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Research Article

Response of Sorghum to Cold Stress at Early Developmental Stage

Amandin Rutayisire,[1,2] **Geoffrey Lubadde**,[3] **Alice Mukayiranga**,[1,2] **and Richard Edema**[1]

[1]*Makerere Regional Center for Crop Improvement (MaRCCI), Department of Agricultural Production, Makerere University, P.O. Box 7062, Kampala, Uganda*
[2]*Rwanda Agriculture and Animal Resources Board, P.O. Box 5016, Kigali, Rwanda*
[3]*Faculty of Agriculture and Animal Science, Busitema University, P.O. Box 236, Tororo, Uganda*

Correspondence should be addressed to Amandin Rutayisire; rutamandin@gmail.com

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Native and adapted to the semiarid tropical regions of Africa, sorghum (*Sorghum bicolor*) is generally sensitive to cold temperatures, especially during the early developmental stages. However, there is genetic variability within the existing germplasm in terms of tolerance to low temperatures. The highland regions of Africa possess important sources of germplasm with adaptation to cold stress, since they tend to be cooler than the low land regions. The goals of the study were to evaluate and identify sorghum lines with tolerance to cold temperature stress and make recommendations on varieties that may be planted in the East African highland regions or used in plant breeding programs for cold tolerance. Forty sorghum genotypes were evaluated for emergence, shoot, and root development at seedling stage under controlled environment in growth chambers and in the field. Significant genotypic differences were detected for all evaluated traits. Correlation between controlled environment experiments and field trial results was mostly significant, suggesting that the growth chambers can be used to predict and identify cold-tolerant genotypes. Results showed that emergence and vigor are the best surrogate traits for selecting cold-tolerant genotypes. Using rank summation index, we were able to identify the best cold-tolerant sorghum genotypes (IS 25557, IS 25558, IS 25546, BM6, BM 29, IESV 90042LT, and Cytanobe) that can be used in future breeding programs and enhance adaptation and expansion of sorghum production further into the highland regions of Africa.

1. Introduction

Chilling stress (0°–15°C) has been recognized among the major abiotic constraints to sorghum (*Sorghum bicolor* (L) Moench) production, especially in the cool regions [1, 2]. Sorghum is a C4 plant that evolved under warm conditions in tropical Africa, where temperatures during the growing season generally remain above 20°C [3]. Therefore, cold stress, observed mostly in higher altitude regions, affects its growth and development [4]. Low temperature occurring during the crop growing season affects nearly all growth stages and consequently leads to yield reduction.

The adverse effects of low temperature stress are commonly visible during early development stages in most crops sensitive to low temperatures [5–7]. Low temperature stress can reduce germination and emergence rate, limit seedling establishment, stunt shoot and root development, negatively affect photosynthesis, and consequently decrease dry matter accumulation [8–10]. In young sorghum plants, survival rate and seedling vigor under low temperature stress have been suggested as indicators for chilling tolerance due to their relationship with emergence and dry matter accumulation [7, 11].

Several studies have indicated the presence of genetic variability in sorghum genotypes in response to low temperature stress at seedling stage, and some cold-tolerant sources have been identified [7, 12–14]. The discovery of several quantitative trait loci (QTL) of interest provided valuable information about chromosomal hotspot regions involved in the performance of cold-related traits, which may be used in marker-assisted selection [7, 15, 16]. Research works on genetic, biochemical, and physiology have been conducted to dissect cold stress effects on emergence, shoot and root development, and dry matter accumulation [4, 9, 10]. However, the process of germination, emergence, and early developmental stages (shoot and root development) were found to be controlled by different genetic mechanisms [3, 4, 7, 8]. Additionally, the interaction of genotype and environment complicates the selection of best performing varieties, since the degree of cold stress can vary with severity, frequency, soil composition, and sunshine period.

In Uganda, sorghum is grown in almost all agricultural regions, including the higher altitude zones, which cover about 25 percent of the arable land, and is considered as an important food security crop. Nevertheless, the high-altitude regions are characterized by cold temperatures, specifically during the crop growing season. Temperatures tend to be low (<15°C) from the beginning up to the middle of the rainy season and gradually increase towards the end of the season. Owing to the lack of improved cold-tolerant sorghum varieties, farmers have adopted escaping strategies by planting sorghum 4–6 weeks before the starting of the rainy season, which begins in the end of February or early March. To help farmers avoid planting too early and synchronize planting with the start of the growing season, it is imperative to develop sorghum varieties with the ability to tolerate cold temperature stress at early growth stages. The objectives of this study were to evaluate and identify sorghum lines with tolerance to cold temperature stress and make recommendations on varieties that may be planted in the East African highland regions or used in plant breeding programs for cold temperature tolerance.

