

Phenotyping a Diverse Collection of Forage Sorghum Genotypes for Chilling Tolerance

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Abstract: Forage sorghum (FS) (*Sorghum bicolor* (L.) Moench) is a warm-season biomass crop used as forage for hay or silage with the potential to become a bioenergy feedstock or for dual-purpose (forage and energy). The objective of this study was to screen potential forage sorghum genotypes for increased chilling tolerance and biomass productivity. Seventy-one genotypes of FS were first ranked for high to low vigor index under controlled conditions at 24, 12, and 10 °C. Field experiments were also conducted on a subset of 12 genotypes in Fargo and Hickson, ND, USA, in 2017 and 2018, using two different 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. Under field conditions, seed mortality and biomass yield were affected by the seeding date and biomass yield correlated with emergence index and normalized vegetative index. Chemical composition of forage sorghum biomass was not affected by the seeding dates. The results of this study suggest that some forage sorghum genotypes carry genetic traits for increased chilling tolerance and produce greater biomass yield when seeded earlier than normal, which could allow for breeding chilling tolerance into forage sorghum.

Keywords: bioenergy feedstock; emergence index; seed mortality; NDVI; biomass yield; nutritive value

1. Introduction

Forage sorghum (*Sorghum bicolor* (L.) Moench) is an annual biomass crop mainly grown for silage, hay and grazing forage. As an alternative crop to maize (*Zea mays* L.), it can also serve as a bioenergy feedstock for biogas, reducing negative environmental impact [1]. Research conducted in the North central region (NCR) has identified forage sorghum as the feedstock for energy (lignocellulosic to ethanol or biogas), with the most potential in this region [2–4]. Because forage sorghum is highly tolerant to heat and drought stress [5], requires low water input due to high-water use efficiency [6,7], and can be grown on marginal land with lower fertilizer input [8,9]. It is well suited to semi-arid conditions [10,11]. However, a main limitation of forage sorghum is its tolerance to temperatures below 15 °C, especially early in the growing season [12,13]. Consequently, in the NCR of the USA, planting of forage sorghum is limited by early-season soil temperatures, which usually do not reach an average of 15 °C until two weeks later than maize planting. This limitation likely involves water imbibition and respiration of sorghum seeds, which slows down at suboptimal temperatures (10 °C) and is often a factor in germination failure [14]. Forage sorghum has the

potential for greater biomass production, as lignocellulosic feedstock for energy, than maize in the NCR [2,3]; thus, improving chilling tolerance could increase biomass, acreage, and productivity of forage sorghum in the NCR.

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 well adapted to the Highlands of Honduras, Kenya, and Mexico, but poorly adapted to the northwestern USA, southern Canada, and West Germany [15,16]. Chinese grain sorghum lines such as Shan Qui Red, Kaoliang, Niu Sheng Zui, and Hong Ke Zi have chilling-tolerance genes [17–19]. However, because these lines generally lack other important agronomic traits, the grain sorghum reference lines BTx623, and SC265 are often used for chilling-sensitive studies in most US laboratories [17].

Many promising quantitative trait loci (QTL) associated with low temperature tolerance have also been identified in grain sorghum [20]. The QTL associated with maintenance of cell division and growth during chilling stress highlight potential prerequisites for improving chilling tolerance of sorghum genotypes [21]. Franks et al. [22] compared 10 Chinese Kaoliang accessions with 10 US inbred parental lines and 10 US commercial grain sorghum hybrids for the trait of chilling-tolerance under growth chamber and field conditions. Although Chinese lines had better performance than both inbred and hybrid US classes in laboratory germination and field emergence rate studies, US hybrids had greater final field stand counts and biomass yield than Chinese lines. These results suggested that Chinese accessions of grain sorghum are a favorable source of germplasm that could be utilized in breeding programs for introgression of early season chilling-tolerant germination traits/genes in grain and forage sorghum.

Phenotyping forage sorghum genotypes for germination rates under controlled conditions is an efficient way for identifying lines adapted to low temperature germination. Under laboratory conditions, a seven-day cold test at 10 °C was a useful predictor for increased emergence in the field, which identified eight novel accessions with potential superior alleles for chilling-tolerance [23]. Measuring shoot growth and germination rate at a controlled temperature of 15 °C is helpful for screening genotypes for chilling-tolerance before final evaluation in the field [24]. Under field conditions, emergence and root establishment of forage sorghum early in the growing seasons also was a key determinant of chilling-tolerance [21]. However, it is important to note that results obtained from growth chamber performance at 15 °C and field performance differed, which included emergence percentage, emergence index, shoot and root dry weight, seedling height, and vigor score [16]. Additionally, control and field studies including different seeding dates (early and late) to screen for chilling-tolerant sorghum genotypes, Kapanigowda et al. [25] identified significant differences in emergence percentage, emergence index, biomass yield, plants height, and leaf number. In that study, late-emerging genotypes produced greater biomass than early emerging genotypes.

Correlations 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 Shan Qui Red genotype [26,27]. The survival and growth performance of sorghum seedlings under cold conditions positively correlated with emergence rate and root development in the field [21].

Chilling tolerant commercial cultivars of forage sorghum and sorghum × sudangrass (*S. bicolor* var. *sudanense*) hybrids are unknown. This study was aimed at identifying and selecting chilling-tolerant genotypes of commercial forage sorghum cultivars and hybrids for greater biomass potential as feedstock for bioenergy.

2. Materials and Methods

2.1. Seed Germination Rate and Vigor Index Evaluation

Sixty-one commercially cultivated forage sorghum or sorghum × sudan genotypes from different seed companies and 10 grain sorghum genotypes (two chilling-sensitive and eight chilling-

tolerant) provided by Kansas State University, Manhattan, KS, USA were tested to measure germination rate. Seed germination rates were evaluated at 24 °C and 12 °C between 4 and 24 March, in 2017, and at 10 °C between 1 and 21 February, in 2018, at the United States Department of Agriculture—Agricultural Research Service (USDA-ARS), Edward T. Schafer Agricultural Research Center in Fargo, ND, USA. 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 baseline germination test at 24 °C, as seeds were stored at cold temperature (4 °C). Germination rate and vigor index was measured for 24 °C, 12 °C, and 10 °C using the following formula:

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

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

$$\text{Corrected vigor index} = \text{Vigor index (at 10 or 12°C)} * \text{germination rate (at 24°C)} / 100$$

In 2017, the eight genotypes ranking the highest in corrected vigor index 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 (BT × 623, 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, had different 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, 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.

