emergence stage, right before anthesis had begun.

Data collection and analysis

Data were collected on a number of growth parameters and yield components in both experiments. In experiment I, data on seedling growth parameters (seedling height, seedling vigor, seedling dry weight and number of leaves per plant) and leaf chlorophyll content were recorded immediately after the treatment application was ended, and plant height and number of leaves per plant were recorded every 10 d thereafter. Seedling vigor was visually rated using a 1 to 5 scale with '1' being excellent and '5' poor vigor. Seedling dry weight was measured from a separate set of plants raised using cone-tainers, and exposed to cold stress treatment in the same way as described above. Upon the end of treatment application, 10 seedlings were sampled from each plot and dried at 120^{o}C for five days, and seedling dry weight was measured as the weight of the dried samples. Number of leaves per plant was determined as the mean of the number of leaves from each of the three plants representing a plot. Leaf chlorophyll content was determined on each of the three plants on the last day of the stress treatment immediately before the plants

were returned to normal temperature. SPAD-502 chlorophyll meter (Spectrum Technologies, Plainfield, IL) was used to measure the leaf chlorophyll content.

Additional parameters including days to flowering, days to maturity, plant height and yield components (panicle weight, number of seeds per panicle, grain yield per panicle, and 1000 kernel weight (TKW)) were measured in both experiment I and II and anthesis duration was also measured in experiment II. Days to flowering was measured as the mean number of days taken by each of the plants in a plot to reach half-bloom stage. Anthesis duration was determined as the number of days taken for each panicle to complete anthesis. Days to maturity were determined as the average of days between planting to when the grains on the lower one-third section of the panicles formed black layer. After physiological maturity, the plants were harvested and the panicles were carefully separated from the stalks for measuring yield components. Panicle weight was determined as the weight of panicles from individual plants detached at the base. Seed weight per panicle was measured as the mean weight of seeds threshed from each panicle. Number of seeds per panicle was determined by counting seeds threshed from each panicle using a laboratory seed counter (Seed counter Model 850-3, International Marketing and Design Corp., San Antonio TX). Thousand kernel weight was estimated as the weight of 250 kernels from each head multiplied by four. Data for seed weight per panicle and 1000 kernel weight were adjusted to 12.5% moisture content before statistical analysis.

The data were subjected to statistical analysis using SAS 9.1 GLM (SAS, Institute, 1989). Analysis of variance was performed for both sets of growth chamber experiment using the GLM procedure in SAS. Individual runs for each set were independently analyzed and then combined over runs. The temperature effect was tested against the main plot error while the genotype and temperature × genotype interaction effects were tested against the overall error. Mean

comparisons for each parameter was performed using the Fisher's Least Significance Difference (LSD) test.

RESULTS

Effect of cold temperature stress on seedling growth and phenology

The analysis of variance for both experiment I and II is presented in Table 1.1. The effect of early-season temperature stress was highly significant for seedling height, seedling vigor and seedling dry weight. Leaf chlorophyll content, days to flowering and days to maturity were also significantly affected by early-season temperature stress (Table 1.1). However, overall number of leaves per plant and plant height were not affected by early stress (Appendix 1). The effect of genotypes under early season cold stress was also highly significant for seedling height, vigor and dry weight as well as days to flowering and maturity. Mid-season temperature stress significantly affected days to maturity. But temperature × genotype interaction effect was significant only for visual seedling vigor score.

Scrutiny of the results presented in Table 1.2 shows that early-season low temperature stress can significantly reduce seedling growth and delay time to flowering and maturity. Since the stress was imposed at mid-growth stage, seedling growth parameters were not measured under experiment II. Reduction of seedling vigor as a result of early season stress was severe enough that the effect persisted until four weeks after the plants were returned to normal temperature (Appendix 1). The impact of the stress, however, was not reflected on total leaf number and plant height at maturity (Table 1.2, Appendix 1). Mean seedling vigor score under cold stress was 3.4 compared to 1.2 under normal temperature (Figure 1.1). Effect on seedling dry weight was similar that plants subjected to the stress had mean seedling dry weight of 0.33 g compared to 1.34 g in those maintained under normal temperature. The impact on both traits, however, was

markedly different among genotypes. Under cold temperature, the tolerant line SQR had a vigor score of 2.8 compared to 4.3 for SRN39 and 3.3 for Pioneer 84G62. But under normal temperature, the three genotypes had a vigor rating of 1, 1.5 and 1.2, respectively. Vigor rating based on seedling dry weight also indicate that the cold tolerant line SQR had the highest seedling dry weight of 0.47 g compared to 0.1 for SRN39 and 0.41 for Pioneer 84G62. Under normal condition seedling dry weight of SQR, Pioneer 84G62 and SRN39 was 1.52, 1.29 and 1.21g, respectively. Likewise, leaf chlorophyll content was significantly reduced by the cold treatment resulting in mean chlorophyll content of 28.83 as compared to 36.59 units in plants grown under normal temperature. Difference in leaf chlorophyll content among genotypes was significant under early season cold stress with the susceptible genotype SRN39 having only 26.7 units as compared to 30 and 29.8 in SQR and Pioneer 84G62, respectively, with no significant difference among the later two. But difference among all the genotypes under normal temperature condition was not significant (Table 1.2).

In addition, early-season cold stress caused significant delay in flowering and maturity in all genotypes. While the average delay was 8 d and 4 d for flowering and maturity, respectively, there was marked variation between genotypes. Unlike for the other parameters, the cold tolerant genotype experienced the longest delay in both flowering and maturity. Under normal temperature, SQR required 62 and 107 d to reach half bloom and physiological maturity, respectively. But exposure to early season cold stress delayed these processes such that it needed 74 d to reach half bloom and 112 d to attain physiological maturity. In Pioneer 84G62, the stress caused flowering and maturity to delay by 8 and 3 d, respectively, while the process in the susceptible genotype SRN39 caused only 4 and 3 d delay.

Effect of cold temperature stress on yield components

The analysis of variance shows that, despite its significant impact on seedling vigor and phenology, early-season temperature stress had no effect on yield components (Table 1.1). But genotypic effect under early-season cold stress was highly significant for all yield components except TKW. The effect of mid-season temperature stress, however, was highly significant for all yield components. Likewise the effect of genotype under mid-season cold stress was highly significant for all yield components. But the effect of genotype × temperature interaction was not significant under both early and mid-season stress. Yield components among genotypes both under early-season cold stress and normal temperature were significantly different. Mean panicle weight, number of seeds per panicle and seed weight per panicle were significantly higher in the commercial hybrid Pioneer 84G62 under both early season stress and normal temperature conditions followed by the cold tolerant line. Thousand kernel weight was not significantly different between genotypes both under early-season cold stress and normal temperature conditions. SRN39 had the least mean yield components under both normal and cold stress conditions (Table 1.3). But across genotype means for all yield components were not significantly different between the temperature regimes.

Unlike the early-season stress, cold temperature episode at flowering significantly reduced all yield components (Table 1.3). Mean panicle weight was 19.1 g under cold stress compared to 32.1 g under normal temperature. Similarly the number of seeds per panicle was 388 for the cold stress vs. 846 under normal temperature. Other yield components such as TKW and seed per panicle were 22.7 and 10.4g, respectively, under cold stress treatment as compared to 28.3 and 32.3g, respectively, under normal temperature. Overall, cold stress episode at flowering resulted

in 40, 54, 28 and 63% reduction in panicle weight, number of seeds per panicle, TKW and seed weight per panicle, respectively.

Yield components were also significantly different among genotypes under both cold and normal temperature conditions. Similar to the results under experiment I, mean yield components were highest for Pioneer 84G62 followed by SQR under both temperature regimes, but the effect of the stress on genotypes was different. In the cold tolerant SQR, mid-season cold stress caused 28% reduction in panicle weight. The reduction in Pioneer 84G62 and SRN39 was 42 and 59%, respectively. At the same time SQR had 48, 18 and 60% reduction in number of seeds per panicle, TKW and seed weight per panicle, respectively, while Pioneer 84G62 had a reduction of 51, 25 and 61% for the same traits in that order. The susceptible line SRN39 had the highest reduction of 76, 48 and 82% for seed number per panicle, TKW and seed yield per panicle, respectively.

The degree of sensitivity and the specific plant traits affected by cold temperature episode depended on the timing of the stress. The average number of days to flowering and maturity were 86 and 121, respectively under early-season cold stress compared to only 78 and 117 under similar stress imposed at flowering (Table 1.4). The effect of timing of the stress was more prominent on yield components. Yield components were more affected by cold stress imposed at flowering stage than early season stress. Mean panicle weight was 33.5 g in early stressed plants compared to 19.2 g in those exposed to same stress at flowering. Likewise mean number of seeds per panicle, TKW and seed weight per panicle were 901, 30 and 27.3 g, respectively under early-season cold stress compared to 388, 22.7 and 10.4 in those exposed to cold temperature at flowering stage (Table 1.4). Yield components in those plants exposed to normal temperature under experiments I and II were very similar

Table 2.1. Combined analysis of variance for reaction of sorghum genotypes to early and mid-season cold temperature stress evaluated under controlled environment condition.

Source of variation	df	Seedling height (cm)	Seedling vigor (1-5)	Seedling dry weight (g)	Leaf chlorophyll content (SPAD units)	Days to flowering	Days to maturity	Panicle weight (g)	Seed number panicle^{-1}	TKW (g)	Seed weight panicle^{-1}
Seedling stage											
Run (R)	2	256.6	0.4	1.3	10.8	80.2	26.9	545.5	393417.8	170.7	241.1
Rep(R)	6	3.1	0.2	0.2	6.9	9.1	78.9	169.1	184237.8	13.8	192.8
Temperature (T)	1	3678.7**	67.2**	23.3**	542.1**	748.2**	240.7**	31.4	101573.4	14.5	9.8
R x T	2	566.3	0.1	1.2	4.3	4.5	50.0	4.3	70402.0	38.1	7.9
Error A	6	262.7	0.1	0.2	2.7	2.6	14.3	291.5	134923.2	95.5	125.3
Genotype (G)	2	469.0**	4.1**	0.8**	9.2	3902.8**	1801.0**	3183.8**	1729758.1**	18.3	1995.6**
T × G	2	22.0	1.5**	0.01	14.3	76.0	23.3	307.1	65504.2	10.7	138.2
Error B	32	7.2	0.1	0.1	3.7	132.7	20.4	236.8	192749.4	46.5	218.7
Flowering stage											
Run (R)	2	-	-	-	-	0.9	33.5	85.8	115330.2	357.2	55.2
Rep (R)	6	-	-	-	-	39.4	48..8	81.7	44713.8	72.0	22.6
Temperature (T)	1	-	-	-	-	20.2	1156.5**	2280.7**	2823576.0**	991.8**	4353.5**
R x T	2	-	-	-	-	6.7	34.4	181.6	30465.5	184.8	87.4
Error A	6	-	-	-	-	16.9	18.1	70.1	20292.3	56.8	35.7
Genotype (G)	2	-	-	-	-	5101.2**	3319.4**	3017.4**	2278133.6**	565.2**	2647.5**
T × G	2	-	-	-	-	32.7	18.5	182.1	151857.6	32.0	347.9
Error B	32	-	-	-	-	60.9	18.0	206.2	92730.3	59.8	104.7

Table 2.2. Phenology and seedling growth parameters as affected by early-season cold temperature stress.

	Seedling height (cm)		Seedling vigor (1-5)		Seedling dry weight(g)		Leaf chlorophyll content (SPAD units)		Days to flowering		Days to maturity		Final plant height (cm)	
	Temperature regimes		Temperature regimes		Temperature regimes		Temperature regimes		Temperature regimes		Temperature regimes		Temperature regimes	
Genotypes	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal
Shan Qui Red	20.91	38.69	2.8	1.0	0.47	1.52	29.95	35.78	74	62	112	107	134.41	138.97
SRN 39	12.62	26.57	4.3	1.5	0.10	1.21	26.7	36.83	99	95	130	127	100.24	103.6
Pioneer 84G62	15.81	33.59	3.3	1.2	0.41	1.29	29.83	37.15	84	78	121	118	110.33	112.06
Mean	16.45	32.95	3.4	1.2	0.33	1.34	28.83	36.59	86	78	121	117	114.99	118.21
LSD	5.05	5.86	0.47	0.93	0.06	0.15	2.49	NS	7.68	3.81	3.72	5.61	21.10	12.31
†LSD	4.68		0.21		0.07		1.72		3.05		2.85		NS	

Table 2.3. Mean yield components of sorghum genotypes as affected by early and mid-season cold temperature stress.