2. Materials and Methods

2.1. Plant Genetic Materials. The forty highland sorghum lines used in this study were provided by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT, Kenya) and included various breeding lines, released varieties, and local landraces. Names, origin, characteristics, and subspecies are given in Appendix.

2.2. Experimental Design

2.2.1. Field Trials. Field trials were conducted at two locations (Kachwekano and Zombo) in Uganda during the seasons 2017B and 2018A. Kachwekano (1° 15'S, 29° 57'E, 2,200 m.a.s.l.) located in the highland of South Western Uganda is characterized by a bimodal rainfall pattern and has a sandy clay loam soil with an annual mean temperature of 18°C. Zombo (2° 30'S, 30° 54'E; 1,705 m.a.s.l.) is located in the northwestern (West Nile) Uganda with heavy clay loam soil and has bimodal rainfall pattern (Table 1). At both sites, a 4 × 10 alpha lattice design with three replications was adopted. Each sorghum genotype was planted in four rows per plot, with 2.5 m row length. Spacing within plots was 60 cm × 25 cm. Field levelling and hand-weeding activities were performed following recommended agronomic practices. Seedlings were treated with insecticides to avoid distortion of results due to insect pests, especially shoot flies.

Thirty days after sowing, emergence rate was recorded as the percentage of emerged seedlings, and vigor was scored on scale of 1–9 (1 as poor, 5 as medium, and 9 as excellent). In addition, 10 representative plants per plot were hand-harvested (from the ground), measured for seedling height, and dried at 120°C to determine shoot dry weight per plant (SDW).

2.2.2. Soil-Based Assay. Forty sorghum lines were evaluated for cold tolerance (13°C/10°C) and at normal temperature (25°C/20°C), using a 12/12 h day/night cycle. Temperatures were chosen based on previous studies [11, 17]. A minimal of 300 μE/m^2 light intensity was provided (Phillips HPI bulbs), and the day/night period was set at 12 h/12 h to reflect the Uganda's day-neutral conditions (Table 2). Twenty seeds of each genotype were planted into pots of 35 × 25 × 16 cm at 2 cm sowing depth.

A randomized complete block design was used with various replications for each experiment (Table 2). After 30 days under cold stress, vigor (scale: 1 as poor, 5 as medium, and 9 as excellent) and emergence rate were assessed. Newly emerged seedlings were counted daily, while final emergence was determined on the 30th day after sowing. Emergence index was determined by using the following formula as described by Smith and Millet [18]:

$$\text{Emergence index} = \frac{\sum Ej \times Dj}{E}, \quad (1)$$

where Ej is the number of newly emerged seedlings on day j, Dj is the number of days after planting, and E is the final seedling stand.

Five randomly selected plants per pot were harvested and rinsed with distilled water to measure shoot length (SL) and primary root length (PRL). Shoot length was measured as the distance from the ground to the longest leaf tip, while the primary root length was determined as the length from the ground to the end of the primary root tip. Afterwards, the total shoot and root matter of the five previously selected plants were dried at 120°C and weighed to determine the average shoot dry weight (SDW) and root dry weight (RDW) per sorghum line.

2.2.3. Filter Paper-Based Assay. Filter paper assay was conducted in order to analyze germination of sorghum seeds under both optimum and cold stress conditions. The seeds of sorghum genotypes were surface sterilized with 70% ethanol, followed by 5% sodium hypochlorite, and washed with distilled water. One hundred seeds were then placed on 15 cm diameter Whatman filter paper in 16 × 2 cm Petri dishes. Two sets of experiments were prepared in the growth room under complete darkness, whereby the first set was placed in the controlled cold room at a constant day and night temperature of 12°C (cold treatment) and the second set at 25°C (optimum temperature). Five milliliters of distilled water were added in each Petri dish after every two days to permit seed germination and maintain the optimum moisture content [19, 20]. Both sets were randomized in a complete block (RCB) design with two replications. Data on

Table 1: Climatic conditions of the field experiments at two locations.

Location	Season	Trial date (sowing-harvest)	Mean temp. (°C)	Mean max. temp. (°C)	Mean min. temp. (°C)	Precipitations (mm)
Kachwekano	2017 B	August 8–September 10	16.3	22.4	12.3	69
	2018 A	February 12–March 13	15.2	21.6	11.5	83
Zombo	2017 B	August 11–September 12	22.4	26.3	16.2	54
	2018 A	February 15–March 17	21.7	24.8	14.6	71

Table 2: Overview of the controlled environment experiments at early development stages.

