# SCREENING FOR FORAGE SORGHUM GENOTYPES WITH CHILLING TOLERANCE

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Swarup Podder

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

June 2019

Fargo, North Dakota

# North Dakota State University
Graduate School

**Title**

SCREENING FOR FORAGE SORGHUM GENOTYPES WITH CHILLING TOLERANCE

**By**

Swarup Podder

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

SUPERVISORY COMMITTEE:

Dr. Marisol Berti
Chair

Dr. James V. Anderson

Dr. Michael S. McMullen

Dr. Qi Zhang

Approved:

06/21/2019
Date

Richard D. Horsley
Department Chair

## ABSTRACT

Forage sorghum (FS) [*Sorghum bicolor* (L.) Moench] is a warm-season biomass crop with the potential to become a bioenergy feedstock. The objective of this study was to screen potential FS genotypes for increased chilling tolerance and biomass productivity. The experiments were conducted in Fargo and Hickson, ND, in 2017 and 2018. Seventy-two genotypes of FS were tested at 24, 12, and 10℃. The genotypes were ranked from high to low vigor index and 12 genotypes were planted on two seeding dates: early (10 May) and late (27 May). Field emergence index values were greater for the late-seeding compared with the early-seeding date. Stand establishment and seed mortality were affected by the seeding date. Biomass yield correlated with emergence index and normalized vegetative index. Some of the genotypes tested had increased chilling tolerance and biomass yield when seeded earlier than normal, and may be used for breeding chilling tolerance into FS.

ACKNOWLEDGEMENTS

I express my deepest sense of gratitude and indebtedness to my honorable advisor, Dr. Marisol Berti, for her affection, inspiration, constant guidance, and valuable suggestions throughout my graduate study and this research work. I am grateful for her patience and hard-work to review and correct this thesis. I am blessed having her as my graduate advisor and mentor.

I express gratitude to my graduate committee members- Dr. James V. Anderson, Dr. Michael S. McMullen, and Dr. Qi Zhang for their co-operation, guidance, valuable suggestions and encouragement in planning, executing and improvement of the thesis. Special thanks to Dr. James V. Anderson for providing necessary facilities to conduct the environmental growth chamber experiments at the USDA-ARS, Edward T. Schafer Agricultural Research Center in Fargo.

From the core of my heart, I express my gratefulness to all the member of NDSU-Forages team- Dulan Samarappuli, Sergio Cabello Leiva, Alan Peterson, Bryce Andersen, and Alex Wittenberg, for their help in counting germinated seeds, preparing field, planting, data collection, harvesting, and all other activities. This research work would never be completed without their physical hard-work and constant support to implement valuable ideas.

I am grateful to Dr. Burton Johnson for valuable suggestion and inspiration. I express special thanks to Rick Eggum, Chad Deplazes, and John F. Nowatzki for providing required technical tools. I would like to extend my thanks to all faculty, office staffs, and graduate friends of Plant Sciences for providing support throughout this journey.

I am grateful to the USDA-National Institute of Food and Agriculture-Agriculture and Food Research Initiative-North Central SunGrant for providing financial and logistic support to conduct this research.

I express my ever gratefulness and indebtedness to my parents (Sukumar Chandra Podder and Puspa Rani Podder), brothers, sisters and other members of my family for their great sacrifice, endless prayers, and blessings and support, so I reach this level of higher education.

Finally, I express my heartfelt gratitude to my beloved life partner Shilpi Rani Das for her love, understanding, great sacrifice, endless prayers, and support to complete this research work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# CHAPTER 1. INTRODUCTION

Global climate change is one of the most widely spoken topic of the last few decades. It is predicted that the sustainability of food systems will be affected and farmers' livelihoods, markets, and food security will face challenges due to the vulnerability to climate change (Wheeler and von Braun, 2013). Development of stress tolerant crops is of interest to the scientific community as climate change and weather conditions are expected to adversely affect agroecosystems worldwide.

Due to the long winter season, the North Central Region (NCR) has a short window of time for growing crops, especially warm-season crops. In addition, crop production becomes challenging due to cold temperatures early in the season, limited rainfall during the growing season, and other adverse weather conditions. Thus, the number of warm-season crops that can be grown in the NCR is limited, and identification of crops tolerant to drought and cold temperatures at planting time should increase crop diversity in the NCR.

Forage sorghum [*Sorghum bicolor* (L.) Moench] is an annual biomass crop mainly grown for silage, hay and forage for grazing. It is highly tolerant to heat and drought stress (Rooney et al., 2007). It requires low water input as it has high-water use efficiency (McCollum et al., 2005). It can be grown in marginal land with lower fertilizer input. It can be an alternative crop to corn (*Zea mays* L.) as a bioenergy feedstock. Several research findings supported that forage sorghum is better suited in semi-arid conditions because of lower irrigation requirements, lower transpiration ratio, and faster recovery after drought stress (Undersander et al., 1990; Perazzo et al. 2017). Howell et al. (2008) reported that forage sorghum has 27% less evapotranspiration and has higher biomass production potentiality than corn in North Dakota (Berti et al., 2011; Samarappuli et al., 2014)

The main limitation of forage sorghum is its low tolerance to temperatures below 15°C, especially early in the growing season (Peacock, 1982; Razmi et al., 2013). Cold soil temperature early in the season in the NCR limits forage sorghum planting until the average soil temperature reaches 15°C or above, which usually happens about two weeks later than corn planting. Improving chilling tolerance in forage sorghum would increase the biomass potential and likely lead to an increase in acreage and productivity of forage sorghum in the NCR.

Genotypes of grain sorghum with chilling tolerance have been identified. But the chilling tolerance in commercial cultivars of forage sorghum and sorghum x sudangrass hybrids are unknown. This study was aimed at identifying and selecting chilling-tolerant genotypes of commercial forage sorghum cultivars and hybrids for higher biomass potential as feedstock for bioenergy. From this study, commercially available chilling-tolerant forage sorghum genotypes were identified and selected, which should enhance the acreage and production of forage sorghum biomass and increase diversification of cropping systems in the NCR.

### 1.1. Objectives

To identify commercially chilling-tolerant forage sorghum commercial cultivars and hybrids with faster emergence at low temperature and higher biomass productivity for North Dakota

# CHAPTER 2. LITERATURE REVIEW

## 2.1. Sorghum description

The genus *Sorghum* is one of about 600 genera in the Poaceae family. It belongs to the subfamily Panicoideae and the tribe Andropogoneae. There are about 30 species in the *Sorghum* genus, some of which are cultivated for grain, used either as fodder plants cultivated, or as part of pasture (New World Encyclopedia, 2015). The mostly cultivated species is *Sorghum bicolor* L. Moench (Venkateswaran et al., 2014).

Sorghum originated in northeast-Africa approximately 5000 years ago (Mann et al., 1983) and spread to other regions such as India, China, Middle East, and Africa via migration of people and traders (Dillon et al., 2007). That early-domesticated sorghum was dispersed throughout a broad range of environments and its widely adapted genetic base has been exploited throughout the agricultural process to create the currently cultivated sorghum cultivars (Dahlberg et al., 2010).

Sorghum has bisexual flowers and it is mostly a self-pollinated crop, but up to 50% cross pollination has been observed (Osuna-Ortega et al., 2003). Sorghum is a diploid ($2n=2x=20$) plant but there are a few polyploid genotypes ($2n=4x=40$) (Celarier, 1959). Sorghum is a C4 plant which requires 30 to 35°C for maximum photosynthetic activity. Sorghum requires 21 to 35ºC for optimum germination, 26 to 34ºC for vegetative growth and development, and 25 to 28ºC for reproductive growth (Maiti, 1996; Prasad et al., 2008).

Sorghum germplasm is widely diversified. The USA sorghum germplasm has more than 41,000 accessions including landraces, cultivars, wild type, and hybrids (Dahlberg et al., 2010). Based on purposes, the following are the most widely cultivated sorghum types of the *Sorghum bicolor* species:

Grain sorghum: It is cultivated for the purposes of harvesting grain. Grain sorghum is generally dwarf in type that can grow 0.6 to 1.5 m tall (Undersander et al., 1990).

Forage sorghum: It includes both open-pollinated cultivars and hybrids which can grow 1.8 to 3.6 m tall. Forage sorghum has relatively wide stems and produces more dry matter than grain sorghum. Forage sorghum is mainly grown for silage and grazing (Undersander et al., 1990; Vendramini et al., 2010).

Sudangrass: It is a subspecies of *Sorghum bicolor; S. bicolor* var. *sudanense.* It has finer stems and tillers more profusely than forage sorghum. Sudangrass is a short-season *Sorghum* species grown for pasture or green feed during mid-summer when perennial grasses are not available. They have superior regrowth ability after cutting or grazing (Undersander et al., 1990; Vendramini et al., 2010).

Sorghum x sudangrass hybrids: Sorghum x sudangrass hybrids are the cross between forage sorghum and sudangrass to combine high productivity and nutritive value. Generally, sorghum x sudan hybrids are used for hay or silage (Undersander et al., 1990; Vendramini et al., 2010).

Sweet sorghum: Sweet sorghum has been bred to produce thick stems with high content of sucrose. It is mainly grown to extract the juice from the stalks for ethanol production, but it is also used as a forage. Typically, sweet sorghum stalks contain up to 75% juice, which has 12 to 23% soluble solids, when grown in the South US. In North Dakota, sweet sorghum soluble solids content in the juice fluctuates from 11-16%, due to the short season and lower temperatures (Berti et al., 2011).

Sorghum is a short-day photoperiod sensitive plant, which has great potential as a biomass feedstock in subtropical and temperate region, as plants continue in the vegetative phase

until they reach short-day length (<12 h). Also, sorghum plants show heterosis for plant height which allows for breeding of taller plants (Ross et al., 1979, Packer and Rooney, 2014). Sorghum plants are highly variable in plant height from 0.5 to 5 m. Plant height is controlled by four unlinked major dwarfing loci (*$Dw_1$*, *$Dw_2$*, *$Dw_3$*, and *$Dw_4$*) where tallness is incompletely dominant (Quinby and Karper, 1954). Flowering time and photoperiod sensitivity of sorghum are controlled by four major maturity loci (*$Ma_1$*, *$Ma_2$*, *$Ma_3$*, and *$Ma_4$*) where dominant alleles are responsible for late-season flowering (Quinby, 1966). For seed production in temperate regions, photoperiod-insensitive parental lines need to be developed through identifying complementary gene action of maturity loci *$Ma_1$*, *$Ma_5$*, and *$Ma_6$* (Rooney and Aydin 1999, Mullet et al., 2010).

2.2. Forage sorghum, sudangrass, sorghum x sudan, biomass yield

Total biomass yield depends on seasonal precipitation and nitrogen application (Ikanovic et al., 2013). Forage sorghum produces higher dry biomass (20.15 Mg $ha^{-1}$) if harvested at physiological maturity stage (Atis, 2012) than earlier in the season. Total one-cut biomass yield averaged across two locations and two years in North Dakota was 17.8 Mg $ha^{-1}$ (Samarappuli et al., 2014; Samarappuli and Berti, 2018). Vinutha et al. (2017) evaluated 36 different forage sorghum genotypes in Pathancheru, India, for forage yield, and ratooning (regrowth) ability. Main crops were harvested at 80 days after planting and second harvest was done 80 days after first harvest to allow for regrowth. The mean dry biomass yield was 8.5 to 22.8 Mg $ha^{-1}$ for the first harvest and the regrowth, respectively. Forage sorghum produced highest amount of dry biomass at late dough stage (11.3) while highest forage nutritive value was observed at mid-vegetative stage. Results indicated that without decreasing dry matter yield, harvesting twice can provide higher quality forage than harvesting only once (McCormick et al., 1995). Sudangrass, generally, produces taller and thinner stems and higher number of leaves, while generally

yielding less than forage sorghum or sorghum x sudan types. Sorghum x sudan produces higher amount of stem biomass than forage sorghum. Biomass yield is positively correlated with stem weight and negatively correlated with leaf weight. Sudangrass produces significantly lower biomass yield compared with forage sorghum and sorghum x sudan hybrids (Ikanovic et al., 2011).

2.3. Forage sorghum composition and quality

Improved forage nutritive value is an important criterion for selecting commercial cultivars of forage sorghum (Akabari and Parmar, 2014). The most widely used parameters of forage nutritive value include crude protein (CP), dry matter digestibility (DMD), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), *in vitro* dry matter digestibility (IVDMD), neutral detergent fiber digestibility (NDFD), prussic or hydrocyanic acid (HCN), and tannins (Ahluwalia, 1977). Utilizing near infra-red technologies is a cost effective and efficient way for measuring chemical composition of forage sorghum (Dahlberg et al., 2011b).

The lignin content of cell walls is an important component for structural strength and an important quality factor. Low lignin content is correlated with high digestibility, also increasing sugar release, which enhances the fermentation process during biofuel production (Vermerris et al., 2007, and Lorenz et al., 2009). Brown mid-rib (BMR) sorghums (*bmr-6* and *bmr-12*) have reduced lignin content. Brown mid-rib sorghum biomass showed 10 to 25% lower lignin content with 8% higher crude protein content compared with non-BMR sorghum (Guragain et al., 2017 and Li et al., 2015). Lignin and cellulose concentration increases as plant matures. Improving forage sorghum genotypes for increased IVDMD is the most rapid way of improvement of high quality forage genotypes (Pedersen, 1982).

Sorghum x sudangrass hybrids produce higher quality forage than forage sorghum because it has higher leaf to stem ratio, and lower lignin content than forage sorghum. Brachytic sorghum types have shorter internode length with higher numbers of nodes and higher leaf to stem ratio. Higher leaf to stem ratio increases the digestibility, as leaves have lower lignin content, and increases crude protein content. Sudangrass produces taller, thinner stems with high lignin content, which reduces forage quality (Undersander et al., 1990). Forage sorghum leaves contains a cyanogenic glucoside, dhurrin, which releases HCN (commonly known as cyanide) when young plants are exposed to frost and or extreme drought (Halkier and Moller, 1991, Hoveland and Monson, 1980). This HCN causes cellular asphyxiation and even death of livestock grazing on forage sorghum exposed to a light frost (Hoveland and Monson, 1980). Selecting forage sorghum cultivars with low HCN content is an important trait of interest. Sudangrass accumulate less HCN than forage sorghum when exposed to frost stress (Vendramini et al., 2010).

2.4. Sorghum as feedstock for bioenergy

The demand for ethanol continues to increase in the USA. According to the Energy Security Act 2007, 30% of fossil fuel needs to be replaced by renewable fuels by 2030 (DOE, 2006). To fulfill this demand, finding alternative sources of biofuels, rather than corn starch, is of interest to the scientific community. Lignocellulosic biofuel production would be a cost-effective way as multiple sources of bioenergy feedstocks are available.

For sustainable bioenergy feedstock production, forage sorghum is an excellent choice as it has high biomass production, which has the potential for high ethanol production from the lignocellulose (Carpita and McCann, 2008). Biofuel production form lignocellulose biomass is commonly known as second generation biofuels. The composition of lignocellulose biomass is

mainly of cellulose, hemicellulose, lignin. The amount of biofuel production depends on the amount of cellulose and hemicellulose where lignin affects biofuel production negatively.

Additionally, forage sorghum biomass can be converted to biogas through anaerobic fermentation to produce heat and power. Samarappuli and Berti (2018) reported 12,400 to 16,400 $Nm^3$ $ha^{-1}$ biogas yield in BMR and non-BMR forage sorghum genotypes in North Dakota, supporting the potential of forage sorghum as an energy crop. Mahmood et al., (2013) found biogas yield with a range of 7649 to 12,880 $Nm^3$ $ha^{-1}$ in forage sorghum, in Germany.

Currently, grain sorghum has been supplying 15 to 20% of the feedstocks for eight ethanol-producing plants in the USA. It has been estimated that grain sorghum can produce in average 6,146 L $ha^{-1}$ of renewable fuels (Dahlberg et al., 2011). Rapid growing, tall sweet sorghum genotypes can have potential for improving bioenergy sorghum (Mocoeur et al., 2015). Sweet sorghum can provide grain and stem which can be utilized for feed, sugar, syrup and ethanol production. Sweet sorghum juice contains 16 to 18% sugar which can be converted to ethanol directly through fermentation (Ratnavathi et al., 2011). Kim and Day (2011) stated that theoretical ethanol yields are 5,804 kg $ha^{-1}$ from sweet sorghum. A sweet sorghum genotype-BMR Elite can produce up to 32 Mg of juice $ha^{-1}$ that could be fermented to ethanol with a yield of 1014 to 1968 L ethanol $ha^{-1}$ (Chmielewska et al., 2012).

2.5. Sorghum germination and field emergence

Germination and field emergence of forage sorghum early in the season are the most important determinants of crop performance. Reduced germination and field emergence, as well as poor seedling growth, occurs when forage sorghum is exposed to below 15ºC soil temperature, especially at the beginning of the season which leads to low stand establishment

(Yu and Tuinstra, 2001). Generally, in the North Central region of the US, forage sorghum is seeded between 20 May to 5 June when soil temperatures reach 15℃ (Undersander et al., 1990).

2.6. Chilling tolerance in sorghum

Under cold stress, a quick reduction in photosynthetic efficiency of forage sorghum seedlings occurs. This is induced by metabolic (non-stomatal) limitation during exposure to suboptimal temperatures and by stomatal limitation after the termination of cold stress (Janowiak et al., 2015). The sorghum plants subjected to cold stress in seedlings stage had lower leaf chlorophyll content and seedling vigor than sorghum seedlings grown without cold stress. Cold stress, applied at flowering stage, delayed maturity and affected yield components (Maulana and Tesso, 2013). Photosynthetic activity of sorghum seedlings decreased under cold stress, indicating measuring photochemical quenching of chlorophyll fluorescence is the efficient way for screening chilling-tolerant sorghum genotypes (Havaux, 1989).

Plant respiration is an important variable to select for chilling-tolerance genotypes. At 25˚C, forage sorghum seeds have higher respiration rate which enhances germination and elongation rates of seedlings. Thus, measuring the respiration rate at 10 to 15˚C is a useful selection method for chilling-tolerance of forage sorghum seeds (Balota et al., 2010). Water imbibition and respiration of sorghum seeds slows down at 10˚C, which leads to germination failure (Patane et al., 2006).

Although some information about chilling-tolerance in grain sorghum genotypes exists, there is limited information about chilling-tolerance in forage sorghum commercial cultivars and hybrids. There are a large number of chilling-tolerant grain sorghum lines that are well adapted to the Highlands of Honduras, Kenya, and Mexico, but poorly adapted to the northwestern USA, southern Canada, and West Germany (Singh, 1985; Yu et al., 2004). In sorghum, several genes

control chilling tolerance. Grain sorghum Shan Qui Red, Kaoliang, Niu Sheng Zui, and Hong Ke Zi Chinese lines have chilling-tolerance genes (Marla et al., 2017, Knoll et al., 2008 and Maulana et al., 2017). However, these lack other agronomic traits. The lines BTx623 and SC265 are the grain sorghum reference lines used for chilling sensitive studies in US laboratories (Marla et al., 2017).

The identification of QTLs associated with maintenance of cell division and growth under chilling stress conditions are an important prerequisite for improving chilling tolerance of forage sorghum genotypes (Bekele et al., 2014). Many promising QTLs associated to low temperature tolerance have been identified (Fiedler et al., 2014). Franks et al. (2006) compared ten Chinese Kaoliang accessions with ten U.S. inbred parental lines and ten U.S. commercial grain sorghum hybrids for the traits of chilling-tolerance under growth chamber and field conditions. Chinese lines had better performance than both inbred and hybrid U.S. classes in laboratory studies on germination and field emergence rates. The U.S. hybrids showed greater final stand counts in the field and greater biomass yield than Chinese lines. The previous study suggested that those Chinese accessions would be a favorable source of chilling-tolerance genes for germination at low temperature conditions that could be utilized for chilling-tolerant forage sorghum breeding programs.

Chilling-tolerant forage sorghum genotypes are essential for early seeding. Screening forage sorghum genotypes through germination tests under controlled conditions is an efficient way for identifying forage sorghum lines adapted to germinate at low temperatures. Fernandez et al. (2014) reported that under laboratory condition, a 7-day cold test at 10°C was a useful predictor of higher emergence in the field. In their study, eight novel accessions with potential superior alleles for chilling-tolerance were identified (Fernandez et al., 2014). Measuring shoot

growth and germination rate at a controlled temperature of 15°C is helpful to screen genotypes for chilling-tolerance before final evaluation in the field (Tiryaki and Andrews, 2001). Field emergence and root establishment of forage sorghum early in the growing seasons is a key determinant of chilling-tolerance (Bekele, 2014). Differences were observed in sorghum genotypes between growth chamber performance and field performance at 15°C for emergence percentage, emergence index, shoot and root dry weight, seedling height, and vigor score (Fiedler et al., 2014).

