Analyses of sorghum [*Sorghum bicolor* (L.) Moench] lines and hybrids in response to early-season planting and cool conditions

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[1]KSU, Agricultural Research Center, Hays, KS 67601, USA; [2]KSU, Northwest Research-Extension Center, Colby, KS 67701, USA; [3]CGAHR-USDA-ARS, Manhattan, KS, 66502, USA; and [4]Department of Plant Pathology, KSU, Manhattan, KS 66506, USA. *Received 4 December 2012, accepted 22 May 2013.*

Kapanigowda, M., H., Perumal, R., Aiken, R. M., Herald, T. J., Bean, S. R. and Little, C. R. 2013. **Analyses of sorghum [*Sorghum bicolor* (L.) Moench] lines and hybrids in response to early-season planting and cool conditions**. Can. J. Plant Sci. **93**: 773–784. Early-season cold tolerance in sorghum contributes to emergence, seedling establishment, and early vegetative growth, and reduces damping-off diseases under chilling conditions. The objectives of this study were to identify cold-tolerant sources and to evaluate and optimize rapid screening techniques under a controlled environment. Field studies involving 48 genotypes, representing phases of the hybrid development process (landraces, elite and advanced breeding lines, recombinant inbred lines (RILs) and hybrids were conducted with early and normal planting dates in 2011 at Hays and Colby, Kansas. Studies under controlled environments were conducted at both locations using 18 genotypes that differ for emergence index (EI) and 30 d after emergence (DAE) shoot biomass based on field studies during 2011. Significant differences among the genotypes were recorded for all seedling traits (emergence percentage, EI, shoot biomass, plant height, and leaf number measured 30 DAE), and agronomic traits (days to 50% flowering, panicle exsertion, panicle length, and plant height at maturity). Eight advanced breeding lines: ARCH10731, ARCH10732, ARCH10736, ARCH10737, ARCH10738, ARCH10739, ARCH10744 and ARCH10749 and one RIL (RTx430/SQR-2) were found to be potential sources of cold tolerance with early EI, higher biomass and relatively early flowering. These genotypes are free from tannin, which helps to increase the feed grain efficiency of livestock, and hence were selected for test hybrid evaluation to assess fertility status, combining ability and yield performance. Significant correlation was observed between EI and biomass during early planting, which indicated that late-emerging genotypes produced greater biomass (30 DAE) compared with early-emerged genotypes. Significant correlation between growth chamber and field study for EI offers a potential and fast preliminary high-throughput screening technique for identification of cold-tolerant sorghum.

Key words: Sorghum, cold-tolerant, high throughput screening

Kapanigowda, M., H., Perumal, R., Aiken, R. M., Herald, T. J., Bean, S. R. et Little, C. R. 2013. **Analyse de la réaction des lignées et des hybrides du sorgho [*Sorghum bicolor* (L.) Moench] à une plantation hâtive et aux températures fraîches**. Can. J. Plant Sci. **93**: 773–784. La tolérance du sorgho au froid, en début de saison, favorise la levée, l'établissement des plantules, une croissance rapide de la végétation ainsi qu'une moins grande fonte des semis attribuable aux maladies quand il fait frais. La présente étude devait identifier les sources de cette tolérance au froid et permettre l'évaluation puis une optimisation des techniques de présélection rapide en milieu contrôlé. Les auteurs ont étudié sur le terrain 48 génotypes représentant les stades de développement d'un hybride (population naturelle, lignées élite et hybrides évolués, lignées autogames de recombinaison) et des hybrides aux dates de plantation précoce et normale, en 2011, à Hays et à Colby (Kansas). Des études en conditions contrôlées ont été réalisées aux deux endroits sur 18 génotypes dont l'indice de levée et la biomasse des pousses 30 jours après la levée n'étaient pas les mêmes, compte tenu des résultats de l'étude effectuée sur le terrain, en 2011. Les génotypes présentent des écarts significatifs pour tous les paramètres de la plantule (pourcentage de levée, indice de levée, biomasse des pousses, hauteur du plant et nombre de feuilles 30 jours après la levée) et les facteurs agronomiques (nombre de jours jusqu'à 50 % de floraison, exsertion du panicule, longueur du panicule et hauteur du plant à maturité). Huit lignées avancées (ARCH10731, ARCH10732, ARCH10736, ARCH10737, ARCH10738, ARCH10739,

Abbreviations: **DAP**, days after planting; **DAE**, days after emergence; **EI**, emergence index; **GDD**, growing degree days; **RIL**, recombinant inbred lines; **SQR**, Shan Qui Red (PI 656025).

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ARCH10744 et ARCH10749) et une lignée autogame de recombinaison (RTx430/SQR-2) pourraient devenir des sources de tolérance au froid avec un indice de levée hâtif, une plus grande biomasse et une floraison relativement précoce. Ces génotypes sont sans tanin, ce qui concourrait à accroître l'assimilation du grain par le bétail. On les a donc retenus pour évaluation, afin en d'en préciser la fertilité, les aptitudes à la combinaison et le rendement. Une corrélation significative a été relevée entre l'indice de levée et la biomasse lors des semis précoces. Cette corrélation indique que les génotypes levant plus tard donnent une biomasse supérieure (30 jours après la levée) à celle des génotypes à levée plus rapide. Une corrélation significative entre l'étude en phytotron et l'étude au champ eu égard à l'indice de levée laisse entrevoir la possibilité d'une technique de présélection rapide à fort débit qui permettrait l'identification du sorgho tolérant au froid.

Mots clés: Sorgho, tolérance au froid, présélection à haut débit

Sorghum (*Sorghum bicolor* L. Moench) is a drought-tolerant and water-efficient cereal crop that originated in Africa and is grown throughout the semi-arid tropical and temperate regions of the world (Doggett 1988; Blum 2004). However, it is vulnerable to freezing temperatures and suffers chilling injury during the seedling stage when subjected to non-freezing temperatures of 15°C or below (Burow et al. 2011). Chilling temperature stresses occur when planting coincides with periods of cool, wet weather especially in heavier soils that do not drain well. It affects normal germination and/or early vegetative growth and results in poor seedling establishment, reduced growth rate after emergence (Forbes et al. 1987; Yu et al. 2004) and increased susceptibility to a number of seedling pathogens including *Pythium* spp., predominantly *P. aphanidermatum*, *P. ultimum*, and *P. arrhenomanes* (Martin 1992). The frost-free growing season extends from May through September in the central Great Plains; a sorghum crop needs to reach physiological maturity within this period. Therefore, chilling tolerance of seedlings and rapid reproductive development would permit earlier sorghum planting, resulting in greater utilization of the frost-free growing season as well as late spring and early summer rainfall.