Experiment	Reps	Light	Temp (°C)	Duration of experiment (days)	Traits scored
Soil-based assay (cold temperature)	4	12 h, 60 W, m^{-2}	13/10°C	30	Emergence rate (E%), emergence index (EI), seedling length (SL), primary root length (PRL), vigor (V), shoot dry weight (SDW), and root dry weight (RDW)
Soil-based assay (normal temperature)	3	12 h, natural light	30/20°C	15	Emergence rate (E%), seedling length (SL), primary root length (PRL), shoot dry weight (SDW), and root dry weight (RDW)
Filter paper-based assay (chilling /optimum)	2	12 h, constant dark	12°C/ 25°C	15/7	Germination rate (%)

germination were recorded on the 7th day for the optimum temperature and on the 14th day for the cold treatment. A germinated seed was recorded when the radicle or coleoptile had extended at least 1mm beyond the seed coat [13]. Germination percentage was determined by dividing the total number of germinated seeds by hundred. The data for germination were transformed by applying arcsine transformation methods [21].

2.3. Statistical Analysis. For the field experiment, restricted maximum likelihood (REML) analysis was used to generate analysis of variance (ANOVA) by utilizing GenStat 18th edition (VSN International, England). Replications, locations, and seasons were considered as random and lines as fixed effects. Pearson correlation coefficient was calculated to determine the level of association between all traits recorded for both controlled experiments and field trials. A correlation heat map was generated by using GenStat 18th edition.

A rank summation index [22] was used to rank the performance of all genotypes and determine the best cold-tolerant sorghum genotypes. Entries were ranked separately from controlled environment and field experiments. Since emergence rate, seedling length, seedling dry weight, and vigor were common in both controlled environment and field experiments, they were used in creating the rank summation index. In addition to that, percentage germination, emergence index, and shoot and root dry weight were used to construct rank summation index for controlled environment. This index was obtained by first ranking genotypes based on their performance on a scale of 1–40, with 1 representing the line with the highest mean of that trait. Afterwards, the rankings of both the growth chamber and field trials were summed for the traits under study. Therefore, the index value obtained by this method characterize rankings from the first (lowest value) to the last (highest value) cold-tolerant sorghum genotype.

3. Results

3.1. Evaluation of Chilling Stress in the Field Trials. Results of the ANOVA for emergence rate, seedling vigor, shoot length, and shoot dry weight under field locations (Zombo and Kachwekano), during two seasons (2017B and 2018A), are presented in Tables 3 and 4. Except the interaction of location x season for emergence rate in the first season, significance differences of genotypes, locations, seasons, and their interactions were observed for all traits recorded. This significant difference observed in the field trials indicated the existence of genetic variability among the tested sorghum genotypes. Since Zombo and Kachwekano had different weather and soil conditions, locations and seasons significantly affected all traits recorded (Table 4).

Generally, Zombo had higher mean values for all traits recorded in both seasons (Table 5). This location was overall characterized by higher temperatures compared to Kachwekano (located in the high-altitude mountains) that endures low temperatures, especially at night. At Zombo, seedling emergence rate ranged from 33.50–72.67% with an average of 54.62% in season 2017B and 27.59–64.01% with an average of 50.61% in season 2018A (Table 5). At Kachwekano, seedling emergence rate ranged from 26.89–65.09% with an average of 47.3% in season 2017B and 4.17–60.06% with an average of 42.17% in season 2018A (Table 5).

Season 2017B registered relatively better emergence rate compared to 2018A at the two locations, and this could be partially explained by the generally lower temperatures and the lengthy heavy rain period that were recorded in the second season. Sorghum lines BM6, IS 11838, IS 25547, and IS 25557 had higher emergence rates, while the most vulnerable lines were IESV 91003LT, IESV 91054LT, IESV 91105LT, and IS 29376, in both locations (Supplementary Document S2).

Significant differences in vigor were observed among sorghum lines at Kachwekano in season 2017B and season

Table 3: Mean squares of field trials for all observed traits and their interactions across all locations and seasons.

Source of variation	d.f.	Emergence (arcsine)	Vigor	Shoot length	Seedling dry weight
Location (L)	1	7,453.62***	102.95***	2,429.55***	36.95***
Season (S)	1	2,503.01***	20.63***	29.35***	10.46***
L × S	1	37.76 ns	2.17 *	240.13***	13.98***
L × S/Rep	8	31.65	0.30	2.51	0.02
Genotype (G)	39	887.40***	13.20***	24.58***	0.18***
G × L	39	40.82***	0.78***	3.70***	0.05***
G × S	39	48.26***	0.71***	1.96**	0.04***
G × L × S	39	36.52***	0.59***	2.81***	0.04***
Pooled error	312	18.55	0.26	1.13	0.01
C.V. (%)	—	8.84	9.38	9.66	4.92

ns, nonsignificant. *Significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$.

Table 4: Mean squares of field trials and their interactions, partitioned into seasons (2017B and 2018A) and locations (Kachwekano and Zombo).