Kapanigowda et al. (2013) conducted a study under controlled and field conditions. Two different seeding dates (early and late) were used to screen chilling-tolerant sorghum genotypes. Significant differences were found in emergence percentage, emergence index, biomass yield, plants height, and leaf number. Eight potential chilling-tolerant genotypes with early emergence index and higher biomass yield were selected. In that study, late-emerged genotypes produced higher biomass than early-emerged genotypes. Correlation between growth chamber and field studies for emergence index can be a useful method for screening chilling-tolerant forage sorghum genotypes.

Under cold conditions, some controlled- and field-grown sorghum lines had significantly higher emergence and seedling growth than the check chilling-tolerant genotype -Shan Qui Red (Chiluwal et al., 2018, and Maulana, and Tesso, 2013). The survival and growth performance of sorghum seedlings under cold conditions positively correlated with emergence rate and root development in the field (Bekele et al., 2014). Genome wide association studies (GWAS) disclosed eight probable markers linked with final emergence percentage and seedling survival rate on chromosome SBI-01, -02, -03, -06, -09, and -10 with probability ($p < 5.7 \times 10^{(-5)}$) where

chromosome SBI-06 strongly associated with field emergence under chilling conditions (Parra-Londono et al., 2018).

# CHAPTER 3. MATERIALS AND METHODS

## 3.1. Collection of germplasm

Sixty-two commercially cultivated forage sorghum genotypes were collected from different seed companies. Genotypes included commercial cultivars and hybrids of forage sorghum, sudangrass, sorghum x sudan hybrids, sweet sorghum, and ten grain sorghum genotypes (2 chilling-sensitive and 8 chilling-tolerant) from Kansas State University

## 3.2. Pre-screening through seed germination test in controlled environment

Seed germination rates were evaluated at 24°C and 12°C between 4-24 March, in 2017, and at 10°C between 1-21 February, in 2018, at the USDA-ARS, Edward T. Schafer Agricultural Research Center in Fargo. In both runs, three Petri dishes with 50 seeds in each were evaluated for 20 days and arranged in a completely randomized design. Evaluations included germination rate, and vigor index. The vigor index at 12°C and 10°C were corrected by the baseline germination rate at 24°C. In the second run seeds were evaluated at 10°C instead of 12°C to get better discrimination for vigor index among the genotypes. In the second run, seeds were not evaluated for base line germination test at 24°C as seeds were stored at cold storage. Germination rate and vigor index was measured for 24°C, 12°C, and 10°C using the following formula.

$$Germination\ rate\ (\%) = \frac{No.of\ seeds\ germinated}{No.of\ seeds\ tested} * 100$$

$$Vigor\ index = \frac{No.of\ seeds\ germinated\ at\ first\ counting\ day}{1} + \frac{No.of\ seeds\ germinated\ at\ second\ counting\ day}{2} + \cdots + \frac{No.\ of\ seeds\ germinated\ at\ day\ n}{n}$$

*Corrected vigor index = Vigor index at 10 or 12°C × (germination rate at 24°C/100)*

3.3. Data analysis

The germination rate and vigor index data were analyzed with SAS 9.4 (SAS Institute, 2012) using GLM procedure to identify chilling-tolerant genotypes from non-chilling tolerant genotypes. The statistical analysis was done separately by run and then combined. Temperature, genotype, and run were fixed effects and replicate was a random effect. A means separation test was performed by LSMEANS and then by the *F*-protected LSD at $P \leq 0.05$. In 2017, the eight genotypes ranking the highest in corrected vigor index (Sweetie BMR, brachytic sorghum, Pampa Triunfo XLT, Green Treat 128, 54126, Hay King, SPX-901, and 1990) were selected to test in the field. However, if among the first ranked genotypes two of them were from the same seed company, we skipped to the next highest ranked genotype from a different seed company source to avoid selecting genotypes with the same genetic base. In addition, 'Forage King' (ranking the lowest in corrected vigor index) and two chilling-sensitive genotypes (BTx623, and SC265) were selected as well, totaling 11 genotypes. In 2018, after the second run of germination, a few of the genotypes selected in 2017 produced corrected vigor index rankings. Thus, a few new genotypes were included in the 2018 field trial, as well as eliminating those which did not perform well in the 2017 field experiments. In 2018, nine genotypes with the highest corrected vigor index (Sweetie BMR, Pampa Triunfo XLT, Hay King, SPX-901, 1990, Pampa Verde BMR-6, Sordan Headless, NK 300, and BMR-90), one genotype with the lowest corrected vigor index (Forage King) and two check chilling-tolerant genotypes (Niu Sheng Zui, and Kaoliang) were selected for field trials in 2018. Six of the genotypes were common in the both years of field experiments.

3.4. Field experimental design

Experiment were conducted in 2017 and 2018 at the North Dakota State University (NDSU) research site in Fargo, ND (-96°817'W, 46°897'N, 274 m elevation) and in Hickson, ND (-96°838'W, 46°634'N, 280 m elevation). Soil type in Fargo is Fargo-Ryan silty clay soil and the soil type in Hickson is Fargo-Hegne silty clay, (Fargo: fine, montmorillonitic, frigid, Vertic Haplaquol with a leached and degraded nitric horizon; Ryan: fine, smectitic, frigid Typic Natraquerts; Hegne: fine, smectitic, frigid Typic Calciaquerts) (Web Soil Survey, 2009). Rainfall and daily temperature were recorded by the North Dakota Agricultural Weather Network (NDAWN, 2018) at both sites (Figure 1).

A composite soil sample was collected from each replicate at 0- to 15-cm and 15- to 60-cm in depth in each site before seeding; in total four composite samples per site. Samples were sent to the soil testing laboratory to measure $NO_3$-N, organic matter, pH, P, and K in the 0- to 15-cm soil samples and only $NO_3$-N was tested in the 15- to 60-cm soil samples using the following methods: i) organic matter, loss on ignition; ii) $NO_3$-N, colorimetric determination by trans-nitration of salicylic acid method (Vendrell and Zupancic, 1990); iii) P, Olsen procedure using Brinkmann PC 910 colorimeter; and iv) K, ammonium acetate method using Buck Scientific Model 210 VGP atomic absorption spectrophotometer.

The experiment was a randomized complete block design with a split-plot arrangement and four replicates. The main plot was the seeding date (early and late), and the sub-plot was the selected genotypes. The previous crops were wheat (*Triticum aestivum* L.) and soybean [*Glycine max* (L.) Merr.] at Fargo and Hickson, respectively in 2016 and 2017. Early seeding was done on 10 May in 2017 and in 2018 and late seeding was done on 27 May in 2017 and 29 May in 2018. The plots size was 7.62-m long with four rows 0.15-m apart. A 4-cone continuous plot drill XL

(Wintersteiger, Salt Lake City, UT) was used. Seeding rate for each genotype was 190,190 pure live seeds ha-1 calculated to target 172,900 plants ha-1. For all genotypes, 1000-seed weight was measured to calculate the exact number of live seeds needed per row. Pure live seeds were calculated using the germination rate obtained in growth chamber experiments. Seeds were sown to 2.5-cm depth.

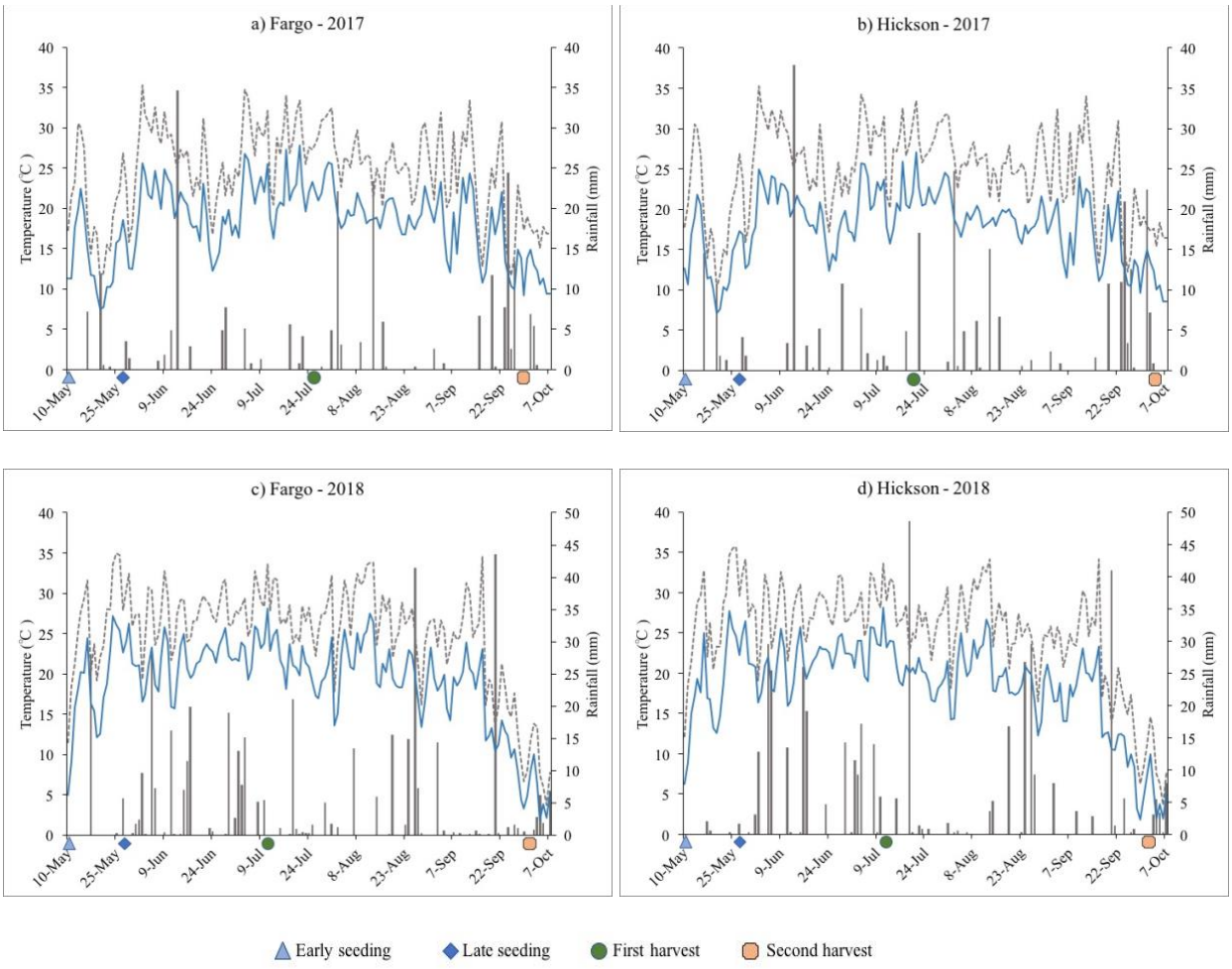


**Figure 1.** Air temperature and rainfall distribution (rainfall events indicated in grey bars) at four environments at Fargo and Hickson, ND, in 2017 and 2018.

The Hickson experiment site was fertilized with 80 kg N $ha^{-1}$ and 50 kg $P_2O_5$ $ha^{-1}$ before seeding in 2017 and 2018. The Fargo experiment site was fertilized with 60 kg N $ha^{-1}$ at the V-5 stages as side dressing in 2017 and 2018. No potassium was applied in any experimental sites.

Weed pressure was greater at the Fargo site compare with the Hickson site. At Hickson, the most common weeds were: common mallow (*Malva neglecta* L.), green foxtail (*Setaria viridis* L.), Canada thistle [*Cirsium arvense* (L.) Scop.], and field bindweed (*Convolvulus arvensis* L.). At the Fargo site, weeds were mostly pigweed (*Amaranthus retroflexus* L.). The fourth replicate area of early-seeding genotypes was affected by high pressure of field bindweed at the Fargo site in 2018. In 2017, hand weeding was done twice at the Fargo site and one time at the Hickson site. In addition, 2,4 D was sprayed at the V-3 stage of sorghum with a rate of 270 g a.i. $ha^{-1}$ at the Fargo site in 2017 and 2018.

3.5. Sampling and data collection

A sensor (5TM water content and temperature sensors, Decagon Devices, Inc., Pullman, WA) was set in the field at 2.5- to 3.5-cm depth in the level of seed to record the soil temperature and soil water content. The sensor has four probes, which were set in a representative way within the plot. The sensor recorded the hourly soil temperature and soil water content throughout the growing season.

One linear meter of each two-center rows was marked for counting the emerged plants. Within the marked area, emerged plants were counted from 5 days after seeding (DAS) and continued up to 15 DAS. Emergence index was calculated using the following formula.

$$Emergence\ index = \frac{No.\ of\ emerged\ plants\ at\ first\ counting\ day}{1} + \frac{No.\ of\ emerged\ plants\ at\ second\ counting\ day}{2} + \cdots + \frac{No.\ of\ emerged\ plants\ at\ day\ n}{n}$$

CANOPEO (a free mobile application developed by Oklahoma State University to measure canopy) was used to measure the canopy coverage at early vegetative stage (Patrignani and Ochsner, 2015). One picture was taken from each experimental unit from the same level of height with an Android phone. Those pictures were run in a computer-based CANOPEO application to measure the percentage of green coverage within a selected area.

Number of plants were counted within one linear meter of each two-center rows at 20 days after each seeding. To calculate stand establishment, total number of counted plants within the two-linear meters was converted to plants per hectare. Seed mortality were calculated using the following formula:

$$\text{Mortality (\%)} = 100 - [(190{,}190 - \text{stand establishment})/190{,}190 \times 100]$$

A chlorophyll meter (SPAD 502 Plus Chlorophyll Meter, Spectrum Technologies, Inc., Plainfield, IL) was used to measure chlorophyll content in the leaves at different stages (36, 42, 49, and 63 DAS in 2017 and 2018). Chlorophyll content was measured from four leaves at the same maturity stage of four different plants per sampling experimental unit (plot). The SPAD meter provided the average readings for each sampling-plot.

Normalized difference vegetation index (NDVI) was measured using a handheld GreenSeeker (Trimble Inc., Sunnyvale, CA) at different growing stages (35 DAS, 49 DAS, 63 DAS and 15 days after first harvest (DAH) in 2017 and 2018)). GreenSeeker was run above the center-row of each sampling-plot. The NDVI is a measurement of plant health based on how a plant reflects sun light at specific wavelengths. To be more specific, $NDVI_{(656, 774)}$ is a measurement of the reflectivity of plants expressed as:

$$NDVI = (NIR - VIS)/ (NIR + VIS)$$

Where, NIR= near-infrared reflectivity at 774 nm

VIS= red reflectivity at 656 nm

Photosynthetically active radiation (PAR) readings under and above the canopy were collected at different growing stages (58 DAS, 68 DAS, 30 DAH, 45 DAH in 2017 and 2018) by placing a ceptometer in between the two-center rows. Three readings were taken from each experimental unit and ceptometer provided the average readings. Intercepted PAR light percentage was calculated using the following formula:

$$\text{Intercepted PAR light (\%)} = (\text{Light readings above the canopy} - \text{Light readings below the canopy}) / (\text{Light readings above the canopy}) \times 100$$

Plant height was measured before each harvest from each sub-plot. Plant height was measured with a measuring stick from the ground level to top level of the arch of the uppermost leaf whose tip is pointing down. Plants growing stage was recorded during each harvest.

The two-center rows of each sub-plot were harvested with a flail forage harvester (Carter, Brookston, IN) in the first harvest, leaving 15-cm stubble to allow regrowth. The second harvest was done cutting stalks by hand with a scythe. First harvest was done on 20 July and on 27 July in 2017 at Hickson and Fargo, respectively, and on 12 July in 2018 at both sites. All the genotypes planted in either date were harvested at the same time. Plant stalks were allowed to regrow and harvested for second time on 28 September and 6 October in 2017 at Fargo and Hickson, respectively. In 2018, the second harvest was done on 1 October at both locations. The second harvest was conducted when growing degree days (GDD) stop accumulating. Growing degree days were calculated from minimum and maximum daily temperatures from the North Dakota Weather Network (NDAWN, 2018). The general base temperature for GDD calculation

is 10°C (Gerik et al., 2005). However, in this research we consider 15°C as base temperature to calculate GDD for forage sorghum.

$$\text{GDD} = \sum \left[ \frac{(\text{maximum temperature} + \text{minimum temperature})}{2} - \text{Base temperature} \right]$$

Total harvested fresh biomass weight from each sub-plot was recorded and then about 1 kg of fresh biomass sample was taken to calculate dry biomass yield. After recording fresh weight, samples were allowed to dry for two weeks in a drier at 45℃. Dry matter content and dry biomass yield were calculated using the following formula.

Dry matter content= (dry weight/fresh weight) ×100

Dry biomass yield = (total harvested fresh weight × dry matter content)/100

Dried biomass samples were ground to a 1-mm mesh with a mill (Wiley Mill Standard Model Nº3, Philadelphia, PA). Samples of the first harvest in 2017 were sent to the Animal Sciences laboratory at North Dakota State University for wet chemistry analysis for N content using the Kjeldahl method, crude protein (CP) according to AOAC Method 2001.11, neutral detergent fiber (NDF) according to ANKOM, 2011.A200 Method 6), acid detergent fiber (ADF) according to ANKOM, 2011. A200 Method 5), acid detergent lignin (ADL), and ash according to AOAC Method 942.05). With the wet chemistry data, a calibration was created for sorghum biomass samples in a near-infrared spectroscopy instrument, XDS analyzer (Foss, Denmark). Samples falling off the calibration ranges in 2018 were sent to the laboratory for wet chemistry analysis. Nitrogen accumulation were calculated by multiplying N content in biomass by the biomass yield.

3.6. Data analysis

All the data collected throughout the season were analyzed separately by year and location using the procedure GLM in SAS 9.4 software (SAS Systems Inc., Cary, NC).

Homogeneity of variance tests were done to determine if environments could be combined. If homogeneous, a combined analysis across four environments, defined as the combination of location-year, was conducted.

To determine the difference among those treatments, procedure mixed of SAS and *F*-protected least squared differences with a significance of 95% level of confidence were used. Regression analysis was used to determine the biomass yield. Linear regression model was used where independent variables were emergence index and NDVI, and dependent variable was biomass yield.

# CHAPTER 4. RESULTS AND DISCUSSION

## 4.1. Growth chamber seed germination and vigor index

Sorghum genotypes varied in germination rate and vigor index at 24, 12, and 10℃ (Table 1). The ranges of germination rate were 52 to 100% at 24℃, 32.7 to 100% at 12℃, and 6.7 to 99.3% at 10℃ (Table 1). Germination rates were higher at 24℃, as that is the optimum temperature for germination of forage sorghum (Table 1). The range of corrected vigor indices were 5.2 to 25.0 at 12℃, and 0.7 to 17.8 at 10℃ (Table 1). Based on the corrected vigor index at 12℃ and 10℃, genotypes were ranked from highest to lowest (Table 1). At 12℃, 48 genotypes had the same corrected vigor index ($P \leq 0.05$) as the highest ranked genotype, showing limited discrimination on the ability of genotypes to germinate at cold temperature. In the second year of the study, the temperature was decreased to 10℃ to exert more cold stress on genotypes. The results produced at 10℃ indicated that only eight genotypes had the same corrected vigor index ($P \leq 0.05$) as he highest ranked genotype. Only six genotypes ranked among the top 11 genotypes at both temperatures, 12℃ and 10℃ (Table 2). Temperatures below 15℃ are considered a cold stress condition in sorghum, which drastically reduces the seed germination rate and seedling growth (Peacock, 1982; Razmi et al., 2013). Razmi et al. (2013) observed 50% germination reduction, vigor index reduction, and 10.5 days delay in germination at 11/8℃ (day/light) compared with a 25/22℃ regime in three sorghum genotypes conducted under growth chamber conditions.

## 4.2. Field experiment

The initial soil test report indicated that K and P in the soil was above critical levels for corn in high smectite soils (> 200 mg $kg^{-1}$ of K and > 16 mg $kg^{-1}$ of P) in the experimental sites at Fargo and Hickson in 2017 and 2018. Nitrogen and P content were higher at the Fargo site

compared with the Hickson site. Soil pH was neutral to alkaline condition in the all-experimental plots (Table 3).