Adapted, cold-tolerant sorghum hybrids can increase competitiveness of sorghum with other crops in terms of acreage in semi-arid cropping systems. Field and controlled-environment studies have demonstrated genetic variability for cold tolerance in sorghum (Thomas and Miller 1979; Soujeole and Miller 1984; Bacon et al. 1986; Brar and Stewart 1994; Yu and Tuinstra 2001; Cisse and Ejeta 2003; Yu et al. 2004; Burow et al. 2011). Seedling traits of cold-tolerant sorghum lines include smaller emergence index (EI) and increased emergence percentage, seedling height, shoot and root dry weight and tolerance to seedling diseases caused by *Pythium* spp. under chilling conditions (Soujeole and Miller 1984; Martin 1992; Yu et al. 2004).

Screening for early-season cold tolerance under field conditions is unreliable since unpredictable climatic conditions from year to year confound selection for chilling temperature stress. Also, the polygenic nature of seedling traits complicates field evaluation for cold tolerance due to the presence of genotype × environment interactions. Yu et al. (2004) and Franks et al. (2006) studied seedling cold tolerance in sorghum under controlled conditions and recommended further research to evaluate greenhouse screening procedures for cold tolerance in combination with field evaluation under multi-environment testing. Pinnell (1949) and Franks et al. (2006) reported that growth chamber assays could serve as a preliminary screen to differentiate the best germplasm for cold tolerance.

Sources of cold tolerance in sorghum have been identified (Soujeole and Miller 1984; Singh 1985; Franks et al. 2006; Knoll and Ejeta 2008). Previous reports have shown that Chinese landraces exhibit higher emergence and improved seedling vigour under cool conditions compared to selected US hybrids and elite lines (Franks et al. 2006). However, most of these landraces are linked with undesirable agronomic traits, which include high grain tannin content. High tannin reduces feed efficiency by 5 to 10% compared with non-tannin sorghums and hence are not preferred by the feedlot industry (Rooney et al. 2004). Thus, it is important to identify tannin free potential sources of germplasm tolerance to cold conditions. Few studies have tested the utility of controlled environments for cold tolerance screening purposes. We hypothesize that there is significant genetic variation for cold tolerance traits within the advanced breeding lines currently used in the breeding programs and landraces and hybrids and these traits could be used to identify the cold-tolerant genotypes as well as to optimize a high throughput screening for cold tolerance. Thus, the objectives of this study were: (1) to evaluate elite and advanced breeding lines, recombinant inbred lines (RIL) developed from the cross T × 430 (cold susceptible)/ SQR (cold tolerant), experimental and commercial hybrids for early-season cold tolerance under field conditions, (2) to identify potential breeding lines tolerant to sub-optimal temperatures, (3) to evaluate a high throughput controlled environments screening procedure in relation to field performance.

MATERIALS AND METHODS

Seedling Emergence

The 48 sorghum genotypes evaluated were composed of six elite lines received from the USDA-ARS, Lubbock, TX, four Chinese landraces [Shan Qui Red (SQR), Gai Gaoliang, Hong Ke Zi and Liang Tang AiR] as

Table 1. Phenotypic mean value of the 48 genotypes evaluated for seedling and agronomic traits at two locations (Hays and Colby, Kansas) for the early planted sorghum (2011 May 02)