Source of variation	d.f.	Season 2017B				Season 2018A			
		Emergence (arcsin)	Vigor	Seedling length	Shoot dry weight	Emergence (arcsin)	Vigor	Seedling length	Shoot dry weight
Location (L)	1	3215.17	37.60	571.03	2.739	4276.21	67.52	2098.64	48.19
Location/Rep	4	31.68	0.21	3.01	0.008	31.62	0.40	2.01	0.03
Genotype (G)	39	376.93***	5.88***	13.48***	0.080***	558.73***	8.03***	13.07***	0.15***
L x S	39	12.94 ns	0.49**	1.65 *	0.016***	64.39***	0.88***	4.87***	0.08***
Pooled error	156	14.18	0.28	1.02	0.002	22.91	0.24	1.23	0.02
C.V. (%)		7.39	9.40	8.99	1.74	10.32	9.34	10.33	7.2
s.e.d.		3.07	0.43	0.82	0.03	3.91	0.40	0.91	0.11
Source of variation	d.f.	Zombo				Kachwekano			
		Emergence (arcsin)	Vigor	Seedling length	Shoot dry weight	Emergence (arcsin)	Vigor	Seedling length	Shoot dry weight
Season (S)	1	962.95	4.704	50.78	0.127	1577.82	18.10	218.70	24.31
Season/Rep	4	29.03	0.5718	3.27	0.007	34.27	0.03	1.75	0.03
Genotype (G)	39	396.8***	8.03***	8.80***	0.105***	531.42***	5.95***	19.48***	0.13***
G x S	39	28.79***	0.51***	1.93***	0.002 *	55.98***	0.79***	2.85**	0.09***
Pooled error	156	13.67	0.232	0.78	0.001	23.42	0.28	1.48	0.02
C.V. (%)	—	7.02	8.21	6.65	1.36	10.81	10.84	13.9	7.80
s.e.d.	—	3.02	0.39	0.72	0.03	3.95	0.43	0.99	0.11

ns, nonsignificant. *Significant at $P < 0.05$; **significant at $P < 0.01$; ***significant at $P < 0.001$.

Table 5: Descriptive statistics traits for all recorded traits from field trials in the two seasons.

Trait	Item	Kachwekano		Zombo	
		2017B	2018A	2017B	2018A
Emergence (arcsin (%))	Mean	47.30	42.17	54.62	50.61
	Min.	26.89	4.17	33.50	27.59
	Max.	65.09	60.06	72.67	64.01
	S.D.	8.17	11.36	7.95	8.87
Vigor (1–9)	Mean	5.23	4.68	6.02	5.74
	Min.	3.03	1.33	3.10	2.40
	Max.	7.33	6.73	7.97	7.80
	S.D.	0.96	1.16	1.10	1.28
Seedling length (cm)	Mean	9.70	7.79	12.78	13.70
	Min.	6.22	1.07	10.57	11.13
	Max.	14.18	11.70	15.67	16.60
	S.D.	1.84	2.02	1.29	1.38
Seedling dry weight (log (mg))	Mean	2.15	1.51	2.36	2.40
	Min.	1.88	0.40	2.06	2.13
	Max.	2.35	1.81	2.65	2.70
	S.D.	0.12	0.24	0.13	0.13

2018A. Vigor scores ranged from 3.03 to 7.33 and 1.33 to 6.73 for the two seasons, respectively. Seedling vigor at Zombo ranged from 3.10 to 7.97 (2017B season) and from 2.40 to 7.80 (2018A season) (Table 5). Lines BM6, IS 11838, and IS 25557 that had high seedling emergence percentage were also shown to be highly vigorous, while lines IESV 91003LT, IESV 91054LT, IESV 91105LT, and IS 29376 had poor performance in both traits (Supplementary Document S1).

3.2. Reaction to Cold Stress under Controlled Environments. Pooled analysis of the two temperature regimes in soil-based assays indicated that temperature had a highly significant effect on all traits measured (Table 6). The results indicated that sorghum lines reacted differently to chilling stress during the early developmental stages. Compared with the normal temperature regime, the overall mean germination percentage in the filter paper-based assay and the soil-based assay were reduced from 68.47% to 47.43% and from 73.92% to 53.04%, respectively, under cold stress. In the soil-based

Table 6: Mean squares from analysis of variance for cold tolerance traits under soil-based conditions (cold and normal temperatures) and the filter paper assay.