Table 1. Mean seed germination and corrected vigor index of forage sorghum genotypes evaluated at different temperatures in controlled environment growth chambers in 2017 and 2018.

| Genotypes | Sorghum type | Fungicide-treated seed | Germination (%) | | | Corrected vigor index | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 24°C | 12°C | 10°C | 12°C | Rank$_{12°C}$ | 10°C | Rank$_{10°C}$ |
| SPX-901 | FSH | Y | 96.7 | 99.3 | 97.3 | **25.1**[†] | **1** | 16.2 | 4 |
| CHR-FS4 | FS | Y | 96.0 | 97.3 | 96.7 | 25.0 | 2 | 14.9 | 14 |
| BTx623 | GS | N | 98.7 | 98.7 | 82.7 | 25.0 | 3 | 10.6 | 45 |
| Sordan Headless | SxS | Y | 97.3 | 98.7 | 94.7 | 24.9 | 4 | 15.7 | 8 |
| Pampa Triunfo XLT | SxS | Y | 94.7 | 97.3 | 95.3 | 24.6 | 5 | **17.8** | **1** |
| NK300 | FSH | Y | 98.0 | 98.7 | 98.7 | 24.4 | 6 | 16.6 | 2 |
| SPX3952 | SxS | Y | 94.0 | 96.0 | 90.7 | 24.4 | 7 | 15.0 | 13 |
| Hay King | Su | Y | 100.0 | 100.0 | 98.0 | 24.4 | 8 | 14.8 | 16 |
| SC265 | GS | N | 99.3 | 98.7 | 53.3 | 24.2 | 9 | 5.4 | 64 |
| Pampa Verde BMR 6 | SxS | Y | 98.0 | 96.7 | 96.7 | 24.2 | 10 | 16.2 | 5 |
| 1990 | FSH | Y | 99.3 | 97.3 | 98.0 | 24.0 | 11 | 16.5 | 3 |
| CHR-FS3 | FS | Y | 99.3 | 99.3 | 96.7 | 23.9 | 12 | 12.3 | 30 |
| 54126 | SW | Y | 90.0 | 94.0 | 86.7 | 23.9 | 13 | 12.2 | 31 |
| SPX-28313 | FSH | Y | 91.3 | 94.0 | 92.0 | 23.9 | 14 | 15.0 | 12 |
| CHR-SS2 | SxS | Y | 95.3 | 96.0 | 86.0 | 23.9 | 15 | 11.5 | 40 |
| X94Z | FS | Y | 96.0 | 96.7 | 94.7 | 23.8 | 16 | 11.7 | 38 |
| Hong Ke Zi | GS | N | 84.0 | 88.7 | 73.3 | 23.8 | 17 | 7.0 | 59 |
| SPX 902 | FSH | Y | 97.3 | 95.3 | 92.0 | 23.8 | 18 | 12.9 | 24 |
| SDH2942 BMR | SxS | Y | 100.0 | 98.0 | 90.0 | 23.6 | 19 | 10.5 | 47 |
| Niu Sheng Zui | GS | N | 87.3 | 92.7 | 80.7 | 23.6 | 20 | 9.2 | 50 |
| SPX 903 | FSH | Y | 95.3 | 96.7 | 90.6 | 23.5 | 21 | 12.1 | 32 |
| Sweetie BMR | SW | Y | 96.0 | 96.0 | 99.3 | 23.5 | 22 | **15.2** | **9** |
| Shan Qui Red | GS | Y | 98.0 | 99.3 | 70.7 | 23.5 | 23 | 6.6 | 61 |
| 36126 | SW | Y | 89.3 | 91.3 | 84.7 | 23.4 | 24 | 13.2 | 23 |
| Green Treat 128 | SxS | Y | 98.7 | 95.3 | 96.7 | 23.4 | 25 | 14.7 | 17 |
| Brachytic sorghum | FS | Y | 97.3 | 96.0 | 86.7 | 23.4 | 26 | 12.1 | 33 |
| SPX 904 | FSH | Y | 92.7 | 88.7 | 87.3 | 23.3 | 27 | 16.0 | 7 |
| SPX-3402 | FS | Y | 97.3 | 96.7 | 93.3 | 23.3 | 28 | 15.0 | 11 |
| Pampa Karamelo | SW | Y | 94.0 | 95.3 | 90.0 | 23.2 | 29 | 12.0 | 34 |
| 36111 | SW | Y | 93.3 | 90.0 | 90.7 | 23.2 | 30 | 14.2 | 19 |
| CHR-FS9 | FS | Y | 98.7 | 96.0 | 98.0 | 23.0 | 31 | 15.1 | 10 |
| Honey Sweet | SxS | Y | 97.3 | 96.7 | 97.3 | 22.9 | 32 | 12.8 | 26 |
| BMR 105 | FS | Y | 97.3 | 98.0 | 96.6 | 22.9 | 33 | 13.2 | 22 |
| SS405 | FSH | Y | 90.7 | 92.0 | 85.3 | 22.9 | 34 | 12.5 | 28 |
| SCI 1345 | GS | N | 94.7 | 98.0 | 87.9 | 22.8 | 35 | 10.5 | 46 |
| Greentreat Dynamo | SxS | Y | 99.3 | 98.7 | 98.0 | 22.5 | 36 | 12.8 | 25 |
| Kaoliang | GS | N | 98.0 | 96.0 | 85.3 | 22.5 | 37 | 8.1 | 54 |

Table 1. Mean seed germination and corrected vigor index of forage sorghum genotypes evaluated at different temperatures in controlled environment growth chambers in 2017 and 2018 (continued).

| Genotypes | Sorghum type | Fungicide-treated seed | Germination (%) | | | Corrected vigor index | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 24°C | 12°C | 10°C | 12°C | $Rank_{12°C}$ | 10°C | $Rank_{10°C}$ |
| Pampa Centurion | FS | Y | 91.3 | 93.3 | 83.3 | 22.5 | 38 | 11.5 | 41 |
| AL 31 BMR | SxS | Y | 80.7 | 84.0 | 74.0 | 22.4 | 39 | 11.8 | 37 |
| BMR 106 | FS | Y | 96.7 | 91.3 | 89.3 | 22.4 | 40 | 13.5 | 21 |
| Trudan Headless | SxS | Y | 94.7 | 94.0 | 81.3 | 22.4 | 41 | 11.1 | 43 |
| CHR-SG1 | Su | Y | 94.7 | 95.3 | 82.0 | 22.4 | 42 | 12.0 | 36 |
| BMR-90 | FS | Y | 99.3 | 94.7 | 98.7 | 22.3 | 43 | 16.1 | 6 |
| Green Treat Plus | SxS | Y | 92.0 | 87.3 | 74.7 | 22.3 | 44 | 12.7 | 27 |
| XAL 53 | SxS | N | 91.3 | 84.7 | 61.3 | 22.3 | 45 | 10.4 | 48 |
| SD-1741 | SxS | Y | 98.7 | 95.3 | 97.3 | 22.2 | 46 | 14.4 | 18 |
| Topper | SW | N | 93.3 | 86.0 | 68.0 | 22.1 | 47 | 7.7 | 55 |
| Sweet Thing BMR | SxS | Y | 98.7 | 96.7 | 96.7 | 22.0 | 48 | 12.4 | 29 |
| Pampa Verde Pacas | SxS | Y | 96.7 | 94.7 | 84.0 | **22.0** | **49** | 8.6 | 52 |
| BMR 108 | FS | Y | 98.7 | 96.7 | 91.3 | 21.3 | 50 | 11.2 | 42 |
| Top 76-6 | SW | N | 89.3 | 89.3 | 76.7 | 21.3 | 51 | 8.8 | 51 |
| PI 453014 | GS | N | 87.3 | 82.7 | 63.3 | 20.9 | 52 | 8..2 | 53 |
| SX17 | SxS | Y | 89.3 | 81.3 | 40.0 | 20.6 | 53 | 5.2 | 65 |
| Nutri Plus | SxS | N | 83.9 | 84.0 | 50.0 | 20.7 | 54 | 7.2 | 58 |
| 59-09 | FS | Y | 98.7 | 90.7 | 94.7 | 20.4 | 55 | 14.1 | 20 |
| 56111 | SW | Y | 94.7 | 89.3 | 84.0 | 20.3 | 56 | 11.6 | 39 |
| Greentreat A + | SxS | Y | 92.7 | 94.7 | 80.7 | 20.2 | 57 | 10.0 | 49 |
| Theis | SW | N | 90.6 | 91.3 | 70.7 | 19.9 | 58 | 6.8 | 60 |
| Sweet Thing | SxS | Y | 90.7 | 87.3 | 86.7 | 19.8 | 59 | 11.1 | 44 |
| SPX 3903 | FSH | Y | 90.7 | 86.6 | 88.7 | 19.5 | 60 | 14.8 | 15 |
| RTx430 | GS | N | 90.7 | 84.0 | 56.7 | 19.1 | 61 | 7.6 | 56 |
| SS M81-E | SW | N | 93.3 | 91.2 | 26.0 | 18.5 | 62 | 2.5 | 70 |
| PI 452841 | GS | N | 86.7 | 76.7 | 49.3 | 18.4 | 63 | 7.4 | 57 |
| Piper (1) | Su | N | 92.7 | 90.0 | 52.0 | 16.2 | 64 | 4.5 | 67 |
| Dale | SW | N | 94.0 | 96.0 | 64.0 | 16.0 | 65 | 5.7 | 63 |
| Special Effort | SxS | N | 71.7 | 63.3 | 30.7 | 15.2 | 66 | 3.8 | 69 |
| Pampa Mijo 11 | PM | Y | 91.3 | 81.3 | 6.7 | 15.0 | 67 | 0.7 | 72 |
| Pacesetter BMR | FS | Y | 94.7 | 92.0 | 87.3 | 14.6 | 68 | 12.0 | 35 |
| FS-5 | FS | Y | 90.0 | 81.3 | 42.0 | 14.6 | 69 | 4.3 | 68 |
| Piper (2) | Su | Y | 98.0 | 92.7 | 60.0 | 14.0 | 70 | 4.9 | 66 |
| Enorma | Su | N | 92.6 | 71.3 | 72.0 | 11.0 | 71 | 6.3 | 62 |
| Forage King | Su | N | 52.0 | 32.7 | 6.7 | 5.2 | 72 | 0.8 | 71 |
| LSD (0.05) | | | 6.3 | 8.1 | 11.5 | 3.5 | | 2.6 | |

FS=forage sorghum, FSH = forage sorghum hybrid, Su= sudangrass, SxS= sorghum x sudangrass, SW= sweet sorghum, PM= pear millet, GS= grain sorghum. BMR=Brown -midrib

†Values in bold in the table indicate the range of values not significantly different from the highest ranked value within a same column.

Table 2. Selected genotypes for field trials based on rank of corrected vigor index at 12℃ in 2017 and 10℃ in 2018.

| Year | Genotypes | $Rank_{12°C}$ | $Rank_{10°C}$ |
|---|---|---|---|
| 2017 and 2018 | Sweetie BMR | 22 | 9 |
| | Pampa Triunfo XLT | 5 | 1 |
| | Forage King | 72 | 71 |
| | Hay King | 8 | 16 |
| | SPX-901 | 1 | 4 |
| | 1990 | 11 | 3 |
| 2017 | Brachytic Sorghum | 26 | 33 |
| | Green Treat 128 | 25 | 17 |
| | 54126 | 13 | 31 |
| | BTx 623 | 3 | 45 |
| | SC265 | 9 | 64 |
| 2018 | Pampa Verde BMR-6 | 10 | 5 |
| | Sordan Headless | 4 | 8 |
| | NK300 | 6 | 2 |
| | BMR-90 | 43 | 6 |
| | Niu Sheng Zui | 20 | 50 |
| | Kaoliang | 37 | 54 |

Table 3. Soil analysis before seeding at Fargo and Hickson in 2017 and 2018.

| Environment | Soil depth | $NO_3$-N | P | K | pH | OM |
|---|---|---|---|---|---|---|
| | cm | kg $NO_3$-N $ha^{-1}$ | ……mg $kg^{-1}$….. | | | mg $kg^{-1}$ |
| 2017 | | | | | | |
| Fargo | 0-15 | 58 | 36 | 348 | 7.50 | 67 |
| | 15-60 | 108 | | | | |
| Hickson | 0-15 | 18 | 6 | 380 | 7.80 | 59 |
| | 15-60 | 30 | | | | |
| 2018 | | | | | | |
| Fargo | 0-15 | 47 | 18 | 324 | 7.48 | 71 |
| | 15-60 | 81 | | | | |
| Hickson | 0-15 | 22 | 12 | 330 | 7.40 | 55 |
| | 15-60 | 20 | | | | |

4.2.1. Soil temperature and soil water content from planting to emergence

Soil temperature at emergence was lower for early-seeded genotypes than genotypes seeded two weeks later (Fig. 2). In 2018, soil temperature was higher than in 2017 during emergence. In 2018, fluctuation of day and night temperature was greater at the planting. Though

soil temperature was above the base temperature for sorghum (15℃) at planting, at the Fargo site in 2018, emergence did not occur, as soil water content was very low (< 200 g $kg^{-1}$). Early-seeded genotypes were exposed to cold temperatures (below base temperature) at the beginning of the season for twelve days in 2017 and only six days in 2018, while late-seeded genotypes were exposed to temperatures below base temperature for only two days in 2017 (Fig. 2). For the late-seeded genotypes, soil temperature was above the base temperature at seeding in both 2017 and 2018 (Fig. 2).

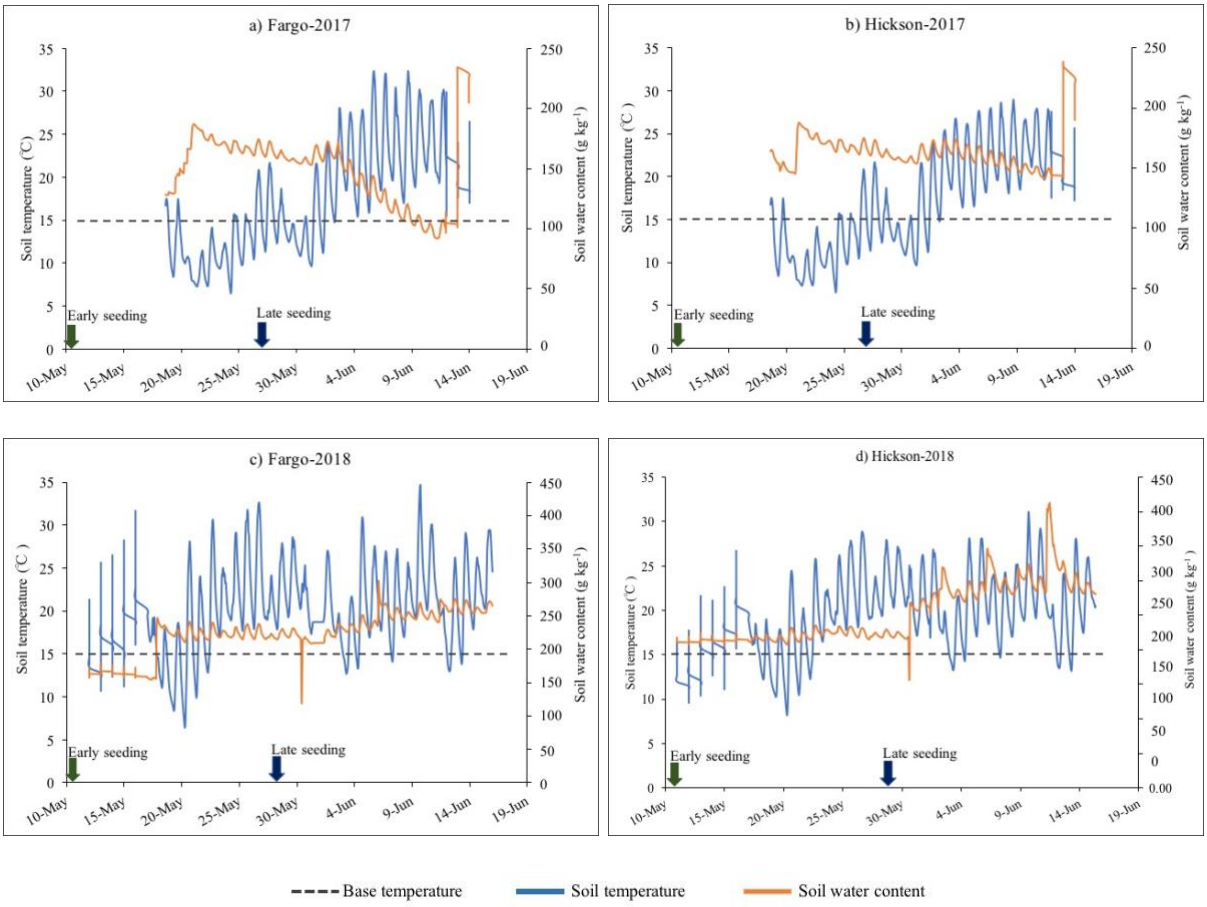


**Figure 2.** Soil temperature and soil water content from planting to the end of emergence at four environments at Fargo and Hickson, ND, in 2017 and 2018.

4.2.2. Air temperature and rainfall

Average air temperature was lower at seeding time in 2017 compared with 2018. At emergence period, average air temperature was below 15℃ for twelve days in 2017 and five days in 2018. At the end of the season, average air temperature decreased below 15℃ on 23 September in 2017 and 17 September in 2018 (Fig. 1). There was less rainfall in 2017 compared with 2018. From seeding to final harvesting total rainfall was 246 mm at Fargo, and 284 mm at Hickson in 2017 and 405 mm at Fargo, and 425 mm at Hickson in 2018 (NDAWN, 2018).

4.2.3. Emergence index

Genotypes emergence index varied significantly. The seeding date main effect and the interaction of genotypes by seeding date were significant for emergence index *(P* ≤0.05) (Table 3). Emergence index was much greater for the second seeding date than the first seeding date (Fig. 3a), as expected, since soil temperatures were greater (Fig. 2). The genotypes Sordan Headless, NK300, and Hay King emerged faster in the early-seeding date compared with the other genotypes. The faster emergence at lower temperatures indicates these genotypes are able to emerge in colder soils, which was the objective of this study. Despite the lower emergence index of the first seeding date compared with the second seeding date, the first seeding date produced better growth and development as indicated by the greater canopy coverage.

Typically, forage sorghum should be planted once the average 10-d soil temperature (0-15-cm in depth) is >15℃ (Marsalis, 2006). Temperature below 15℃ inhibits seedling emergence and decreases emergence rate in forage sorghum (Pinthus and Rosenblum, 1961; Singh, 1985; Marsalis, 2006). Kapanigowda et al. (2013) found significant variation in early-emergence index and emergence percentage. These research findings supported the results in our current study,

observing lower emergence index in the earlier seeding. Thus, field emergence index is an efficient way of screening forage sorghum genotypes for chilling tolerance.

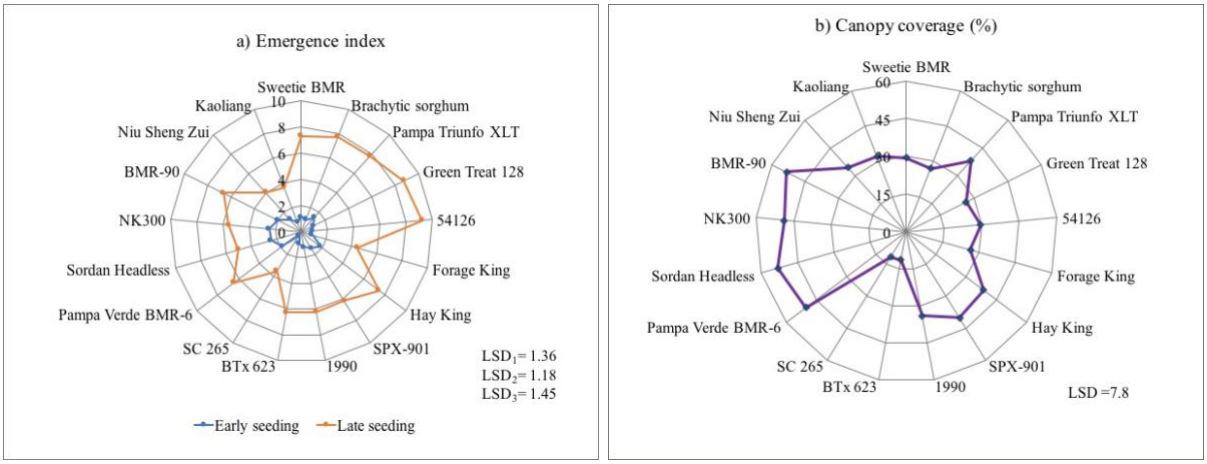


**Figure 3.** a) Mean emergence index at early and late seeding, and b) mean canopy coverage at 46 DAS in forage sorghum genotypes averaged across two seeding dates and four environments in Fargo and Hickson, ND, in 2017 and 2018. ($LSD_1$= to compare among genotypes within the same seeding date, $LSD_2$= to compare same genotype between different seeding date, $LSD_3$= to compare among genotypes on different seeding dates).