		Seedling traits			Agronomic traits				
Entry	Lines/hybrids	Emergence (%)	EI (days)	Biomass (g plant^{-1})^{z}	Leaf number^{z}	Days to flower	Panicle exertion (cm)	Panicle length (cm)	Plant height (cm)
	Elite lines (6)								
1	RTx2737	18.30	11.42	0.85	5.92	95.00	4.15	30.13	100.00
2	BTx3042	43.62	19.78	1.34	7.06	79.67	2.78	23.56	84.17
3	BTx623	59.73	9.97	0.84	6.78	79.00	−1.56	24.28	89.50
4	BTx398	38.92	19.49	1.37	7.03	92.67	1.84	24.13	88.31
7	TX430R	20.83	12.70	1.07	6.17	93.00	−0.53	28.50	106.75
44	Redbine58B	39.98	12.38	0.69	6.83	86.33	10.33	24.83	96.39
	Chinese land races (4)								
5	Gai Gaoliang	43.33	11.48	0.52	6.28	79.00	15.42	24.44	192.39
6	Hong Ke Zi	51.13	12.73	1.00	6.22	94.50	0.48	28.78	207.17
8	SQR	41.65	10.74	1.17	6.50	83.33	2.56	22.92	127.75
15	LiangTangAiR	55.57	13.51	1.09	6.67	81.33	2.61	16.53	99.86
	Recombinant inbred lines (6)								
9	RTx430/SQR-2	30.28	16.58	1.52	6.72	84.00	4.72	24.00	108.33
10	RTx430/SQR-58	28.60	12.42	0.50	5.83	74.67	2.53	23.11	94.81
11	RTx430/SQR-60	13.90	12.72	0.70	6.20	89.50	14.05	23.73	116.03
12	T RTx430/SQR-83	42.48	11.47	0.89	6.83	81.33	1.61	25.25	115.14
13	RTx430/SQR-92	23.33	12.46	0.77	6.28	83.33	−1.88	25.59	109.19
14	RTx430/SQR-116	9.34	13.75	0.17	6.33	76.00	3.37	23.53	92.24
	Advanced breeding lines, Hays, KS (21)								
16	ARCH10730 (PI574570R/4/KS120B-1)	49.43	16.07	1.18	6.72	76.67	7.92	24.14	112.78
17	ARCH10731 (PI574578 R/3/KS118B-1)	41.67	10.82	0.99	7.06	77.67	1.24	21.47	73.22
18	ARCH10732 (PI574568R/TXArg-1B)	39.17	12.67	1.03	6.83	74.00	3.81	30.83	100.14
19	ARCH10733 (PI574562 R/4/ KS116B)	36.38	13.04	1.05	6.39	72.67	2.33	20.92	79.33
20	ARCH10734 (PI574586R/4/KS119B-1)	40.57	13.83	0.93	6.22	77.00	4.62	20.47	121.81
21	ARCH10735 (PI574570R/4/KS120B-2)	54.17	14.01	1.19	6.95	74.00	8.31	22.33	97.53
22	ARCH10736 (PI 574570R/4/KS120B-3)	26.12	11.98	0.82	6.33	73.33	6.53	19.71	79.41
23	ARCH10737 (PI574578R/3/KS118B-2)	31.10	13.28	1.31	6.89	75.33	11.28	20.14	94.28
24	ARCH10738 (PI574578R/3/KS118B-3)	33.32	11.17	1.27	6.60	73.50	7.00	20.63	91.13
25	ARCH10739 (PollenCompT4C4-210R/PI574554R-1)	51.93	14.87	1.03	6.50	72.00	13.33	22.97	106.39
26	ARCH10740 (Pollen CompT4C4-210R/PI 574554R-2)	36.97	16.49	1.25	7.11	78.00	4.42	20.74	84.61
27	ARCH10741 (PollenCompT4C4-210R/PI574554R-3)	31.42	16.52	1.62	6.61	76.00	4.28	25.42	88.50
28	ARCH10742 (PollenCompT4C4-210R/PI574554R-4)	45.28	14.42	1.20	6.67	78.00	1.81	25.97	91.83
29	ARCH10743 (PollenCompT4C4-210R/PI574554R-5)	62.50	15.92	1.35	7.11	76.00	9.14	22.17	101.43
30	ARCH10744 (PI574590R/3/KS118B)	37.22	13.17	1.07	6.78	72.67	4.68	20.00	89.11
31	ARCH10745 (PollenCompT4C4-279R/PI 574594R)	44.45	12.24	0.66	6.86	90.67	−1.33	21.94	111.57
32	ARCH10746 (PI574578R/3/KS118B-4)	33.07	12.58	1.50	6.45	76.33	1.33	23.56	104.56
33	ARCH10747 (PI574599R/B35-6B)	39.45	19.75	1.96	7.22	80.33	3.94	20.50	79.61
34	ARCH10748 (PI574560R/B803B)	31.67	15.99	0.61	6.07	73.00	5.89	25.22	84.56
35	ARCH10749 (PI574586R/4/KS119B-2)	47.22	11.39	1.37	6.56	67.00	11.48	22.54	90.65
36	ARCH10750 (PI574558R//B1B/B9501B-V60B)	26.95	12.94	0.97	6.89	76.50	3.25	22.84	88.61
	Hybrids involving Redbine58A (7)								
37	Redbine58A// PI574570 R/4/ KS120B	51.68	21.87	2.74	7.28	77.33	8.53	24.92	104.25
38	Redbine58A//Pollen CompT4C4-279 R/PI 574594R	32.50	22.95	2.86	6.72	94.00	6.58	24.94	122.96
39	Redbine58A//PI574586R/4/KS119B	43.87	21.75	1.52	6.83	77.33	7.04	26.25	120.50
40	Redbine58A//PI 574568R/TXArg-1B	37.23	21.65	3.26	7.17	79.00	5.83	28.86	99.43
41	Redbine58A//ARCH 10731	50.85	21.70	3.40	7.11	76.33	10.22	24.81	104.06

Table 1 (*Continued*)

Entry	Lines/hybrids	Seedling traits			Agronomic traits				
		Emergence (%)	EI (days)	Biomass (g plant^{-1})^z	Leaf number^z	Days to flower	Panicle exsertion (cm)	Panicle length (cm)	Plant height (cm)
42	Redbine58A//ARCH 10737	44.72	20.31	1.50	7.20	78.00	6.61	24.67	95.06
43	Redbine58A//PollenCompT4C4-210R/PI574554R	40.82	21.93	1.74	6.39	78.33	7.72	24.28	104.17
	Commercial hybrids checks (4)								
45	Pioneer 8500	70.02	12.55	0.82	6.78	79.00	0.89	24.83	88.22
46	Pioneer86G32	48.62	15.29	1.48	6.61	75.33	0.19	27.11	97.03
47	Pioneer 85G03	66.95	13.81	1.05	6.72	80.33	1.08	25.93	94.01
48	Sorghum Partners NK7633	48.32	11.21	1.11	6.67	82.67	5.22	21.72	93.22
	LSD	12.38	3.24	0.65	0.66	5.51	3.15	1.89	6.12

^{z}Measured 30 d after emergence.

cold-tolerant sources (Franks et al. 2006), six RILs (Mitch Tuinstra, Purdue University), 21 advanced breeding lines developed for cold tolerance at Hays, KS, seven hybrids having a cold-tolerant female line in their pedigree, and four commercial hybrid checks (Pioneer 8500, 86G32, 85G03 and NK7633) (Table 1). The purpose of including advanced breeding lines and RILs (obtained from a cross between two contrasting parents for cold tolerance, Tx430 and SQR) in this study was to evaluate the phenotypic response and to select lines with improved agronomic traits. In addition, hybrids with a cold-tolerant female line (Redbine58A; Smith and Frederiksen 2000) were included to know the comparative and improved performance under cold conditions. Field studies were conducted at the Agriculture Research Center, Hays, KS (lat. 38.979°N, long. 99.326°W; 611 m elevation above the sea level) and at the Northwest Research-Extension Center, Colby, KS (lat. 39.396°N, long. 101.051°W; 956 m elevation above sea level). There were two planting dates: an early planting on 2011 May 02 (daily average soil temperature approximately 14°C) and a normal planting (standard planting period for sorghum in the Central Great Plains) on 2011 May 31 (daily average soil temperature approximately 21°C). Early planting provided cold temperature stress for sorghum seeds during emergence and seedling growth while normal planting provided a basis for comparison under non-stressful temperature (Maulana and Tesso 2013).