Source of variation	d.f.	Emergence (arcsin)	Emergence index (days)	Vigor (1–9)	Shoot lengtd (cm)	Shoot dry weight (mg)	Primary root lengtd (cm)	Root dry weight (mg)
Soil-based assay (cold temperature)								
Rep	3	47.65	11.43	0.23	1.75	0.60	0.05	0.30
Genotype	39	720.48***	24.50***	7.43***	15.31***	19.80***	13.67***	6.93***
Error	117	22.86	4.91	0.19	0.36	0.50	0.56	0.17
C.V. (%)	—	10.08	12.71	8.35	9.87	10.43	12.43	12.64
s.e.d.	—	3.38	1.57	0.31	0.42	0.49	0.53	0.29
Soil-based assay (normal temperature)								
Source of variation	d.f.	Emergence (arcsin)	Seedling lengtd (cm)	Shoot dry weight (mg)	Primary root lengtd (cm)	Root dry weight (mg)	—	—
Rep	2	330.56	12.33	4.75	2.87	1.21	—	—
Genotype	39	105.39***	27.64***	14.04***	24.64***	12.01***	—	—
Error	78	42.34	3.50	3.80	2.32	1.93	—	—
C.V. (%)	—	9.50	9.78	7.93	6.41	7.41	—	—
s.e.d.	—	5.31	1.53	1.59	1.25	1.39	—	—
Filter paper assay								
Source of variation	d.f.	Germination % (optimum)	Germination % (cold)	—	—	—	—	—
Rep	1	5.89	11.12	—	—	—	—	—
Genotype	39	58.17***	142.25***	—	—	—	—	—
Error	39	15.9	19.28	—	—	—	—	—
C.V. (%)	—	5.44	8.55	—	—	—	—	—
s.e.d.	—	3.99	4.39	—	—	—	—	—

ns, nonsignificant. *** A significant mean square value at p-value <0.1%.

assay under cold stress, emergence percentage ranged from 17.21% to 69.83%, as compared to normal temperatures (where the range was from 59.16% to 82.14%). Across the lines, the mean vigor score was 5.24, while the genotypic values ranged from 2.68 for IESV 91105LT to 7.93 for IS11838 under cold temperature regime. IESV 91105LT, IESV 91054LT, N2, and IS 29376 had the poorest vigor under cold stress, while IS11838, IS25557, BM6, BM29, and IESV 90042LT were the most vigorous. As expected, these vigorous genotypes were also among the best for seedling height and primary root length under both cold and normal temperature regimes. Under normal temperature conditions, sorghum genotypes IESV 91105LT and N2 were the best performers for seedling length and primary root length, despite performing poorly under cold stress. Sorghum genotypes IESV 91054LT, IS 25563, and IS 29376 performed poorly under cold and normal temperature regimes.

Compared with normal temperature, cold stress decreased both dry weight of shoot (from 24.59 to 6.76 g) and root (from 18.81 to 3.24 g). Additionally, under cold stress, large ranges in shoot dry weight (1.68–11.33 g) and root dry weight (1.33–6.75 g) were observed. Under the cold temperature regime, sorghum genotypes lines IESV 90042LT, BM6, BM29, IS11838, IS 25546, and IS25557 were the top ranking, while lines IS 25547, IS 25558, and IESV 91105LT were the best under normal temperature regime. The genotypes IS 29376 and IESV 91073LT had the lowest shoot and root dry weight under both cold and normal temperature regimes. These results indicated a significant negative effect of cold stress on both shoot and root dry weight, which impacts growth and development of seedlings at early developmental stage.

3.3. Association between Traits from Controlled Environment Experiments and Field Trials. Under controlled conditions, the Pearson correlation analysis showed a highly significant ($P < 0.01$) correlation between traits evaluated in the soil-based assays (SBA) experiments. Moreover, medium-to-high correlations were also observed between soil-based assay and field trials at both locations (Figure 1).

Emergence rate, shoot length, and vigor (SBA) showed medium-to-high correlation with other traits evaluated under controlled experiments and field trials. As expected, emergence index (SBA) was significantly negatively correlated ($P < 0.05$) with most traits. Germination rate under cold stress (filter paper-based assay) showed a medium correlation with most traits evaluated under cold stress conditions. Field experiments revealed high significant correlation between emergence and vigor at both locations, while shoot dry weight showed low-to-medium correlations with other traits. Interestingly, vigor at Zombo was closer related to root dry weight in the soil-based assay.

3.4. Rank Summation Index. A relative ranking of cold tolerance responses for all 40 sorghum genotypes was based on rank summation index values derived from traits recorded in both controlled environment and field trials (Table 7). In the soil-based assay, means for emergence percentage, emergence index, shoot length, root and shoot