4.2.4. Canopy coverage at 46 days after the early-seeding date (DAS)

Canopy coverage at 46 DAS or 30 days after the late seeding was significant for both the seeding date and genotype main effects ($P \leq 0.05$). No interaction between seeding date and genotype was observed (Table 4). The genotypes Sordan Headless, Pampa Verde BMR-6, NK300, BMR-90, and SPX-901 had higher canopy coverage at 46 DAS (Fig. 3). These results indicate that those genotypes were performing better early in the season as greater canopy coverage at earlier stages of development indicates rapid growth. Chung et al. (2017) measured canopy coverage and found a significant correlation between plant height and percentage of green color of four different sorghum genotypes in field condition. It has been reported that sorghum growth rate is reduced if exposed to cold condition (<15℃), especially early in the season (Pinthus and Rosenblum, 1961; Singh, 1985). Therefore, genotypes that perform better early in the season are potential genetic sources for the chilling tolerance trait.

Table 4. Combined analysis of variance and mean square values for emergence index (EI), canopy coverage, stand establishment, and seed mortality in forage sorghum genotypes (G) planted on two seeding dates (SD) at four environments (Env) in Fargo and Hickson, ND, in 2017and 2018.

| SOV | df | Emergence index | Canopy coverage | Stand establishment | Seed mortality |
|---|---|---|---|---|---|
| Env | 3 | 36.8* | 9629* | 7191442199* | 1988* |
| Rep(env) | 12 | 10.0* | 736* | 3948330526* | 1092* |
| SD | 1 | 2183.8* | 47199* | 34741414396 | 9604 |
| Env x SD | 3 | 76.8* | 2698* | 6479096519 | 1791 |
| Env x rep x SD | 12 | 10.0* | 385* | 4734974747* | 1309* |
| G | 16 | 17.2* | 1121* | 10034580177* | 2774* |
| Env x G | 26 | 3.4* | 122* | 1887739575 | 522 |
| SD x G | 16 | 9.0* | 136 | 3301141624* | 913* |
| Env x SD x G | 26 | 3.1 | 92 | 1487027324 | 411 |
| Residual | 252 | 2.2 | 74 | 1391976645 | 385 |
| CV, % | | 38.7 | 25 | 28 | 62 |

*Significant at $P \leq 0.05$ levels of probability.

4.2.5. Stand establishment

The genotype and seeding date main effects and the interaction between seeding date and genotypes were significant for stand establishment (Table 4). Lower stand establishment was observed among most of the early-seeded genotypes as compared with late-seeded genotypes. The interaction occurred because the brachytic sorghum, Hay King, SPX-901, and BTx623 genotypes showed similar stand establishment at both seeding dates while Sordan Headless and NK300 showed higher stand establishment at the early-seeding date. These results suggest that the genotypes Sordan Headless and NK300 likely have better ability to tolerate lower temperatures early in the growing season (Fig. 4).

Low stand establishment was observed in the chilling-tolerant check genotype- Kaoliang regardless of the seeding date. Chiluwal et al., (2018), and Maulana and Tesso (2013) also

reported lower emergence and lower vigor in another Chinese chilling-tolerant genotype-Shun Qui Red, compared with other sorghum lines in controlled and field condition. Although these lines contain genes for chilling tolerance, they do not exhibit many of the agronomic traits observed for commercial cultivars.

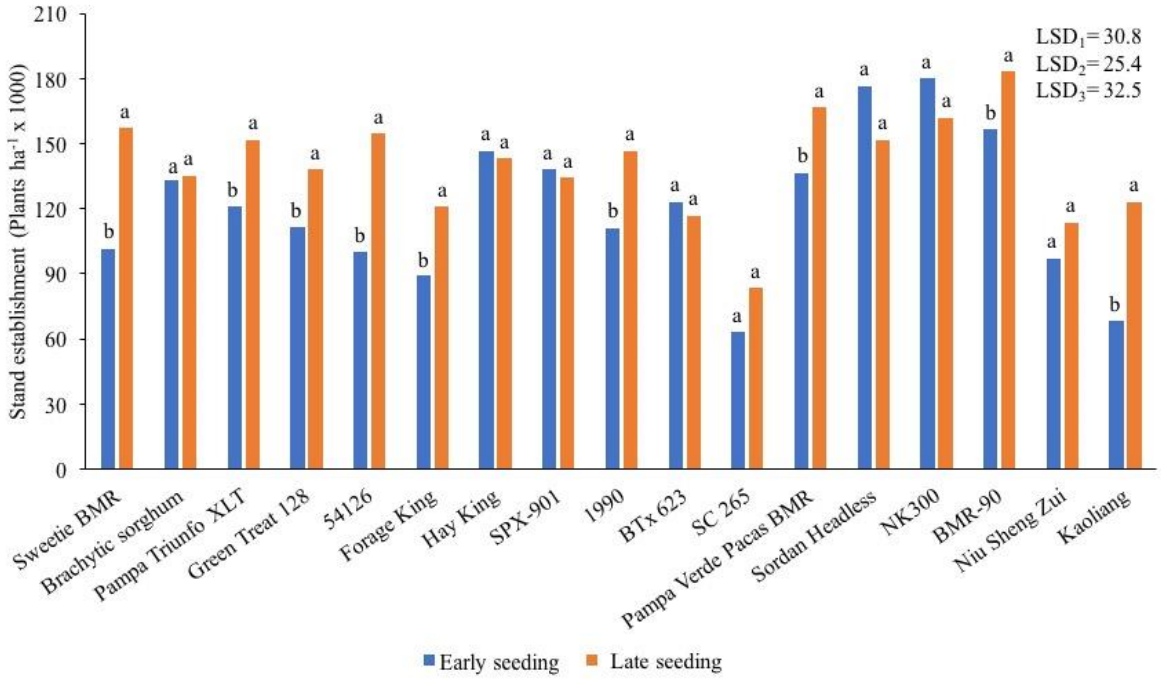


**Figure 4.** Mean stand establishment in forage sorghum genotypes planted on two dates averaged across four environments at 20 days after each planting in Fargo and Hickson, in 2017 and 2018. ($LSD_1$= to compare among genotypes within the same seeding date, $LSD_2$= to compare same genotypes between different seeding date, also indicated as small case letters for each genotype; $LSD_3$= to compare among genotypes on different seeding date).

Yield potentiality and profit from annual crops depend on emergence percentage and optimum stand establishment (Parera and Cantliffe, 1994; Pirasteh-Anosheh and Hamidi, 2013). However, a good stand establishment of forage sorghum is not always indicative of higher biomass yield and excessive plant density can lead to lodging, especially in low lignin BMR sorghum types (Marsalis, 2006). Low soil temperature (<15℃) significantly reduces sorghum stand establishment (Pinthus and Rosenblum, 1961; Singh, 1985), the early-seeded genotypes

that had increased or similar stand establishment, compared with late-seeded genotypes, could also be indicative of potential chilling tolerance.

4.2.6. Seedling mortality

Seedling mortality was significant for genotype and the interaction between genotype and seeding date. Higher mortality was observed at the early seeding compared with the late seeding for most genotypes. Sweetie BMR, Pampa Triunfo XLT, Green Treat 128, 54126, Forage King, 1990, SC 265, Niu Sheng Zui, Kaoliang had higher mortality percentage at the early seeding compared with the same genotypes seeded later. This indicates that cold soils imposed a stress in seedlings, increasing mortality. Even if seedling mortality was high when seeded early, the ability of the surviving plants to grow once temperatures increased is desired. Though Niu Sheng Zui and Kaoliang are widely known for their chilling tolerance, their seed mortality at the early-seeding date was 45-60% and not different than many other genotypes on the same seeding date. Seedling mortality in cold soils can also be due to pathogens. Soil temperature below 15℃ promotes susceptibility to molding and other pathogen infections like *Pythium* spp., *P. aphanidermatum, P. ultimum,* and *P. arrhenomanes* etc. that ultimately increases the chances for germination failure early in the season (Martin, 1992). The chilling-tolerant lines were not coated with fungicides as were most of the other commercial cultivars and hybrids tested. The genotypes NK300 and Sordan Headless had lower seed mortality than most genotypes (<25%) at both seeding dates. At early-seeding, seed mortality was not different from that of the late-seeding for these same genotypes. Interestingly, these two genotypes had the highest emergence index and highest stand establishment at early-seeding, which is explained with lower seed mortality. Generally, up to 65-70% seed emergence may occur during emerging period at optimum seeding time depending on field moisture (Marsalis, 2006).

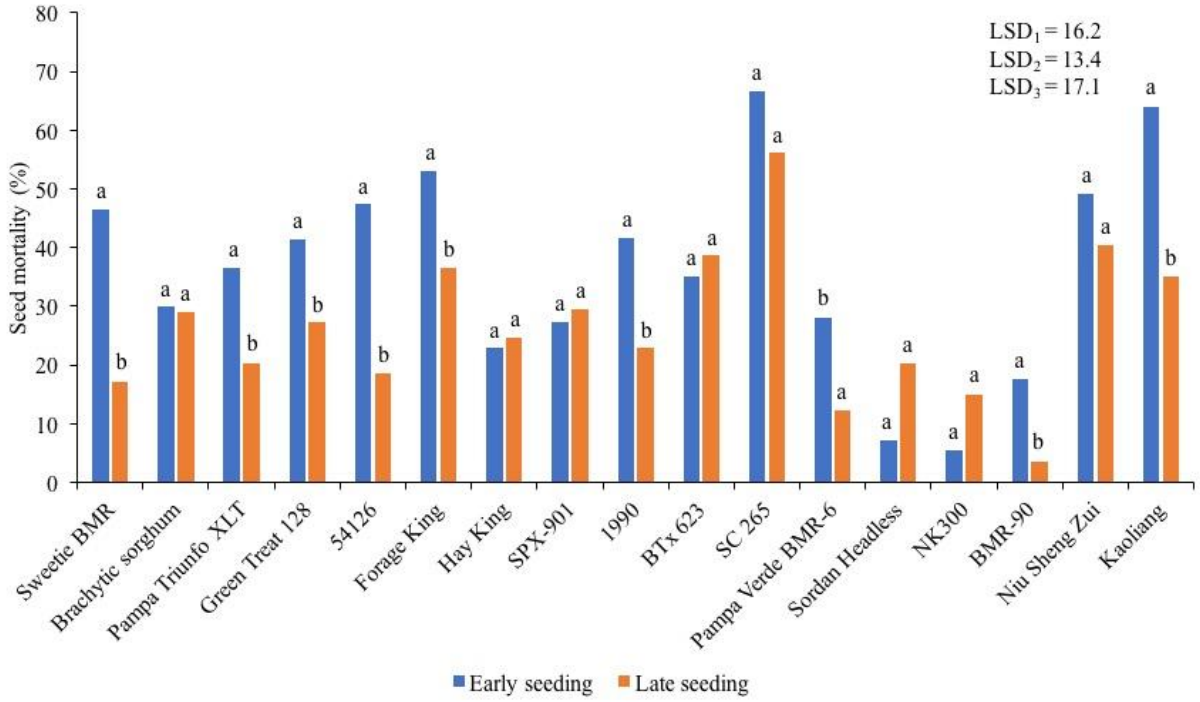


**Figure 5.** Mean seed/seedling mortality rate in forage sorghum genotypes planted on two seeding dates averaged across four environments at 20 days after each planting in Fargo and Hickson, in 2017 and 2018. ($LSD_1$= to compare among genotypes within the same seeding date, $LSD_2$= to compare same genotypes between different date and also different small case letters show significant differences, $LSD_3$= to compare among genotypes on different seeding date)

4.2.7. Normalized difference vegetation index (NDVI)

The genotype main effect was significant at all stages of evaluation ($P \leq 0.05$). The seeding date main effect for NDVI was significant only at 49 DAS. A significant interaction between genotype and seeding date for NDVI was observed at 35 DAS and 63 DAS (Table 4). A higher NDVI value indicated those genotypes had greater cover and likely were growing faster than the other genotypes seeded earlier, but genotype's plant architecture was not homogeneous, which will likely influence NDVI. At 49 DAS, some of the genotypes seeded on the second date had similar NDVI values to those seeded on the earlier seeding date.

The analysis indicated there were no significant differences between the seeding date main effect or the interaction between seeding date and genotype at 20 DAH (Table 5). The NDVI differences among genotypes were mainly due to the differences in plant height and

coverage (Fig. 5). The possible cause of this may be the genotypes seeded in the second date were younger than the early-seeded genotypes at the first harvest. These results indicate NDVI may be better for predicting differences among genotypes cover at the early vegetative stages than later stages of development.

In field conditions, aerial measurement of NDVI was found to be a potential tool to evaluate the phenotypic variation among sorghum genotypes for chilling response at 30 to 60 days after emergence (Chiluwal et al., 2018).

Table 5. Combined analysis of variance and mean square values for normalized difference vegetation index (NDVI) in forage sorghum genotypes (G) seeded on two dates (SD) and evaluated at 35, 42, 49, and 63 days after the first seeding date (DAS) and 20 days after first harvest (DAH) in four environments (Env), in Fargo and Hickson, ND, in 2017 and 2018.

| SOV | df | NDVI | | df | NDVI | | |
|---|---|---|---|---|---|---|---|
| | | 35 DAS† | 42 DAS | | 49 DAS | 63 DAS | 20 DAH†† |
| Env | 1 | 0.00750* | 0.124* | 3 | 0.768* | 0.255* | 0.185* |
| Rep(env) | 6 | 0.00150* | 0.017* | 12 | 0.035* | 0.030 | 0.011 |
| SD | 1 | 0.01400 | 0.081 | 1 | 1.948* | 0.00003 | 0.127 |
| Env x SD | 1 | 0.00012 | 0.005 | 3 | 0.202* | 0.002 | 0.024* |
| Env x rep x SD | 6 | 0.00040* | 0.006* | 12 | 0.014* | 0.025 | 0.006* |
| G | 10 | 0.00420* | 0.024* | 16 | 0.103* | 0.028* | 0.070* |
| Env x G | 10 | 0.00170 | 0.003* | 26 | 0.008* | 0.013 | 0.001* |
| SD x G | 10 | 0.0004* | 0.002 | 16 | 0.014 | 0.037* | 0.001 |
| Env x SD x G | 10 | 0.00005 | 0.009 | 26 | 0.007* | 0.014 | 0.003* |
| Residual | 120 | 0.00011 | 0.001 | 252 | 0.004 | 0.025 | 0.002 |
| CV, % | | 7.33 | 14.89 | | 14.3 | 21.72 | 5.85 |

* Significant at $P \leq 0.05$ levels of probability. Environment was a random effect.

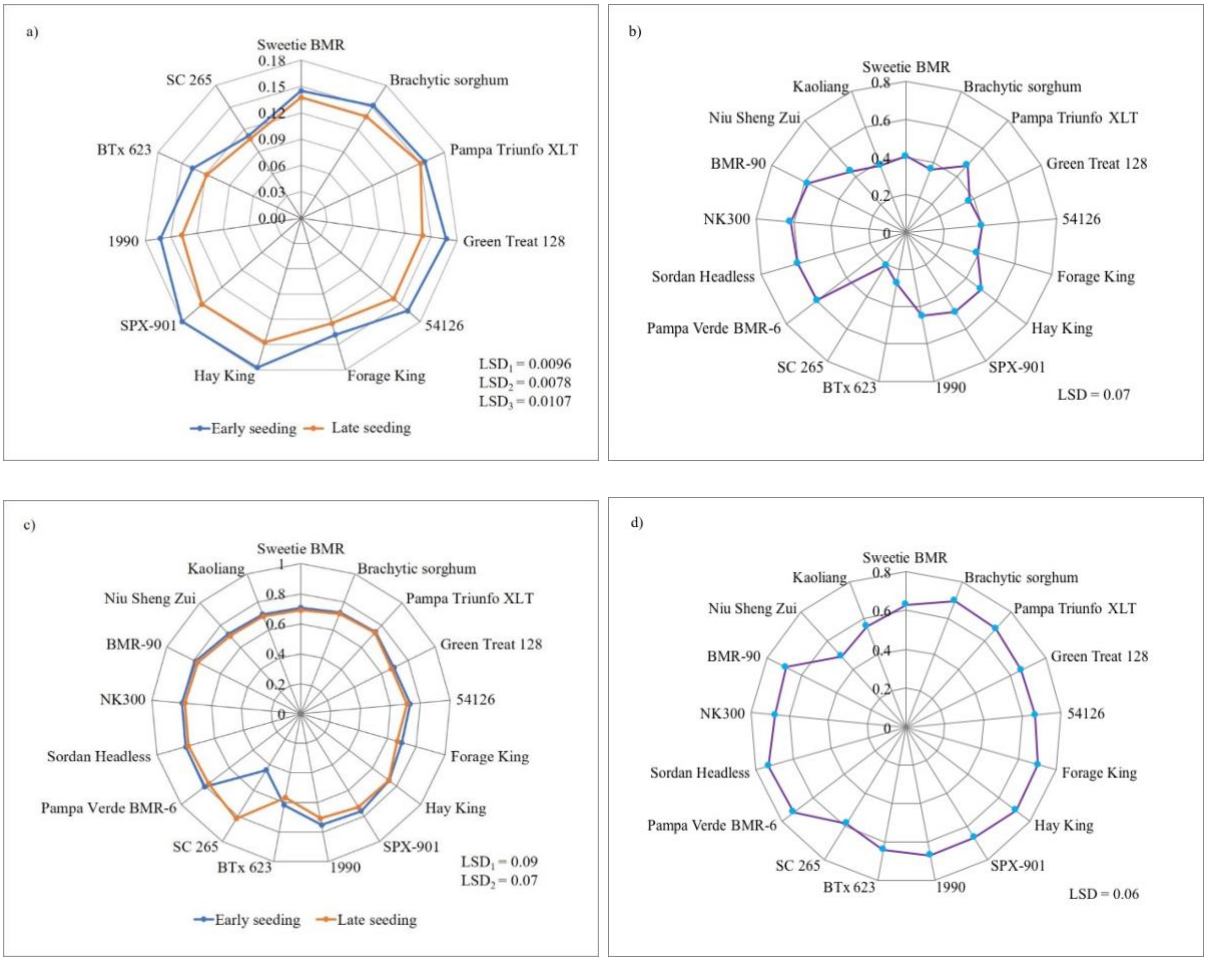


**Figure 6.** Mean normalized difference vegetation index (NDVI) evaluated at a) 35 DAS, b) 49 DAS, c) 63 DAS, and d) 20 days after first harvest (DAH) in forage sorghum genotypes averaged across four environments, Fargo and Hickson, ND, in 2017 and 2018. ($LSD_1$= to compare among genotypes within the same seeding date, $LSD_2$= to compare same genotypes between different date, $LSD_3$= to compare among genotypes on different seeding date)

4.2.8. Leaf chlorophyll content

Though genotypes varied for leaf chlorophyll content, chlorophyll content between seeding dates or the interaction between seeding dates and genotypes was not significant. Leaf chlorophyll content was not a good indicator of growth rate or soil coverage as shown for NDVI (Table 6). It was expected chilling-tolerant genotypes would have higher chlorophyll content than non-tolerant genotypes at the first sampling date, but this did not occur (Fig. 6). However, chilling stress, conducted under controlled environments, was reported to produce less

chlorophyll in leaves of sorghum than in non-stressed leaves (Maulana and Tesso, 2013). The lack of differences in chlorophyll content was probably due to temperature fluctuation and other environmental conditions in the field. Temperature was above base temperature (15℃) at the stage of measurement and it is likely plants were not exposed to enough chilling stress to show a response in chlorophyll content. In addition, sorghum plants can overcome earlier chilling-induced reduction of chlorophyll content when reaching favorable temperature (Maulana and Tesso, 2013).