2.2. Field Establishment and Experimental Design

Experiments were conducted in 2017 and 2018 at the North Dakota State University (NDSU) research site in Fargo, ND, USA (46°89'7989" N, −96°81'7342" W, 274 m elevation) and in Hickson, ND, USA (46°634' N, −96°838' W, 280 m elevation). The 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) [28]. Rainfall and daily temperature were recorded by the North Dakota Agricultural Weather Network (NDAWN) [29] at both sites (Figure 1).

A composite (include three separate soil cores) soil sample was collected from each replicate at 0–15 cm and 15–60 cm in depth in each site before seeding; in total four composite samples per site, using a closed tube, hand-held soil probe. Soil samples from 0 cm to 15 cm were tested for pH, organic matter (OM), soil nitrate (NO₃-N), phosphorus (P), and potassium (K) by the North Dakota State University (NDSU) Soil Testing Laboratory (Fargo, ND, USA) [30]. The nitrate (NO₃-N) analysis was the only test performed on the soil samples taken at the 15–60 cm depth (Table 1). Soil samples were tested using the following methods: i) organic matter, loss on ignition; ii) NO₃-N, colorimetric determination by trans-nitration of salicylic acid method [31,32]; iii) P Olsen procedure using a Brinkmann PC 910 colorimeter (Brinkmann Instruments, Westbury, NY, USA) [33]; and iv) K ammonium acetate method using a Buck Scientific Model 210 VGP atomic absorption spectrophotometer (Buck Scientific, East Norwalk, CT, USA) [34].

Table 1. Soil analysis before seeding at Fargo and Hickson, ND, USA in 2017 and 2018.

Environment	Soil depth	NO ₃ -N †	P	K	pH	OM
	Cm	kg 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	-	-	-	-

† pH, organic matter (OM), phosphorus (P), potassium (K), nitrate nitrogen (NO₃-N).

The experiment was a randomized complete block design (RCBD) 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 four-cone continuous plot drill XL (Wintersteiger, Salt Lake City, UT, USA) 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 percentage obtained in growth chamber experiments. Seeds were sown to 2.5 cm depth.

Forage biomass was harvested two times. 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 Hickson experiment site was fertilized with 80 kg N ha⁻¹ and 50 kg P₂O₅ ha⁻¹ before seeding in 2017 and 2018. The Fargo experiment site was fertilized with 60 kg N ha⁻¹ at the V-5 stage of sorghum, as side dressing in 2017 and 2018. The Fargo experiment site was fertilized later with lower rate as initial soil test report showed higher NO₃-N content than Hickson site. No K was applied in any experimental sites since soil test results were above 200 mg kg⁻¹, which is considered an adequate level [30]. Weed pressure was greater at the Fargo site compared 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 dichlorophenoxyacetic acid (2,4 D) was sprayed at the V-3 stage of sorghum with a rate of 270 g active ingredient (a.i.) ha⁻¹ at the Fargo site in 2017 and 2018.

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 °C for twelve days in 2017 (Figure 1a,b) and five days in 2018 (Figure 1c,d). At the end of the season, average air temperature decreased below 15 °C on 23 September in 2017 and 17 September in 2018. There was less rainfall in 2017 (Figure 1a,b) compared with 2018 (Figure 1c,d). From seeding to final harvesting, total rainfall was 246 mm at Fargo and 284

mm at Hickson in 2017 (Figure 1a,b) and 405 mm at Fargo and 425 mm at Hickson in 2018 (Figure 1c,d) [29].

A sensor (5TM water content and temperature sensors, Decagon Devices, Inc., Pullman, WA, USA) was set in the field at 2.5–3.5 cm depth at 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.

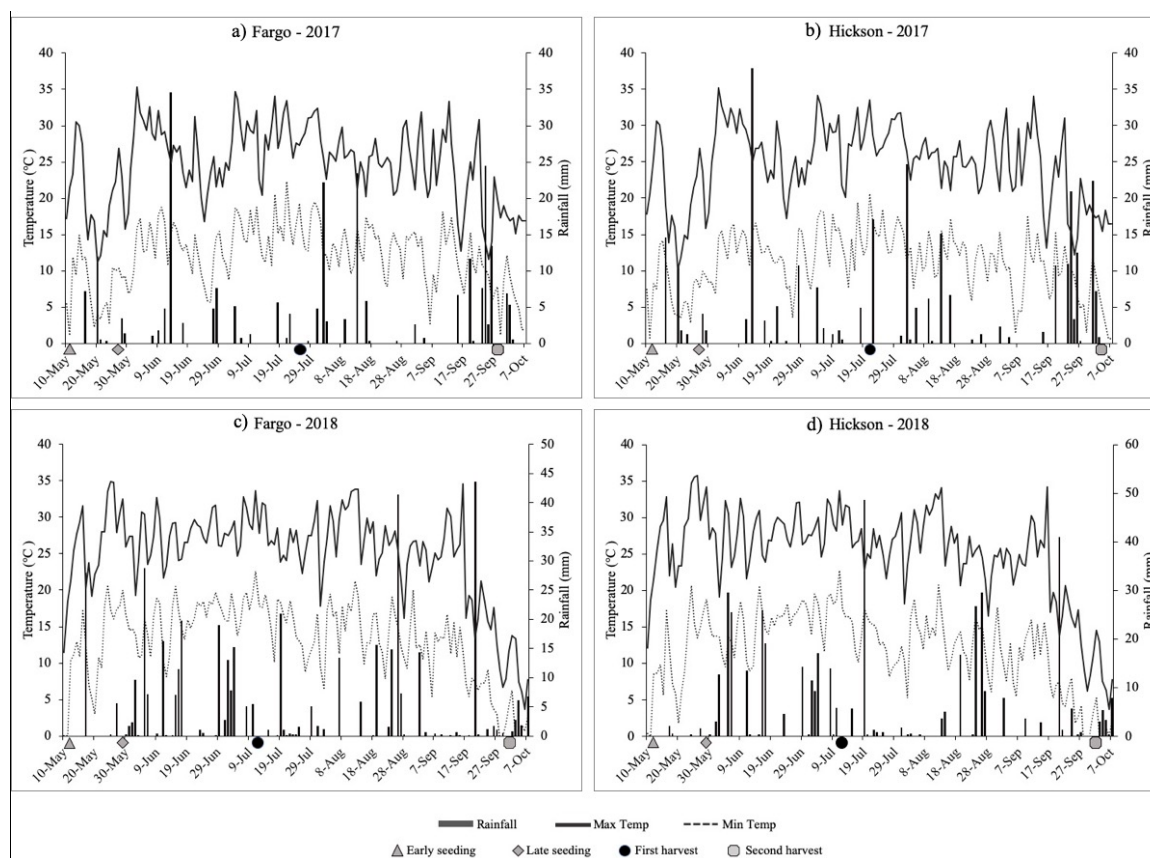


Figure 1. Air temperature and rainfall distribution (rainfall events indicated in grey bars) at four environments at (a) Fargo 2017, (b) Fargo 2018, (c) Hickson, 2017, and (d) Hickson 2018, ND, USA.