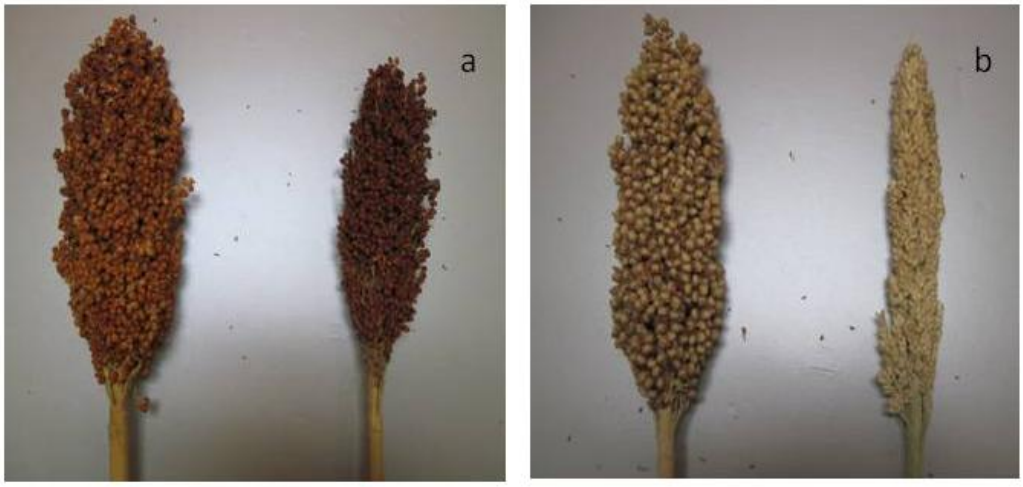
	Panicle weight(g)		Seed number panicle^{-1}		TKW(g)		Seed weight panicle^{-1}(g)	
Genotypes	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal
Seedling stage								
Shan Qui Red	35.98	26.57	1024	806	30.06	30.54	29.63	23.68
SRN 39	21.35	19.11	553	493	28.38	28.22	17.24	15.58
Pioneer 84G62	43.17	50.24	1126	1144	31.5	30.9	34.94	39.99
Mean	33.5	31.97	901	814	29.98	29.89	27.27	26.42
LSD	12.67	17.9	360	521	NS	NS	11.07	18.85
† LSD	NS		NS		NS		NS	
Flowering stage								
Shan Qui Red	22.12	30.54	440	843	26.33	32.15	11.01	27.25
SRN 39	7.21	17.52	96	403	15.29	29.49	2.29	12.4
Pioneer 84G62	28.1	48.37	629	1291	26.43	35.19	17.87	45.35
Mean	19.14	32.14	388	846	22.68	32.28	10.39	28.33
LSD	11.06	17.44	286	341	7.32	NS	6.13	13.31
† LSD	3.03		129		3.57		4.09	

Table 2.4. Phenology and yield components of sorghum genotypes subjected to cold temperature stress at seedling and flowering stages.

	Days to flowering	Anthesis duration (days)	Days to maturity	Panicle weight (g)	Seed number panicle^{-1}	TKW(g)	Seed weight panicle^{-1} (g)
Seedling stage							
Cold	86	7	121	33.50	901	30.00	27.27
Normal	78	7	117	31.97	814	29.90	26.42
Mean	82	7	119	32.74	858	29.9	26.85
LSD	3.05	NS	2.85	NS	NS	NS	NS
Flowering stage							
Cold	78	11	117	19.14	388	22.68	10.39
Normal	77	7	108	32.14	846	32.28	28.33
Mean	78	9	113	25.64	617	27.48	14.88
LSD	NS	1.0	7	4.09	129	3.57	3.03
† LSD	3.84	0.57	2.31	6.38	172	3.82	4.71

Cold (15/13^{o}C) Normal (25/23^{o}C)

Figure 2.1. Effect of early-season cold temperature stress on seedling vigor.



Normal Cold Normal Cold

Figure 2.2. Effect of mid-season cold temperature stress on seed setting and grain development: (a) tolerant genotype exposed to normal (left) and cold (right), and (b) susceptible genotype exposed to normal (left) and cold (right) temperature treatments.

DISCUSSION

Native to the sub-Saharan environment, sorghum has been less exposed to extreme cold temperature conditions that it is generally more sensitive to low temperature stress than related species such as maize. Reduced germination and seedling vigor are the common responses when the crop is planted in to cold soils. Though the other extreme temperature (heat stress) appears to be more important in sorghum production, cold temperature in late spring and low night temperature during grain filling period may have serious impact on sorghum yield in the Great Plains.

In the present study, we observed that exposing sorghum seedlings to cold temperature for 10 d halted growth markedly reducing seedling growth as noted from poor visual vigor score and seedling dry weight. This appears to have resulted from reduced cellular functions and low photosynthetic activity imposed by cold temperature event (Brouwer et al., 1973; Ku et al., 1978). But once the plants were returned to regular temperature condition, normal growth resumed such that by the end of the growing period, the plants have attained optimum leaf number and plant height. Similar result was reported by Major et al. (1982). Though growth was reduced in all genotypes, differences in genotypic response to cold stress were evident that the tolerant genotype SQR was little affected by the stress compared to the cold susceptible African line SRN39. Reduction in seedling dry weight was 69% in the SQR as opposed to 92% in SRN39. This variation represents the difference in relative sensitivity of plant growth processes to low temperature stress between the genotypes. Though no attempt was made to measure the difference in photosynthetic activity between these genotypes, the marked difference in leaf chlorophyll content measured at the end of early-season cold treatment may explain the effect of cold stress on photosynthetic activity. The across

genotype reduction in mean leaf chlorophyll content was significant, 28.8 vs. 36.6, under cold stress and normal temperature, respectively. Early season cold stress in SQR resulted in only 16% reduction in leaf chlorophyll content. But the same stress in SRN39 reduced leaf chlorophyll content by 28% and it is possible that photosynthetic rates might have followed the same trend. Studies conducted on other related cereals reported the negative impact of cold stress on photosynthesis. In maize, photosynthetic efficiency measured in a range of genotypes subjected to cold temperature of 15ºC was shown to be markedly lower than those grown under normal temperature (25ºC) and the extent of the reduction was different between entries with cold sensitive genotypes showing the greatest reduction (Fracheboud et al., 1999). Difference in leaf chlorophyll content among genotypes was not significant under normal temperature.

Though active growth resumed after the plants were returned to normal condition, the temporary halt on growth imposed by the stress significantly delayed flowering and maturity in all genotypes with the delay being longer for early-maturing lines regardless of their response to cold temperature. It seems that genotypes that had longer recovery period after the stress was removed (long maturing types) had enough time to compensate for the growth arrest and relatively had shorter delay than those with inherently short growth duration. Accordingly, the early-maturing cold tolerant line SQR had 12 and 7 d delay to flowering and maturity, respectively, while the longer maturing susceptible line SRN39 had only 4 and 3 d delay to reach flowering and maturity, respectively. The exact mechanism for delayed reproductive development is not well understood. But reduction in physiological activities including megasporogenesis and microsporogenesis and photosynthesis are expected under sub-optimal temperature and hence reducing the rate at which these processes are

accomplished. Findings from previous work on other species agree with the present result. In wheat, cold temperature stress at seedling stage has been reported to cause reduced growth and development thereby resulting in prolonged onset of flowering (Subedi et al., 1998). Nevertheless, early-season cold temperature stress in this study did not cause significant reduction in any of the yield components (Table 1.3). Perhaps the same developmental response that compensated for final leaf number and plant height was responsible for compensation for yield components. Previous reports on sorghum agree with the current results (Major et al., 1982).

On the other hand, cold temperature at flowering seems to be more detrimental to yield of sorghum. All yield components including panicle weight, number of seeds per panicle, seed weight per panicle and TKW were severely affected by mid-season cold stress. The stress was imposed immediately before anthesis when both male and female floral organs are expected to be viable (Prasad et al., 2008) hence the effect on yield components appears to be primarily due to the impact of the stress on anthesis, pollination or fertilization. In our observation during routine growth chamber supervision, we noticed slow anthesis and markedly reduced anther dehiscence which may have likely resulted in low amount of pollen. Although both the anther and stigma have fully extended, the low temperature may have impacted receptivity of the stigma, germination and growth of pollen tube or fertilization resulting in reduced seed-set and lower number of seeds per panicle (Downes and Marshall, 1971). Studies conducted on the sister crop (maize) have shown that cold stress imposed just before or at the start of flowering reduced the number of tassel branches and spikelets and also seed size (Bechoux et al., 2000). The stress was also reported to cause structural and functional abnormalities in reproductive organs that lead to either failure of fertilization or

premature abortion of florets, induced flower abscission, pollen sterility, pollen tube distortion, ovule abortion and thus reduced seed-set ultimately leading to lower yield (Thakur et al., 2010). Earlier reports have shown that low night temperature during flowering causes significant reduction in spikelet and flower fertility in rice (Jiang et al., 2002; Pereira da Cruz et al., 2006).

CONCLUSION

Cold temperature stress at seedling stage may temporarily reduce seedling vigor, height and number of leaves per plant, and also interfere with photosynthesis through inducing chlorophyll degradation. As a result both days to flowering and maturity may be delayed. However, such brief exposure to cold temperature at seedling stage does not necessarily result in significant reduction in yield. From the current result it can be concluded that provided that germination and emergence are not an issue, sorghum in the mid-west can be planted earlier than the current practice to allow extended grain fill period without sacrificing yield due to reduced seedling vigor. Nevertheless, low temperature stress at flowering stage appears to be more detrimental to yield mainly through affecting seed number and seed weight perhaps due to its effect on pollen shedding, fertilization or grain setting and development. Future studies need to focus on examination of the specific biological mechanisms sensitive to cold stress.

Chapter 3 - SCREENING OF SORGHUM GERMPLASM FOR TOLERANCE TO EARLY-SEASON COLD TEMPERATURE STRESS

INTRODUCTION

Early-season cold temperature stress is one of the major abiotic factors that undermines sorghum production in temperate regions. Low soil temperature in late spring prevents early planting resulting in narrow growing window. While this affects all summer crops including maize, sorghum is particularly sensitive to cold stress because of its inherent adaptation to hot and dry climate. Low-temperature stress of less than 20ºC can affect sorghum growth and productivity; and non-freezing temperatures of 10 to 15^{o}C can cause chilling injury (Peacock, 1982). Seed germination, emergence and seedling vigor in sorghum are particularly sensitive to cold stress (Pinthus and Rosenblum, 1961; Singh, 1985; Harris et al., 1987; Ander and Pinter, 1994) and temperatures lower than 23ºC can significantly reduce germination of sorghum seeds (Kanemasu et al., 1975). Therefore, rapid and high germination percentage and seedling vigor under cold temperature are important traits associated with early season cold tolerance (Keim and Gardner, 1984; Revilla et al., 2000; Cisse and Ejeta, 2003) and these traits are generally positively correlated among themselves (Mendoza-Onofre et al., 1979; Brar and Stewart, 1994). Seedling height and seedling dry weight has also been shown as indicators of early-season cold tolerance (Acevedo et al., 1991; Regan et al., 1992; Cisse and Ejeta, 2003) though they are also noted to have association with plant stature, mature plant height, leaf number and biomass (Maiti et al., 1981).