The soil type at the Hays experimental site was a Harney silt loam (fine smectitic mesic Typic Argiustoll) and at the Colby a Keith silt loam (fine-silty, mixed, mesic Aridic Argiustolls). Seeds of each genotype were treated with fungicide [ethanethiol or ethyl mercaptan (Captan)], at the rate of 2 mL kg^{-1} seed and were planted in single-row plots 6.09 m long and 0.76 m wide with 60 seeds per row (10 909 seeds ha^{-1}). Stand counts were recorded three times during the week after emergence until no additional emergence was measureable. Emergence percentage and EI were calculated as described by Yu et al. (2004) as follows:

$$\text{Emergence \%} = (\text{total seedlings emerged}/\text{total seeds planted}) \times 100 \tag{1}$$

$$\text{Emergence index (EI)} = \Sigma[\text{n}_\text{i} \times \text{DAP}_\text{i}]/\text{total seedlings emerged} \tag{2}$$

$$\text{Growing degree days (GDD)} = ((\text{daily max. air temp.} + \text{daily min. air temp.})/2) - \text{base temp.}) \tag{3}$$

Where, n_i is the number of seedlings emerged on the ith day after planting (DAP_i). Early emergence was indicated by a small EI value. Final stand counts taken at 35 DAP were used to calculate emergence percentage.

Three seedlings per entry were flagged on the day of emergence and harvested at 30 d after emergence (DAE) to determine above-ground dry biomass and the number of leaves as indicated by collar (ligule) formation. Shoots were cut at soil level, oven dried at 60°C, and weighed. At Hays, plant height was measured from the base of the plant to the tip of the final leaf at 30 DAE. Agronomic traits including panicle exsertion, panicle length, and plant height at maturity were recorded for the two planting dates at both locations; days to 50% flowering was observed at Hays for both planting dates. Grain samples were analysed for tannin content for all 48 genotypes collected from early planting using the vanillin hydrochloric acid method (Makkar and Becker 1993). Growing degree days were calculated using Eq. 3, where base temperature limit of sorghum was 10°C, while the upper limit for GDD accumulation was 30°C (Vanderlip and Reeves 1972).

High Throughput Cold Tolerance Screening

Based on the early planting mean performance in the field over two locations, all 48 genotypes were grouped into low (10 to 14 d) and high (15 to 20 d) groups for EI and low (0.5 to 1.0 g), moderate (1.1 to 1.5 g) and high (1.6 to 2.0 g) for biomass (30 DAE). A total of 18 genotypes (Table 2) were selected, six each from low/low, low/moderate and high/high EI/biomass (30 DAE) group to evaluate a high throughput controlled environment screening procedure. These lines were evaluated for EI under controlled conditions in a growth chamber at Hays, KS, and biomass at 30 DAE in greenhouses at Hays and Colby, KS, between November 2011 and March 2012.

Table 2. List of 18 selected genotypes for high throughput cold screening

Entry	Lines	Greenhouse and growth chamber studies[z]
	Elite lines	
1	RTx2737	X*
2	BTx3042	X***
3	BTx623	X*
4	BTx398	X***
	Chineese land race	
8	SQR	X**
	Recombinant inbred lines	
9	RTx430/SQR-2	X***
10	RTx430/SQR-58	X*
12	RTx430/SQR-83	X*
	Advanced breeding lines from Hays, KS	
17	ARCH 10731	X*
21	ARCH 10735	X**
22	ARCH 10736	X*
23	ARCH 10737	X**
24	ARCH 10738	X**
27	ARCH 10741	X***
29	ARCH 10743	X***
32	ARCH 10746	X**
33	ARCH 10747	X***
35	ARCH 10749	X**

[z] Selected 18 lines, six from each of the three groups (* low/low, ** low/medium, *** high/high) based on emergence index/biomass (30 d after emergence).

Emergence Index

The selected 18 lines were planted in tubs (540 mm × 380 mm) on 2011 Nov. 15, filled to a depth of 70 mm with finely screened sand (passed through a 2-mm mesh) using two randomized complete blocks. Growth chamber and cold table facilities (insulated rectangular metal table with the dimension of 146 cm width × 307 cm length × 17 cm depth, where temperature was maintained by water bath, Model SB-135, Series 78A6018.1, Percival Mfg. Co., Bonne, IA) installed inside the greenhouse were used in this study. The dimension of the cold table allows to screening large number of 600 to 700 genotypes simultaneously. Hence, both growth chamber and cold table were used to standardize the high-throughput screening procedure and to compare plant growth under controlled environments. Before planting, sand was leveled and rows were made using a parallel array of 25-mm angle iron by pressing into the sand (406 × 610 mm, 38 mm row spacing). Each sorghum line was planted into a 406-mm row with 20 seeds line^{-1}. The same method was followed for both cold table and growth chamber studies by maintaining the same temperatures regime at 15/12°C day/night on a 12-h cycle. After planting, seeds were covered with 3 mm of sand. A water mist was sprayed as required to maintain surface moisture. The sand-filled tubs were placed on an insulated cold table and covered with closed-cell foam insulation (25 mm thick). Supplemental heat was provided when greenhouse ambient temperatures reached 12.7°C and ventilation when temperatures exceeded 18.3°C. The temperature inside the greenhouse was regularly monitored to make sure the set temperature in the cold table is accurately maintained. Both growth chamber and cold table facilities were used with an assumption that both methods do not show differences in plant response under controlled environments. Seedling emergence was counted from both methods when a minimum of 2 mm of shoot was visible; observations commenced a week after planting and were measured three times a week until 21 DAP and EI was calculated using Eq. 2.

Seedling Biomass

Seeds from the 18 lines were germinated in Petri dishes under dark condition for 24 h at 22°C in an incubator. Three to four emerged seedlings were transplanted to cone-tainers (38/203 mm diameter/deep) with 50-mm × 50-mm spacing containing soil (Harney silt loam) and vermiculite (Sungrow® Horticulture Distribution Inc., USA) (1:1) as the potting mix. The experimental design was a randomized complete block with four replications and two planting dates (2011 Nov. 15 and Dec. 05) and in two greenhouses (Hays, KS and Colby, KS). Seedlings were thinned to one per cone-tainer after establishment. The open bases of the cone-tainers were placed in plastic tubs containing nutrient solution 0.5 g L^{-1}, 24:8:16 Miracle Grow (The Scotts MiracleGro Company, Marysville, OH) approx. 100 mm deep; the solution was replaced weekly. The greenhouse temperature was maintained at a minimum of 12.7°C and ventilated when temperatures exceeded 18.3°C. Measurements were made on emergence, seedling survival until 30 DAE and shoot biomass at 30 DAE.