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

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

CANOPEO (a free mobile application developed by Oklahoma State University, Stillwater, OK, USA, to measure canopy) was used to measure the canopy coverage at early vegetative stage [35]. One picture was taken from each experimental unit from the same level of height with an Android phone. Those pictures were analyzed in a computer-based CANOPEO application to measure the percentage of green coverage within a selected area.

The number of plants was 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 was calculated using the following formula:

$$\text{Mortality (\%)} = 100 - \frac{(190,190 - \text{stand establishment})}{190,190 * 100}$$

Normalized difference vegetation index (NDVI) was measured using a handheld *GreenSeeker* (Trimble Inc., Sunnyvale, CA, USA) at 49 days after seeding (DAS) in 2017 and 2018. The *GreenSeeker* instrument 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

$$\text{NDVI} = \frac{\text{NIR} - \text{VIS}}{(\text{NIR} + \text{VIS})}$$

where NIR = near-infrared reflectivity at 774 nm and VIS = red reflectivity at 656 nm

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

$$\text{Intercepted PAR light (\%)} = \frac{\text{PAR above the canopy} - \text{PAR below the canopy}}{\text{PAR above the canopy} * 100}$$

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 of stubble to allow regrowth. The second harvest was done cutting stalks by hand with a scythe. 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 °C. Dry matter content and dry biomass yield were calculated using the following formula.

$$\text{Dry matter content} = \frac{\text{Dry weight}}{\text{Fresh weight}} * 100$$

$$\text{Dry biomass yield} = \frac{\text{Total harvested fresh weight} * \text{Dry matter content}}{100}$$

Dried biomass samples were ground to a 1-mm mesh with a mill (Wiley Mill Standard Model N°3, Philadelphia, PA, USA). Samples of the first harvest in 2017 were analyzed at the Animal Sciences laboratory at NDSU, Fargo, ND, USA, to measure N content using the Kjeldahl method [36]; crude protein (CP) according to Association of Official Agricultural Chemists (AOAC) Method 2001.11; neutral detergent fiber (NDF) according to ANKOM, 2011.A200 (ANKOM Technology, Macedon, NY, USA) 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 these wet chemistry data, a calibration was created for forage sorghum biomass samples in an XDS near-infrared reflectance (NIR) rapid content analyzer (Foss, Denmark), following the methods described by Abrams et al. [37]. Samples falling off the calibration ranges in 2018 were sent to the laboratory for wet chemistry analysis. Nitrogen accumulation was calculated by multiplying nitrogen content in biomass by the dry matter yield.

2.3. Data Analysis

The germination rate and corrected vigor index data were analyzed with statistical analysis software (SAS) [38] using MIXED 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. Treatment means separation was determined by *F*-protected least significant difference (LSD) comparisons at the $p \leq 0.05$ probability level.

All the field data collected throughout the season were analyzed separately by year and location. Homogeneity of variance tests were done to determine if environments (defined as the combination of location–year) could be combined. If homogeneous, a combined analysis across four environments was conducted. To determine the difference among those treatments, *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.

2.3.1. 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 (Figure 2a–d). In 2018, soil temperature was higher than in 2017 during emergence. In 2018, fluctuation of day and night temperature was greater at planting. Though soil temperature was above the base temperature for sorghum (15 °C) at planting, at the Fargo site in 2018 (Figure 2c,d), emergence did not occur, as soil water content was very low ($< 200 \text{ g kg}^{-1}$). Early seeded genotypes were exposed to cold temperatures (below base temperature) at the beginning of the season for twelve days in 2017 (Figure 2a,b) and only six days in 2018 (Figure 2c,d), while late-seeded genotypes were exposed to temperatures below base temperature for only two days in 2017 (Figure 2a,b). For the late-seeded genotypes, soil temperature was above the base temperature at seeding in both 2017 and 2018 (Figure 2a–d).