Early-planting of sorghum has numerous advantages; it allows the use of full season hybrids and thus increases biomass accumulation which ultimately results in improved grain

and biomass yields (Cisse and Ejeta, 2003). Besides permitting extended biomass accumulation, the use of cold tolerant materials also allows expansion of sorghum production in space and time and also reduces risks of seedling diseases often associated with reduced vigor. Early planting also assist farmers to take advantage of high soil moisture early in the spring and reduce risk of terminal drought in the summer (Franks et al., 2006). Besides, the great potential for the use of sorghum as lignocellulosic feedstock for biofuel production has increased the importance of cold tolerance that a number of programs are engaged in the improvement of the trait. Efforts made to date have been highly dependent on limited sources of cold tolerance from the Chinese kaoliangs. These materials have relatively high germination percentage and improved seedling vigor under cold stress compared to the tropical sources (Franks et al., 2006; Qingshan and Dahlberg, 2001). Selected genotypes from these sources are being utilized in breeding programs. But despite the high germination and improved seedling vigor the materials are highly susceptible to leaf diseases and contain high concentration of tannin in the grain. Most of the promising cold tolerant lines derived from populations based on these sources have been found to contain high levels of tannin in the grain and this has limited their use for hybrid grain production. Tannin is a polyphenolic compound; in sorghum it is found in the testa layer of the seed (Gisele de Oliveira et al., 2007). Though recent reports underline the health benefit of tannin (Woodward et al., 2001; Makkar, 2003), the compound has been reported to have anti-nutritional property and thus market demand for high tannin sorghum grains is low. Animal feed made from sorghums with high tannin content are low in protein availability and this reduces the value of sorghum as feed grain (Silanikove et al., 2001; Gisele de Oliveira et al., 2007). Recent unpublished results show that genomic regions associated with improved cold tolerance and germination

in these sources have been shown to closely overlap with QTLs for tannin content. Further study is needed to determine the nature of the relationship between tannin accumulation and early-season cold tolerance. While the existing high tannin cold tolerance sources can be utilized for improving the trait in sweet or high biomass sorghums, low tannin cold tolerance sources are required in grain sorghums. The objective of this experiment was to identify additional sources of early-season cold tolerance for use in improvement of the trait in food and feed grain sorghums.

MATERIALS AND METHODS

Field experiment

The experiments were conducted in the field under early planting and in controlled environment (greenhouse/growth chamber). The field experiment was conducted at Kansas State University, Agronomy research farm near Manhattan, KS during the 2010 main season. The experimental materials consisted of 150 sorghum genotypes obtained from the national gene bank. The list of genotypes is provided in Appendix 2. The genotypes were selected based on center of origin, adaptation and photoperiod response. All of them are from colder regions of the world and photoperiod insensitive. The genotypes were acquired in spring 2009 and seeds were increased during the 2009 main season at Manhattan. Prior to planting, the seeds were manually cleaned and surface sterilized using standard sorghum seed treatment. The materials were subjected to cold and normal temperatures by manipulating the planting time. The cold treatment was imposed by planting early in the season (April 13th, 2010) as opposed to the normal temperature that was planted on May 26th, 2010. The soil temperature during the planting time was 20.1/13.4ºC max/min for the cold temperature and 30.0/20.4ºC max/min for the normal temperature (Appendix 3 and 4). The experiment was

laid in a randomized complete block design with three replications. The plots were 5 m long single rows spaced 0.75 m apart. Fifty high quality seeds were drilled in to each row using a cone planter. Standard weed control and fertilizer management practices were applied.

Data were collected on emergence percentage, seedling vigor, seedling height, number of leaves per plant, and seedling dry weight. Emergence data was collected by counting the number of emerged seedlings in each plot on day 14 after planting. On 28^{th} d after planting, plot-based visual seedling vigor scores were taken using a 1-5 scale with score '1' representing excellent vigor and score '5' representing poor vigor as described by Maiti (1996). Seedling height and number of leaves per plant were determined based on ten random plants selected from each plot. Seedling height was measured as the mean length of all ten samples measured from the soil-surface to the tip of the longest leaf. Number of leaves per plant was determined as the mean of total number of leaves from ten seedlings. For seedling dry weight, ten randomly selected plants were harvested from each plot and the weights recorded after the samples were dried at 120^{o}C for five days.

Controlled environment experiment

The top 15% of the genotypes (twenty-four accessions) selected based on emergence percentage and seedling vigor score from the field experiment were further studied. The standard cold tolerant and susceptible checks were also included. Two sets of experiment were conducted; one for germination and the other for seedling vigor. For germination study, seeds of the selected genotypes were surface-sterilized with ethanol (70%) followed by 5% sodium hypochlorite, and washed with double distilled water. This process was repeated three times to avoid fungal infection. Fifty surface-sterilized seeds were then placed on a 9 cm whatman filter paper in 10 by 1.5 cm petri-dishes. Moisture was supplied by wetting the

filter paper with double distilled water. Two sets of test samples were prepared for each genotype. The first set was placed in a growth chamber calibrated to a constant day and night temperature of 15^{o}C (cold treatment) and the second set at 25^{o}C (normal temperature) under complete darkness (Brar and Steward, 1994; Radford and Henzell, 1990; Harris et al., 1987; Brar et al., 1992). Relative humidity was kept between 65 and 70%. Four milliliters of double distilled water was applied to each petri-dish on the first day and were rewetted every 2 days thereafter. The experiment was repeated three times, each run serving as one replication. Germination scores were taken on 14 d after planting. The seed was regarded germinated when the radicle or coleoptile has extended at least 1mm beyond the seed coat (Franks et al., 2006). Germination percentage was determined by dividing the number of seeds germinated by the total number of seeds on each petri-dish and then multiplying by hundred.

Growth chamber experiment-seedling vigor test

The same set of genotypes was used for evaluating seedling vigor. Before planting, seeds were surface sterilized using standard sorghum seed treatment to protect against fungal infection. Planting was done under normal temperature in the greenhouse using SC-10 Super Cell Cone-tainers ^{TM} (Hummert International, Earth City, MO) filled with Metro-Mix 360 growing medium (Sun Gro, Bellevue, WA). Two seeds were planted in each container, and each experimental unit consisted of seven containers. A randomized complete block design with three replications was used. After emergence, the seedlings were thinned to one plant per cone-tainer. Three days after emergence, the seedlings were transferred to growth chambers, calibrated at 15/13^{o}C day/night (cold temperature) and 25/23^{o}C day/night (normal temperature).The temperature transition time between day and night was thirty minutes. The relative humidity was kept at 70% in both growth chambers, and the diurnal cycle was 14/10

h (day/night). The temperature treatments lasted 10 d. Data were collected on seedling vigor, number of leaves per plant, seedling height and seedling dry weight using the procedure described above. The experiment was repeated three times.

Statistical analysis

Data from both field and controlled environment experiments were subjected to statistical analysis using SAS 9.1 (SAS Institute 1989). The general linear model (GLM) procedure in SAS was used to estimate variation among genotypes under both cold and normal temperature plantings. Genotypes and blocks were treated as random effects in all experiments. Mean comparisons for each parameter were performed using the Fisher's Least Significance Difference (LSD) test at α=0.05. Correlations between germination percentage and seedling traits for controlled environment and field conditions were conducted separately. Correlation between field and controlled environment data was also performed to determine the similarity in expression of cold tolerance traits under the two conditions.

RESULTS

Field experiment

The analysis of variance for emergence percentage and seedling vigor traits under the field experiment is presented in Table 2.1. The results show that both temperature and genotype effects were highly significant for all traits. The temperature × genotype interaction effect was also highly significant for emergence percentage and seedling height, but not significant for seedling vigor, number of leaves per plant and seedling dry weight.

Seedling emergence was generally 43% lower under cold planting compared to normal planting. Under cold temperature, average seedling emergence was 38.3% as

compared to 67.4 % under normal temperature. But the range was 4.7 to 57.3% under early planting and 50 to 88.7% for normal planting (Table 2.3). Though significant genotype × temperature interaction was noted (Table 2.1), five of the top six genotypes with respect to seedling emergence were also among the top six under normal temperature condition. Under early planting, genotypes MN2735, K-385, IS12740, ETS3638, San Er Sui, and IS12744, in that order, had mean emergence percentage that is significantly higher than the overall mean of 38.3%. Likewise, the top genotypes under normal planting include ETS3638, Durra Belaya, K-385, San Er Sui, IS12740 and IS12744 (Table 2.3). The cold tolerant and susceptible checks had mean emergence percentage of 15 and 4.7% under early planting and 65.3 and 62.7% under normal planting, respectively.

Similarly differences in seedling vigor among genotypes ranged from 1 to 5 under cold temperature and 1 to 2.33 under normal temperature. Some of the genotypes that had high emergence percentage under cold temperature were also shown to have high seedling vigor and these include San Er Sui and IS12744 with vigor score of 1, and IS27935 and K-385 with vigor score of 2. Mean seedling vigor of the cold tolerant check SQR is similar to the top genotypes despite its lower emergence percentage. The susceptible line, SRN39, had the poorest mean seedling vigor of 5. Under normal planting, the range in seedling vigor among genotypes is narrower but genotypes that exhibited improved seedling vigor under cold stress continued to express the highest seedling vigor under normal temperature as well (Table 2.3). Seedling dry weight showed more or less similar trend with visual seedling vigor except few genotypes not among the top in visual vigor score came in to picture. Again the cold tolerant check SQR was among the top 15% with respect to seedling dry weight and the susceptible check, SRN39, was the lowest. The results are consistent under both temperature

regimes except change of ranks among the top genotypes. Mean seedling height and leaf number among genotypes ranged from 8.1 to 14.7 cm and 3 to 5, respectively, under early planting. The range under normal planting was 39.8 to 81.6 cm for seedling height and 6 to 7 for leaf number. Again several of the genotypes that were among the top with respect to seedling emergence and vigor were among the top for seedling height and leaf number under both early and normal planting. This was expected since seedling height and leaf numbers are among the criteria for scoring seedling vigor.

Controlled environment

Similar to the field experiment, the effects of both temperature and genotype on germination percentage and seedling traits (seedling vigor, seedling height, number of leaves per plant and seedling dry weight) under the growth chamber experiment were also highly significant (Table 2.1 and Appendix 5). The genotype × temperature interaction effect was highly significant for all traits. Mean germination percentage across genotypes was only 43.3% under cold stress as compared to 89.6 % under normal temperature (25ºC) indicating significant effect of temperature on germination. But the range in germination percentage was again wider under cold stress with the values ranging from 1.8% in the highly susceptible genotypes to 87.1% in the relatively tolerant types (Table 2.4). The range under normal temperature condition was from 72.7 to 100%. The markedly variable response of genotypes under cold stress condition indicates the potential for improving the trait through selection. Many of the genotypes that ranked among the top with respect to germination under controlled environment such as Da Qing Ye, Kaoliang-Wx, Ping Ding Xiang were not among the top for other traits scored under field condition except IS12744 that was among genotypes with the highest score for seedling vigor under field experiment. When compared

with germination percentages under normal condition, these top genotypes, Da Qing Ye, Kaoliang-Wx and Ping Ding Xiang, had mean germination percentage reduction of 12.9, 11.8, and 18.5 %, in that order, and the known cold tolerant check SQR had 22.2% reduction. IS12744 showed germination reduction of 27.1% and was in the top 2 with respect to seedling vigor and in the top 8 for all traits measured in the field.

Mean seedling vigor scores among genotypes under cold temperature ranged from 1.8 in San Chi San to 4.6 in susceptible genotype, SRN39. Under normal temperature the range was from 1.23 to 3.13. San Chi San, IS12744 and Ping Ding Xiang were more vigorous under both cold and normal temperatures. These genotypes were also among the top for seedling height and number of leaves per plant under both cold and normal temperature regimes. The susceptible genotype, SRN39, and another line, L1999B-13, had the poorest seedling vigor of all genotypes under cold stress. Besides those, additional Chinese lines, Da Qing Ye (Yang Qu), Ping Ding Xiang and Kei Ko She Jen Hing had among the tallest seedlings at the time of scoring. Again, the susceptible genotypes, SRN39 and L1999B-13 were the shortest plants at the time of scoring.

Like germination and other seedling traits, the difference in seedling dry weight among genotypes was significant under both cold and normal temperature regimes. The genotypic values ranged from 0.12g to 0.41g under cold stress, and from 0.43 to 1.02 g under normal temperature. Across genotypes, the average was 0.25g under cold stress and 0.7g under normal temperature. Genotypes, IS12750, IS12741, San Chi San, IS27935 and Da Guan Dong had the highest seedling dry weight and are slightly higher or comparable with the tolerant line SQR. The susceptible lines, SRN39 and L1999B-13 again had the lowest seedling dry weight. Genotypes, IS12744, Bai Nian Gao Liang, Da Qing Ye and IS27929

were among those with the highest seedling dry weight under normal temperature. But SRN39 and L1999B-13 continued to have the lowest seedling dry weight under normal temperature as well.

Association among cold tolerance traits

Results of genotypic performance under field and controlled experiments tended to show similar trend but were not strictly consistent. Only five of the genotypes that ranked among the top 10 under field condition maintained those ranks under controlled experiments (Table 2.5). The other five genotypes ranked between 11^{th} and 20^{th}.