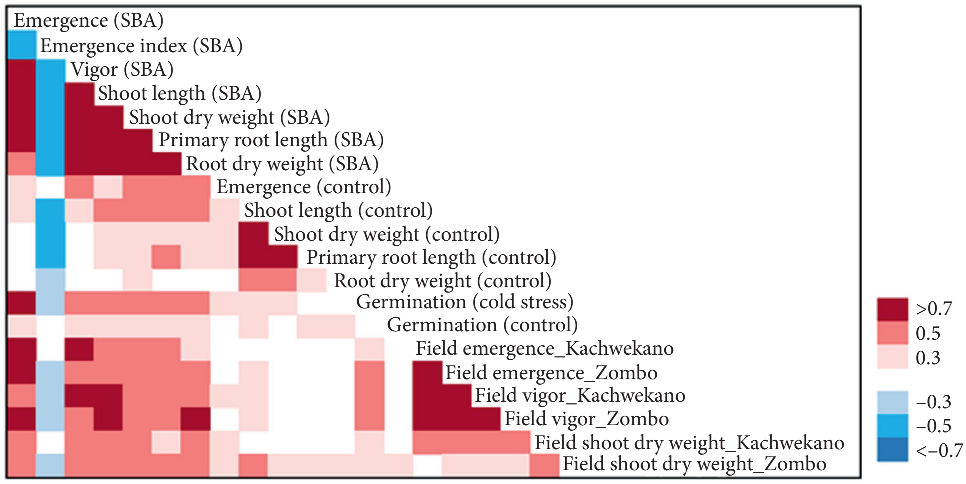

Figure 1: Heat map showing Pearson's correlation (r) between cold tolerance traits evaluated under soil-based conditions and field trials at both locations. SBA, soil-based assay.

Table 7: Relative ranking of sorghum lines based on rank summation index values from soil-based assay and field trial.

Entry	Soil-based assay							Field trials				
	E	EI	Vigor	Shoot length	SDW	RDW	Germ. (cold)	E	Vigor	Shoot length	SDW	Rank summation index
IS 25557	2	3	2	4	2	2	11	1	1	4	5	37
IS 11838	1	9	1	1	1	3	5	4	3	2	12	42
BM 6	6	8	5	5	3	6	4	5	2	8	8	60
BM 29	3	4	4	6	4	1	22	6	4	3	4	61
IESV 90042 LT	4	5	3	9	8	4	3	8	7	7	6	64
IS 25546	5	7	7	2	6	7	6	10	9	1	16	76
CYTANOBE	8	14	15	3	11	5	7	7	6	5	3	84
IS 25558	12	2	10	7	7	12	1	13	8	9	18	99
IS 25547	10	15	6	15	9	10	20	2	5	6	2	100
NDAMOGA	7	6	14	8	5	9	8	19	17	26	25	144
BM 21	9	13	23	12	18	19	30	15	11	29	14	193
BM 16	23	28	12	24	24	15	19	12	12	10	22	201
BM 27	13	17	20	19	15	23	14	22	26	31	1	201
MB 30	14	12	21	10	10	17	26	18	30	23	24	205
IS 11442	30	37	29	11	25	11	21	3	14	14	11	206
IS 25561	15	19	18	27	21	32	27	14	19	20	10	222
N 12	27	21	30	20	17	13	32	9	15	22	17	223
ABALESHYA	21	23	11	22	20	14	31	25	24	15	21	227
NYUNDO	25	16	26	16	12	18	2	28	21	35	31	230
AMASUGI	11	25	19	29	31	29	9	24	22	13	28	240
E 1291	16	30	9	33	33	30	29	16	13	24	15	248
IS 25563	18	35	13	38	28	28	16	11	18	19	26	250
S 87	32	27	27	14	16	8	38	35	34	11	13	255
IESV 91073 LT	29	26	31	25	34	38	15	17	16	17	23	271
IS 25562	20	20	16	17	30	27	35	20	29	30	29	273
IS 29415	19	18	22	18	27	39	18	27	10	38	37	273
IS 11612	22	36	17	13	14	21	36	23	31	27	35	275
IESV 91075 LT	35	24	33	21	13	35	34	21	20	18	32	286
IKINYARUKA	28	22	24	26	35	24	17	29	25	25	33	288
IESV 91018 LT	33	38	34	23	19	22	39	33	35	12	9	297
IS 11721	17	11	25	36	39	36	24	31	27	16	39	301
IESV 91003 LT	37	33	35	28	23	16	12	38	38	28	19	307
IS 25545	24	34	8	31	29	25	28	34	36	39	20	308
N 2	38	39	38	34	22	33	13	26	23	21	27	314
IESV 91105 LT	40	1	40	39	38	31	10	40	39	33	7	318
IESV 90015 LT	26	32	28	32	26	26	37	36	28	34	34	339
IESV 91069 LT	31	29	32	30	32	20	33	30	33	40	36	346
IESV 91071 LT	34	31	36	35	37	37	25	32	32	32	40	371
IESV 91054 LT	36	40	37	37	36	34	23	39	40	36	30	388
IS 29376	39	10	39	40	40	40	40	37	37	37	38	397

E, emergence; EI, emergence index; Germ., germination rate; SDW, shoot dry weight; RDW, root dry weight.

Table 8: List of sorghum accessions, origins, and characteristics used in the study.