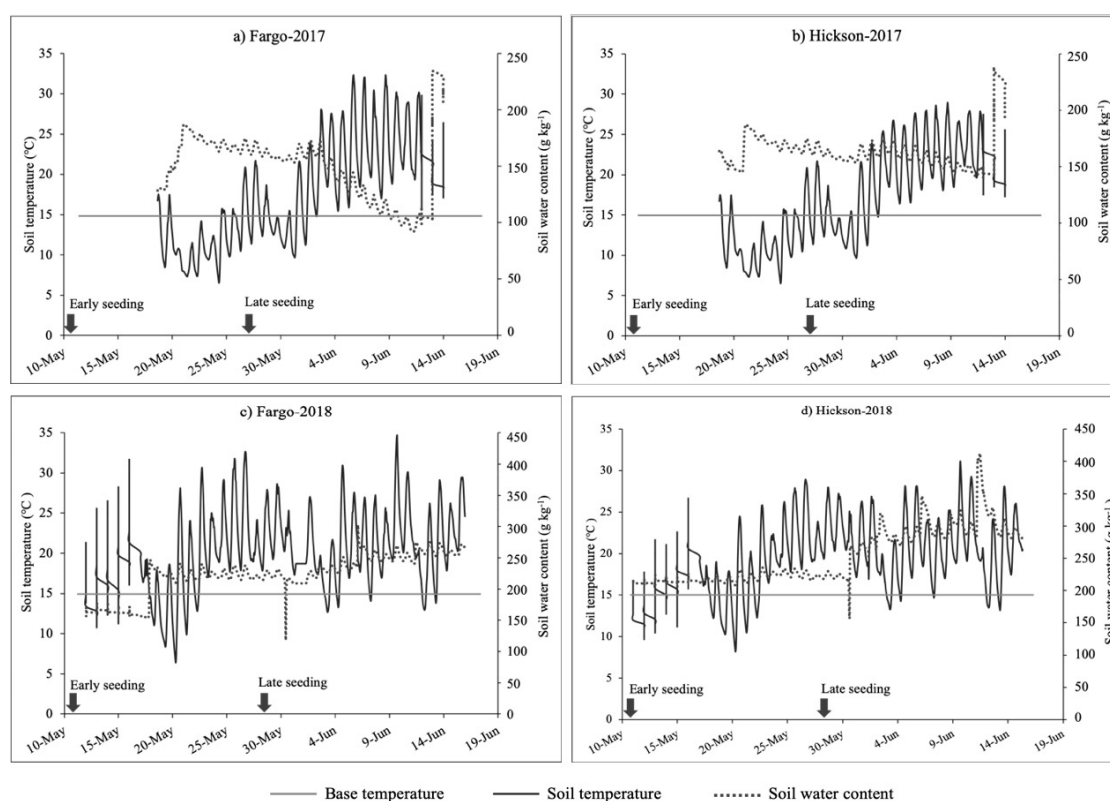


Figure 2. Soil temperature and soil water content from planting to the end of emergence at four environments at (a) Fargo 2017, (b) Hickson 2017, (c) Fargo, 2018, and (d) Hickson 2018, ND, USA.

3. Results and Discussion

3.1. Growth Chamber Seed Germination and Vigor Index

Sorghum genotypes varied in germination rate and vigor index at 24, 12, and 10 °C (Table 2). The ranges of germination rate were 52 to 100% at 24 °C, 32.7 to 100% at 12 °C, and 6.7 to 99.3% at 10 °C. Germination rates were greater at 24 °C, as that is the optimum temperature for germination of forage sorghum [39]. The range of corrected vigor indices were 5.2 to 25.0 at 12 °C, and 0.7 to 17.8 at 10 °C. Based on the corrected vigor index at 12 °C and 10 °C, genotypes were ranked from highest to lowest. At 12 °C, corrected vigor indices were statistically indistinguishable ($p \geq 0.05$) for the 1 to 48 ranked genotypes. In the second year of the study, the temperature was decreased to 10 °C to exert more cold stress on genotypes. The results produced at 10 °C indicated that only eight genotypes had the same corrected vigor index ($p \geq 0.05$) as the highest ranked genotype. Only six genotypes ranked among the top 11 genotypes at both temperatures, 12 °C and 10 °C. Temperatures below 15 °C are considered a cold stress condition in sorghum, which drastically reduces the seed germination rate and seedling growth [12,13]. Razmi et al. [12] observed 50% germination reduction, vigor index reduction, and a 10.5 days delay in germination at 11/8 °C (day/light) compared with a 25/22 °C regime in three sorghum genotypes conducted under growth chamber conditions.

Table 2. 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	SorghumType	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 ^{ab}	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 ^a	GS	N	98.7	98.7	82.7	25.0	3	10.6	45
Sordan Headless ^b	SxS	Y	97.3	98.7	94.7	24.9	4	15.7	8
Pampa Triunfo XLT ^{ab}	SxS	Y	94.7	97.3	95.3	24.6	5	17.8	1
NK300 ^b	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 ^{ab}	Su	Y	100.0	100.0	98.0	24.4	8	14.8	16
SC265 ^a	GS	N	99.3	98.7	53.3	24.2	9	5.4	64
Pampa Verde BMR 6 ^b	SxS	Y	98.0	96.7	96.7	24.2	10	16.2	5
1990 ^{ab}	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 ^a	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 ^b	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 ^{ab}	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 ^a	SxS	Y	98.7	95.3	96.7	23.4	25	14.7	17
Brachytic sorghum ^a	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 ^b	GS	N	98.0	96.0	85.3	22.5	37	8.1	54
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 ^b	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
Pacesetter BMR	FS	Y	94.7	92.0	87.3	14.6	67	12.0	35
FS-5	FS	Y	90.0	81.3	42.0	14.6	68	4.3	68
Piper (2)	Su	Y	98.0	92.7	60.0	14.0	69	4.9	66
Enorma	Su	N	92.6	71.3	72.0	11.0	70	6.3	62
Forage King ^{ab}	Su	N	52.0	32.7	6.7	5.2	71	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 × sudangrass, SW = sweet sorghum, GS = grain sorghum, BMR = brown-midrib. ^a Selected for field experiment in 2017, ^b Selected for field experiment in 2018, ^{ab} Selected for field experiment in both 2017 and 2018. † Values in bold in the table indicate the range of values not significantly different from the highest ranked value within a same column.

3.2. Seeding Date Study in Field Conditions

Genotypes field emergence index varied significantly. The seeding date main effect and the interaction of genotypes by seeding date were significant for emergence index ($p \leq 0.05$) (Figure 3a). Emergence index was much greater for the second seeding date than the first seeding date (Figure 3a), as expected, since soil temperatures were greater at second planting (Figure 2a–d). The genotypes Sordan Headless, NK300, and Hay King emerged faster in the early-seeding date compared with the other genotypes. The faster germination and emergence at lower temperatures suggested these genotypes as having better tolerance to chilling temperatures, 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 in all genotypes evaluated (Figure 3b).

Temperature below 15 °C inhibits seedling emergence and decreases emergence rate in forage sorghum [16,40,41]. Thus, forage sorghum is typically planted once the average 10-d soil temperature (0–15 cm in depth) is >15 °C [40]. However, significant variation in early-emergence index and emergence percentage has reported previously by Kapanigowda et al. [25], which supports the results in this current study where a lower emergence index was observed with earlier seeding. Thus, field emergence index is an efficient way of screening forage sorghum genotypes for chilling tolerance.

Canopy coverage at 46 days after seeding (DAS) (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. The genotypes Sordan Headless, Pampa Verde BMR-6, NK300, BMR-90, and SPX-901 had greater canopy coverage at 46 DAS (Figure 3b), indicating those genotypes performed better early season as greater canopy coverage at earlier stages of development indicates rapid growth. Chung et al. [42] measured canopy coverage and found a significant correlation between plant height and percentage of green color of four different sorghum genotypes in field conditions. It has been reported that sorghum growth rate is reduced if exposed to temperatures of <15 °C, especially early in the season [16,41]. Therefore, genotypes that grow faster early in the season are potential genetic sources for the chilling tolerance trait.

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 [43]. 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 [27]. 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 [44]. Chlorophyll fluorescence was measured on our study, but no differences were observed among genotypes at any date or location, thus the data is not shown.