Pearson correlation coefficients among the different cold tolerance traits are presented in Tables 2.6 and 2.7. Many of the traits under the controlled experiment were significantly correlated with each other. Germination percentage in the growth chamber had significant correlation with seedling vigor (r = -0.33) and number of leaves per plant (r = -0.13). Seedling vigor was also significantly correlated with seedling height (r = -0.32), but not correlated with leaf number and seedling dry weight (Table 2.6). However, seedling height, leaf number per plant and seedling dry weight were positively and significantly correlated with each other. Under field conditions, all seedling traits were highly significantly correlated with each other except between emergence percentage and leaf number for early planting. Emergence percentage had highly significant correlation with seedling vigor (r = -0.43), seedling height (r = 0.54) and seedling dry weight (r = 0.32). Whereas, the correlation of seedling vigor was highly significant with seedling height (r = -0.59), number of leaves per plant (r = -0.40), and seedling dry weight (r = -0.49). Additionally, seedling height had highly significant and positive correlations with leaf number per plant (r = 0.28), and

seedling dry weight (r = 0.64). Furthermore, number of leaves per plant had a highly significant and positive correlation with seedling dry weight (r = 0.37).

Results of correlations between cold tolerance traits scored under the controlled conditions and in the field are presented in Table 2.7. Many of the traits scored under the controlled environment did not have significant correlation with those scored in the field. Accordingly germination and leaf number per plant under controlled experiment had significant correlation with seedling dry weight and emergence percentage scored in the field, respectively. But seedling vigor under the growth chamber did significantly correlate with seedling vigor, seedling height and seedling dry weight in the field. Correlation with other seedling traits in the field such as emergence percentage and leaf number per plant was not significant. Likewise, seedling height under growth chamber significantly correlated with all of the traits scored in the field except leaf number.

Table 3.1.Combined analysis of variance for cold tolerance traits under growth chamber and field conditions evaluated at Manhattan

Source of variation	df	Emergence (%)	†Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}	Seedling dry weight (g)
Growth chamber						
Run (R)	2	12838.1	25.5	249.6	3.8	5.6
Rep (R)	6	2267.1	1.7	15.9	0.2	0.2
Temperature (T)	1	250339.7**	77.5**	30277.8**	224.1**	24.5**
R x T	2	2888.1	3.9	1233.0	13.9	4.4
Error A	6	1430.4	1.1	30.0	1.0	0.4
Genotype (G)	25	4018.6**	5.2**	192.4**	0.8**	0.1**
T × G	25	1789.4**	1.8**	51.4**	0.6**	0.1**
Error B	400	212.0	0.7	11.0	0.1	0.03
Field conditions						
Block	2	5.6	0.6	16.3	1.1	1.0
Environment (E)	1	12907.4**	41.0**	89967.4**	95.5**	287.2**
Error A	2	478.38	3.7	27.5	0.1	1.5
Genotype (G)	25	1178.8**	2.4**	158.7**	0.4*	1.0**
E × G	25	372.16**	0.7	99.4**	0.2	0.8
Error B	50	169.4	0.8	36.3	0.2	0.5

*, ** Significant at $P \leq 0.05$, $P \leq 0.01$, levels of probability, respectively; †Seedling vigor: 1 = excellent, 5= poor.

Table 3.2.Mean performance of cold tolerance traits and the number of genotypes significantly higher than Shan Qui red under early planting in field conditions

Traits	Mean	Range	Standard deviation	Number of genotypes significantly higher than SQR under early planting
Emergence (%)	22	1-57	8.42	53
Seedling vigor (1-5)	3.8	1-5	0.82	3
Seedling height (cm)	10.8	7.7-14.7	1.10	30
Seedling dry weight (g)	0.38	0.12-0.84	0.11	17

Table 3.3.Mean cold tolerance related traits among the top 24 accessions evaluated under cold (early planting) and normal (regular) field planting at Manhattan, 2010.

	Emergence (%)		†Seedling vigor (1-5)		Seedling height (cm)		Leaf number seedling^{-1}		Seedling dry weight (g)	
Genotypes	Early	Normal	Early	Normal	Early	Normal	Early	Normal	Early	Normal
Durra Belaya	45.33	82.00	2.67	1.67	11.89	60.43	4	6	0.38	3.03
ETS3638	54.00	88.67	2.67	1.67	11.46	65.01	4	6	0.34	2.24
IS 12741	42.67	70.00	3.30	1.67	11.33	57.25	5	6	0.39	2.66
K-517	34.67	55.00	2.67	1.67	12.38	53.78	4	6	0.56	3.14
IS 27935	36.67	65.00	2.00	1.67	13.12	54.08	5	6	0.67	2.89
IS 12750	40.67	55.67	2.67	2.33	10.38	47.40	4	6	0.40	2.49
Kei ko She jen hing	41.33	64.67	3.33	1.33	12.73	59.02	4	6	0.40	2.51
Da Qing Ye (Yang Qu)	42.00	60.67	3.00	1.33	12.47	59.93	4	6	0.62	3.48
Ping Ding Xiang	45.33	73.00	3.33	1.33	12.76	81.64	4	6	0.49	3.98
IS 27929	33.33	71.67	2.33	1.33	12.35	60.47	4	6	0.43	4.53
K-385	57.33	79.33	2.00	1.00	14.73	71.70	4	6	0.51	5.00
MN 2735	50.67	68.33	2.67	1.00	14.00	68.31	5	6	0.51	3.33
Kaoliang-Wx	32.67	64.00	3.33	2.33	9.83	51.82	4	6	0.40	2.96
Susu zairai shu	38.00	63.00	3.33	2.00	9.82	56.51	5	6	0.50	3.25
PI563943	42.00	71.00	2.33	1.00	13.75	77.36	4	6	0.48	4.15
Da Guan Dong	24.67	56.67	3.33	1.33	12.37	57.95	4	6	0.44	3.03
San Er Sui	54.00	78.33	1.00	1.00	14.41	71.22	4	6	0.62	4.22
San chi San	33.33	65.00	2.67	2.00	10.81	52.69	4	6	0.59	3.25
Japanese dwarf Broomcorn	32.67	67.33	3.33	1.67	12.53	59.89	4	7	0.50	2.81
Bai Nian Gao Liang (Jin Xi)	42.00	66.00	2.67	1.67	12.53	54.98	5	6	0.56	2.99
IS 12744	53.33	73.67	1.00	1.67	13.96	60.35	5	6	0.58	3.13
IS 12740	54.67	76.33	2.33	1.33	12.29	67.04	4	6	0.48	3.68
L 1999B-13	11.33	50.00	3.33	3.00	9.87	39.76	5	5	0.39	1.83
Dao Zai Tou	34.67	60.00	2.33	1.33	12.19	64.45	4	7	0.43	2.74
Shan Qui Red	14.67	65.33	2.00	2.00	10.21	55.44	5	6	0.31	3.38
SRN39	4.67	62.67	5.00	3.67	8.07	52.53	3	6	0.22	2.05
Mean	38.34	67.44	2.72	1.69	12.01	60.04	4	6	0.47	3.18
LSD	13.5	14.10	1.68	1.13	2.04	13.82	0.76	NS	0.26	1.67

NS = non-significant; †Seedling vigor: 1= excellent, 5=poor.

Table 3.4.Mean cold tolerance related traits among the top 24 accessions evaluated under cold (15/13°C day/night) and normal (25/23°C day night) temperature under growth chamber condition.

	Germination (%)		†Seedling vigor (1-5)		Seedling height (cm)		Leaf number plant^{-1}		Seedling dry weight (g)	
Genotypes	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal
Durra Belaya	14.67	72.67	2.92	2.16	17.57	35.46	3	5	0.19	0.77
ETS3638	32.00	96.00	3.08	3.08	15.71	27.59	3	4	0.18	0.51
IS 12741	42.22	98.67	2.21	1.43	18.32	36.24	3	5	0.36	0.78
K-517	33.78	88.67	2.83	2.13	18.20	31.66	3	4	0.26	0.74
IS 27935	43.56	94.67	2.49	1.59	19.29	33.20	3	5	0.31	0.76
IS 12750	60.89	98.67	2.67	2.08	15.44	32.69	3	4	0.41	0.73
Kei ko She jen hing	14.67	80.67	2.24	1.37	19.53	36.61	3	5	0.28	0.78
Da Qing Ye (Yang Qu)	87.11	100.00	2.31	1.73	20.59	35.17	3	4	0.28	0.87
Ping Ding Xiang	78.22	96.67	1.98	1.51	20.14	40.43	3	4	0.25	0.78
IS 27929	13.78	82.67	3.17	1.42	16.09	36.23	3	4	0.25	0.80
K-385	34.67	67.33	3.92	2.60	13.42	34.89	3	4	0.13	0.60
MN 2735	28.00	94.00	3.14	2.13	15.26	32.97	3	5	0.19	0.62
Kaoliang-Wx	79.56	91.33	2.39	1.92	18.11	32.99	3	5	0.28	0.71
Susu zairai shu	49.78	88.67	3.52	2.49	14.45	29.01	3	4	0.22	0.65
PI563943	36.00	96.67	3.31	2.29	16.36	35.82	3	4	0.29	0.63
Da Guan Dong	7.11	76.00	2.92	2.21	14.83	30.54	3	4	0.31	0.74
San Er Sui	58.22	98.00	2.63	2.50	17.45	33.30	3	4	0.25	0.53
San chi San	51.11	94.67	1.77	2.72	19.17	25.48	3	4	0.33	0.57
Japanese dwarf Broomcorn	21.33	98.67	3.73	2.43	14.47	30.58	3	5	0.19	0.65
Bai Nian Gao Liang (Jin Xi)	63.56	86.67	3.12	1.42	14.89	33.68	3	5	0.20	0.94
IS 12744	71.56	98.67	1.83	1.23	19.74	39.92	3	5	0.25	1.02
IS 12740	31.56	91.33	2.27	1.80	18.58	36.38	3	4	0.19	0.71
L 1999B-13	60.00	83.33	4.22	3.13	7.94	22.78	2	5	0.12	0.43
Dao Zai Tou	42.22	88.67	2.94	2.18	17.54	32.65	3	5	0.20	0.67
Shan Qui Red	69.11	91.33	3.10	2.43	17.50	27.42	3	4	0.29	0.52
SRN39	1.78	74.00	4.56	2.12	11.04	26.22	2	6	0.17	0.77
Mean	43.31	89.56	2.90	2.08	16.60	32.69	3	4	0.25	0.70
LSD	20.05	8.51	0.93	0.67	3.47	2.70	0.30	0.32	0.17	0.19

†Seedling vigor score: 1 = excellent and 5 = poor.

Table 3.5.Relative ranking of genotypes based on germination percentage and seedling vigor under controlled environment and field conditions.

	Growth chamber experiment				Field experiment			
	Germination[a] (%)		†Seedling vigor[b]		Emergence[a] (%)		†Seedling vigor[b]	
Genotypes	(Cold)	(Normal)	(Cold)	(Normal)	(cold)	(Normal)	(cold)	(Normal)
Da Qing Ye (Yang Qu)	1	1	7	7	10	20	5	2
Kaoliang-Wx	2	8	8	9	19	17	7	5
Ping Ding Xiang	3	4	3	5	8	7	7	2
IS 12744	4	2	2	1	5	6	1	3
Bai Nian Gao Liang (Jin Xi)	6	10	18	3	10	13	4	3
IS 12750	7	2	11	10	12	23	4	5
L 1999B-13	8	11	25	24	23	25	7	6
San Er Sui	9	3	10	20	4	4	1	1
San chi San	10	6	1	22	18	15	4	4
Susu zairai shu	11	9	22	19	13	18	7	4
IS 27935	12	6	9	6	14	15	2	3
IS 12741	13	2	4	4	9	10	6	3
Dao Zai Tou	14	9	15	15	16	21	3	2
PI563943	15	4	21	17	10	9	3	1
K-385	16	16	24	21	1	3	2	1
K-517	17	9	12	12	15	24	4	3
ETS3638	18	5	16	23	3	1	4	3
IS 12740	19	8	6	8	2	5	3	2
MN 2735	20	7	19	13	6	11	4	1
Japanese dwarf Broomcorn	21	2	23	18	20	12	7	3
Durra Belaya	22	15	13	14	7	1	4	3
Kei ko She jen hing	23	12	5	2	11	16	7	2
IS 27929	24	11	20	3	17	8	3	2
Da Guan Dong	25	13	14	16	21	22	7	2
Shan Qui Red	5	8	17	18	22	14	2	4
SRN39	26	14	26	11	24	25	8	7

[a]Genotypes with the same rank have the same germination or emergence percentage; [b]Genotypes with the same rank have the same seedling vigor scores; Cold and normal temperature under field condition refers to early and regular planting; Cold and normal temperature under controlled environment corresponds to 15/13°C and 25/23°C day/night, respectively.