Accession name	Origin	Status	Subspecies
ABALESHYA	Rwanda	Fixed line	Caudatum
AMASUGI	Rwanda	Fixed line	Durra
BM 16	Uganda	Fixed line	Caudatum
BM 21	Uganda	Fixed line	Bicolor-caudatum
BM 27	Kenya	Fixed line	Caudatum
BM 29	Kenya	Fixed line	Bicolor-caudatum
BM 6	Kenya	Fixed line	Caudatum
CYTANOBE	Uganda	Fixed line	Bicolor
NYUNDO	Rwanda	Fixed line	Caudatum
E 1291	Kenya	Fixed line	Bicolor-caudatum
IESV 90015 LT	Kenya	Breeding line	Bicolor
IESV 90042 LT	Kenya	Breeding line	Caudatum
IESV 91003 LT	Kenya	Breeding line	Caudatum
IESV 91018 LT	Kenya	Breeding line	Kafir
IESV 91054 LT	Kenya	Breeding line	Kafir
IESV 91069 LT	Kenya	Breeding line	Caudatum
IESV 91071 LT	Kenya	Breeding line	Bicolor
IESV 91073 LT	Kenya	Breeding line	Bicolor-caudatum
IESV 91075 LT	Kenya	Breeding line	Caudatum
IESV 91105 LT	Kenya	Breeding line	Caudatum
IKINYARUKA	Rwanda	Fixed line	Caudatum
IS 11141	Kenya	Breeding line	Bicolor
IS 11612	Kenya	Breeding line	Bicolor
IS 11721	Kenya	Breeding line	Caudatum
IS 11838	Kenya	Breeding line	Bicolor-caudatum
IS 25546	Kenya	Breeding line	Caudatum
IS 25547	Kenya	Breeding line	Caudatum
IS 25557	Kenya	Breeding line	Bicolor-caudatum
IS 25558	Kenya	Breeding line	Bicolor
IS 25561	Kenya	Breeding line	Caudatum
IS 25562	Kenya	Breeding line	Durra
IS 25563	Kenya	Breeding line	Kafir
IS 29415	Kenya	Breeding line	Kafir
IS 25545	Kenya	Breeding line	Bicolor
MB 30	Kenya	Fixed line	Caudatum
N 12	Uganda	Fixed line	Bicolor-caudatum
N 2	Uganda	Fixed line	Bicolor
NDAMOGA	Uganda	Fixed line	Caudatum
S 87	Kenya	Breeding line	Caudatum
IS 11442	Kenya	Breeding line	Bicolor

Source, Rutayisire et al. [34].

dry weight, as well as emergence rate, vigor, shoot length, and shoot dry weight, in the field trials (for one season of 2017B) were used to generate rank summation index values. The correlation between rank summation indices for performance in the soil-based assay and field trials was significant ($r = 0.81$), indicating that the rankings were almost similar in both experiments. Therefore, five sorghum genotypes (IS 25557, IS 11838, BM 6, BM 29, and IESV 90042LT) were ranked best across both the controlled environment experiments and field trials, while IESV 90015LT, IESV 91069LT, IESV 91071LT, IESV 91054LT, and IS 29376 were the worst. Genotype IS 25557 was ranked first in the field trials but second in the soil-based assay, while IS 118383 was first in the controlled experiments but ranked second in the field trial.

4. Discussion

4.1. Genetic Potential of Sorghum Genotypes Adapted to Highland Regions of Uganda. Low temperature has been listed among important abiotic stresses that interfere with plant growth and development, lowering productivity and limiting production of certain crops, especially cereals, to specific geographical locations [23]. Therefore, improved chilling tolerance in sorghum is an important trait for the long term outlook and consequently may increase production in high-altitude regions where the crop may be not grown currently. Interestingly, some highland regions of Eastern Africa are believed to harbor the origin of an important source of sorghum lines adapted to cold environmental conditions [12, 24]. In the present study, various phenotyping approaches were used to evaluate the effect of chilling stress at early developmental stages and to identify potential sources of tolerance to chilling stress among sorghum lines tested. Germination, emergence rate, and seedling vigor have proven their importance under cold stress and therefore can be used as potential surrogate traits for breeding and selection. In addition, the results indicated significant negative effects of cold stress on shoot and root dry weight, which hamper growth and development of seedlings at juvenile stage.

It was observed that genetic variability exists among evaluated sorghum genotypes for traits associated with early-stage chilling tolerance. Out of the 40 genotypes evaluated, 8 showed superior performance for emergence and biomass-related traits under both controlled environments and field trials, based on rank summation indices that were below 100 (Table 7). It is important that sorghum genotypes express high emergence rate and strong vigor under prolonged cold stress to compete with adapted weeds and therefore enable field establishment and further growth and developmental processes [11, 17, 25]. Therefore, the sorghum genotypes identified as the best performers under cold stress are potential parental materials that can be used in breeding programs aiming at improving cold tolerance.