Table 6. Combined analysis of variance and mean square values for leaf chlorophyll content (CHL) in forage sorghum genotypes (G) seeded on two dates (SD) and evaluated at 36, 42, 49, and 63 days after the first seeding date (DAS) at four environments (Env), in Fargo and Hickson, ND, in 2017 and 2018.

| SOV | df | CHL | df | CHL | df | CHL | df | CHL |
|---|---|---|---|---|---|---|---|---|
| | | 36 DAS† | | 42 DAS | | 49 DAS | | 63 DAS |
| Env | 3 | 105.5* | 1 | 230.9* | 3 | 1220.1* | 1 | 65.3* |
| Rep(env) | 12 | 15.1* | 6 | 28.8* | 12 | 31.7* | 6 | 20.4* |
| SD | 1 | 328.9 | 1 | 26.3 | 1 | 244.9 | 1 | 0.8 |
| Env x SD | 1 | 26.9 | 1 | 27.1 | 3 | 62.9* | 1 | 3.2 |
| Env x rep x SD | 6 | 25.3* | 6 | 10.2 | 12 | 9.6 | 6 | 9.3 |
| G | 16 | 26.5* | 10 | 30.6* | 16 | 33.6* | 10 | 102.7* |
| Env x G | 26 | 3.8 | 10 | 3.3 | 26 | 12.8 | 10 | 15.3 |
| SD x G | 10 | 13.1 | 10 | 13.8 | 16 | 8.7 | 10 | 24.1 |
| Env x SD x G | 10 | 6.5 | 10 | 16.1 | 26 | 8.7 | 10 | 8.4 |
| Residual | 186 | 7.1 | 120 | 8.7 | 252 | 7.8 | 120 | 8.9 |
| CV, % | | 8.7 | | 9.6 | | 6.93 | | 7.92 |

*Significant at $P \leq 0.05$ level of probability.
† Days after early seeding (DAS)

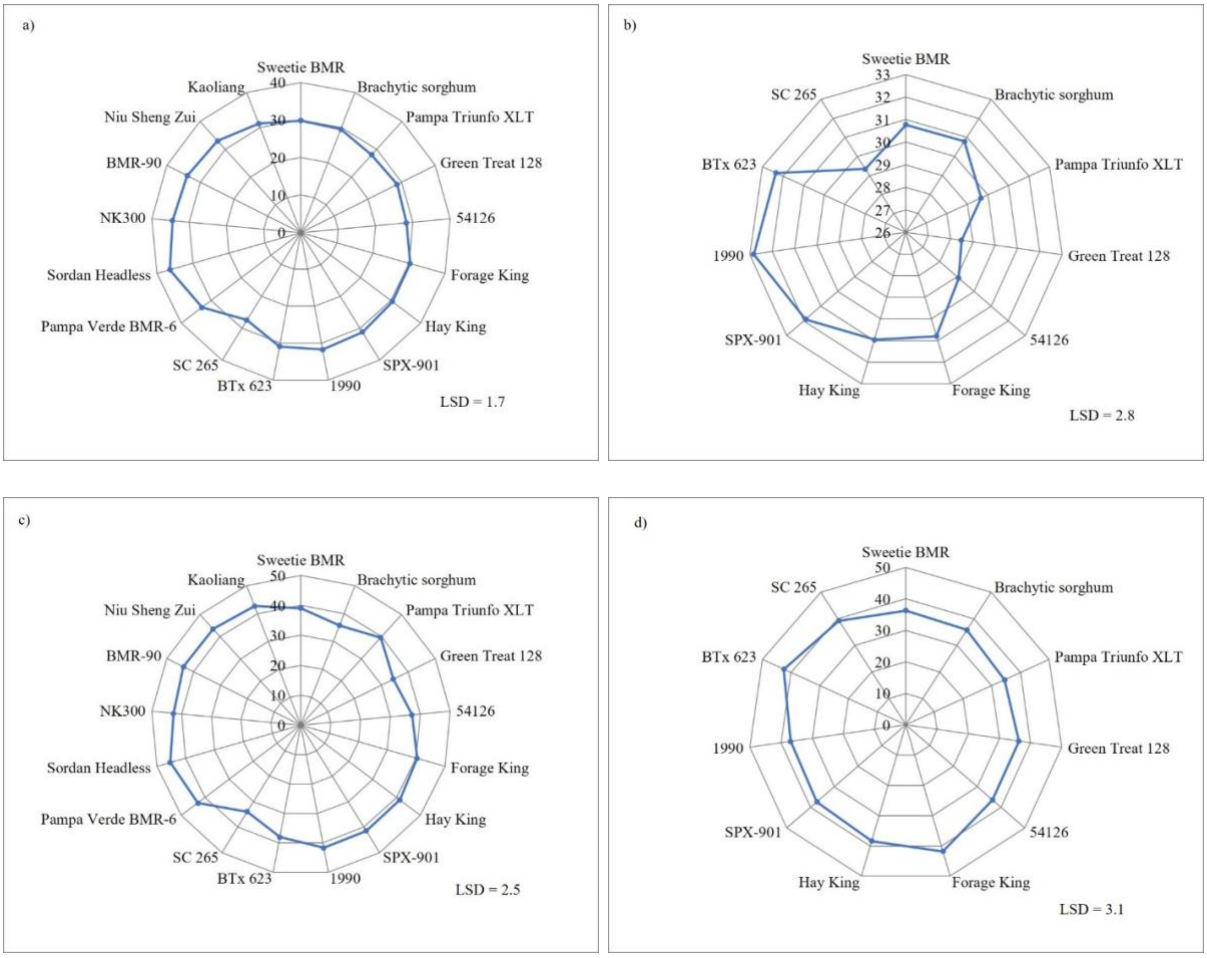


**Figure 7.** Mean leaf chlorophyll content at a) 36 DAS, b) 42 DAS, c) 49 DAS, and d) 63 DAS in forage sorghum genotypes averaged across two seeding dates and four environments, in Fargo and Hickson, ND, in 2017 and 2018.

4.2.9. Intercepted photosynthetically active radiation (IPAR)

Seeding date main effect was significant for IPAR at 58 DAS and at 30 DAH only and genotype main effect was significant ($P \leq 0.05$) at all sampling dates (Table 7). No interaction was observed between the genotypes and seeding dates at any sampling date. Before the first harvest, IPAR ranged between 25 to 84% at 58 DAS and 78 to 95% at 68 DAS. Sordan Headless, NK300, Pampa Verde BMR-6, and BMR-90 had higher IPAR at 58 DAS than the other genotypes, indicating greater growth and canopy. Kaoliang and Niu Sheng Zui showed had lower IPAR as they had reduced tiller number and low stand establishment compared with other

genotypes. After the first harvest, IPAR ranged between 39 to 81% at 30 DAH and 77 to 95% at 45 DAH (Fig. 7). In later growing stages, IPAR was similar for all genotypes as ground was covered by a high number of tiller regrowth after the first harvest. These results suggest that IPAR predicted differences of genotypes growth at early vegetative stages better than at later growth stages. Maughan et al. (2012) found similar results with forage sorghum reaching up to 95% IPAR in central and southern Illinois and Meki et al. (2017) reported over 90% IPAR in forage sorghum in a field trial at Temple, TX.

Table 7. Combined analysis of variance and mean square values for intercepted photosynthetically active radiation (IPAR) at 58 and 68 days after the first seeding date (DAS), at 30 and 45 days after the first harvest (DAH), and for plant height at two harvest dates in forage sorghum genotypes seeded on two dates (SD) and four environments, at Fargo and Hickson, ND, in 2017 and 2018.

| SOV | df | IPAR | df | IPAR | df | IPAR | | Plant height | |
|---|---|---|---|---|---|---|---|---|---|
| | | 58 DAS | | 68 DAS | | 30 DAH | 45 DAH | H-1 | H-2 |
| Env | 3 | 41359* | 1 | 3177* | 3 | 6356* | 468* | 2.39* | 3.16* |
| Rep(env) | 12 | 1522* | 6 | 328* | 12 | 396* | 82* | 0.10* | 0.04* |
| SD | 1 | 16586* | 1 | 47 | 1 | 2219* | 672 | 4.43* | 0.97* |
| Env x SD | 3 | 492 | 1 | 77 | 3 | 222 | 116 | 0.44* | 0.08 |
| Env x rep x SD | 12 | 335* | 6 | 64 | 12 | 105* | 37* | 0.03* | 0.04* |
| G | 16 | 1564* | 10 | 553* | 16 | 1836* | 357* | 0.7* | 1.79* |
| Env x G | 26 | 177* | 10 | 64 | 26 | 169* | 41* | 0.03* | 0.06* |
| SD x G | 16 | 81 | 10 | 43 | 16 | 54 | 32 | 0.01 | 0.02 |
| Env x SD x G | 26 | 116* | 10 | 54 | 26 | 39 | 17 | 0.01 | 0.02 |
| Residual | 252 | 69 | 120 | 74 | 252 | 58 | 15 | 0.01 | 0.02 |
| CV, % | | 14 | | 10 | | 10 | 4 | 6.52 | 7.10 |

*, Significant at $P \leq 0.05$ level of probability.
† Days after early seeding (DAS), †† Days after first harvesting (DAH)

4.2.10. Plant height

Plant height at both harvests were significant among genotypes and seeding dates. However, no interaction was observed for the genotype by seeding date interaction (Table 8). Plant height was greater for all genotypes in the second harvest than first harvest (Fig. 9). Late-seeded genotypes were shorter at first harvest as they had less time to grow compared with early-

seeded genotypes. After the first harvest, 15-cm of stalk was left for regrowth. As the plants were already established, m it is likely that with high temperatures they were able to uptake more nutrients and water from soil, which made the plants grow taller at the second harvest, however nutrient and water uptake was not measured. The genotypes brachytic sorghum, BTx623, and Sc 265 are dwarf types (Fig. 9).

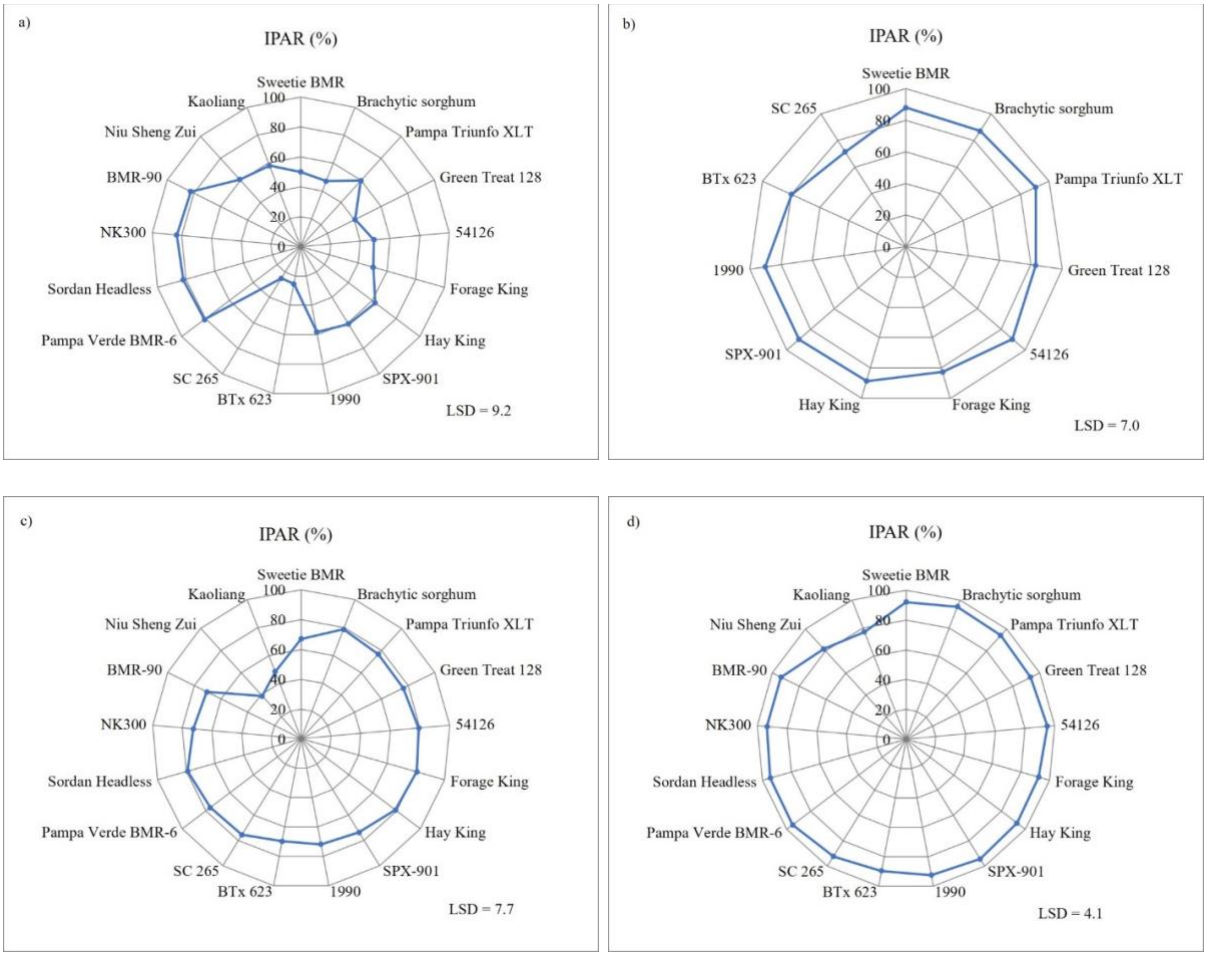


**Figure 8.** Mean intercepted photosynthetically active radiation (IPAR) evaluated at a) 56 DAS, b) 68 DAS, c) 30 DAH, and d) 45 DAH in forage sorghum genotypes averaged across two seeding dates and four environments, in Fargo and Hickson, ND, in 2017 and 2018.

4.2.11. Growth stage at harvest

Growth stage for each genotype at each harvest time is shown in Table 8. Except for Forage King all other genotypes were in the vegetative stage at the first harvest and most of the

genotypes were in the reproductive stages at the second harvest. Forage King was the earliest-maturing genotype compared with all other genotypes. Forage sorghum can provide the greatest dry biomass yield if harvested at hard dough stage (Pedersen and Rooney, 2004). Photoperiod sensitive genotypes- Sordan Headless, 1990, and Pampa Verde BMR-6, reached the reproductive stages later in the season when photoperiods reached less than 12 h and 20 min (McCollum, 2005), which happened after 19 September at Fargo (timeanddate.com).

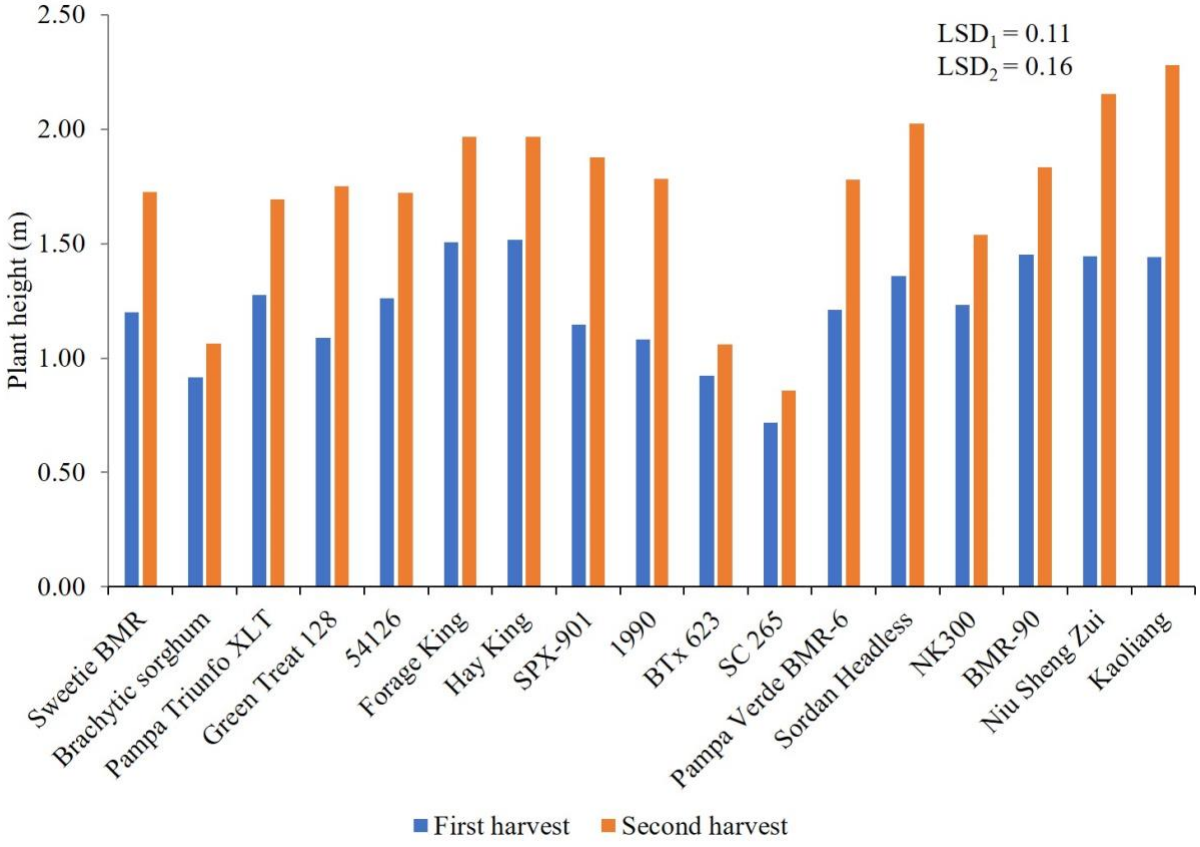


**Figure 9.** Mean plant height at each harvest in forage sorghum genotypes averaged across two seeding dates and four environments (env), Fargo and Hickson in 2017 and 2018. ($LSD_1$= to compare among genotypes within first harvest, $LSD_2$= to compare among genotypes within second harvest) ($P \leq 0.05$)

Table 8. Growing stages of forage sorghum genotypes at each harvest (H-1, H-2) seeded on two dates at four environments, Fargo and Hickson, ND, in 2017 and 2018.

| Genotypes | Photo-period sensitive | Growth stage | | | |
|---|---|---|---|---|---|
| | | 2017 | | 2018 | |
| | | H-1 | H-2 | H-1 | H-2 |
| | | Early seeding | | | |
| Sweetie BMR | N | 7 | 11.2 | 7 | 10 |
| Brachytic sorghum | N | 7 | 11.2 | . | . |
| Pampa Triunfo XLT | N | 7 | 9 | 8 | 10 |
| Green Treat 128 | Y | 6 | 9 | . | . |
| 54126 | N | 7 | 9 | . | . |
| Forage King | N | 10 | 11.2 | 9 | 11.2 |
| Hay King | N | 8 | 11.1 | 8 | 11.1 |
| SPX-901 | Y | 6 | 9 | 6 | 9 |
| 1990 | Y | 6 | 8 | 6 | 8 |
| BTx623 | N | 6 | 11.2 | . | . |
| SC 265 | N | 6 | 11.2 | . | . |
| Pampa Verde BMR-6 | Y | . | . | 8 | 8 |
| Sordan Headless | Y | . | . | 8 | 8 |
| NK300 | N | . | . | 8 | 11 |
| BMR-90 | N | . | . | 8 | 11 |
| Niu Sheng Zui | N | . | . | 8 | 11.2 |
| Kaoliang | N | . | . | 7 | 11.2 |
| | | Late -seeding | | | |
| Sweetie BMR | N | 6 | 11 | 6 | 10 |
| Brachytic sorghum | N | 6 | 11.2 | . | . |
| Pampa Triunfo XLT | N | 6 | 10 | 7 | 10 |
| Green Treat 128 | Y | 6 | 9 | . | . |
| 54126 | N | 6 | 9 | . | . |
| Forage King | N | 9 | 11.2 | 6 | 11.2 |
| Hay King | N | 7 | 10 | 6 | 11.1 |
| SPX-901 | Y | 6 | 9 | 6 | 9 |
| 1990 | Y | 6 | 9 | 6 | 9 |
| BTx623 | N | 6 | 11.2 | . | . |
| SC 265 | N | 6 | 11.2 | . | . |
| Pampa Verde BMR-6 | Y | . | . | 6 | 9 |
| Sordan Headless | Y | . | . | 6 | 8 |
| NK300 | N | . | . | 6 | 10 |
| BMR-90 | N | . | . | 6 | 10 |
| Niu Sheng Zui | N | . | . | 6 | 11.2 |
| Kaoliang | N | . | . | 6 | 11.2 |

Stages of sorghum growth: Stage 0: 0.0 planting; 0.1 start of imbibition; 0.5 radicle emergence from seed (caryopsis); 0.7 coleoptile emergence from seed (caryopsis); 0.9 leaf at coleoptile tip; Stage 1: emergence; Stage 2: first leaf visible; Stage 3: third leaf sheath visible; Stage 4: fifth leaf sheath visible; Stage 5: Panicle differentiation and start of tillering; 5.1 main shoot and one tiller; 5.9 main shoot and several tillers; Stage 6: stem elongation (late vegetative stage); Stage 7: flag leaf visible, whorl; Stage 8: booting (end of vegetative stage); Stage 9: panicle just showing, inflorescence emergence; Stage 10: anthesis (50% of panicle flowering); Stage 11: maturity; 11.1 grains at milk stage; 11.2 grains at early dough stage; 11.3 grains at late dough stage; 11.4 grains at physiological maturity (black layer, approximately 30% seed moisture); 11.5 mature grain (seed moisture approximately 15%). (Marsalis and Bean, 2010).