Table 3.6.Pearson correlation coefficients among cold tolerance traits of sorghum germplasm accessions evaluated under growth chamber and field conditions.

Growth chamber (Cold)	Germination	Seedling vigor	Seedling height	Leaf number plant^{-1}	Seedling dry weight
Germination		-0.33**	-0.02	-0.13*	-0.07
Seedling vigor			-0.32**	-0.06	-0.07
Seedling height				0.75**	0.59**
Leaf number plant^{-1}					0.57**
Field (early planting)					
Emergence		-0.43**	0.54**	0.09	0.32**
Seedling vigor			-0.59**	-0.40**	-0.49**
Seedling height				0.28**	0.64**
Leaf number plant^{-1}					0.37**

*,** Significant at P≤ 0.05, P≤ 0.01 levels of probability, respectively.

Table 3.7. Pearson correlation coefficients between cold tolerance traits scored under growth chamber and field conditions.

	Traits	Field (early planting)				
		Emergence	Seedling vigor	Seedling height	Leaf number plant^{-1}	Seedling dry weight
Growth chamber, Cold (15/13°C day/night))	Germination	0.16	-0.18	-0.01	0.18	0.22*
	Seedling vigor	0.18	0.35**	-0.29**	-0.14	-0.29**
	Seedling height	0.41**	-0.36**	0.30**	0.02	0.26*
	Leaf number plant^{-1}	-0.35**	-0.20	0.04	-0.02	0.04
	Seedling dry weight	0.12	0.13	0.04	-0.18	0.13

*,** Significant at P≤ 0.05, P ≤ 0.01 levels of probability, respectively.

DISCUSSION

Germination, emergence and seedling vigor are the most important traits for early-season cold tolerance. In sorghum, these traits are associated with good stand establishment and increased biomass accumulation early in the season which ultimately leads to increased grain yield. Low soil temperature significantly affects these traits and as a result sorghum planting is often delayed such that it does not fully benefit from late spring moisture and sunlight. The use of early-season cold tolerant sorghum hybrids are thought to address this problem by allowing early planting. In the present study we observed significant genetic variability among sorghum germplasm materials for traits associated with early-season cold tolerance (Table 2.1). This result which is based on relatively broader set of genotypes affirms the findings reported by earlier investigators (Knoll, 2007; Cisse and Ejeta, 2008; Singh, 1985). From the 150 genotypes evaluated for various seedling traits in the field 53, 3, 17 and 30 of them were shown to be superior to the cold tolerant line SQR with respect to emergence, seedling vigor, seedling dry weight and seedling height, respectively (Table 2.2 and Appendix 6). This shows that there is wide opportunity to identify new alternative sources of cold tolerance for use in breeding programs.

Looking further into the field and controlled environment results, one can note that mean emergence percentage was generally lower under field condition compared to the growth chamber though this may not be the case for every genotype. This could be perhaps due to the fact that plants under field conditions are subjected to multiples of factors other than the assigned treatments that may somewhat affect their performance (Tiryaki et al., 2001; Yu et al., 2001). Sezegen and Carena (2009) also indicated that expression of traits

associated with cold tolerance is dependent on environmental conditions. McConnell and Gardener (1979b) failed to observe any improvement in emergence and seedling vigor among maize genotypes under field conditions after repeatedly selecting for improved germination under cold temperature and they attributed this to weather variability in the field. Thus, it is very imperative that screening for early season cold tolerance be conducted under both field and controlled environment conditions in order to have better and consistent results. However, successful screening for cold tolerance under field conditions alone has been reported (Dai et al., 2004; Zenna and Berhe, 2009).

Though the seeds were treated prior to planting, emergence percentage of some of the genotypes including the cold tolerant line SQR was very low under field condition despite its excellent performance in the growth chamber. But few other genotypes such as Ping Ding Xiang, IS12744 and San Er Sui, however, had consistent performance and ranked among the top ten genotypes under both growth chamber and field conditions (Table 2.5). One of these lines, Ping Ding Xiang, has also been reported elsewhere as having excellent germination under low soil temperature (Qingshan and Dahlberg, 2001). On the other hand, many other lines including Da Qing Ye (Yang Qu), Kaoliang-Wx, Bai Nian Gao Liang (Jin Xi), IS12750, and L1999B-13 had higher germination percentages under the controlled conditions but low emergence percentage under early planting in the field. Conversely, other genotypes such as K-385, IS12740, ETS3638, and MN2735 that had high emergence percentage in the field were low in terms of germination in the growth chamber. This appears to be due to differential response of genotypes to factors other than temperature and should be considered when screening genotypes for these traits.

Similar to germination and emergence, variability in seedling vigor among genotypes was remarkable. It is possible that the difference in germination between field and growth chamber conditions might have reflected on the vigor of the seedlings under the two conditions. However, many of the genotypes that had the best seedling vigor under controlled environment condition also had excellent performance in the field though many other lines did not consistently perform under the two conditions. Moreover some of the lines reported to have excellent seedling vigor both in the field and controlled environment such as San Er Sui and IS12744 also had good level of germination under both conditions.

These results are better expressed using the correlation coefficients performed on both the field and growth chamber data. It is clear that for almost all of the traits association between the traits was stronger among the batches evaluated under similar condition, either growth chamber or field (Table 2.6). Only seedling height and seedling vigor from field and growth chamber scores had significant correlation (Table 2.7). Association between the other three traits, emergence, leaf number per plant and seedling dry weight was not significant. This presents challenges to identification and use of potential cold tolerance sources in breeding program. While thorough field screening followed by laboratory confirmation is important, we suggest that decisions on selection of a suitable cold tolerance source should mainly consider the field response of genotypes in the event field and laboratory data fail to agree.

CONCLUSION

The present study indicates that there is genetic variability for early-season cold tolerance among sorghum germplasm. Significant differences for germination percentages, seedling vigor, seedling height and seedling dry weight were observed among the genotypes under both controlled environment and field conditions.

Although not the case for majority of the genotypes, we observed some of the genotypes express both higher germination percentages and seedling vigor than other entries under both controlled cold and field conditions. This indicates that genotypes that combine multiple seedling cold tolerance characteristics can be identified. Genotypes, Ping Ding Xiang, IS12744, San Er Sui and Da Qing Ye (Yang Qu) are some of the top genotypes that combine multiple cold tolerance characteristics.

In contrast, genotypes, IS27935, IS12741, IS12740 and Kei ko she jen king expressed high seedling vigor but had low germination percentages. While tannin content has been implicated as one of the factors affecting germination and seedling vigor, the likely causes for improved germination and low vigor in some genotypes and low germination and high vigor in others may need further investigation.

While genotypes that consistently express seedling cold tolerance characteristics under both field and controlled environment condition are the ideal sources, those may not be widely available given the low correlation between field and controlled environment performance of genotypes. In such cases we suggest that while controlled environment data is valuable, selection for the best sources should largely be based on field response of genotypes.

REFERENCES

Acevedo, E., P.O. Crawfurd, R.B.Austin, and P. Perez-Marco. 1991. Traits associated with high yield in barley in low-rainfall environments. J.Agric. Sci.116:23-36.

Alegre De La Soujeole, A., and F. Miller. 1984. Cold tolerance of sorghum of sorghum during early developmental stages. p.18-32. Proc. Annu. Corn and Sorghum Res. Conf., 39th , Chicago, IL.5-6 Dec.1984. Am. Seed Trade Assoc., Washington, DC.

Ali, M.G., R.E.L. Naylor, and S.Mathews. 2006. Distinguishing the effects of genotype and seed physiological age on low temperature tolerance of rice (*Oryza sativa L.*), Exp. Agric. 42:337-349.

Allen, D.J., and D.R.Ort. 2001. Impacts of chilling temperatures on photosynthesis in warm climate plants. Trends Plant Sci. 6:36-42.

Anda, A., and L. Pinter.1994.Sorghum Germination and Development as Influenced by Soil Temperature and Water content. Agron.J. 86: 621-624.

Andaya, V.C., D.J. Mackill.2003. Mapping of QTLs associated with cold tolerance during the vegetative rice. J.Exp. Bot.54:2579-2585.

Aroca, R., F. Tognoni, J.J.Irigoyen, M. Sanchez-Diaz, A. Pardossi. 2001.Difference in root low temperature response of two maize genotypes differing in chilling sensitivity. Plant Physiol. Biochem. 39: 1067-1075.

Basnayake, J., V. Sihathep, Sipaseuth, P.Sonekham, M.Senthonghae, V.Sibounheuang, V. Phamixay, Sengkeo, M. Chanphengxay, and S. Fukai. 2003. Effects of time of planting on agronomic and yield performance of several rice cultivars under various temperature conditions in Lao PDR, Proc. 11thAgron. Conf. Melbourne, 1February-6 February. 2003. URL: *http\\www.regional.org.au/au/asa/2003/*

Bechoux, N., G. Bernier, and P. Lejeune. 2000. Environmental effects on the early stages of tassel morphogenesis in maize (*Zea mays* L.). Plant Cell Environ. 23:91–98.

Board, J.E., M.L.Peterson, E.Ng. 1980. Floret sterility in rice in a cool environment. Agron. J. 72: 483-487.

Bradbury, M., N.R. Baker.1983. Analysis of the induction of chlorophyll fluorescence in intact leaves and isolated thylakoids: contributions of photochemical and non-photochemical quenching. Proc. Royal Soc. London B 220: 251-264.

Brar, G.S., and B.A.Stewart.1994.Germination under controlled temperature and field emergence of 13 sorghum cultivars. Crop Sci.34:1336-1340.

Brouwer, R., A. Kleinendorst, and J.T. Locher.1973. Growth responses of maize plants to temperature. In: R.O.Slatyer (Editor), Plant responses to climatic factors. UNESCO, Paris, p.169-173.

Cisse, N., and G. Ejeta 2003. Genetic Variation and Relationships among Seedling Vigor Traits in Sorghum. Crop Sci. 43:824-828.

Clarke, H.J., K.H.M.Siddique. 2004. Response of chickpea to low temperature stress during reproductive development. Field Crops Res. 90: 323-334.

Condon, .A. G., R. A. Richards, and G. D. Farquhar. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field grown wheat. Crop Sci. 27:996-1001.

Cooper, P. J. M., P. J. Gregory, J. D. H. Keatings, and S. C. Brown. 1987. Effects of fertilizer, variety, and location on barley production under rainfed conditions in Northern Syria. II Soi1 water dynamics and crop water use. Field Crops Res. 16:67-84.

Cruz, R.P.,S.C.K., Milach, L.C. Federizzi.2006. Inheritance of rice cold tolerance at the germination stage.Genet.Mol.Biol.29:314-320.

Dai, L.Y., X.H.Lin,C.R.Ye,K.Ise, K.Saito, A.Kato, F.R.Xu, T.Q.Yu and D.P.Zhang.2004. Identification of quantitative trait loci controlling cold tolerance at the reproductive stage in Yunnan landrace of rice, Kunmingxiaobaigu.Breed.Sci.54:253-258.

Doggett, H. 1970. Sorghum. Longman Green & Co. Ltd., London.

Downes, R. W., and D.R. Marshall.1971. Low temperature induced male sterility in *Sorghum bicolor*.Aust. J. Exp. Anim.Husb. 11: 352-356.

Engels, C. 1994. Nutrient acquisition by plants and its limitations by low temperatures in maize. In: Dorffling, K., B.Brettschneider, H. Tantau, K. Pithan (eds) Crop adaptation to cool climates. COST 814 workshop. ECSP-EEC-EAEC, Brussels, pp 503-510.

Engels, C., and H. Marschner.1990. Effect of sub-optimal root zone temperatures on at varied nutrient supply and shoot meristem temperature on growth and nutrient concentrations in maize seedlings (*Zea mays L.*). Plant Soil. 126:215-225.

FAO. 2004. Food and Agriculture Organization of the United Nations, Statistics Division: *http:// www.fao.org/es/ess/index_en.asp*.

Farell et al., T.C. Farrell, K.M.Fox, R.L. Williams, S. Fukai, R.F. Reinke, and L.G. Lewin. 2001. Temperature constraints to rice production in Australia and Lao : a shared problem in: S.Fukai., and Basnayake,Editors, ACIAR Proc. 101; Increased lowland rice production in the Mekong Region, Australian centre for Int. Agric. Res., GPO Box 1571, Canberra, ACT 2601:129-137.