The genotype main effect was significant at all stages of evaluation for normalized difference vegetation index (NDVI) ($p \leq 0.05$). A higher NDVI value indicated those genotypes had greater cover and likely were growing faster than the other genotypes seeded earlier, but the genotypes' plant architecture was not homogeneous and likely influenced NDVI. The NDVI differences among genotypes were mainly due to the differences in plant height and coverage (Figure 3c). 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. Similar results were found by Chiluwal et al. [26] in field conditions using aerial measurement of NDVI. This provides a potentially useful tool to evaluate the phenotypic variation among sorghum genotypes for chilling response at 30 to 60 days after emergence [26].

Seeding date main effect and genotype main effect were significant for intercepted photosynthetically active radiation (IPAR) only at 58 DAS ($p \leq 0.05$). Before the first harvest, IPAR ranged between 25% and 84% at 58 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 (Figure 3d). Kaoliang and Niu Sheng Zui lines had lower IPAR resulting from reduced tiller number and lower stand establishment compared with other genotypes. After the first harvest, IPAR ranged between 39% and 81% at 30 days after harvest (DAH) and 77% to 95% at 45 DAH (not shown). In later growing stages, IPAR was similar for all genotypes as ground was covered by tillers 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. [45] found similar results with forage sorghum reaching up to 95% IPAR in central and southern Illinois, and Meki et al. [46] reported over 90% IPAR in forage sorghum in a field trial at Temple, TX.

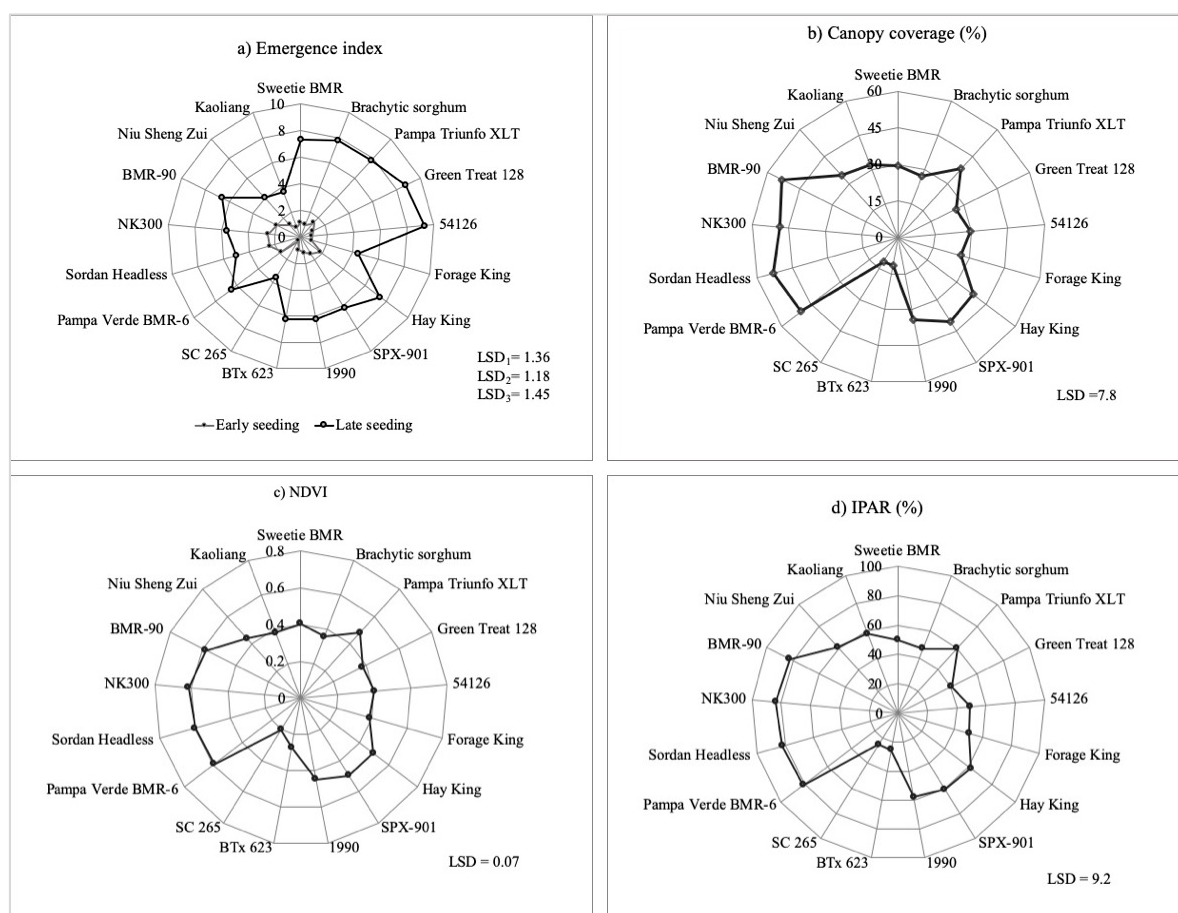


Figure 3. (a) Mean emergence index at early and late seeding, and (b) mean canopy coverage at 46 days after seeding (DAS), (c) mean normalized difference vegetation index (NDVI) evaluated at 49 DAS, (d) mean intercepted photosynthetically active radiation (IPAR) evaluated at 58 DAS in forage sorghum genotypes averaged across two seeding dates and four environments in Fargo and Hickson, ND, USA, in 2017 and 2018. Least significant differences (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, LSD in Figure 3b, 3c, and 3d are to compare among genotypes-mean averaged across seeding date within the same figure.

3.2.1. Seed Mortality

Mortality of pure live seeds was significant for genotype and the interaction between genotype and seeding date ($p \leq 0.05$). 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, and Kaoliang genotypes had higher mortality percentage at the early seeding compared with the same genotypes seeded later (Figure 4). This indicates that cold soils imposed a stress in seedlings, increasing mortality. Even if 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 (Figure 4). Seed mortality in cold soils can also be due to pathogens. Soil temperature below 15 °C promotes susceptibility to molding and other pathogen infections like *Pythium* spp., *P. aphanidermatum*, *P. ultimum*, *P. arrhenomanes*, etc. that ultimately increases the chances for germination failure early in the season [47]. Most of the commercial cultivars and hybrids tested seed had added fungicides. The chilling-tolerant lines-Niu Sheng Zui and Kaoliang were not coated with fungicides (Table 2), which could have stimulated higher seed mortality for those lines. 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 by the lower seed mortality. Generally, up to 65–70% seed emergence may occur during the optimum seeding time, depending on field moisture [40].