Data Analysis

Forty-eight sorghum genotypes were used in the field seedling emergence studies. For the controlled environment study, 18 genotypes were selected that differed in EI and shoot biomass. A randomized complete block experimental design was used with three replications. Treatment design was split-split plot with location as whole plot, planting date as split plot and genotype as split-split plot. Analysis of variance was carried out over the two planting dates and locations using the PROC GLM procedure of SAS software (SAS, Institute, Inc., version 9.1.3). Main effects (genotype, location and planting date) and their interactions were treated as random effects and tested using the appropriate F-test. Mean separations of main effects were performed using the ‘‘means’’ option with Scheffe’s multiple comparison adjustment at $P<0.05$ significance level. Comparative analyses of genotypes between the selected groups were performed using Scheffe’s multiple comparison at $P<0.05$ significance level and confirmed with orthogonal contrast analysis. Pearson correlation coefficients were obtained for emergence, EI, biomass, plant height, leaf number and days to flowering to evaluate the relationship among genotypes between cold-tolerant traits and growth parameters. To evaluate the efficacy of the high throughput, controlled environment screening procedure, Pearson correlation coefficients were calculated for percent emergence and EI between early in the field (seedling emergence), cold table, and growth chamber studies (high throughput screening). Spearman’s Rank Correlation coefficients (ρ) were calculated to confirm the relationship between field and controlled environments screening and to measure the strength of association between two ranked variables where:

$$\rho = 1 - [(6\Sigma di^2)/n(n^2 - 1)], \tag{4}$$

and d = difference between the ranks of the each observation on two variables; n = number of lines used.

RESULTS

Environmental Conditions

Mean daily maximum and minimum soil temperatures to 5 cm depth and air temperature for 45 d after planting as well as mean air temperature during the greenhouse studies are given in Supplementary Table 1. Soil profile temperatures were ~6 to 7°C cooler during early planting of sorghum than normal planting at Colby and Hays, KS, in 2011. Average minimum/maximum air temperatures in early planting were, respectively, 12.0/28.1°C and 8.9/25.6°C at Hays and Colby, KS. This clearly indicated that the soil and air temperature recorded 45 d at both locations was favorable for cold stress screening of sorghum at emergence and seedlings stages.

Seedling Emergence

Phenotypic and Genotypic Variation

ANOVA results for the effect of genotype, location, planting date, and their interactions for seedling traits (emergence, EI, biomass and leaf number) are presented in Supplementary Table 2. Planting date affected seedling traits associated with cold temperature stress at both locations. Genotype × planting date interacted significantly for EI. Location, planting date and genotype effects interacted to influence emergence and seedling biomass at 30 DAE (Supplementary Table 2).

Separate analysis of variance calculations for each location were conducted to account for interaction effects and to evaluate differences among genotypes. Results revealed significant differences among genotypes and planting date for emergence and EI at both locations. Within locations, significant differences among the mean value of emergence and EI were observed between early and normal planting. Emergence was decreased by 10% and EI increased by 4 d during early planting at Hays relative to Colby, which may have contributed to the significant interaction effect between location and planting date. The overall means of the EI over two locations in early plantings for lines and hybrids involving Redbine58A were 13 and 22 d, respectively. These hybrids recovered faster and compensated for delayed seedling emergence with rapid seedling growth. The average number of days to flowering was greater for the early planting (79 DAP), relative to the normal planting period (67 DAP). Few differences were observed between early and normal planting for other agronomic traits, such as panicle exsertion, panicle length and plant height at maturity (data not shown).

Early planting decreased emergence by 27% and increased EI by 6 d (Table 3). In addition, decreased seedling biomass and leaf number at 30 DAE were observed during early compared with normal planting. Early planting delayed days to 50% bloom by 12 to 15 d (data not shown). Phenotypic mean values (averaged over both sites) for seedling, agronomic and seed quality traits for the 48 lines evaluated under cold temperature stress are presented in Table 1. Among the elite lines BT × 623 had decreased EI and increased emergence percentage. Of the 48 lines evaluated, hybrids involving Redbine58A had increased EI with larger shoot biomass compared to RILs and advanced breeding lines.

Table 3. Cold tolerance traits (mean emergence, emergence index, biomass, and leaf number) of 48 sorghum genotypes planted on early or normal dates at two locations in 2011

Treatment		Emergence (%)	Emergence index (d)	Biomass[z] (g plant^{-1})	Leaf number[z]
Planting date	Early (2011 May 02)	40.35*b*	14.85*a*	1.27*b*	6.67*b*
	Normal (2011 May 31)	51.53*a*	8.96*b*	9.41*a*	11.50*a*
Location	Hays, KS	40.17*b*	12.72*a*	5.53*a*	8.69*b*
	Colby, KS	51.70*a*	11.07*b*	4.66*b*	9.30*a*

[z] Measured at 30 d after emergence.

a, *b* For mean values within columns for planting date and location, means followed by the same letters are not significantly different according to Scheffe ($P<0.05$).

Comparative analysis among the groups (Table 4) showed greatest emergence for commercial hybrids and least emergence for RILs. For EI, the hybrids involving Redbine58A had delayed emergence relative to the other groups, which were similar. Hybrids of Redbine58A also had greatest shoot biomass (30 DAE); elite lines, RILs and advanced breeding lines from Hays, KS, had smaller shoot biomass.

Cold tolerance traits exhibited inter-correlation. Shoot biomass (30 DAE) increased with EI for early planting (Table 5). EI and shoot biomass was significantly correlated with plant height and leaf number during early planting. No correlation was observed between the seedling traits (EI, biomass, plant height and leaf number) and other agronomic traits (days to flowering, panicle exsertion, panicle length and plant height at maturity).