Fennell, A., A.H. Markhart.1998.Rapid acclimation of root hydraulic conductivity to low

temperature. J.Exp. Bot.49:879-884.

Forbes, G.A., O.Ziv, R.A. Frederiksen.1987. Resistance in sorghum to seedling disease caused by *Pythium arrhenomanes*. Plant Disease.71: 145-148.

Fracheboud, Y., P. Haldimann, J. Leipner, and P.Stamp. 1999. Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). J. Exp. Bot.50: 1533-1540.

Franks, C.D., G.B. Burrow, and J.J. Burke. 2006. A comparison of U.S. and Chinese sorghum germination for early season cold tolerance. Crop Sci. 46: 1371-1376.

Fryer, M.J., J.R. Andrews, K. Oxborough, D.A. Blowers, and N.R. Baker. 1998. Relationship between CO_2 assimilation, photosynthetic electron transport, and active O_2 metabolism in leaves of maize in the field during periods of low temperature. Plant Physiol.116: 571-580.

Gardner, C.O., M.A. Thomas-Campton, T.L.Gocken, and K.D.Eichelberger.1987. Selection for cold and freeze tolerance in corn: Evaluation of original and selected populations.p.126-140.Proc. Annu. Corn and Sorghum Res. Conf., 42nd, Chicago, IL.10-11 Dec.1987. Am. Seed Trade Assoc., Washington, DC.

Gisele de Oliveira, S., T.T.Berchielli, M.S.Pedreira, O. Primavesi, R. Frighetto and M.A. Lima.2007. Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. Anim. Feed Sci.Technol. 135:236-248.

Gomez, L.D., H.Vanacker, P.Buchner, G.Noctor, and C.H. Foyer. 2004. Intercellular distribution of glutathione synthesis in maize leaves and its response to short-term chilling. Plant physiol. 134:1662-1671.

Greaves, J.A.1996.Improving suboptimal temperature tolerance in maize. The search for variance. J.Exp. Bot.47:307-323.

Gregory, **P. J.,** D. Tennant, and R. R. Belford. 1992. Root and shoot growth, and water and light use efficiency of barley and wheat crops grown on a shallow duplex soi1 in a mediterranean type environment. Aust. J. Agri. Res. 43 :555-573.

Gunawardena, T.A., S.Fukai, and P.Blamey. 2003a. Low temperature induced spikelet sterility in rice. I. Nitrogen fertilization and sensitive reproductive period. Aust. J. Agri. Res.54: 937-946.

Gunawardena, T. A., S.Fukai, and P.Blamey. 2003b. Low temperature induced spikelet sterility in rice.II. Effects of panicle and root temperature, Aust. J. Agric. Res. 54:947- 956.

Haldimann, P.1998. Low growth temperature-induced changes to pigment composition photosynthesis in *Zea mays* genotypes differing in chilling sensitivity. Plant, Cell and Environ. 21: 200-208.

Haldimann, P., Y. Fracheboud, and P. Stamp. 1995. Carotenoid composition in *Zea mays* developed at suboptimal temperature and different light intensities. Physiol. Plantarum 95: 409-414.

Haldimann, P., Y. Fracheboud, and P. Stamp. 1996. Photosynthetic performance and resistance to photoinhibition of Zea *mays*. L. leaves grown at suboptimal temperature. Plant, Cell and Environ. 19: 85-92.

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

Hodgins, R., and R.V. Huystee.1986.Porphyrin metabolism in chill stressed maize (*Zea mays* L.). J. Plant Physiol. 25: 326-336.

Keim, K.R.,and C.O.Gardner.1984. Genetic variation for cold tolerance in selected and unselected maize populations. Field Crops Res.8:143-151.

Kanemasu, E. T., D. L. Bark, and E. C.Choy. 1975. Effect of soil temperature on sorghum emergence. Plant Soil. 43:411-417.

Khan, D.R., D.J. Mackill, and B.S. Vergara. 1986. Selection for tolerance to low temperature induced spikelet at anthesis in rice. Crop Sci. 26:694-698.

Knoll, J., and G.Ejeta. 2008. Marker-assisted selection for early–season cold tolerance in sorghum: QTL validation across populations and environments. Theor. Appl. Genet. 116:541-53.

Knoll, J., N. Gunaratna, and G. Ejeta. 2008. QTL analysis of early-season cold tolerance in sorghum. Theor. Appl. Genet. 116:577-87.

Ku, S.B., G.E. Edwards, and D. Smith. 1978. Photosynthesis and nonstructural carbohydrate concentration in leaf blades of *Panicum virgatum* as affected by night temperature. Can. J. Bot. 56:63-68.

Lawlor, H.J., Siddique, K.H.M., R.H. Sedgley, N. Thurling.1997. Improving cold tolerance and insect resistance in chickpea and the use of AFLPs for the identification of molecular markers for these traits. Acta Hort.461:185-190.

Lee, E. A., M.A.Staebler, and M. Tollenaar.2002. Genetic variation in physiological discriminators for cold tolerance-early autotrophic phase of maize development. Crop Sci.42: 1919-1929.

Lee, M.H. 2001. Low temperature tolerance in rice: the Korean experience. In: Fukai S.

and J. Basnayake (eds), ACIAR Proc. 101; Increased lowland rice production in the Mekong Region, Australian Centre for Int. Agric. Res., GPO Box 571,Canberra, p.138-146.

Lopez-Castaneda, C., R. A. Richards, and G. D. Farquhar. 1995. Variation in early vigor between wheat and barley. Crop Sci. 35:472-479.

Ludlow, M. M. and R. C, Muchow. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. in Agron. 43 : 107- 153.

Lyons, J.M. 1973. Chilling stress in plants. Annu. Rev. Plant Physiol. 24: 445-446.

Maiti, R.K. 1996. Germination and seedling establishment. p.41-98.In R.K. Maiti (ed.) Sorghum science. Science Publishers, Inc., Lebanon, NH.

Maiti, R.K., P.S. Raju, and F.R. Bidinger. 1981. Evaluation of visual scoring for seedling vigor in sorghum.Seed Sci. Technol.9: 613-622.

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

Makkar, H.P.S., 2003 .Effect and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds, Small Rumin. Res. 49: 241–256.

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

McConnell, R.L. C.O.Gardner.1979.Selection of cold germination in two corn populations. Crop Sci.19:765-768.

Mendoza-Onofre, L., J.D. Eastern, C.Y.Sullivan, and W.M.Ross.1979. Relations between

laboratory and field observations of sorghum seedlings grown under cold temperature. p.137-145. In Development of improved high-yielding sorghum cultivars.Ann.Rep. No.9.Univ.of Nebraska, ARS, USDA, Lincoln, NE.

Menkir, A., and E.N.Larter.1985. Growth-responses of early maturing inbred lines of corn to suboptimal temperatures. Can. J. Plant Sci. 65:79-86.

Mock, J.J., A.A. Bakri. 1976. Recurrent selection for cold tolerance in maize Crop Sci. 16: 230-415.

Nahar, K., J. K. Biawas, A.M.M. Shamsuzzaman, M. Hasanuzzaman, and H.N. Barman. 2009. Screening of indica rice (*Oryza sativa* L.) Genotypes against low temperature stress. Botany Res. Int. 2:295-303.

Nie, G.Y., S.P. Long, and N.R. Baker. 1992. The effects of development at suboptimal growth temperatures on photosynthetic capacity and susceptibility to chilling–dependent photoinhibition in *Zea mays*. Physiol. Plantarum 85: 554-560.

Nilanyana, T.R.,I.R.P.Singh, N.K.Singh.2003. Evaluation of germination, cold tolerance, and seedling vigor of boro rice germination. Int. Rice Res. Notes 28:19-20.

Nishiyama, I.1995. Damage due to extreme temperatures. In: T. Matsuo, K. Kumazawa, R. Ishii, H. Ishihara and H. Hirata, Editors, Science of the Rice Plant, Food and Agric. Policy Res. Center, Tokyo, Japan p. 769–812.

Oliver, S.N., J.T.V. Dongen, and S.C. Alfred. 2005. Cold-induced repression of rice anther-specific cell wall invertase gene OSINV4 is correlated with sucrose accumulation and pollen sterility. Plant cell and environ. 28: 1534-1551.

Peacock, J.M. 1982. Response and tolerance of sorghum to temperature stress. p.

143-160. In Sorghum in the eighties. Proc.Int. Symp. On Sorghum, Patancheru, AP, India. 2-7 Nov.1981. ICRISAT, Patancheru, AP, India.

Pereira da Cruz, R.,S.C.K. Milach, and L.C. Federizzi. 2006. Rice cold tolerance at the reproductive stage in a controlled environment, Sci. Agric. 63: 255–261.

Pereira da Cruz, R., and S.C.K. Milach. 2004. Cold tolerance at the germination stage of rice : Methods of evaluation and characterization of genotypes. Sci. Agric. 61: 1-8

Pinthus , M.J., and J. Rosenblum.1961. Germination and seedling emergence of sorghum at low temperatures. Crop Sci. 1:293-296.

Pramod, K., and M.Vinay.2007. Effect of low temperature stress on photosynthesis, total soluble sugars, grain filling rate and yield in rice (*Oryza sativa* L.). Indian J. Plant Physiol.12: 253-260.

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

Qingshan, L., and J.A. Dahlberg. 2001. Chinese sorghum genetic resources.Econ.Bot.55(3): 401-425.

Quinby, J.R., J.D. Hesketh, and R.L. Voigt. 1973. Influence of temperature and photoperiod on floral initiation and leaf number in sorghum. Crop Sci. 13:243–246.

Radford, B. J.,and R. G. Henzell. 1990. Temperature affects the mesocotyl and coleoptile length of grain sorghum genotypes. Aust. J. Agric. Res. 41:79-87.

Regan, K.L.,K.H.M. Siddique, N.C.Turner, and B.R. Whan.1992.Potential for increasing early vigor and total biomass in spring wheat.2.Characteristics associated with early vigor. Aust.J.Agric. Res. 43:541-553.

Revilla, P., A.Butron, M.E. Cartea, R.A.Malvar, and A.Ordas.2005. Breeding for cold tolerance. In M.Ashraf and P.J.C. Harris (ed.) Abiotic stresses: Plant resistance through breeding and molecular approaches. Haworth Press, New York.

Revilla, P., J.R. Hotchkiss, and W.F. Tracy.2003.Cold tolerance evaluation in a diallel among open-pollinated sweet corn cultivars. Hort.Sci.38:88-91.

Revilla, P.,A.Butron, R.A.Malvar, and A. Ordas. 1999. Relationships among kernel weight, early vigor, and growth in maize. Crop Sci.39:654-658.

Revilla, P., R.A.Malvar, M.E. Cartea, A. Butron, and A.Ordas.2000.Inheritance of cold tolerance at emergence and during early season growth in maize. Crop Sci. 40:1579-1585.

Robertson, E.J., N.R. Baker, and R.M. Leech. 1993. Chloroplast thylakoid protein changes induced by low growth temperature in maize revealed by immunocytology. Plant, Cell and Environ. 16: 809-818.

Rodriquez, V.M., A.Butron, G.Sandoya, A. Ordas and P.Revilla. 2007. Combining Maize Base Germplasm for Cold Tolerance Breeding.Crop Sci.47: 1467-1474.

Rymen, B., F. Fabio, Fatma, V. Klaas, I. Dirk, Gerritt, and T.S.Beemster. 2007. Cold nights impair leaf growth and cell cycle progression in maize through transcriptional changes of cell cycle genes. Plant physiol.143:1429-1439.
Exp. Bot. 67: 429-443.

Sally, L. D., F.M. Shapter, R.J. Henry, G. Cordeiro, L. Izquierdo, and L.S. Lee. 2007. Domestication to Crop Improvement: Genetic Resources for Sorghum and Saccharum (Andropogoneae). Ann Bot. 100(5): 975-989.

SAS User's guide., 1989. Statistics. SAS Institute, Cary, NC.

Satake, T., H. Hayase. 1970. Male sterility caused by cooling treatment at the young microspore stage in rice plants.V: Estimations of pollen developmental stage and the most sensitive stage to coolness. Proc.Crop Sci.Jpn. 39: 468-473.

Satya, P., and A.Saha.2010. Screening for low-temperature stress tolerance in boro rice. Int. Rice Res. Notes.

Savithri, K.S ., P.S., Ganapathy, S.K., Sinha.1980. Sensitivity to Low Temperature in Pollen Germination and Fruit-Set in *Cicer arietinum* L.J.Exp.Bot.31:475-481.