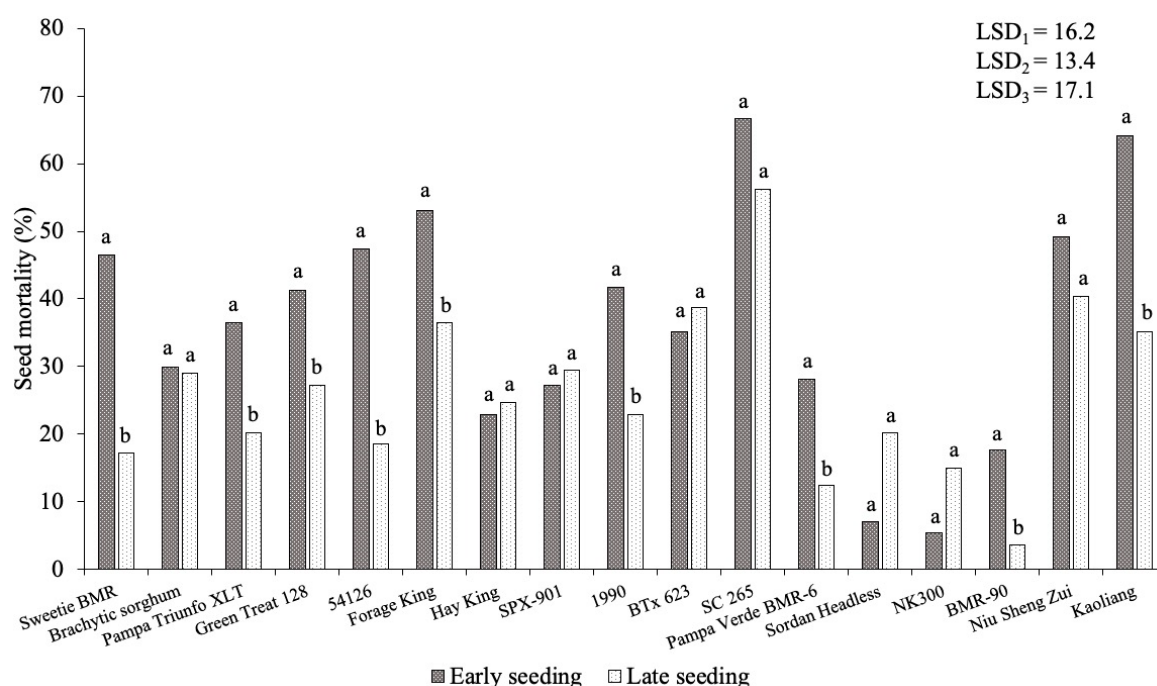


Figure 4. Mean seed mortality rate in forage sorghum genotypes planted on two seeding dates averaged across four environments at 20 days after each planting in Fargo and Hickson, ND, USA, in 2017 and 2018. Least significant differences (LSD_1) to compare among genotypes within the same seeding date, 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).

3.2.2. Plant Height

Plant height at both harvests was significant among genotypes and seeding dates ($p \leq 0.05$). However, no interaction was observed for the genotype by seeding date interaction. Plant height was greater for all genotypes in the second harvest than the first harvest (Table 3). 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, it is possible that increased temperatures following the first harvest allowed uptake of more nutrients and water from soil, which allowed the plants to grow taller by the second harvest. However, in this study, nutrient and water uptake were not measured. The genotypes brachytic sorghum, BTx623, and Sc 265 are dwarf types (Table 3).

Table 3. Mean plant height, biomass yield and dry matter content of two harvests (H-1 and H-2) in forage sorghum genotypes seeded on two dates averaged across four environments, in Fargo and Hickson, ND, USA, in 2017 and 2018.

Genotype	Plant Height (m)		Biomass Yield (Mg ha ⁻¹)			Dry Matter (%)	
	H-1	H-2	H-1	H-2	Total	H-1	H-2
Early Seeding							
Sweetie BMR	1.31	1.68	3.2	8.5	11.7	17.2	22.0
Brachytic sorghum	0.94	1.07	3.2	8.8	12.0	18.6	23.0
Pampa Triunfo XLT	1.38	1.65	3.3	9.2	12.5	16.9	23.0
Green Treat 128	1.10	1.74	3.2	10.4	13.6	19.1	19.6
54126	1.28	1.70	3.8	12.3	16.1	17.3	20.9
Forage King	1.64	1.95	3.1	10.4	13.4	19.0	32.9
Hay King	1.61	1.95	3.9	10.5	14.5	18.6	26.3
SPX-901	1.28	1.79	4.0	11.0	15.0	18.2	22.5
1990	1.20	1.67	4.0	10.5	14.5	18.3	21.1
BTx623	0.96	1.02	2.7	7.2	9.9	17.1	25.9
SC 265	0.72	0.87	0.7	5.6	6.3	15.5	28.3
Pampa Verde BMR-6	1.39	1.69	4.1	10.0	14.2	15.2	21.9
Sordan Headless	1.57	1.94	5.3	12.7	18.0	16.4	22.5
NK300	1.41	1.45	5.6	10.3	15.9	17.7	24.1
BMR-90	1.64	1.73	4.6	9.7	14.4	16.6	26.2
Niu Sheng Zui	1.68	2.04	2.1	5.3	7.4	14.9	30.9
Kaoliang	1.67	2.19	1.6	7.2	8.7	16.6	36.2
Late Seeding							
Sweetie BMR	1.09	1.77	2.5	11.0	13.5	16.9	23.6
Brachytic sorghum	0.90	1.06	3.2	10.5	13.7	18.4	22.8
Pampa Triunfo XLT	1.17	1.74	2.6	10.6	13.2	16.7	23.4
Green Treat 128	1.07	1.76	3.1	13.1	16.2	19.4	20.5
54126	1.25	1.74	3.6	14.0	17.6	17.8	21.5
Forage King	1.37	1.98	2.2	11.1	13.3	17.6	30.1
Hay King	1.43	1.98	2.9	11.6	14.5	17.5	26.4
SPX-901	1.02	1.97	2.9	13.1	16.0	18.4	22.7
1990	0.97	1.89	2.7	13.0	15.7	17.9	21.7
BTx623	0.89	1.10	1.3	5.1	6.4	17.0	25.8
SC 265	0.71	0.85	1.1	5.9	7.0	15.9	27.5
Pampa Verde BMR-6	1.03	1.87	2.5	12.3	14.8	15.3	20.1
Sordan Headless	1.14	2.11	2.6	13.3	15.9	15.0	20.4
NK300	1.05	1.63	3.2	12.5	15.6	16.4	25.6
BMR-90	1.26	1.94	3.0	13.9	17.0	14.6	28.3
Niu Sheng Zui	1.21	2.27	1.2	5.8	7.0	13.5	31.9
Kaoliang	1.21	2.37	1.1	6.3	7.3	14.3	31.6
LSD ₁ (0.05)	0.11	0.16	0.6	2.2	2.4	1.5	2.3
LSD ₂ (0.05)			0.5	1.2	1.4		

Least significant differences (LSD₁) to compare genotypes within the same or different seeding date, LSD₂ to compare same genotypes within different seeding date within each harvest date.