Among the advanced breeding lines, eight advanced lines (ARCH10731, ARCH10732, ARCH10736, ARCH10737, ARCH10738, ARCH10739, ARCH10744 and ARCH10749) exhibited favorable cold tolerance traits: mean values of EI less than 13, shoot biomass (30 DAE) greater than 0.7 g plant^{-1} and days to 50% bloom fewer than 75 (Fig. 1 and Table 1). Chinese land race SQR showed rapid emergence and slow growth. Line RTx430/SQR-83 showed medium emergence with satisfactory growth during early planting. When compared with lines, all hybrids (commercial checks and hybrids) involving Redbine58A as seed parent emerged late, but exhibited rapid compensatory growth (Fig. 1).

High Throughput Screening

Results of the high throughput, controlled environment screening procedure were consistent with field data (Supplementary Table 2 and Table 6). Genotypes differed in emergence under both cold table and growth chamber study and for EI in the cold table study (Table 6). These results were consistent for both planting dates. Average emergence ranged from 40 to 100% in the cold table and from 27 to 97% in the growth chamber. The EI ranged from 7 to 13 d in cold table and from 11 to 16 d in the growth chamber, which was comparable to the field studies. Pearson correlation coefficients showed significant relationship for EI between the early planted field study at Colby and the cold table ($r=0.49$) and growth chamber ($r=0.82$) studies (Table 7). This significant relationship was further confirmed by Spearman rank correlation for EI between Colby field and growth chamber ($\rho=0.62$) and between Hays field and growth chamber ($\rho=0.56$) (data not shown). These results indicate that high throughput screening for the cold tolerance EI trait can be done under controlled conditions.

The seedling biomass study showed significant effects of genotypes on seedling survival; no significant planting date main effects or genotype × planting date interactions were detected under controlled conditions at Colby.

Table 4. Mean value, mean square and significance level from the comparative analyses of emergence, emergence index, and shoot biomass of genotypes between the selected groups (Table 1) and against commercial checks from field studies in 2011

Group	Emergence (%)	Emergence index (d)	Shoot biomass[z] (g plant^{-1})
Elite lines	40.35*d* ± 2.58[y]	11.78*b* ± 0.55	4.67*b* ± 0.36
Chinese land race	51.38*bc* ± 2.15	10.34*b* ± 0.41	5.47*ab* ± 0.50
Recombinant inbred lines	9.67*e* ± 1.81	10.95*b* ± 0.43	4.63*b* ± 0.38
Advanced breeding lines	45.90*cd* ± 1.15	11.30*b* ± 0.25	4.77*b* ± 0.19
Redbine58A hybrids	52.44*b* ± 1.90	16.04*a* ± 0.74	6.11*a* ± 0.36
Commercial checks	62.01*a* ± 2.44	10.83*b* ± 0.51	5.58*ab* ± 0.48

[z] Measured 30 d after emergence.

[y] Standard error.

*, **, *** $P<0.05$, $P<0.01$, and $P<0.001$ significance levels, respectively.

a–e Means followed by same letter are not significantly different according to Scheffe ($P<0.05$).

Table 5. Pearson correlation coefficients among the seedling and agronomic traits using 48 sorghum genotypes evaluated under early planting in the field at Hays and Colby, Kansas in 2011

	Seedling traits		Agronomic traits		
Traits	Emergence index	Biomass^z	Plant height^z	Leaf number^z	Panicle length
Hays, Kansas					
Emergence	0.31*	0.23	0.31*	0.45**	0.14
Emergence index		0.74***	0.62***	0.62***	0.18
Biomass			0.83***	0.70***	0.16
Plant height				0.62***	0.28
Leaf number					0.15
Days to flowering					0.30*
Panicle exsertion					−0.17
Colby, Kansas					
Emergence	−0.19	−0.06	–	0.43**	−0.26
Emergence index		0.59***	–	0.06	0.11
Biomass				0.06	0.01
Leaf number					−0.35*

^{z}Measured 30 d after emergence.
*, **, *** $P<0.05$, $P<0.01$, and $P<0.001$ significance levels, respectively.

However, at Hays, planting date had significant effect on emergence and seedling survival as well as on shoot weight (Supplementary Table 3). However, shoot biomass (30 DAE) showed negative rank correlation coefficients between Colby field and greenhouse studies ($\rho = -0.14$) and Colby field and Hays greenhouse studies ($\rho = -0.10$) indicating that cone-tainers failed to provide information relevant to seedling performance under field conditions.

DISCUSSION

The present study showed wide genetic variability among the sorghum genotypes for cold tolerance traits. Planting date had a significant effect on emergence and EI resulting in decreased values of each seedling trait associated with cooler temperatures in both locations compared with normal planting. A significant effect of location was observed for emergence and EI, possibly due to variation in temperature, rainfall and soil type, as well as irrigation provided at Colby, KS. Early planting took 66% longer for 100% emergence compared with the normal planting time (Table 3). Cold temperature stress resulted in a significant reduction in seedling biomass and leaf number. These results were smaller than those reported by Yu et al. (2004). These authors observed a decrease in emergence from 37% to doubling of EI in early-planted sorghum. Two- and three-way interactions between locations, planting date, and genotype had significant effects on EI indicating the significant effect of environment on genotypes in complete emergence. Similar findings of a significant genotype effect on cold tolerance traits (emergence, EI, and seedling biomass) have been well documented from growth chamber assays as well as in-field tests (Thomas and Miller 1979; Bacon et al. 1986; Brar and Stewart 1994; Tiryaki and Andrews 2001; Yu and Tuinstra 2001; Cisse and Ejeta 2003; Yu et al. 2004; Burow et al. 2011).

Early planting delayed days to flowering relative to the normal planting period, indicating that the same genotypes required 12 to 15 d longer to flower when growth was initiated under chilling conditions. However, similar values of cumulative GDD were observed from average emergence index to average days to flowering for early (847°C-d) and normal (875°C-d) planting, indicating that early planting did not alter average thermal requirement for flowering. Despite delayed flowering, the actual calendar dates of expected 50% bloom would be Jul. 16 and Jul. 30 for genotypes with 75-day bloom periods (early planting) when planted on May 02 and May 31, respectively. Thus, expected maturity of early-planted genotypes would occur prior to first freeze, which normally occurs in early October in Kansas.