Schoper, J. B., R. J. Lambert, and B. L. Vasilas. 1987. Pollen viability,pollen shedding and combining ability for tassel heat tolerance in maize. Crop Sci. 27: 27-31.

Sezegen, B., and M. J.Carena. 2009. Divergent recurrent selection for cold tolerance in two improved maize populations. Euphytica. 167: 237-244.

Shimono et al., H. Shimono, T.Hasegawa, S. Fujimura, and K. Iwama.2004. Responses of leaf photosynthesis and plant water status in rice to low water temperature at different growth stages. Field Crops Res. 89:7183.

Shimono, H.Okada, E.Kanda, and I.Arakawa. 2007. Low temperature induced sterility in rice: evidence for the effects of temperature before panicle initiation. Field Crops Res. 101: 221-231.

Siddique, K.H.M., R.H.Sedgley.1986. Chickpea (*Cicer arietinum* L.), a potential grain legume for south-western Australia: seasonal growth and yield. Aust. J.Agric. Res.37: 245-261.

Singh, S. 1985. Sources of cold tolerance in grain sorghum. Can. J. Plant Sci. 65:251-257.

Sipaseuth, J., S.Basnayake, T.C. Fukai, M.Farrell, Senthonghae, S. Sengkeo, B.

Phamixay, Linguist and M.Chanphengsay.2007. Opportunities to increasing dry season rice productivity in low temperature affected areas. Field Crops Res. 102 (2):87-97.

Silanikove, N., A. Perevolotsky, F.D. Provenza. 2001. Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. Anim. Feed Sci. Technol. 91: 69–81.

Smillie, R.M., R.Nott, S.E.Hetherington and G. Oquist. 1987. Chilling injury and recovery in detached and attached leaves measured by chlorophyll fluorescence. Physiol. Plantarum 69: 419-428.

Srinivasan, A.,C. Johansen, N.P. Saxena.1998. Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): characterization of stress and genotypic variation in pod set. Field Crops Res. 60: 209-222.

Stack, J. 2000. Sorghum Ergot in the Northern Great Plains.

Stamp, P.1984. Chilling tolerance of young plants demonstrated on the example of maize (*Zea mays* L.). J.Agron. and Crop Sci.7: 1-83.

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

Stone, P.J., I.B. Sorensen, and P.D. Jamieson. 1999. Effect of soil temperature on phenology, canopy development, biomass and yield of maize in a cool temperature climate. Field Crops Res. 63:169-178.

Subedi, K.D., P.J. Gregory, R.J. Summerfield, and M.J. Gooding.1998. Cold temperatures and boron deficiency caused grain set failure in spring wheat (*Triticum aestivum* L.). Field Crops Res. 57**:** 277–288.

Tiryaki, I., and D. J. Andrews. 2001. Germination and seedling cold tolerance in sorghum: I. Evaluation of rapid screening methods. Agron. J.93:1386-1391.

Tiryaki, I., and D.J. Andrews.2001. Germination and seedling cold tolerance in sorghum: II. Parental lines and hybrids. Agron. J. 93: 1391-1397.

Thakur, P., S. Kumar, J.A. Malik, J. D. Berger, and H. Nayyar.2010. Cold stress effects on reproductive development in grain crops: Environ. and Exp. Bot. 67:429-443.

Verheul, M.J., C. Picatto, and P. Stamp.1996. Growth and development of maize (*Zea mays L*.) seedlings under chilling conditions in the field. Eur. J. Agron.5: 31-43.

Whan, B. R., G. P. Carlton, and W. K. Anderson. 1991. Potential for increasing early vigor and total biomass in spring wheat. 1. Identification of genetic improvements. Aust. J. Agric. Res. 42:343-361.

Wise, R.R., and A.W.Naylor.1987. Chilling enhanced photooxidation. Plant Physiol.83:278-282.

Woodward, S.L., G.C. Waghorn, M.J. Ulyatt and K.R. Lassey.2001. Early indications that feeding lotus will reduce methane emissions from ruminants. Proc. of the The New Zealand Soc. Anim. Prod., ACIAR, Adelaide pp. 23–26.

Yang, J.C., J.H.Zhang. 2006.Grain filling of cereals under soil drying. N. Phytol.169:223-236.

Yan, S.P., Q.Y.Zhang, Z.C.Tang, W.A.Su, W.N.Sun.2006. Comparative proteomic analysis provides new insights into chilling stress responses in rice. Mol. Cell. Proteom. 5:484-496.

Ying, J., E.A.Lee, M. Tollenaar. 2002. Response of leaf photosynthesis during the grain-

filling period of maize to duration of cold exposure, acclimation, and incident PPFD. Crop Sci.42: 1164-1172.

Yoshida, S., 1981. Fundamentals of Rice Crop Science. Intl. Rice Res. Inst. Los Banos, Laguna, Filipinas.

Yu, J., M.R. Tuinstra, M.Classesn, W.B.Gorgon, and M.D. Witt .2001. Genetic analysis of seedling growth under cold temperature stress in grain sorghum. Crop Sci. 41: 1438-1443.

Yu, J., M.R. Tuinstra, M.M. Claassen, W.B.Gordon, and M.D.Witt.2001.Analysis of cold tolerance in sorghum under controlled environments. Field Crops Res.85:21-30.

Zenna, N., and T. Berhe. 2009. Cold tolerant rice evaluation in Ethiopian highlands. African Crop Sci. Proc. 9: 309-311.

Zinn, K.E., M.T.Ozdemir, and J.F. Harper. 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. J. Exp. Bot. 61: 1959-1968.

Appendix 1. Mean number of leaves and plant height of sorghum genotypes as affected by early season cold temperature stress.

Genotype	LNO1		LNO2		LNO3		LNO4		Plant height (cm)	
	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal	Cold	Normal
Shan Qui Red	3	3	5	6	9	10	14	15	161.25	168.17
SRN39	2	3	4	6	9	9	13	14	111	105.53
Pioneer 84G62	3	4	5	7	9	11	15	16	119	123.33
Mean	2.5	3.4	4.6	6.2	8.8	10.7	14.3	14.8	130.42	132.34
LSD	0.36		0.36		0.51		NS		NS	
†LSD	0.76		0.76		NS		1.31		16.12	
‡LSD	0.76		0.76		0.93		1.19		60.57	

LNO1 = mean leaf number soon after cold stress; LNO2 = mean leaf number 2weeks after cold stress; LNO3= mean leaf number 4 weeks after cold stress; LNO4 = final mean leaf number per plant; † LSD for genotype means under cold temperature; ‡ LSD for genotype means under normal temperature, and NS = Nonsignificant.

Appendix 2. List of sorghum genotypes with their respective country of origin used in this study.

Genotype	Designation	Origin
Brown Kaoliang	PI23231	China
Japanese Dwarf Broomcorn	PI30204	United States
Hemaise	PI55123	Sudan
White Kaoliang	PI63923	China
Kei ko she jen hing	PI68003	China
Lo leung mai	PI71309	China
North West Gold Kaoliang	PI76407	China
She-jen (snake eye)	PI76409	China
Migna	PI81216	Yemen
Kaoliang-Wx	PI82335	Korea
Bomususu	PI87355	Korea
Mokutakususu	PI88000	Korea
Susu zairai shu	PI88004	Korea
Kaoliang	PI90267	Korea
Red Kaoliang	PI90769	China
IS 12740	PI92260	China
IS 12741	PI92261	China
MN 2735	PI92263	China

Genotype	Designation	Origin
IS 12744	PI92264	China
Dwarf Yellow Milo	PI92267	China
Brown Kaoliang	PI92268	China
MN 2740	PI92270	China
IS 12749	PI92271	China
IS 12750	PI92272	China
Katengu	PI192876	Indonesia
Nai- Shaker	PI220636	Afghanistan
IS 1024	PI246699	India
MN 4116	PI250230	Pakistan
IS 13238	PI266962	China
K-11	PI267105	Former SU
K-64/II	PI267106	Former SU
K-637/II	PI267109	Former SU
K-819	PI267112	Former SU
K-892	PI267113	Africa
K-34	PI267115	Former SU.
K-540	PI267117	Former SU
K-47	PI267120	Former SU
K-357	PI267126	Former SU

Genotype	Designation	Origin
K-24	PI267127	Former SU
K-403	PI267129	China
ETS 3633	PI455541	Ethiopia
Big Yellow Umbrella	PI511832	China
Ping Ding Xiang	PI542739	China
Jilin Hei Long Jiang- 22	PI542764	China
Bai Ruan Gao Liang	PI547915	China
Bai Li Gao Liang	PI547919	China
Da Luo Chui	PI547928	China
Dao Zai Tou	PI547991	China
Huang Luo Mian	PI548014	China
Nian Gao Liang	PI548029	China
Durra Belaya	PI550610	Syria
Tunis grain	PI562729	United States
IS 2033	PI562749	United States
IS 2212	PI562755	United States
IS 2216	PI562756	United States
Purdue 81659-2	PI562769	United States
IS 9145	PI563203	United States
Culum Abiad	PI563234	Uganda

Genotype	Designation	Origin
IS 10497	PI563402	United States
IS 10505	PI563404	United States
IS 10731	PI563433	United States
LV 129	PI563576	-
LR 423	PI563632	China
LR 427	PI563634	China
LR 431-1	PI563634	China
LR 431-2	PI563638	China
LR 431-1	PI563639	China
L 1097B	PI563643	China
L 1603B	PI563650	China
L1791B	PI563656	China
L 1985B	PI563657	China
L 1999B-11	PI563666	China
L 1999B-13	PI563667	China
L 1999B-14	PI563668	China
LR 2410	PI563673	China
LR 2412-2	PI563675	China
LR 2417(a)	PI563676	China
LR 2433	PI563686	China

Genotype	Designation	Origin
LR 2462-2	PI563689	China
LR 2463	PI563690	China
LR 2470-1	PI563692	China
LR 2480-2	PI563698	China
LR 2483-1	PI563699	China
LR 2483-2	PI563700	China
LR 2490-1	PI563701	China
LR 2490-2	PI563702	China
LR 2490-3	PI563703	China
LR 2505	PI563705	China
LR 2556-1	PI563725	China
LR 2556-2	PI563726	China
LR 2572	PI563727	China
LR 2820	PI563800	-
Danyang Local	PI567795	South Korea
Pyungchang Local	PI567797	South Korea
Bai Nian Gao Liang (Jin Xi)	PI567911	China.
Bai She Yan (Sui Zhong)	PI567911	China
Da Qing Ye (Yang Qu)	PI567929	China
Da Guan Dong	PI567974	China

Genotype	Designation	Origin
Bai Ri Hong	PI568044	China
San Sui Jiao Zi	PI568047	China
FS73015-D001	PI574605	United States
IS 24666	PI585372	Lebanon
IS 24692	PI585378	India
IS 27667	PI586404	Cameroon
Blackhull Kafir	PI586445	Hungary
Cody	PI586448	Hungary
Framida	PI586451	Hungary
Leoti	PI586454	Hungary
IS 27929	PI586524	China
IS 27931	PI586526	China
IS 27935	PI586529	China
IS 27938	PI586532	China
MP 346	PI601918	United States.
San Er Sui	PI610730	China
Jiao Zi	PI610743	China

Appendix 3**.** The daily maximum and minimum soil temperatures during early planting in the field experiment at Manhattan, 2010.

Early planting				
	2 inches		4 inches	
Date	Max. soil temperature (^{o}C)	Min. soil temperature (^{o}C)	Max. soil temperature (^{o}C)	Min.soil temperature (^{o}C)
4/13/2010	20.1	13.4	19.5	13.8
4/14/2010	23.0	13.9	21.0	14.0
4/15/2010	20.5	14.0	20.3	14.4
4/16/2010	17.1	12.2	17.0	12.5
4/17/2010	18.0	9.9	18.0	10.1
4/18/2010	16.6	9.7	16.5	9.7
4/19/2010	20.3	10.9	20.3	11.8
4/20/2010	21.0	10.9	18.8	11.0
4/21/2010	20.8	13.7	20.9	14.0
4/22/2010	14.9	11.9	15.0	12.9
4/23/2010	20.3	14.4	20.2	14.2
4/24/2010	20.9	13.7	19.0	13.9
4/25/2010	16.0	12.7	15.9	13.0
4/26/2010	15.7	11.6	15.6	11.7
4/27/2010	20.1	10.8	16.9	11.8
4/28/2010	18.6	10.6	18.0	12.0

Early planting				
	2 inches		4 inches	
Date	Max. soil temperature (^oC)	Min. soil temperature (^oC)	Max. soil temperature (^oC)	Min. soil temperature (^oC)
4/29/2010	21.4	13.8	21.3	13.9
4/30/2010	21.3	14.9	21.0	14.9
5/1/2010	21.2	13.3	21.0	13.4
5/2/2010	22.4	12.7	22.0	13.2
5/3/2010	23.7	14.3	22.9	15.1
5/4/2010	23.6	14.7	22.4	14.8
5/5/2010	24.4	14.4	23.4	15.4
5/6/2010	20.6	13.5	20.1	14.7
5/7/2010	20.5	12.9	20.1	14.8
5/8/2010	19.7	8.8	19.5	10.7
5/9/2010	17.5	12.3	16.9	12.4
5/10/2010	13.6	10.2	14.1	11.7
5/11/2010	19.1	12.1	18.7	12.6
5/12/2010	16.2	13.9	16.2	14.2

Appendix 4. The daily maximum and minimum soil temperatures during normal planting in the field experiment at Manhattan, 2010.