3.2.3. 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 3). 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, genotypes 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 3). The increased biomass observed for early seeding dates identified the genotype Sordan Headless as able to grow in colder soils, which was the objective of this study.

3.2.4. 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 (Table 3), which indicated that those genotypes were in more advanced growth stages than the other genotypes. The dry matter content is important if forage sorghum silage will be used for biogas production. Forage sorghum genotypes with greater dry matter at harvest have increased starch and sugar content and that will increase methane yield during anaerobic digestion [48]. This is very important if forage sorghum is produced as feedstock for bioenergy, whether it is for anaerobic digestion or lignocellulosic biochemical conversion [1]. Breeding programs in energy sorghum select genotypes with high biomass productivity, low lignin content, adaptability to low temperature, drought tolerance, 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 concentration (measured by Brix) in the stem and juice yield in sweet sorghum [49]. Forage sorghum harvested with high moisture content (>70%) promotes seepage in the silo leading to nutrient losses, lower digestibility, and production of butyric acid as wet silage promotes higher fermentation losses and lower intake by cows [40].

3.3. Biomass Yield Prediction

Emergence index was a good predictor of biomass yield of the first harvest for both seeding dates (early-seeding, $r^2=0.74$ (Figure 5a); late-seeding, $r^2=0.60$ (Figure 5b), indicating earlier emerging genotypes have greater vigor and better biomass yield at first harvest. Similarly, NDVI at 49 DAS ($r^2=0.67$) (Figure 5c) and 20 DAH ($r^2=0.61$) (Figure 5d) were good predictors of biomass yield of the first and second harvest, respectively. Tagarakis et al. [50] 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. [51] reported 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.

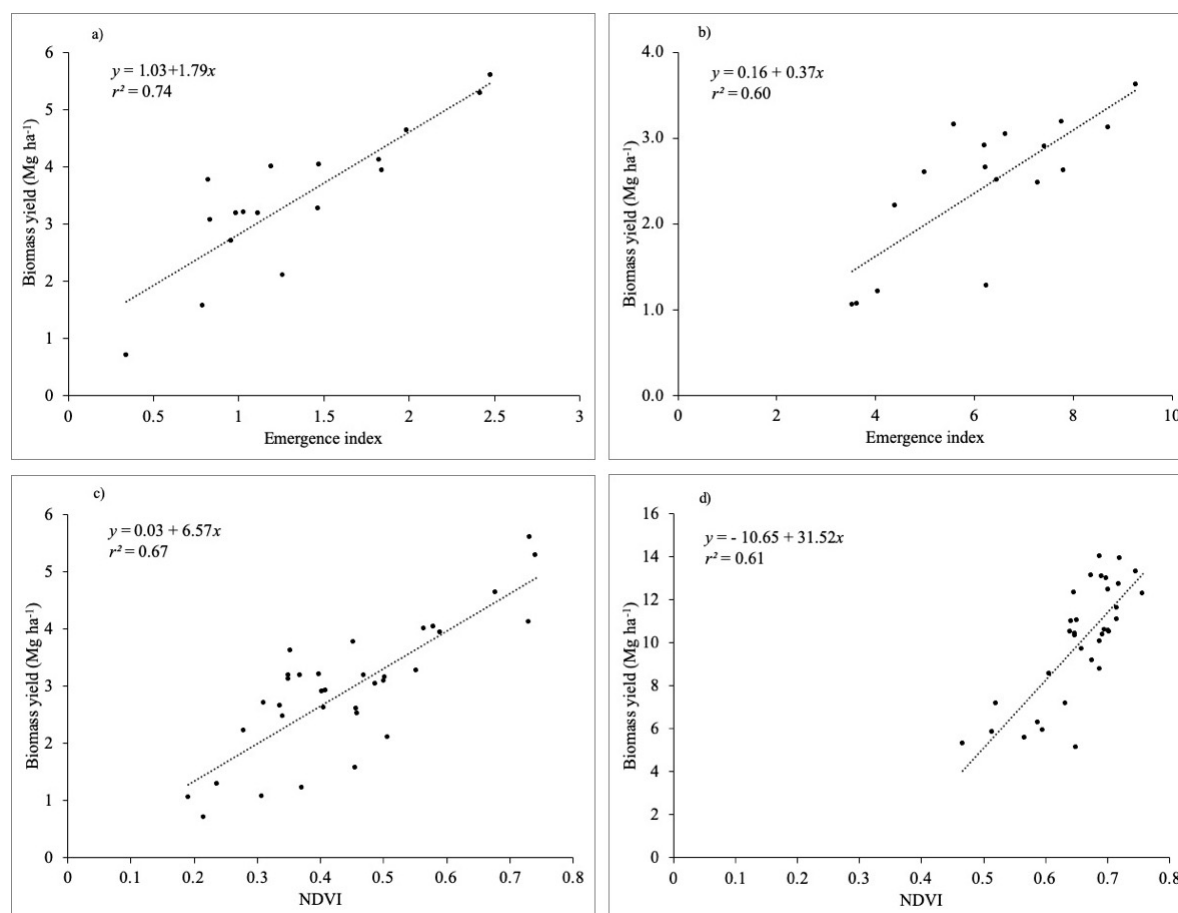


Figure 5. (a) Emergence index vs. biomass yield for early-seeding at first harvest, (b) emergence index vs. biomass yield for late-seeding at first harvest, (c) normalized difference vegetation index (NDVI) at 49 days after seeding (DAS) vs. biomass yield for first harvest, and (d) NDVI at 20 days after harvest (DAH) vs. biomass yield for second harvest in forage sorghum genotypes (G) averaged across four environments, Fargo and Hickson, ND, USA, in 2017 and 2018.