4.2.12. Accumulated growing degree days (AGDD)

Total accumulated growing degree days (AGDD) varied between the two seeding dates for the four environments. Total AGDD were greater in 2018 than in 2017. At first harvest, the early-seeding date had 20 and 78 AGDD more than the later-seeding date in 2017 and 2018, respectively. The second harvest was done once AGDD accumulation stopped (average daily temperature below 15℃). From the first to second harvest, AGDD were the same for both seeding dates as all genotypes were harvested at the same time (Table 9).

Table 9. Accumulated growing degree-days (GDD) with 15℃ and 10℃ base temperature, from seeding to first harvest (H-1), from H-1 to second harvest (H-2), and total from seeding to H-2 in forage sorghum seeded on two dates (SD) at four environments, Fargo and Hickson, ND, in 2017 and 2018.

| Environment | Accumulated GDD (base 15/10℃) | | |
|---|---|---|---|
| | H-1 | H-2 | Total |
| | Early seeding | | |
| 2017 | | | |
| Fargo | 357 /694 | 258 /548. | 615 /1243 |
| Hickson | 255 /550 | 250 /577 | 505 /1127 |
| 2018 | | | |
| Fargo | 404 /708 | 366 /713 | 770 /1421 |
| Hickson | 391 /700 | 310 /645 | 701 /1345 |
| | Late- seeding | | |
| 2017 | | | |
| Fargo | 332 /622 | 258 /548 | 590 /1170 |
| Hickson | 240 /497 | 250 /577 | 490 /1075 |
| 2018 | | | |
| Fargo | 323 /553 | 366 /713 | 689 /1266 |
| Hickson | 316 /550 | 310 /645 | 626 /1195 |

GDD base temperature for forage sorghum is 10℃ (Gerik et al., 2005). However, in this study GDD base temperature for forage sorghum was considered 15℃ as well to compare with 10℃.

4.2.13. Biomass yield

Genotype and seeding date main effects and their interaction were significant for biomass yield at each harvest. The interaction between genotypes and seeding dates occurred because genotypes had a different response to early-seeding (Table 10). The genotypes Sweetie BMR,

Brachytic sorghum, 54126, Green Treat 128, and BMR 90 produced less biomass yield in the early-seeding dates compared with late-seeding dates, which indicated that those genotypes were affected negatively by the conditions early in the season. The genotypes Pampa Triunfo XLT, Forage King, Hay King, SPX-901, 1990, SC 265, Pampa Verde BMR-6, NK300, and Niu Shen Zui biomass yield was the same for both seeding dates. The genotypes Sordan Headless, BTx623, and Kaoliang had greater biomass yield in early-seeding dates compared with late-seeding dates. However, BTx623 and Kaoliang had less seasonal biomass yield than all other genotypes as those are grain type sorghums with less tillering capacity. The genotype Sordan Headless had greater biomass yield (18 Mg $ha^{-1}$) in the early-seeding date than all other genotypes (Table 10). The increased biomass observed for early-seeding dates indicates this genotype is able to grow in colder soils, which was the objective of this study.

4.2.14. Dry matter content

The genotype main effect was significant for dry matter content at each harvest, while an interaction between genotype and seeding date was observed only for the second harvest. Seeding date main effect was not significant for dry matter content. Kaoliang, Niu Shen Zui, and Forage King had higher dry matter content for both seeding dates in the second harvest, which indicated that those genotypes were in more advanced growth stages than the other genotypes. The dry matter content is important if the forage sorghum will be used for silage for biogas production. Sorghum genotypes with greater dry matter at harvest have increased starch and sugar content and that will increase methane yield during anaerobic digestion (Mahmood et al., 2013). This is very important if forage sorghum is produced as feedstock for bioenergy whether it is for anaerobic digestion or lignocellulosic biochemical conversion. Breeding programs in energy sorghum select genotypes with high biomass productivity, low lignin content,

adaptability to low temperature, drought tolerant, high water use efficiency, photosensitivity for increasing cycle duration, higher dry matter content, lower leaf to stem ratio in the fall before frost, and high soluble solids (measured by Brix) in the stem and juice yield in sweet sorghum (Braconnier et al., 2011).

Forage sorghum harvested with high moisture content (>70%) promotes seepage in the silo which leads to nutrient loss, lower digestibility, and production of butyric acid as wet silage promotes higher fermentation losses and lower intake (Marsalis, 2006).

Table 10. Combined analysis of variance and mean square values for biomass yield and dry matter content at harvest in forage sorghum genotypes (G) seeded on two dates (SD) at four environments (env), in Fargo and Hickson, ND, in 2017 and 2018.

| SOV | df | Biomass yield | | | Dry matter content | |
|---|---|---|---|---|---|---|
| | | H-1† | H-2‡ | Total | H-1 | H-2 |
| Env | 3 | 28.3* | 687.8* | 788.0* | 367.8* | 64.6* |
| Rep(env) | 12 | 3.1* | 14.6* | 24.0* | 4.2* | 18.1* |
| SD | 1 | 78.4* | 160.4* | 14.5 | 30.6 | 4.2 |
| Env x SD | 3 | 7.5* | 13.9* | 2.2 | 4.6 | 6.8 |
| Env x rep xSD | 12 | 0.9* | 2.5 | 5.5 | 6.9* | 9.2 |
| G | 16 | 15.2* | 109.1* | 198.2* | 24.5* | 326.4* |
| Env x G | 26 | 0.6* | 13.5* | 15.1* | 4.0* | 11.2 |
| SD x G | 16 | 1.5* | 9.8* | 11.5* | 2.1 | 16.1* |
| Env x SD x G | 26 | 0.5* | 4.2 | 5.2 | 3.8* | 7.1 |
| Residual | 252 | 0.3 | 5.3 | 6.2 | 1.4 | 9.4 |
| CV, % | | 19.5 | 22.5 | 18.8 | 6.9 | 12.3 |

* Significant at $P \leq 0.05$, level of probability
†First harvest, ‡Second harvest

Table 11. Mean biomass yield and dry matter content of two harvests in forage sorghum genotypes seeded on two dates averaged across four environments, in Fargo and Hickson, ND, in 2017 and 2018.

| | Biomass yield (Mg ha$^{-1}$) | | | Dry matter (%) | |
|---|---|---|---|---|---|
| Genotype | H-1 | H-2 | Total | H-1 | H-2 |
| | Early seeding | | | | |
| Sweetie BMR | 3.2 | 8.5 | 11.7 | 17.2 | 22.0 |
| Brachytic sorghum | 3.2 | 8.8 | 12.0 | 18.6 | 23.0 |
| Pampa Triunfo XLT | 3.3 | 9.2 | 12.5 | 16.9 | 23.0 |
| Green Treat 128 | 3.2 | 10.4 | 13.6 | 19.1 | 19.6 |
| 54126 | 3.8 | 12.3 | 16.1 | 17.3 | 20.9 |
| Forage King | 3.1 | 10.4 | 13.4 | 19.0 | 32.9 |
| Hay King | 3.9 | 10.5 | 14.5 | 18.6 | 26.3 |
| SPX-901 | 4.0 | 11.0 | 15.0 | 18.2 | 22.5 |
| 1990 | 4.0 | 10.5 | 14.5 | 18.3 | 21.1 |
| BTx623 | 2.7 | 7.2 | 9.9 | 17.1 | 25.9 |
| SC 265 | 0.7 | 5.6 | 6.3 | 15.5 | 28.3 |
| Pampa Verde BMR-6 | 4.1 | 10.0 | 14.2 | 15.2 | 21.9 |
| Sordan Headless | 5.3 | 12.7 | 18.0 | 16.4 | 22.5 |
| NK300 | 5.6 | 10.3 | 15.9 | 17.7 | 24.1 |
| BMR-90 | 4.6 | 9.7 | 14.4 | 16.6 | 26.2 |
| Niu Sheng Zui | 2.1 | 5.3 | 7.4 | 14.9 | 30.9 |
| Kaoliang | 1.6 | 7.2 | 8.7 | 16.6 | 36.2 |
| | Late seeding | | | | |
| Sweetie BMR | 2.5 | 11.0 | 13.5 | 16.9 | 23.6 |
| Brachytic sorghum | 3.2 | 10.5 | 13.7 | 18.4 | 22.8 |
| Pampa Triunfo XLT | 2.6 | 10.6 | 13.2 | 16.7 | 23.4 |
| Green Treat 128 | 3.1 | 13.1 | 16.2 | 19.4 | 20.5 |
| 54126 | 3.6 | 14.0 | 17.6 | 17.8 | 21.5 |
| Forage King | 2.2 | 11.1 | 13.3 | 17.6 | 30.1 |
| Hay King | 2.9 | 11.6 | 14.5 | 17.5 | 26.4 |
| SPX-901 | 2.9 | 13.1 | 16.0 | 18.4 | 22.7 |
| 1990 | 2.7 | 13.0 | 15.7 | 17.9 | 21.7 |
| BTx623 | 1.3 | 5.1 | 6.4 | 17.0 | 25.8 |
| SC 265 | 1.1 | 5.9 | 7.0 | 15.9 | 27.5 |
| Pampa Verde BMR-6 | 2.5 | 12.3 | 14.8 | 15.3 | 20.1 |
| Sordan Headless | 2.6 | 13.3 | 15.9 | 15.0 | 20.4 |
| NK300 | 3.2 | 12.5 | 15.6 | 16.4 | 25.6 |
| BMR-90 | 3.0 | 13.9 | 17.0 | 14.6 | 28.3 |
| Niu Sheng Zui | 1.2 | 5.8 | 7.0 | 13.5 | 31.9 |
| Kaoliang | 1.1 | 6.3 | 7.3 | 14.3 | 31.6 |
| $LSD_1$ (0.05) | 0.6 | 2.2 | 2.4 | 1.5 | 2.3 |
| $LSD_2$ (0.05) | 0.5 | 1.2 | 1.4 | | |

$LSD_1$ = to compare genotypes within the same or different seeding date, $LSD_2$= to compare same genotypes within different seeding date within each harvest date.

4.2.15. Biomass yield relationship with emergence index and NDVI

Emergence index was a good predictor of biomass yield of the first harvest for both seeding dates (early-seeding, $r^2$=0.74; late-seeding, $r^2$=0.60), indicating earlier emerging

genotypes have greater vigor and better biomass yield at first harvest. Similarly, NDVI at 49 DAS ($r^2$=0.67) and 20 DAH ($r^2$=0.61) were good predictors of biomass yield of the first and second harvest, respectively. Tagarakis et al. (2017) found the best prediction of biomass yield of forage sorghum by NDVI at 49 days after planting when plants were 0.76-m tall. Foster et al. (2017) reported a weak correlation between narrow-band NDVI and final biomass yield of perennial grasses and a strong correlation ($r$=0.72) between narrow-band NDVI and biomass yield of sorghum measured on June to July in 2012 and 2013, in Oklahoma.

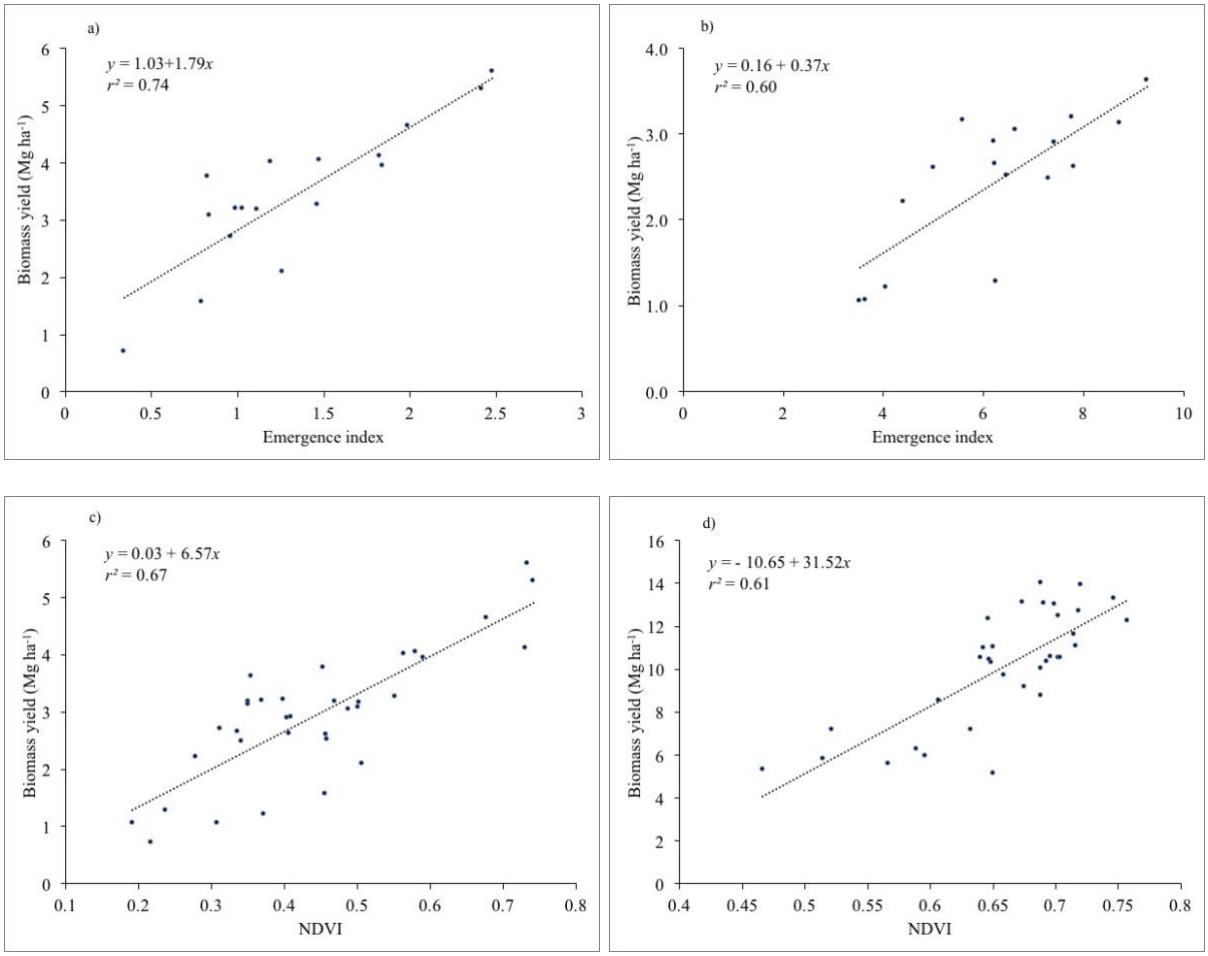


**Figure 10.** Emergence index vs. biomass yield for a) early-seeding at first harvest, b) late-seeding at first harvest, c) NDVI at 49 DAS vs. biomass yield for first harvest, and d) NDVI at 20 DAH vs. biomass yield for second harvest in forage sorghum genotypes (G) averaged across four environments, Fargo and Hickson, ND, in 2017 and 2018.

Table 12. Combined analysis of variance and mean square values for leaf, stem, inflorescence weight, and leaf-to-stem ratio in forage sorghum genotypes (G) seeded on two dates (SD) at two environments (env), Fargo and Hickson, ND, in 2018.

| Source of variation | df | Leaf weight | Stem weight | Inflorescence weight | Leaf-stem ratio |
|---|---|---|---|---|---|
| Env | 1 | 45.1 | 1840.8* | 542.4* | 0.69* |
| Rep(env) | 6 | 201.9 | 470.8* | 58.9 | 0.33* |
| SD | 1 | 136.7 | 473.5 | 61.1 | 0.70 |
| Env x SD | 1 | 266.0 | 65.9 | 132.8 | 0.03 |
| Env x rep x SD | 6 | 166.8 | 135.3 | 25.3 | 0.24 |
| G | 11 | 2811.3* | 1354.5* | 535.0* | 3.31* |
| Env x G | 11 | 143.8 | 278.3 | 153.8 | 0.15 |
| SD x G | 11 | 85.5 | 127.7 | 33.3 | 0.34 |
| Env x SD x G | 11 | 95.7 | 81.1 | 18.3 | 0.14 |
| Residual | 132 | 108.4 | 157.7 | 36.6 | 0.10 |
| CV, % | | 27.7 | 33.4 | 95.0 | 29.64 |

* Significant at $P \leq 0.05$ level of probability

In forage sorghum and sudangrass, leaf-stem ratio decreases by 50% with advancing plant maturity at the end of the season (Smith and Frederiksen, 2000; Pedersen and Rooney, 2004). Because many of the leaves are lost after frost and during the collection of the biomass before processing, energy sorghum, with its low leaf- to-stem ratios, are preferred for lignocellulosic energy conversion. Perennial biomass crops for lignocellulosic ethanol are generally harvested late in the fall to allow for a lower N content in the biomass and to allow remobilization of C and N for the following seasons (McKinley et al., 2018). Though remobilization reduces biomass yield by 20 to 30%, it reduces transportation cost due to higher dry matter content as well as increasing the biomass stability. In contrast, sweet sorghum harvest is done before leaf senescence when sucrose content, and water content in the stem, is greater (McKinley et al., 2018).

Low nitrogen content in biomass is preferred as it is associated with producing fuel impurities like emission of $NO_X$ during the combustion process (Robbins et al., 2012) and higher nitrogen can be problematic for the quality of liquid fuel from fast pyrolysis (Wilson et al., 2013).

Table 13. Mean dry weight of leaf, stem, inflorescence, and leaf-to-stem ratio in forage sorghum genotypes (G) seeded on two dates (SD) at two environments (env), Fargo and Hickson, ND, in 2018.

| Genotype | Leaf | Stem | Inflorescence | Leaf to stem ratio |
|---|---|---|---|---|
| | ----------g---------- | | | |
| Sweetie BMR | 50.2 | 51.0 | 9.6 | 1.03 |
| Pampa Triunfo XLT | 32.4 | 35.8 | 3.4 | 0.93 |
| Forage King | 10.6 | 21.4 | 5.7 | 0.51 |
| Hay King | 22.8 | 36.8 | 8.4 | 0.66 |
| SPX-901 | 52.2 | 36.1 | 2.0 | 1.59 |
| 1990 | 61.3 | 32.3 | - | 2.11 |
| Pampa Verde BMR-6 | 36.4 | 24.7 | - | 1.54 |
| Sordan Headless | 38.3 | 31.9 | - | 1.25 |
| NK300 | 33.8 | 42.4 | 16.7 | 0.83 |
| BMR-90 | 35.1 | 44.9 | 14.0 | 0.86 |
| Niu Sheng Zui | 39.3 | 47.3 | 12.3 | 0.93 |
| Kaoliang | 39.3 | 46.8 | 4.4 | 0.86 |
| LSD (0.05) | 9.8 | 12.0 | 8.3 | 0.34 |

4.2.16. Forage nutritive value

For all the forage nutritive value parameters, the genotype main effect was significant ($P \leq 0.05$) in each harvest while the seeding date main effect was significant only for NDF and ADF in the first harvest (Table 14). No interaction between genotype and seeding date was observed for any quality parameters.