The positive correlation between shoot biomass (30 DAE) and EI during early planting, indicates that late-emerged seedlings produced greater biomass relative to early-emerged seedlings (Table 5). This might be due to greater accumulation of GDD with late-emerged seedlings due to exposure to warmer growing conditions. Seedlings harvested on 2011 Jun. 10 and 2011 Jun. 30 (30 DAE for early and normal planting) were exposed to cumulative GDD of 243°C-d and 349°C-d for Colby and 305°C-d and 410°C-d for Hays, respectively. Seedling planted at the normal date encountered 44 and 34% more cumulative GDD (at 30 DAE) than seedlings planting early. Since the seedling growth increases exponentially with GDD (Craufurd et al. 1998), rapid growth and greater seedling biomass were observed in late-emerged compared with early-emerged seedlings. Cisse and Ejeta (2003) observed a significant positive correlation between emergence and seedling dry

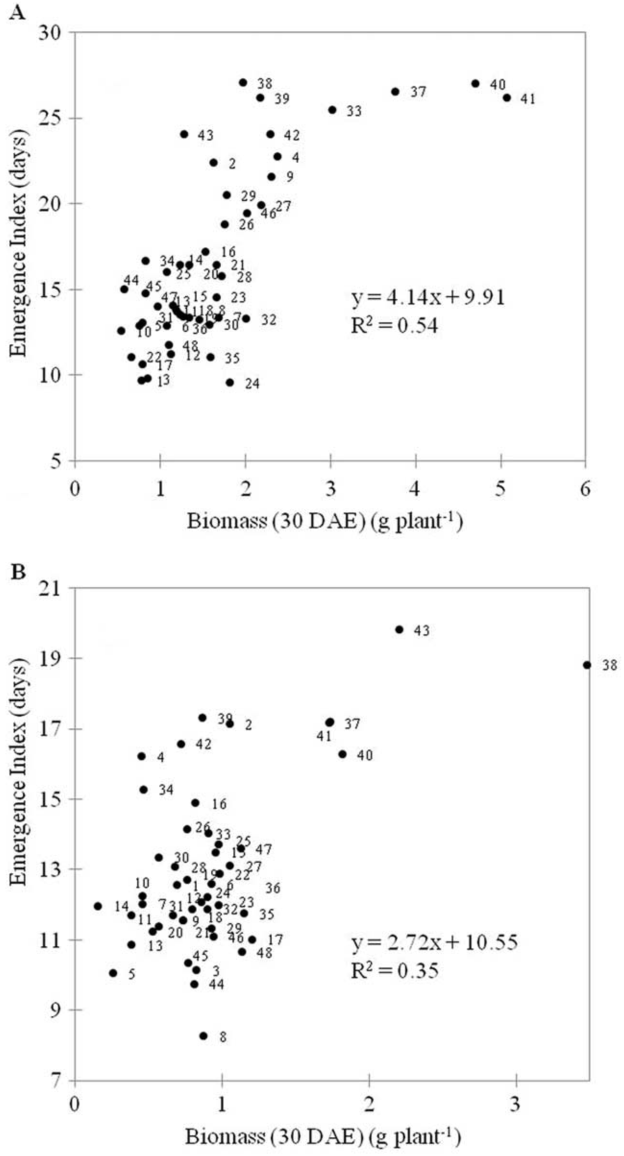

Fig. 1. Comparison of mean values of emergence index and 30 d after emergence (DAE) biomass of 48 sorghum genotypes during early planting at (A) Hays and (B) Colby, KS in 2011 Note: Entry numbers correspond to genotype name in Table 1.

weight under controlled conditions in sorghum. However, Yu et al. (2004) reported that EI decreased with shoot dry weight, root dry weight, and seedling height using a soil-based growth chamber assay.

Significant correlation was observed among emergence, EI and biomass with plant height and leaf number (30 DAE) during early planting (Table 5). It supports the observation that the late-emerged seedlings (hybrids formed with Redbine58A) grew rapidly, and produced greater biomass with a greater leaf number (Fig. 1). The lack of correlation between the seedling traits (EI, biomass, plant height and leaf number) and other agronomic traits (days to flowering, panicle exsertion, panicle length) indicates that cold tolerance did not affect the expression of other agronomic traits in the later part of the growing season. Based on the correlation studies, selecting for reduced EI, increased biomass at 30 DAE and early flowering should lead to breeding lines with positive cold tolerance traits. Evaluating the impact of cold stress on agronomic traits, including grain yield, will require hybrid formation with selected breeding lines, which could be submitted for multi-location test hybrid evaluation trials.

Our data show that eight advanced lines ARCH10731, ARCH10732, ARCH10736, ARCH10737, ARCH10738, ARCH10739, ARCH10744 and ARCH10749 clearly separated from other genotypes with decreased EI, satisfactory shoot biomass (30 DAE) and relatively early flowering. These genotypes were free from tannin content (data not shown). In a companion study, significantly higher cumulative emergence and seedling biomass were found in advanced lines ARCH10735, ARCH10738, and ARCH10749 under the cold temperature regime after *P. aphanidermatum* inoculation (data not shown). These results indicate that these lines are tolerant to *Pythium* spp. as well as to cold temperature stress. These genotypes could be used in developing cold-tolerant sorghum. Chinese landrace SQR showed increased emergence and slow growth. Cisse and Ejeta (2003) concluded that SQR had a greater percentage of germination with good seedling height and recommended it as a potential source for cold tolerance. Yu and Tuinstra (2001) reported a similar conclusion; however, Tiryaki and Andrews (2001) found that the agronomic traits of Chinese landraces were not acceptable.

Results of the high throughput, controlled environment screening procedure for EI, but not biomass (30 DAE) were consistent with field data. Brar and Stewart (1994), Yu et al. (2004), and Franks et al. (2006) demonstrated the utility of controlled conditions to screen for cold tolerance and to predict field performance. Brar and Stewart (1994) observed 77% association between mean emergences in the field to laboratory results. In addition, Yu et al. (2004) reported a significant correlation ($r = +0.67$) between the rank-summation indices for mean performance of cold tolerance traits in the growth chamber and the field. The present study provides further evidence for a strong correlation ($r = +0.82$; $P < 0.0001$) between growth chamber and field studies for EI, which indicates that the growth chamber can provide preliminary high-throughput screening for sorghum cold tolerance compared with the cold table in the greenhouse. Further work should be undertaken to develop shoot biomass assays that are predictive of genotype performance under chilling field conditions.