3.4. Forage Nutritive Value

Crude protein (CP) content varied among genotypes and harvest dates but not between seeding dates ($p \leq 0.05$). 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 4). 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 [1]. 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. 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 4). The range of CP was 133–214 g kg⁻¹ in the first harvest and 63–106 g kg⁻¹ in the second harvest (Table 4). Crude protein requirement in mature beef cattle ranges between 70–90 g kg⁻¹; thus, in both harvests, most sorghum genotypes meet this requirement [52]. In the first harvest, the highest CP values were observed in SC 265 and in BTx623, as those are grain-type genotypes and are shortest in height (Table 4). Pampa Verde BMR-6 had the highest CP content in the second harvest (Table 4), 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; with the low-quality, high-biomass yield in the second harvest utilized for bioenergy production. Samarappuli and Berti [1] reported 104–105 g kg⁻¹ CP in a single-harvest for bioenergy production in September of non-brown mid rib (BMR) and BMR forage sorghum in North Dakota.

Nitrogen (N) accumulation averaged across genotypes was much greater in the second harvest. Biomass yield in the second harvest was 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 compared with the other genotypes (Table 4). Samarappuli and Berti [1] observed the highest nitrogen accumulation in non-BMR forage sorghum genotypes in North Dakota, which is similar with the current study. Anfinrud et al. [4] 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. [4] reported; however, only if the second harvest (one-cut system) is taken into account was N accumulation similar. Forage sorghum is known as an efficient soil nitrogen scavenger, especially where nitrogen accumulation depends on multiple factors such as sorghum types and hybrids, stage of harvest, and availability of nitrogen in soil [53]. Pedersen et al. [53] found nitrogen accumulation ranged between 137 kg N ha⁻¹ and 283 kg N ha⁻¹ in 18 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 (23–33 g kg⁻¹) compared with the second harvest (11–17 g kg⁻¹).

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 are high in organic matter (50–60 g kg⁻¹). Mineralization is a process of decomposing organic nitrogen from crops residues, to ammonium. Mineralization depends on soil temperature (20 to 35 °C), soil water, and oxygen availability in the soil [54]. 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, USA [54]. Kaur et al. [55] conducted an eight-week incubation study from 0 cm to 15 cm in depth of soil and found cumulative nitrogen mineralized ranged between 0.34 N kg⁻¹ and 2.15 mg N kg⁻¹ for the Glyndon soils and 0.45 N kg⁻¹ to 3.41 mg kg⁻¹ for the Fargo soils, respectively.

Low lignin content is a desired character for high nutritive value forage. Brown mid-rib (BMR) genotypes had lower lignin content than non-BMR sorghum, as expected. In the first harvest, the range of acid detergent lignin (ADL) content was 29–36 g kg⁻¹ and in the second harvest ADL was 17–54 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 4). 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 4). Lee et al. [56] reported 80 g kg⁻¹ ADL in non-BMR forage sorghum evaluated in South Dakota. Samarappuli and Berti [1] 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, USA. Lignin content increases with the progressing plant maturity. In the current study, most of the genotypes were in vegetative stage at first harvest and in early reproductive stage at second harvest, which explains the ADL content observed.

Genotypes main effect was significant for neutral detergent fiber (NDF) and acid detergent fiber (ADF) 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 265–304 g kg⁻¹ and 284–348 g kg⁻¹ in the first and second harvest respectively (Table 4). The range of NDF was 525–579 g kg⁻¹ and 546–652 g kg⁻¹ in the first and second harvest, respectively (Table 4). Samarappuli and Berti [1] reported 304–331 g kg⁻¹ ADF and 542–572 g kg⁻¹ NDF in two forage sorghum genotypes in North Dakota. Anfinrud et al. [4] reported 251–366 g kg⁻¹ ADF and 518–634 g kg⁻¹ NDF in forage sweet sorghum and sorghum × sudangrass in North Dakota, which are similar to the ranges observed in our study. The NDF consists of all the fibers (hemicellulose, cellulose, and lignin) in the plant cell walls and only a fraction of NDF is partially digestible. In a feed ration calculation, 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 is partially digestible and consists of cellulose and lignin. Digestion of forage decreases as ADF increases. Low NDF and low ADF are desired for high nutritive value forage. Prime quality forage for beef cattle consists of <400 g kg⁻¹ NDF and <310 g kg⁻¹ ADF [57]. Effective fiber expressed as eNDF is necessary to maintain the rumen function and pH level in optimum condition

[57]. 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 [57]. In our study, the ADF was within the desired range while NDF value was higher than prime quality forages. It was indicated that the lignin content was lower, which mean higher digestible forages.

Genotypes main effect was significant for ash content at first harvest and second harvest. The range of ash content was 99–165 g kg⁻¹ at first harvest and 76–127 g kg⁻¹ at second harvest (Table 4). Ash content was lower at second harvest compared with first harvest. The ash content was greater than that reported by Samarappuli and Berti [1] (63–65 g kg⁻¹). Mahmood et al. [48] stated that ash content in biomass was affected by cultivar and by site with a range of 61–98 g kg⁻¹. Ash content in the biomass varies with soil type and harvesting process [58]. Ash is considered as waste byproduct and an anti-quality factor in biomass conversion to energy. Biogas production from biomass is negatively affected by high ash content as microorganisms cannot decompose the ash [1], while in thermochemical conversion systems, biomass cannot be heated above 400 °C; otherwise, the ash melts and clogs the reactor [59]. That is a reason a lower ash content is desired in bioenergy feedstocks.

Table 4. Mean value of biomass quality in forage sorghum genotypes in the first and second harvest averaged across two seeding dates and four environments, Fargo and Hickson, ND, USA, in 2017 and 2018.

	CP	Nacc	N	ADL	ADF	NDF	Ash
Genotypes	g kg ⁻¹	kg ha ⁻¹			g kg ⁻¹		
First Harvest							
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 ₁ (0.05)	13	16.5	2	3	9	15	14
Second Harvest							
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 ₂ (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); Least significant differences (LSD₁) to compare genotypes within the first harvest, LSD₂ to compare same genotypes within the second harvest.

4. Conclusions

Forage sorghum had lower germination percentages and slower germination rates at 10 °C compared with 12 °C and 24 °C. Early in the season, forage sorghum was affected by cold soil conditions, which reduced the emergence index, 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 normalized difference vegetation index 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. These genotypes can be evaluated in breeding programs for introgression of the early-season chilling-tolerant trait into high biomass producing forage sorghum. Chemical components of forage sorghum 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—can be potential feedstocks for bioenergy. From this study, commercially available chilling-tolerant forage sorghum genotypes were identified, which should enhance the acreage and production of forage sorghum biomass and increase diversification of cropping systems in the north central region (NCR) of the USA.

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