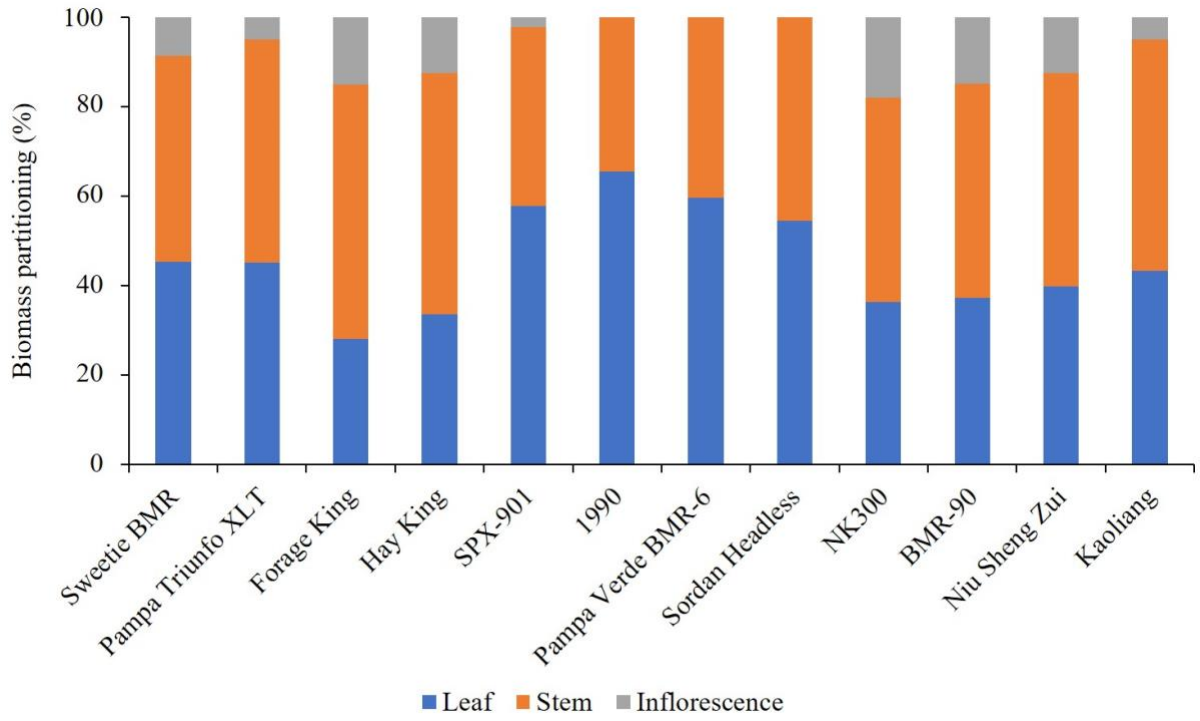


**Figure 11.** Dry biomass partitioning of leaf, stem, and inflorescence in forage sorghum genotypes averaged across two dates and two environments in Fargo and Hickson in 2018.

Crude protein (CP) content varied among genotypes and harvest dates but not between seeding dates (Table 14). Crude protein content was much greater in the first harvest, which was expected because of the higher leaf-to-stem ratio at harvest and that most plants were still in vegetative stage (Table 13). Plants were harvested when the first genotypes, in either seeding date, reached 1.4 m tall. This is the recommended harvest date for high quality hay and for grazing because of the high nutritive value of the forage at this stage (Berti et al., 2018). Genotypes with higher CP values were those shortest at harvest. Crude protein in the second harvest was about half of that of the first harvest for all genotypes, since plants had lower leaf-to-stem ratios and plants were in more advanced growth stages (Table 8). In the first harvest, greater CP values were observed among the late-seeded genotypes, as those were younger in stage at harvest compared with early-seeded genotypes (Table 15). The range of CP was 133 to 214 g $kg^{-1}$ in the first harvest (Table 15) and 63 to 106 g $kg^{-1}$ in the second harvest (Table 17).

Crude protein requirement in mature beef cattle ranges between 70-90 g $kg^{-1}$, thus in both harvests most sorghum genotypes meet this requirement (Seimens et al., 1999). In the first harvest, the highest CP values were observed in SC 265 and in BTx623, as those are grain type genotypes and shortest in height (Table 15). Pampa Verde BMR-6 had the highest CP content in the second harvest (Table 17), although not different from NK300, BMR 90, BTx623, SC 265, and Niu Sheng Zui. In the two-cut system, forage sorghum produced high quality forage in the first harvest, which can be utilized for beef cattle, and the low-quality, high biomass yield in the second harvest can be utilized for bioenergy production. Samarappuli and Berti (2018) reported 104 to 105 g $kg^{-1}$ CP in non-BMR and BMR forage sorghum at North Dakota.

Nitrogen accumulation was much greater across genotypes in the second harvest. Biomass yield in the second harvest were three to four times higher than the first harvest hence the greater N accumulation. Among the early-seeded genotypes, Sordan Headless and NK300) had the highest nitrogen accumulation with both harvests summed (Table 15 and Table 17). Samarappuli and Berti (2018) observed the highest nitrogen accumulation in a non-BMR forage sorghum genotypes in North Dakota, which is similar with the current study. Anfinrud et al. (2013) reported average nitrogen uptake by forage sorghum with a single harvest, ranging between 50 to 150 kg N $ha^{-1}$ depending on nitrogen fertility level at Fargo and Prosper, ND. In the current study, nitrogen accumulation was much higher than Anfinrud et al (2013) reported; however, only if the second harvest (one- cut system) is taken into account then N accumulation is similar. Forage sorghum is known as an efficient soil nitrogen scavenger, especially where nitrogen accumulation depends on multiple factors like sorghum types and hybrids, stage of harvest, availability of N in soil (Pedersen et al., 1995). Pedersen et al. (1995) found nitrogen accumulation ranged between 137 and 283 kg N $ha^{-1}$ in eighteen genotypes of six different types

of sorghum. In the current study with a two-cut system, N concentration in biomass was greater at first harvest (23 to 33 g $kg^{-1}$) compared with the second harvest (11 to 17 g $kg^{-1}$), which represented the higher seasonal nitrogen accumulation.

Total accumulated nitrogen exceeded the sum of N-fertilizer input and soil nitrogen at 60 cm. It was assumed that the excess nitrogen could be from the mineralized nitrogen during the season, since these soils were high in organic matter (5-6%). Mineralization is a process of decomposing organic nitrogen from crops residues, to ammonium. Mineralization depends on soil temperature (20 to 35℃), soil water, and oxygen availability in the soil (Johnson et al., 2005). It is estimated that around 67 to 90 kg N $ha^{-1}$ is mineralized from organic soil in each year in a study conducted in New York state (Johnson et al., 2005). Kaur et al. (2018) conducted an eight-week incubation study from 0- to15-cm in depth of soil and found cumulative nitrogen mineralized ranged between 0.34 and 2.15 mg N $kg^{-1}$ and 0.45 to 3.41 mg $kg^{-1}$ for the Glyndon and Fargo soils, respectively.

Low lignin content is the desired characters for high quality forage. Brown mid-rib (BMR) genotypes had lower lignin content than non-BMR sorghum, as expected. In the first harvest, the range of ADL content was 29 to 36 g $kg^{-1}$ and in the second harvest ADL was 17 to 54 g $kg^{-1}$. In the first harvest, lignin content was higher among the early-seeded non-BMR genotypes such as Forage King, Sordan Headless, NK300, and Kaoliang (Table 15). Low lignin content was observed in each harvest among the BMR genotypes like Sweetie BMR, Green Treat 128, Brachytic, Pampa Triunfo XLT, and BMR 90 (Table 15 and 17). Lee et al. (2007) reported 80 g $kg^{-1}$ ADL in forage sorghum evaluated at South Dakota. Samarappuli and Berti (2018) observed 58 g $kg^{-1}$ and 68 g $kg^{-1}$ ADL in a BMR forage sorghum and non-BMR forage sorghum, respectively, in Fargo, Prosper, and Carrington, ND. Lignin content increases with the

progressing plant maturity. In the current study, mostly all the genotypes were in vegetative stage at first harvest and in early reproductive stage at second harvest, which appears to have influenced the low ADL content.

Genotypes main effect was significant for ADF and NDF in both harvests where seeding date main effect was significant for ADF and NDF in the first harvest only. Among the genotypes, the range of ADF was 265 to 304 g $kg^{-1}$ and 284 to 348 g $kg^{-1}$ in the first and second harvest respectively (Table 15 and Table 17). The range of NDF was 525 to 579 g $kg^{-1}$ and 546 to 652 g $kg^{-1}$ in the first and second harvest, respectively (Table 15 and Table 17). Samarappuli and Berti (2018) reported 304 to 331 g $kg^{-1}$ ADF and 542 to 572 g $kg^{-1}$ NDF in two forage sorghum genotypes in North Dakota. Anfinrud et al. (2013) reported 251 to 366 g $kg^{-1}$ ADF and 518 to 634 g $kg^{-1}$NDF in forage sweet sorghum and sorghum x sudangrass in North Dakota, which are similar to the ranges observed in our study. The NDF consists of all the fibers (hemicellulose, cellulose, lignin) in the plant cell walls and only a fraction of NDF is partially digestible. In the ration, NDF is considered to determine the total feed intake. The NDF increases with plant maturity and with the increase of NDF feed intake decreases. The ADF consists of cellulose and lignin, which is also partially digestible. Digestion of forage decreases as ADF increases. Low NDF and low ADF is desired for high quality forage. Prime quality forage for beef cattle consists of <400 g $kg^{-1}$ NDF and <310 g $kg^{-1}$ ADF (Parish and Rhinehart, 2008). Effective fiber expressed as eNDF is necessary to maintain the rumen function and pH level in optimum condition (Parish and Rhinehart, 2008). Depending on feeding management, up to 250 g $kg^{-1}$ eNDF is needed for beef cattle to maintain the optimum pH (>5.7) level for maximum digestion and microbial growth (Parish and Rhinehart, 2008). In this study, the ADF is within the

desired range where NDF value is higher than prime quality forages. It was indicated that the lignin content is lower, which mean higher digestible forages.

Table 14. Combined analysis of variance and mean square values for biomass quality in forage sorghum genotypes (G) seeded on two dates (SD) in the first harvest, at four environments, Fargo and Hickson, ND, in 2017 and 2018.

| SOV | df | CP | Nacc | N | ADL | ADF | NDF | Ash |
|---|---|---|---|---|---|---|---|---|
| Env | 3 | 663.6* | 13434* | 17.0* | 4.5* | 91.5* | 106.4* | 417.3* |
| Rep(env) | 12 | 23.8* | 3027* | 0.6* | 0.3* | 6.3* | 12.5* | 3.5 |
| SD | 1 | 386.5 | 17632 | 9.9 | 3.7 | 133.0* | 236.3* | 0.3 |
| Env x SD | 3 | 44.0* | 2427* | 1.1* | 0.5 | 5.4 | 8.0 | 10.0 |
| Env x rep x SD | 12 | 7.3* | 632* | 0.2* | 0.2* | 2.6* | 7.0* | 3.7 |
| G | 16 | 35.6* | 8675* | 0.9* | 1.1* | 7.6* | 22.6* | 9.4* |
| Env x G | 26 | 3.4* | 553* | 0.1* | 0.2* | 2.2* | 5.3 | 4.4* |
| SD x G | 16 | 2.8 | 775 | 0.1 | 0.2 | 1.1 | 1.3 | 2.1 |
| Env x SD x G | 26 | 2.5 | 410* | 0.1 | 0.1 | 1.1 | 2.3 | 2.6 |
| Residual | 252 | 1.9 | 251 | 0.1 | 0.1 | 1.2 | 2.4 | 2.2 |
| CV, % | | 8.0 | 20 | 8.0 | 8.5 | 3.9 | 2.8 | 11.5 |

* Significant at $P \leq 0.05$ levels of probability
Crude protein (CP), nitrogen accumulated in biomass (Nacc), nitrogen (N), acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF)

Genotypes main effect were significant for ash content at first harvest and second harvest. The range of ash content was 99 to 165 g $kg^{-1}$ at first harvest and 76 to 127 g $kg^{-1}$ at second harvest. Ash content was lower at second harvest compared with first harvest. The ash content was greater than that of reported by Samarappuli and Berti (2018) (63 to 65 g $kg^{-1}$). Mahmood et al (2012) stated that ash content in biomass was affected by cultivar and by site with a range of

61 to 98 g $kg^{-1}$. Ash content in the biomass varies with soil type and harvesting process (Stefaniak et al, 2012). Ash is considered as waste byproduct and an anti-quality factors in energy conversion. Biogas production from biomass is negatively affected by high ash content as microorganisms cannot decompose the ash (Samarappuli and Berti, 2018), while in thermochemical conversion biomass it cannot be heated above 400℃ otherwise the ash will melt and clog the reactor (Zhao et al., 2017). That is why lower ash content is desired in bioenergy feedstocks.

Table 15. Mean value of biomass quality in forage sorghum genotypes in the first harvest averaged across two seeding dates and four environments, Fargo and Hickson, ND, in 2017 and 2018.

| Genotype | CP | Nacc | N | ADL | ADF | NDF | Ash |
|---|---|---|---|---|---|---|---|
| | g $kg^{-1}$ | kg $ha^{-1}$ | ……g $kg^{-1}$..... | | | | |
| Sweetie BMR | 172 | 75.8 | 28 | 29 | 279 | 543 | 130 |
| Brachytic sorghum | 169 | 86.2 | 27 | 32 | 278 | 552 | 102 |
| Pampa Triunfo XLT | 170 | 78.6 | 27 | 32 | 279 | 549 | 133 |
| Green Treat 128 | 168 | 84.7 | 27 | 29 | 285 | 543 | 99 |
| 54126 | 162 | 94.5 | 26 | 34 | 278 | 543 | 110 |
| Forage King | 183 | 73.4 | 29 | 36 | 277 | 546 | 118 |
| Hay King | 166 | 87.7 | 27 | 32 | 279 | 541 | 121 |
| SPX-901 | 162 | 88.7 | 26 | 35 | 288 | 562 | 127 |
| 1990 | 166 | 86.2 | 27 | 34 | 290 | 570 | 124 |
| BTx623 | 208 | 63.2 | 33 | 35 | 272 | 532 | 106 |
| SC 265 | 209 | 29.2 | 33 | 34 | 272 | 541 | 112 |
| Pampa Verde BMR-6 | 174 | 88.0 | 28 | 33 | 285 | 552 | 165 |
| Sordan Headless | 159 | 95.2 | 25 | 35 | 288 | 560 | 158 |
| NK300 | 146 | 99.0 | 23 | 35 | 289 | 571 | 146 |
| BMR-90 | 161 | 96.5 | 26 | 32 | 281 | 554 | 151 |
| Niu Sheng Zui | 170 | 40.1 | 27 | 32 | 289 | 564 | 143 |
| Kaoliang | 155 | 29.0 | 25 | 34 | 292 | 567 | 141 |
| LSD (0.05) | 13 | 16.5 | 2 | 3 | 9 | 15 | 14 |

Crude protein (CP), nitrogen accumulated in biomass (Nacc), nitrogen (N), acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF)

Table 16. Combine analysis of variance for biomass quality in forage sorghum genotypes (G) planted on two dates (SD) during second harvest at four environments (env), Fargo and Hickson in 2017 and 2018.

| Source of variation | df | CP | Nacc | N | ADL | ADF | NDF | Ash |
|---|---|---|---|---|---|---|---|---|
| Env | 3 | 128.3* | 71398* | 3.3* | 41.1* | 207.5* | 33.0* | 147.8* |
| rep(env) | 12 | 10.5* | 6275* | 0.3* | 1.6* | 2.9 | 5.4 | 6.2* |
| SD | 1 | 0.00002 | 36767 | 0.000001 | 0.1 | 0.1 | 14.3 | 4.6 |
| Env x SD | 3 | 19.4* | 8120* | 0.5* | 1.8* | 8.7 | 8.0 | 24.2* |
| Env x rep xSD | 12 | 3.0 | 1230 | 0.1 | 0.4 | 2.9 | 6.0 | 1.5 |
| G | 16 | 21.7* | 18412* | 0.6* | 7.7* | 69.1* | 127.9* | 21.0* |
| Env x G | 26 | 3.8 | 2816* | 0.1 | 0.5 | 7.1* | 12.9* | 4.4 |
| SD x G | 16 | 3.1 | 1423 | 0.1 | 0.1 | 4.3 | 6.3 | 1.3 |
| Env x SD x G | 26 | 4.1 | 1625 | 0.1 | 0.6 | 4.3 | 10.4 | 2.9 |
| Residual | 252 | 3.1 | 1719 | 0.1 | 0.7 | 3.5 | 8.0 | 3.3 |
| CV, % | | 21.2 | 31 | 21.3 | 22.7 | 5.9 | 4.7 | 19.5 |

* Significant at $P \leq 0.05$, level of probability.
Crude protein (CP), nitrogen accumulated in biomass (Nacc), nitrogen (N), acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF)

Table 17. Mean biomass quality components in forage sorghum genotypes averaged across two dates and four environments for the second harvest, Fargo and Hickson, ND, in 2017 and 2018.

| Genotype | CP | Nacc | N | ADL | ADF | NDF | Ash |
|---|---|---|---|---|---|---|---|
| | g $kg^{-1}$ | kg $ha^{-1}$ | ……g $kg^{-1}$..... | | | | |
| Sweetie BMR | 79 | 119 | 13 | 28 | 309 | 585 | 91 |
| Brachytic sorghum | 86 | 133 | 14 | 26 | 311 | 585 | 83 |
| Pampa Triunfo XLT | 87 | 138 | 14 | 33 | 303 | 587 | 94 |
| Green Treat 128 | 69 | 121 | 11 | 17 | 328 | 591 | 83 |
| 54126 | 66 | 139 | 11 | 32 | 301 | 551 | 76 |
| Forage King | 69 | 117 | 11 | 46 | 344 | 641 | 80 |
| Hay King | 71 | 120 | 11 | 36 | 329 | 615 | 82 |
| SPX-901 | 81 | 148 | 13 | 38 | 330 | 623 | 91 |
| 1990 | 89 | 162 | 14 | 35 | 322 | 617 | 97 |
| BTx623 | 91 | 83 | 15 | 36 | 313 | 580 | 102 |
| SC 265 | 98 | 87 | 16 | 33 | 302 | 580 | 95 |
| Pampa Verde BMR-6 | 104 | 186 | 17 | 38 | 285 | 569 | 127 |
| Sordan Headless | 88 | 183 | 14 | 46 | 302 | 596 | 115 |
| NK300 | 93 | 169 | 15 | 47 | 296 | 607 | 96 |
| BMR-90 | 94 | 178 | 15 | 42 | 294 | 587 | 106 |
| Niu Sheng Zui | 96 | 83 | 15 | 52 | 325 | 630 | 102 |
| Kaoliang | 84 | 85 | 13 | 54 | 339 | 645 | 99 |
| LSD (0.05) | 15 | 35 | 2 | 5 | 18 | 25 | 14 |

Crude protein (CP), nitrogen accumulated in biomass (Nacc), nitrogen (N), acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF)

# CHAPTER 5. CONCLUSIONS

Forage sorghum had lower germination percentages and slower germination rates at 10℃ compared with 24℃. Early in the season, forage sorghum was affected by cold soil conditions, which reduced the emergence index, stand establishment, and increased seed mortality. Earlier and fast emerging genotypes had better growth performance and produced greater biomass yield at earlier planting in the field. Emergence index and NDVI had better prediction of biomass yield. Commercial forage sorghum cultivars- Sordan Headless, NK300, Hay King, and SPX-901 have the potential to grow early in the season.

Chemical components were not affected by seeding date. Nutritive value depended on harvesting stages of forage sorghum. Forage sorghum with a two-cut system can be utilized to get better quality forage without reducing biomass yield. Commercial forage sorghum cultivars- Hay King, Forage King, NK300, and BMR-90 would be a potential feedstock for bioenergy.

In future research, measuring emergence index in controlled environmental conditions would be better than measuring germination rates to screen forage sorghum genotypes for chilling tolerance.

# REFERENCES

Ahluwalia M. 1977. Forage sorghum breeding for animal production. Indian J. Gen. Pl. Br. 37(2): 241-244.

Akabari, V.R., and H.P. Parmar. 2014. Heterosis response and combining ability for green fodder yield and quality traits in forage sorghum. J. Progressive Agricul. 5: 9-14.

Anfinrud, R., L. Cihacek, B.L. Johnson, Y. Ji, and M.T. Berti. 2013. Sorghum and kenaf biomass yield and quality response to nitrogen fertilization in the northern Great Plains of the USA. Ind. Crops Prod. 50:159-165.

Atis, I., O. Konuskan, M. Duru, H. Gozubenli, and S. Yilmaz. 2012. Effect of harvesting time on yield, composition and forage quality of some forage sorghum cultivars. IJAB. 14: 879-886.

Balota, M., W.A. Payne, S.K. Veeragom, B.A. Stewart, and D.T. Rosenow. 2010. Respiration and its relationship to germination, emergence, and early growth under cool temperatures in sorghum. Crop Sci. 50: 1414-1422.