In summary, early planting should contribute to a longer growing season, effective utilization of late spring and early summer rainfall, minimum tillage and enhanced yield potential. Significant positive correlation between

Table 6. Seedling emergence and emergence index mean squares, significance levels (*P*), and mean ranges from ANOVAs for 18 selected sorghum genotypes evaluated under cold table and growth chamber

		Cold table				Growth chamber	
Source	df	Emergence	Emergence index	Source	df	Emergence	Emergence index
Planting date	1	1422.22	7.13	Block	1	69.44	1.71
Block (date)	2	247.22	6.62	Genotype	17	755.88**	3.25
Genotype	17	553.59*	6.51**	Error	35	166.5	1.79
Genotype × planting date	17	191.39	1.93				
Error	34(32^z)	146.48	3.47				
		Mean range				Mean range	
		(%)	(days)			(%)	(days)
1st planting (Nov. 05)		40–100	7.45–12.7			27.50–97.50	11.03–15.98
2nd planting (Dec. 05)		25–100	7.33–19.00				

^{z}Error df for emergence index (EI) for cold table.
*, **, *** $P<0.05$, $P<0.01$, and $P<0.001$ significance levels, respectively.

Table 7. Pearson correlation coefficients of cold tolerance traits between early planting field studies conducted at Hays and Colby, KS, in 2011, cold table, and growth chamber for the selected 18 sorghum genotypes

	Hays (field)	Colby (field)		Hays (cold table)		Hays (growth chamber)	
	EI^z	E^z	EI	E	EI	E	EI
E (Hays, field)^z	0.19	0.77***	−0.19	0.39	−0.25	0.52*	−0.28
EI (Hays, field)^z		0.22	0.53*	0.26	0.13	−0.02	0.39
E (Colby, field)			−0.21	0.38	−0.04	0.46	−0.27
EI (Colby, field)				−0.17	0.49*	−0.51*	0.82***
E (Cold table, Hays)					−0.12	0.79***	−0.40
EI (Cold table, Hays)						−0.31	0.43
E (Growth chamber, Hays)							−0.69**

^{z}EI, emergence index; E, emergence percentage.
*, **, *** $P<0.05$, $P<0.01$, and $P<0.001$ significance levels respectively.

EI and 30 DAE biomass during early planting indicates that late-emerged seedlings produced greater biomass relative to early-emerged seedlings. Nine advanced breeding lines: ARCH10731, ARCH10732, ARCH10736, ARCH10737, ARCH10738, ARCH10739, ARCH10744 and ARCH10749 and RTx430/SQR-2 were found to have decreased EI, satisfactory shoot biomass and early flowering with no tannin content. These genotypes can provide potential sources for improvement of tolerance to cold temperature stress in future sorghum breeding. Of these nine lines, ARCH10738 and ARCH10749 showed tolerance to *Pythium* spp. infection. Significant correlations showed that fast preliminary screening for EI using a growth chamber to simulate cold stress environment offers effective and efficient selection of potential lines for cold tolerance in sorghum.

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Supplementary Table 1. Average minimum and maximum soil (5-cm depth) and air temperatures for 45 d after sorghum planting for early season cold tolerance at Hays and Colby, Kansas, 2011

		45-d average soil temperature (°C, 5 cm)		Average air temperature (°C)	
Treatment	Planting date	Min.	Max.	Min.	Max.
2011, Field experiments					
Early planting	2011 May 02 (Hays)	15.6	28.3	12.0	28.1
	2011 May 02 (Colby)	15.6	21.1	8.9	25.6
Normal planting	2011 May 31 (Hays)	22.0	35.7	18.9	34.5
	2011 May 31 (Colby)	21.7	27.2	15.6	32.2
2011–2012, Greenhouse experiments					
Hays	Nov. 2011			15.6	26.0
	Dec. 2011			19.5	25.7
	Jan. 2012			20.3	27.8
	Feb. 2012			19.4	27.4
Colby	Nov. 2011			15.1	16.8
	Dec. 2011			13.0	14.9
	Jan. 2012			13.4	15.4

Supplementary Table 2. Mean squares, significance levels (*P*) and mean values of 48 sorghum genotypes from combined ANOVAs over two planting dates and two locations (Hays and Colby, KS 2011) for the seedling traits related to cold tolerance

		Seedling traits			
Source	df	Emergence	Emergence index	Biomass	Leaf number
Location	1	20115.98*	385.04*	180.21	43.45
Block (location)	2	694.46	12.20	18.76	15.20
Planting date	1	18967.38*	4919.49**	23010.74*	2766.84**
Block (planting date)	2	475.22	3.92	311.23	19.34
Location × planting date	1	272.81	519.19***	2.60	134.87***
Genotype	47	2039.33***	55.55	23.91	5.33
Genotype × location	47	289.26	18.63**	15.38	4.40
Genotype × planting date	47	234.60	30.31***	18.36	5.01
Genotype × location × planting date	47	301.69***	8.60**	15.17**	4.34
Error	375 (1360^z)	113.92	4.62	8.39	5.00

*, **, *** $P<0.05$, $P<0.01$ and $P<0.001$ significance levels, respectively.

^{z}Error df for biomass; Note: Main effects and their interaction were treated as random effects and obtained using the appropriate F-test.

Supplementary Table 3. Mean squares and significance levels (*P*) from ANOVAs from greenhouse, Colby and Hays, KS during 2011 related to seedling biomass potting mix study in 18 sorghum lines.

	Colby				Hays			
Source	df	Emergence %	Seedling survival	Shoot weight	df	Emergence %	Seedling survival	Shoot weight
Block	3	0.003	0.11	0.02	3	0.06	0.32	3E-05*
Planting date	1	0.003	0.17	0.12	1	0.34**	3.06*	0.001**
Block × Planting date	3	0.053	0.21	0.02	3	0.01	0.22	2E-05
Genotype	17	0.08*	0.55***	0.02	17	0.09	0.19	5E-05***
Planting date × genotype	15	0.041	0.15	0.02	14	0.06	0.25	4E-05***
Error	50	0.045	0.14	0.01	102 (41^z)	0.07	0.22	8E-06

*$P<0.05$, **$P<0.01$, and ***$P<0.001$ significance level.

^{z}*df* for shoot weight.