Normal planting				
	2 inches		4 inches	
Date	Max. soil temperature (^oC)	Min.soil temperature (^oC)	Max. soil temperature (^oC)	Min. soil temperature (^oC)
5/26/2010	30.0	20.4	29.8	20.6
5/27/2010	31.0	20.4	31.0	20.9
5/28/2010	31.6	17.8	31.3	20.6
5/29/2010	32.4	18.8	32.2	20.5
5/30/2010	30.6	20.3	30.1	21.4
5/31/2010	30.6	18.8	29.8	19.8
6/1/2010	32.1	21.9	31.5	22.5
6/2/2010	29.4	20.3	29.4	21.5
6/3/2010	33.0	20.3	29.4	21.5
6/3/2010	33.1	21.7	32.9	22.2
6/4/2010	32.1	23.9	30.8	24.0
6/5/2010	33.3	20.8	33.0	21.5
6/6/2010	26.2	22.5	25.4	23.2
6/7/2010	27.6	21.7	27.1	21.7

Normal planting				
	2 inches		4 inches	
Date	Max. soil temperature (^{o}C)	Min. soil temperature (^{o}C)	Max. soil temperature (^{o}C)	Min. soil temperature (^{o}C)
6/8/2010	30.7	22.2	30.6	22.3
6/9/2010	30.5	22.5	29.4	22.5
6/10/2010	31.0	23.8	30.7	23.9
6/11/2010	29.2	23.2	28.7	23.3
6/12/2010	29.8	22.3	29.8	22.4
6/13/2010	26.0	21.9	25.9	21.6
6/14/2010	29.8	22.3	29.5	21.6
6/15/2010	30.9	22.2	30.4	23.0
6/16/2010	31.8	22.2	30.8	22.8
6/17/2010	32.3	24.3	32.3	24.4
6/18/2010	30.5	24.7	30.0	24.7
6/19/2010	30.9	22.1	30.7	22.9
6/20/2010	31.6	23.3	31.3	23.4
6/21/2010	33.3	23.3	33.1	24.7
6/22/2010	32.5	24.6	32.3	25.3
6/23/2010	34.0	23.2	33.7	23.3

Appendix 5. Mean germination percentages of 26 sorghum genotypes at three time intervals evaluated under controlled cold conditions.

	Laboratory (15^{o}C)		
	Germination (%)		
Genotypes	8 DAP	10 DAP	14DAP
Da Quing Ye (Yang Qu)	47.11	79.11	87.11
Bai Nian Gao Liang (Jin Xi)	36.00	54.67	63.56
Ping Ding Xiang	30.22	61.33	78.22
Kaoliang-Wx	25.78	63.56	79.56
PI563943	24.44	27.11	36.00
IS 27935	23.11	38.22	43.56
San Er Sui	22.22	42.22	58.22
IS 12750	20.44	52.00	60.89
K-385	16.89	27.56	34.67
L1999B-13	16.89	44.44	60.00
K-517	11.11	19.11	33.78
Susu zairai shu	9.33	32.00	49.78
ETS 3638	8.00	18.22	32.00
IS 12744	7.56	38.67	71.56
MN 2735	3.56	8.00	28.00
IS 12741	3.36	22.22	42.22
Dao Zai Tou	3.11	14.67	42.22
San chi san	2.67	24.00	51.11
Kei ko she jen hing	2.22	6.22	14.67
IS 27929	1.78	4.89	13.78
Durra Buleya	1.33	5.33	14.22
IS 12740	0.00	5.78	31.56
Japanese dwarf broomcorn	0.00	2.67	21.33
Da Guan Dong	0.00	0.00	7.11
Shan Qui Red	31.33	40.44	69.11
SRN39	1.78	1.78	1.78
Mean	13.48	28.24	43.31
LSD	13.27	16.76	20.05

Appendix 6. Mean cold tolerance related traits of sorghum genotypes evaluated under early planting in the field conditions at Manhattan, 2010.

Genotypes	Emergence (%)	Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}	Seedling dry weight (g)
San chi san	33	2.7	10.8	4	0.59
Bai She Yan (Sui Zhong)	29	2.7	10.6	5	0.44
Bai Nian Gao Liang (Jin Xi)	42	2.7	12.5	5	0.57
Da Luo Chui	36	2.7	11.9	4	0.46
IS 12750	41	2.7	10.4	4	0.40
PI92268	47	3.0	13.0	4	0.43
Da Qing Ye (Yang Qu)	35	3.0	12.5	4	0.62
MN 2740	39	3.0	11.2	4	0.39
Jiao Zi	35	3.0	10.7	5	0.40
Pyungchang Local	24	3.3	12.1	4	0.34
K-357	33	3.3	10.5	4	0.48
Da Guan Dong	25	3.3	12.4	4	0.44
IS 12741	43	3.3	11.4	5	0.38
PI 607409	29	3.3	12.1	4	0.39
L 1999B-13	11	3.3	9.9	4	0.39
K-11	23	3.3	11.1	5	0.71

Genotypes	Emergence (%)	Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}	Seedling dry weight (g)
San Er Sui	54	1.0	14.4	5	0.62
IS 12740	55	1.0	14.0	5	0.62
IS 12744	53	1.0	14.0	4	0.58
IS 27935	37	2.0	13.1	5	0.67
K-385	57	2.0	14.7	4	0.51
IS 13238	42	2.0	12.2	4	0.50
Bai Ri Hong	29	2.3	12.4	4	0.46
San Sui Jiao Zi	34	2.3	12.3	5	0.43
PI563943	42	2.3	13.8	4	0.48
Culum Abiad	43	2.3	12.5	4	0.48
IS 27929	33	2.3	12.4	4	0.43
Dao Zai Tou	35	2.3	12.2	5	0.43
MN 2735	51	2.7	14.0	5	0.51
K-517	35	2.7	12.4	5	0.56
Durra Belaya	45	2.7	11.9	4	0.38
ETS3638	54	2.7	11.5	4	0.34

Genotypes	Emergence (%)	Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}
Kei ko she jen hing	41	3.3	12.8	4
Susu zairai shu	38	3.3	9.8	5
Ping Ding Xiang	45	3.3	12.8	4
Japanese dwarf Broomcorn	33	3.3	12.6	5
Kaoliang-Wx	33	3.3	9.8	5
K-64/II	47	3.7	10.9	5
North West Gold Kaoliang	25	3.7	10.4	4
Red Kaoliang	39	3.7	11.4	4
Mokutakususu	41	3.7	10.7	5
Jilin Hei Long Jiang-22	25	3.7	12.5	4
SDS 1412	15	3.7	8.7	4
Brown Kaoliang	39	3.7	11.2	4
IS 4225	12	3.7	12.3	4
IS 2216	17	3.7	9.6	4
She-jen (snake eye)	24	3.7	12.3	4
MP 346	7	3.7	12.1	4
Bai Li Gao Liang	39	3.7	11.4	4

Genotypes	Emergence (%)	Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}	Seedling dry weight (g)
Kaoliang	24	4.0	11	4	0.35
White Kaoliang	23	4.0	10.6	4	0.23
LV 129	35	4.0	11.3	4	0.30
Tx378	23	4.0	9.3	4	0.37
PI550666	19	4.0	8.9	4	0.30
PI607407	17	4.0	10.3	4	0.30
Gao Gaoliang	20	4.0	10.9	5	0.29
IS 12749	39	4.0	12.6	5	0.50
Migna	11	4.0	11.3	4	0.41
Bomususu	43	4.0	10	5	0.44
K-892	5	4.0	11	4	0.38
PI266961	31	4.0	12	4	0.37
LR 2505	13	4.0	10.2	4	0.34
LR 2490-1	7	4.0	11	4	0.32
IS 27938	22	4.0	10.8	4	0.40
IS 27931	33	4.0	10.9	4	0.37
Lo leung mai	23	4.0	9.9	4	0.38
K-540	33	4.0	8.6	4	0.37
PI607404	13	4.3	12.7	5	0.44

Genotypes	Emergence (%)	Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}	Seedling dry weight (g)
Huang Luo Mian	13	4.3	11.8	4	0.43
LR 2556-2	17	4.3	10.9	3	0.34
Danyang Local	17	4.3	10.0	4	0.33
K-637/II	49	4.3	11.0	5	0.46
PI 607408	21	4.3	11.3	5	0.41
LR 2820	5	4.3	8.9	4	0.25
K-24	31	4.3	9.2	4	0.33
VA 110	13	4.3	10.2	4	0.39
ETS 3633	26	4.3	9.6	4	0.45
PI607403	18	4.3	11.4	4	0.36
IS 10497	9	4.3	9.4	4	0.33
IS 2212	9	4.3	8.8	4	0.28
PI234456	19	4.3	9.5	4	0.32
Blackhull Kafir	23	4.3	8.8	4	0.30
PI607402	9	4.3	11.6	5	0.38
Nian Gao Liang	22	4.3	10.2	4	0.25
Dwarf Yellow Milo	25	4.3	10.1	4	0.37
PI550859	8	4.3	10.8	4	0.35
LR 423	10	4.3	9.6	4	0.27

Genotypes	Emergence (%)	Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}	Seedling dry weight (g)
IS 10731	9	4.3	9.3	4	0.43
LR 2462-2	7	4.3	11.2	4	0.38
Tx3042	30	4.3	9.2	5	0.38
Katengu	24	4.3	11.1	4	0.34
L 1791B	6	4.3	10.2	4	0.31
Tx399	21	4.7	10.2	5	0.37
Bai Ruan Gao Liang	30	4.7	9.4	4	0.29
PI 501620	34	4.7	12.1	4	0.34
LR 2433	8	4.7	9.6	4	0.29
LR 2490-2	11	4.7	9.6	4	0.31
Framida	21	4.7	9.3	4	0.30
L 1999B-14	7	4.7	9.2	4	0.29
Hemaise	10	4.7	10.2	4	0.34
Leoti	25	4.7	9.9	4	0.31
BP9517	18	4.7	9.1	4	0.34
Purdue 81659-2	14	4.7	7.7	4	0.22
LR 2410	10	4.7	10.5	4	0.22
Big Yellow Umbrella	16	5.0	11.7	3	0.31
FS73015-D001	17	5.0	10.5	4	0.23

Genotypes	Emergence (%)	Seedling vigor (1-5)	Seedling height (cm)	Leaf number plant^{-1}	Seedling dry weight (g)
IS 24692	10	5.0	8.5	3	0.19
IS 9145	17	5.0	7.9	3	0.27
Tx436	9	5.0	9.4	4	0.12
PI217896	15	5.0	9.4	4	0.20
Nai-Shaker	19	5.0	10.1	4	0.28
MN 4116	32	5.0	9.3	4	0.21
LR 2417(a)	10	5.0	8.8	4	0.26
K-403	35	5.0	9.2	5	0.31
LR 2490-3	6	5.0	10.0	4	0.22
K-47	32	5.0	9.4	4	0.31
IS 1024	24	5.0	10.5	4	0.28
LR 431-2	9	5.0	8.4	4	0.28
R-45	9	5.0	8.2	4	0.23
Tunis grain	39	4.3	7.7	4	0.26
Tx2911	14	5.0	9.8	4	0.34
LR 2556-1	11	5.0	10.2	3	0.21
Shan Qui red	15	2.0	10.2	5	0.31
SRN39	5	5.0	6.7	3	0.12
Mean	22	3.8	10.8	4	0.38
LSD	11.7	1.3	1.8	0.8	0.17