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

Berti, M.T., R. Nudell, R. Anfinrud, D. Samarappuli, and B.L. Johnson, 2011. Forage resources as feedstocks for the biofuel industry in North Dakota. *In*: European Biomass Conference Berlin, Germany, 6-9 June 2011. Available at: https://www.ag.ndsu.edu/plantsciences/research/forages/docs/ForageResourcesAsFeedstocksForBiofuel.pdf (verified 29 December 2018).

Braconnier, S., G. Trouche, S. Gutjahr Gutjahr, D. Luquet, and M. Dingkhun. 2011. Development of new sorghum ideotypes to meet the increasing demand of bioethanol. *In* International Conference on Crop Improvement, Ideotyping and Modeling for African Cropping Systems under Climate Change (CIMAC), 7-9 February 2011, Stuttgart, Germany.

Carpita, N.C., and M.C. McCann. 2008. Maize and sorghum: Genetic resources for bioenergy grasses. Trends Plant Sci. 13:415-420.

Celarier, R.P. 1959. Cytotaxonomy of the Andropogonea. III. Sub-tribe sorgheae, genus *Sorghum*. Cytologia. 23:395-418.

Chiluwal, A., R. Bheemanahalli, R. Perumal, A.R. Asebedo, E. Bashir, A. Lamsal, D. Sebela, N.J. Shetty, and S.V.K Jagadish. 2018. Integrated aerial and destructive phenotyping differentiates chilling stress tolerance during early seedling growth in sorghum. Field Crops Res. 227: 1-10. doi:10.1016/j.fcr.2018.07.011.

Chmielewska, J., J. Sowinski and P. Nowakowski. 2012. Processing sweet sorghum to bioethanol. Przem. Chem. 91: 703-705.

Chung, Y.S., S.C. Choi, R.R. Silva, J.W. Kang, J.H. Eom, and C. Kim. 2017. Case study: Estimation of sorghum biomass using digital image analysis with Canopeo. Biomass Bioenerg. 105: 207-210. doi:10.1016/j.biombioe.2017.06.027.

Dahlberg, J., J. Berenji, V. Sikora, and D. Latkovi. 2010. Assessing sorghum [*Sorghum bicolor* (L.) Moench] germplasm for new traits: food, fuels & unique uses. Maydica. 56(1750): 85-92.

Dahlberg, J., E. Wolfrum, B. Bean, and W.L. Rooney. 2011. Compositional and agronomic evaluation of sorghum biomass as a potential feedstock for renewable fuels. J. Biobased Mater. Bioenergy. 4: 1-7.

Dillon, S.L., 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. 100:975-989.

DOE (2006) Breaking the cellulosic barriers of cellulosic ethanol: a joint research agenda. Available online: https://www.doegenomestolife.org/biofuels/

Fernandez, M.G.S., G.R. Schoenbaum, and A.S. Goggi. 2014. Novel germplasm and screening methods for early cold tolerance in sorghum. Crop Sci. 54: 2631-2638.

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

Foster, A.J., V.G. Kakani, and J. Mosali. 2017. Estimation of bioenergy crop yield and N status by hyperspectral canopy reflectance and partial least square regression. Precis. Agric. 18: 192-209.

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

Guragain, Y.N., P.S. Rao, P.V.V. Prasad, and P.V. Vadlani. 2017. Evaluation of brown midrib sorghum mutants as a potential biomass feedstock for 2,3-Butanediol Biosynthesis. Appl. Biochem. Biotechnol. 183: 1093-1110. doi:10.1007/s12010-017-2486-4.

Halkier, B.A., and B.L. Moller. 1991. Involvement of cytochrome p450 in the biosynthesis of dhurrin in *Sorghum bicolor* (L.) Moench. Plant Physiol. 96:10-17.

Havaux, M. 1989. Fluorimetric determination of the genetic-variability existing for chilling tolerance in sweet sorghum and sudangrass. Plant Breeding. 102: 327-332. doi:10.1111/j.1439-0523.1989.tb01264.x.

Howell, T.A., S.R. Evett , J.A. Tolk, K.S. Copeland, P.D. Colaizzi, and P.H. Gowda. 2008. Evapotranspiration of corn and forage sorghum for silage. In World Environmental and Water Resources Congress 2008. Ahupua'a. Honolulu, Hawaii, USA. 12-16 May 2008.

Hoveland, C.S., and W.G. Monson. 1980. Genetic and environmental effects on forage quality. p. 139-168. *In* Crop quality, storage and utilization. American Soc. of Agronomy and Crop Science Society of America, Madison, WI.

Ikanovic, J., D. Glamoclija, R. Maletic, V. Popovic, D. Sokolovic, M. Spasic, and S. Rakic. 2011. Path analysis of the productive traits in sorghum species. Genetika-Belgrade. 43: 253-262. doi:10.2298/gensr1102253i.

Ikanovic, J., V. Popovic, V. Trkulja, L. Zivanovic, Z. Lakic, and S. Pavlovic. 2013. Morphological characteristics of the interspecies hybrid between sorghum and sudan grass under intensive nitrogen nutrition. Genetika-Belgrade. 45: 31-40. doi:10.2298/gensr1301031i.

Janowiak, F., K. Kaczanowska, H.C. Jing, W.A. Bekele, and R.J. Snowdon. 2015. Metabolic limitations to photosynthetic efficiency of sorghum seedling leaves at low temperature. Agriculture and Climate Change - Adapting Crops to Increased Uncertainty (Agri 2015). 29: 277-278. doi:10.1016/j.proenv.2015.07.208.

Johnson, C., G. Albrecht, Q. Ketterings, J. Beckman, and K. Stockin. 2005. Nutrient Management Spear Program. Agronomy Fact Sheet Series. Fact Sheet: 2. Available at http://nmsp.css.cornell.edu (verified 27 May 2019)

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

Kaur, J., L.J. Cihacek, and A. Chatterjee. 2018.Estimation of nitrogen and sulfur mineralization in soils amended with crop residues contributing to nitrogen and sulfur nutrition of crops in the North Central U.S,Communications in Soil Science and Plant Analysis, 49 (18): 2256-2266, doi: 10.1080/00103624.2018.1499761

Kim, M., and D.F. Day, 2011. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. J. Ind. Microbiol. Biotechnol. 38:803-807. doi: 10.1007/s10295-010-0812-8.

Knoll, J., N. Gunaratna, and G. Ejeta. 2008. QTL analysis of early-season cold tolerance in sorghum. Theor. Appl. Genet. 116: 577-587. doi:10.1007/s00122-007-0692-0.

Lee, D., V.N. Owens, A. Boe, and P. Jeranyama. 2007. Composition of herbaceous biomass feedstocks. p. 16 Bull. SGINC1-07. North Central Sun Grant Center. South Dakota State University, Brookings, SD.

Li, Y., P.S. Mao, W.X. Zhang, X.G. Wang, Y.L. You, H.M. Zhao, L. Zhai, and G. Liu. 2015. Dynamic expression of the nutritive values in forage sorghum populations associated with white, green and brown midrid genotypes. Field Crops Res. 184: 112-122. doi:10.1016/j.fcr.2015.09.008.

Lorenz, A.J., J.G. Coors, N. de Leon, E.J. Wolfrum, B.R. Hames, A.D. Sluiter, and P.J. Weimer. 2009. Characterization, genetic variation, and combining ability of maize traits relevant to the production of cellulosic ethanol. Crop Sci. 49:85-98.

Mahmood, A., H. Ullah, M. Ijaz, M.M. Javaid, A.N. Shahzad, and B. Honermeier, 2013. Evaluation of sorghum hybrids for biomass and biogas production. Australian J. Crop Sci. 7: 1456e1462.

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

Mann, J.A., C.T. Kimber, and F.R. Miller, 1983. The origin and early cultivation of sorghums in Africa. Bull. 1454.Texas Agricultural Experiment Station.

Marla, S.R., S. Shiva, R. Welti, S.Z. Liu, J.J. Burke, and G.P. Morris. 2017. Comparative transcriptome and lipidome analyses reveal molecular chilling responses in chilling-tolerant sorghums. Plant Genome. 10: 16. doi:10.3835/plantgenome2017.03.0025.

Marsalis, M.A. 2006. Sorghum forage production in New Mexico (Guide A-332). Las Cruces, NM: New Mexico State University Cooperative Extension Service. Retrieved 4 June 2019, from https://aces.nmsu.edu/pubs/_a/A332/

Marsalis, M.A. and B. Bean. 2010. Western sorghum production guide. United Sorghum Checkoff Program Board. Sorghum Farmer, Prairie View, Kansas.

Martin, F.M. 1992. Pythium Pages 3949 in L.L. Singleton, J.D. Milhail, and C.M. Rush, eds. Methods for research on soilborne phytopathogenic fungi. APS Press, St. Paul, MN.

Maughan, M., T. Voigt, A. Parrish, G. Bollero, W. Rooney, and D.K. Lee, 2012. Forage and energy sorghum responses to nitrogen fertilization in central and southern Illinois. Agron. J., 104(4): 1032-1040.

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

Maulana, F., D. Weerasooriya, and T. Tesso. 2017. Sorghum landrace collections from cooler regions of the world exhibit magnificent genetic differentiation and early season cold tolerance. Front. Plant Sci. 8: 12. doi:10.3389/fpls.2017.00756.

McCollum, T., K. McCuistion, and B. Bean. 2005. Brown mid-rib and photoperiod sensitive forage sorghums. Texas A&M Univ., Agric. Res. and Ext. Center, Amarillo, TX. Available at: http://amarillo.tamu.edu/files/2010/11/brownmidrib. pdf (verified 20 April 2017).

McCormick, M.E., D.R. Morris, B.A. Ackerson, and D.C. Blouin. 1995. Ratoon cropping forage sorghum for silage - yield, fermentation, and nutrition. Agron. J. 87: 952-957. doi:10.2134/agronj1995.00021962008700050030x.

McKinley, B.A., S.N. Olson, K.B. Ritter, D.W. Herb, S.D. Karlen, F. Lu, J. Ralph, W.L. Rooney, and J.E. Mullet. 2018. Variation in energy sorghum hybrid TX08001 biomass composition and lignin chemistry during development under irrigated and non-irrigated field conditions. Plos One 13(4): e0195863. doi:10.1371/journal.pone.0195863

Meki, M.N., R.M. Ogoshi, J.R. Kiniry, S.E. Crow, A.H. Youkhana, M.H. Nakahata, and K. Littlejohn. 2017. Performance evaluation of biomass sorghum in Hawaii and Texas. Ind. Crops Prod. 103: 257-266. doi:10.1016/j.indcrop.2017.04.014.

Mocoeur, A., Y.M. Zhang, Z.Q. Liu, X. Shen, L.M. Zhang, S.K. Rasmussen, and H.C. Jing. 2015. Stability and genetic control of morphological, biomass and biofuel traits under temperate maritime and continental conditions in sweet sorghum (*Sorghum bicolor*). Theor. Appl. Genet. 128:1685-1701.

Mullet, J.E., W.L. Rooney, P.E. Klein, D. Morishige, R. Murphy, and J.A. Brady. 2010. Discovery and utilization of sorghum genes (Ma5/Ma6). US Patent Application Publication (Pub. No. US 2010/0024065 A1).

North Dakota Agricultural Weather Network (NDAWN). 2018. NDAWN Center. North Dakota State Univ., Fargo, ND. Available at: http://ndawn.ndsu.nodak.edu (accessed 06 Dec. 2018).

New World Encyclopedia contributors. Sorghum [Internet]. New World Encyclopedia, 2015. Available at http://www.newworldencyclopedia.org/p/index.php?title=Sorghum&oldid=991242 (verified 27 May 2019).

Osuna-Ortega, J., M.C. Mendoza-Castillo, and L.E. Mendoza-Onofre. 2003. Sorghum cold tolerance, pollen production and seed yield in the Central High Valleys of Mexico. Maydica. 48: 125-132.

Packer, D.J., and W.L. Rooney. 2014. High-parent heterosis for biomass yield in photoperiod-sensitive sorghum hybrids. Field Crops Res. 167: 153-158.

Parera C.A., and D.J. Cantliffe. 1994. Pre-sowing seed priming. Hort. Rev. 16: 109-141.

Parish, J.A., and J.D. Rhinehart. 2008. Fiber in beef cattle diets. Mississippi State University Extension Service. Available at:https://extension.msstate.edu/sites/default/files/publications/publications/p2489.pdf (verified 27 May 2019).

Parra-Londono, S., K. Fiedler, M. Kavka, B. Samans, S. Wieckhorst, A. Zacharias, and R. Uptmoor. 2018. Genetic dissection of early-season cold tolerance in sorghum: genome-wide association studies for seedling emergence and survival under field and controlled environment conditions. Theor. Appl. Genet. 131: 581-595. doi:10.1007/s00122-017-3021-2.

Patane, C., V. Cavallaro, G. Avola, and G.D. Agosta. 2006. Seed respiration of sorghum [*Sorghum bicolor* (L.) Moench] during germination as affected by temperature and osmoconditioning. Seed Sci. Res. 16: 251-260.

Patrignani A. and T. Ochsner. 2015. Canopeo: A powerful new tool for measuring fractional green canopy cover. Agron. J. 15:150.

Peacock, J.M. 1982. Response and tolerance of sorghum to temperature stress. p. 143-159 *In* House LR, et al. (Eds.), Sorghum in the Eighties. Proc. of the International Symposium on Sorghum, Patancheru, India, November 2-7, 1981. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India.

Pedersen, J.F., H.J. Gorz, F.A. Haskins, and W.M. Ross. 1982. Variability for quality and agronomic traits in forage sorghum hybrids. Crop Sci. 22:853-856.

Pedersen, J.F., K.J. Moore, S. Schroth, and D.T. Walters. 1995. Nitrogen accumulation of six groups of sorghum grown on a municipal biosolids use site source: Water Environment Research 67(7):1076-1080.

Pedersen, J.F., and W.L. Rooney 2004. Sorghums. *In* L.E. Mosher, B.L. Burton, and L.E. Sollenberger (Eds.). Warm-Season (C4) Grasses. ASA/CSSA/SSSA. Madison, WI.

Perazzo, A.F., G.G.P. Carvalho, E.M. Santos, H.F.C. Bezerra, T.C. Silva, G.A. Pereira, R.C.S. Ramos, and J.A.S. Rodrigues. 2017. Agronomic evaluation of sorghum hybrids for silage production cultivated in semiarid conditions. Front Plant Sci. 8:1088. doi: 10.3389/fpls.2017.01088. eCollection 2017.

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

Pirasteh-Anosheh H., and R. Hamidi. 2013. Does seed chemical priming improves germination and early growth of oil rapeseed? Inter. J. Agron. Plant Product. 4 (4): 805-808.

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.

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.

Quinby, J.R. 1966. Fourth maturity gene locus in sorghum. Crop Sci. 6: 516-518.

Quinby, J.R., and R.E. Karper. 1954. Inheritance of height in sorghum. Agron. J. 46: 211-216.

Ratnavathi, C.V., S. Kalyana Chakravarthy, V.V. Komala, U.D. Chavan, J.V. Pati. 2011. Sweet sorghum as feedstock for biofuel production: A review. Sugar Tech.13(4):399-407. doi:10.1007/s12355-011-0112-2

Razmi, Z., R. Hamidi, and H. Pirasteh-Anosheh. 2013. Seed germination and seedling growth of three sorghum (*Sorghum bicolor* L.) genotypes as affected by low temperatures. International J. Farming and Allied Sciences (IJFAS). 20(2):851-856. Available online at www.ijfas.com

Robbins, M.P., G. Evans, J. Valentine, I.S. Donnison, and G.G. Allison 2012. New opportunities for the exploitation of energy crops by thermochemical conversion in northern Europe and the UK. Prog. Energy.Combust. Sci. 38: 138-155 10.1016/j.pecs.2011.08.001

Rooney, W.L., and S. Aydin. 1999. Genetic control of a photoperiod sensitive response in *Sorghum bicolor* (L.) Moench. Crop Sci. 39:397-400.

Rooney, W.L., J. Blumenthal, B. Bean, and J.E. Mullet, 2007. Designing sorghum as a dedicated bioenergy feedstock. Biofuels Bioprod. Bioref. 1:147e157.

Ross, W.M., H.J. Gorz, F.A. Haskins, and K.D. Kofoid. 1979. Combining ability in forage sorghum hybrids. Maydica. 24:83-93.

Samarappuli, D. and M.T. Berti. 2018. Intercropping forage sorghum with maize is a promising alternative to maize silage for biogas production. J. Clean Prod. 194: 515-524.

Samarappuli, D.P., B.L. Johnson, H. Kandel, and M.T. Berti. 2014. Biomass yield and nitrogen content of annual energy/forage crops preceded by cover crops. Field Crops Res. 167: 31-39.

SAS Institute, 2012. SAS Institute SAS User's Guide: Statistics. SAS Inst., Cary, NC.

Siemens M.G., D.M. Schaefer, R.J.Vatthauer 1999. Rations for beef cattle. Univ. of Wisconsin-Ext.Publ. A2387. Available at: http://learningstore.uwex.edu/assets/pdfs/A2387.PDF. (verified 2 June 2019).

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

Smith, C. W., and R.A. Frederiksen (Eds.). 2000. Sorghum: Origin, history, technology, and production (Vol. 2). John Wiley & Sons.

Stefaniak, T.R., J.A. Dahlberg, B.W. Bean, Nilesh Dighe, E.J. Wolfrum, and W.L. Rooney. 2012. Variation in biomass composition components among forage, biomass, sorghum-sudangrass, and sweet sorghum types. Crop Sci. 52:1949-1954. doi: 10.2135/cropsci2011.10.0534.

Tagarakis, A.C., Q.M. Ketterings, S. Lyons, and G. Godwin. 2017. Proximal sensing to estimate yield of brown midrib forage sorghum. Agron. J. 109 (1): 109-114.

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.

Undersander, D.J., L.H. Smith, A.R. Kaminski, K.A. Kelling, and J.D. Doll. 1990. Forage sorghum. Alternative field crops manual. Univ. of Wisconsin Ext., Cooperative Ext..

Undersander, D.J., L.H. Smith, A.R. Kaminski, K.A. Kelling, and J.D. Doll. 2010. Sorghum forage. Alternative field crops manual. Univ. of Wisconsin-Ext. Wisconsin Cooperative Ext. Madison, WI. Available at http://www.hort.purdue.edu/newcrop/afcm/forage.html (verified 11 Dec. 2018).

Vendramini, J., Y. Newman, J. Erickson, W. Vermerris, and D. Wright. 2010. Forage sorghum. SS-AGR-333, Agronomy Department, UF/IFAS Ext..

Vendrell, P.F., and J. Zupancic. 1990. Determination of soil nitrate by transnitration of salicylic acid. Comm. Soil Sci. Plant Anal. 21(13):1705-1713. doi: 10.1080/00103629009368334.

Venkateswaran, K., M. Muraya, S.L. Dwivedi, and S.K. Singh. 2014. Wild sorghums- their potential use in crop improvement. p. 56-89. *In* Wang Y-H., Upadhyaya H.D., C. Kole (eds.). Genetics, genomics and breeding of sorghum. Taylor & Francis, Boca Raton, FL,

Vermerris, W., A. Saballos, G. Ejeta, N.S. Mosier, M.R. Ladisch, and N.C. Carpita. 2007. Molecular breeding to enhance ethanol production from corn and sorghum stover. Crop Sci. 47:142-153.

Vinutha, K.S., G.S.A. Kumar, M. Blummel, and P.S. Rao. 2017. Evaluation of yield and forage quality in main and ratoon crops of different sorghum lines. Trop. Grassl.-Forrajes Trop. 5: 40-49.

Wall, J.S., and W.M. Ross. 1970. Sorghum production and utilization. Westport, CT (USA): AVI. pp. 702.

Wheeler, T., and J. von Braun. 2013. Climate change impacts on global food security. Science. 2013.341(6145):508-13. doi: 10.1126/science.1239402.

Wilson, D.M., D.L. Dalluge, M. Rover, E.A. Heaton, and R.C. Brown. 2013. Crop management impacts biofuel quality: influence of switchgrass harvest time on yield, nitrogen and ash of fast pyrolysis products. Bioenerg. Res. 6:103-113.

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

Yu, J., and M. Tuinstra. 2001. Genetic analysis of seedling growth under cold temperature stress in grain sorghum. Crop Sci. 41:438-1443.

Zhao, X., H. Zhou, V.S. Sikarwar, M. Zhao, A.A. Park, P.S. Fennell, L. Shen, and L. Fan. 2017. Biomass-based chemical looping technologies: the good, the bad and the future. Energy Environ. Sci. 10: 1885-1910. doi:10.1039/c6ee03718f.