4.2. Emergence and Seedling Vigor as Important Traits to Enable Adaptation of Sorghum in the Highland Regions. Crops such as sorghum, native in the warm temperature regions, are likely to be sensitive to chilling stress, especially during the emergence process and seedling growth and development at early developmental stages [7, 26, 27]. Therefore, for the crop to develop and reach adequate plant size, germination and emergence are critical target traits to enable adaptation to cold temperatures [28]. In the present study, correlations were observed between emergence rate with other traits (under controlled environment experiments) and field experiments. The finding that emergence index correlated with shoot and root biomass traits implies that cold-tolerant genotypes tend to emerge faster and therefore initiate the autotrophic phase earlier, which permits faster accumulation of dry matter [29]. However, larger values of emergence index were observed among evaluated sorghum genotypes, as well as longer duration to final sprouting of the seedlings, due to chilling stress in both field trials and cold treatment experiments. Similar results were reported by Balota et al. [24] and Bekele et al. [7] in seedling emergence tests.

Early-stage seedling vigor is an essential factor for crop growth in several temperature-related environments [12, 30, 31]. Seedling vigor is associated with crop performance and yield, through its relationship with vegetative growth resulting in adequate accumulation of dry matter into plant organs linked to production [17]. Vigor traits include seedling height, shoot and root dry weight, and growth rate. Therefore, vigor can be regarded as an important indicator of crop establishment in various chilling environments. However, chilling stress reduces vigor, even for the cold-tolerant genotypes, because the rate of biomass accumulation is significantly reduced [7, 9, 11, 25, 32]. In this study, significant correlations were found between vigor and biomass-related traits, indicating that highly ranked cold-tolerant genotypes tended to develop shoot and root organs better than susceptible ones. Similar results have been reported by other researchers [7, 11]. Although vigor score is a subjective measurement, it is cost-effective in the identification of cold-tolerant genotypes under chilling stress both in controlled environment and field experiments.

4.3. Controlled Environment Experiments Can Be Used for Preselection Prior to Field Trials. Selection of appropriate temperature for use in abiotic stress experiments is an important step for identifying cold-tolerant genotypes [9]. The range of 13°C (day)/10°C (night) has been reported as one of the best thresholds to evaluate sorghum under chilling stress [14, 17]. The highland regions in Uganda display a bimodal rainfall pattern, and in most cases, cold temperatures (below 15°C) are observed during the rainy period. The temperature regime (13°C/10°C) was almost similar to the field conditions, which resulted in the medium-to-strong correlation between controlled experiments and field trials. This indicates that controlled environmental conditions may be used for preselection prior to field testing, though field conditions can be biased due to daily temperature fluctuations and unpredictable climatic changes.

Emergence rate under controlled conditions was found to correlate with field emergence, indicating that growth rooms can be used to predict field experiment outputs. Similar results were observed by Windpassinger et al. [11] and Salas-Fernandez et al. [14] who found significant correlations between growth chamber and field experiments, though the temperature regimes used for cold stress were slightly different. Similar trends were observed for other traits in the screen house, such as vigor, shoot length, and root dry weight that showed high correlation with field vigor and field shoot dry weight. Therefore, the findings that genotypes with greater emergence rates are stronger and faster during the early development stages suggest that simultaneous multitrait improvement is feasible. Though days to onset of emergence and growth rate of shoots and roots are compromised under chilling stress during heterotrophic stage [7, 20], vegetative growth rate at autotrophic stage under field conditions is an important key factor that enables sorghum to survive under cold stress conditions when chlorophyll biosynthesis becomes important for the plant [8, 29, 33]. Nevertheless, temperatures in the field are varying and gradually rise towards the end of the growing season. Consequently, researchers may need to develop different strategies for efficiently evaluating cold tolerance in sorghum.

5. Conclusion

Cold tolerance at early developmental stages is a paramount goal for successful adaptation of sorghum in the highland of East Africa. Eight sorghum lines were identified as potential sources of cold tolerance based on their emergence and seedling development and thus can be used in sorghum breeding programs for this type of abiotic stress resilience. Our results demonstrated that controlled environmental conditions with low temperature imposed at seedling stages would be useful to screen and identify the best chilling tolerant sorghum genotypes. Additionally, the significant positive correlation observed between controlled environments and field trials indicated that the growth chambers set up at low temperatures can be used to select genotypes with the best cold tolerance characteristics at early developmental stages, since field experiments are subjected to unpredictable weather. In this era of climate change and unfavorable temperatures, further research studies are therefore needed to provide more insight for helping breeding to mitigate negative effects of this stressor.

Appendix

Data Availability

The data used to support the findings of this study are included within the supplementary information files 1 and 2.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Document 1. Cold-tolerant related traits in 40 sorghum genotypes evaluated under controlled conditions. Supplementary Document 2. Field trials at early developmental stages in two locations for two seasons. (*Supplementary Materials*)

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