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Evaluation of sweet sorghum accessions for seedling cold tolerance using both lab and field cold germination test

Ming Li Wang[1*], Zhanguo Xin[2], Gloria Burow[2], Junping Chen[2], Phiffie Vankus[1], David Pinnow[1], Brandon Tonnis[1], Hugo Cuevas[3], Jianming Yu[4]

[1]USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, Georgia 30223, United States

[2]USDA-ARS, Plant Stress and Germplasm Development Unit, Lubbock, Texas 79415, United States

[3]USDA-ARS, Tropical Agriculture Research Station, Mayaguez, Puerto Rico 00680, United States

[4]Department of Agronomy, Lowa State University, Ames, IA 50011, United States

Abstract

Objective: Seedling cold tolerance is one of the important traits for sweet sorghum production. This study is to evaluate the method for identification of sweet sorghum accessions with seedling cold tolerance using both lab cold germination and field early-spring cold planting.

Methodology: Sorghum seeds were germinated in growth chamber (under lab cold condition) at a constant 12°C for five weeks. After two weeks, germinated seeds and germination rates were counted and calculated at weekly intervals for four times. The same seed lots for lab cold test were also planted 45 days earlier than the normal planting time in the field (early-spring cold planting). In addition to seedling dry weight, germinated seeds and seed germination rates were also counted and calculated at weekly intervals for three times.

Results: In this study, a high correlation coefficient between lab germination rate and field germination rate (R2=0.503, p<0.0001) was observed. In general, lab germination rate can predict the field germination performance; but some discrepancies between field and lab tests were also observed for some accessions. Among 212 sweet sorghum accessions tested, several sweet sorghum accessions with seedling cold tolerance were identified from both lab and field tests. These accessions will be useful materials for development of sweet sorghum cultivars with early spring cold tolerance.

Conclusion: Compared with the field test, the lab test is less labor intensive. For a large-scale screening of seedling cold tolerance, the lab test may be conducted initially followed by selection of superior accessions from the lab test for further evaluation under field conditions.

Keywords: Sweet sorghum germplasm, Seed germination rate, Seedling cold tolerance.

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Introduction

Early planting (or early sowing) may increase yield of sugar, grain or biomass; but it depends on many factors (e.g. rainfall, soil types and temperatures, cultivars, and crops). For example, early planting soybean in southwestern Japan can increase grain yield [1]. But planting peanut in early to mid-April instead of early to mid-May in the state of Georgia can increase the risk of tomato spotted wilt virus (TSWV) incidence [2] and lead to yield reduction. Sorghum seeds can be planted in early April for achieving a higher yield or earlier harvesting time for a double-cropping system in Texas, but selection of cultivars with early spring cold tolerance is important. Sorghum cultivars with early spring cold tolerance have several obvious advantages: faster and better seedling emergence and establishment, an extended growing season in the same region, and slightly expanded planting zones from the south to north regions [3]. Significant seedling cold tolerance exists in sorghum. Several studies have been conducted on seedling cold tolerance in grain sorghum [3,4]. Three SSR markers for different QTL for early-season cold tolerance have been identified, and they are mapped in different chromosome regions [5-7]. However, little research has been conducted on cold tolerance in sweet sorghum. The U.S. sweet sorghum collection is maintained by the Plant Genetic Resources Conservation Unit (PGRCU) at Griffin, GA. These sweet sorghum accessions are used as experimental materials for screening seedling early-spring cold tolerance under both lab and field conditions. Therefore, the objectives of this study were to (i) determine the germination rates of sweet sorghum accessions under cold conditions in both lab and field; (ii) determine the correlation coefficients among investigated traits; (iii) determine the correlation coefficients of germination rates between lab and field conditions; and (iv) identify accessions with cold tolerance under both lab and field conditions and recommend them to sweet sorghum breeders as parental materials for developing new cultivars.

Materials and Methods

Sweet sorghum accessions: Seed regeneration for sweet sorghum accessions was conducted every year from 2013 to

2015 at St. Croix, Puerto Rico. Fresh seeds, after breaking dormancy, were used for germination testing. Four checks (Rio, a good sweet sorghum cultivar; BTx623, a good grain sorghum cultivar; GT26056, a good early spring cold tolerance cultivar; and PI 610727, a very good early spring cold tolerant cultivar from Shanxi, China) were used for comparison with the sweet sorghum accessions. A total of 212 sweet sorghum accessions plus four checks used for both field and lab germination tests are listed in (Table 1).

Detailed information about sweet sorghum accessions can be obtained from the USDA-ARS Germplasm Research Information Network (GRIN) website.

Table 1. *Information on PI number and identifier for the selected sweet sorghum accessions.*

PI	Identifier	PI	Identifier
PI 17548	RED AMBER	PI 173971	JAWAR
PI 22913	CHINESE AMBER	PI 174381	KARADARI
PI 48191	SACCALINE	PI 175919	IS 12833
PI 52606	MN 2680	PI 176766	MN 2873
PI 88000	Mokutakususu	PI 177156	MN 2742
PI 88007	Bangu manguisusu	PI 177553	AKDARI
PI 92270	MN 2740	PI 177554	MN 2894
PI 144134	Inyangentombi	PI 179504	AKDARI
PI 144331	ISIDOMBA	PI 179747	JAWAR
PI 145619	ISIDOMBA	PI 179749	Juar
PI 145622	Jiba	PI 180004	JAWAR
PI 145632	TEGEVINI	PI 180005	JAWAR
PI 145633	Tugela Ferry	PI 180348	Juar
PI 146890	SUGAR DRIP	PI 180489	Juar
PI 147026	Nagro	PI 181077	DEPAR
PI 147200	W. 21	PI 181080	HONEY SORGHUM
PI 147224	B. 35	PI 181083	KAMANDRI
PI 147573	MN 600	PI 181899	Aleppo No. 41
PI 149830	IS 2462	PI 181971	MN 2939
PI 149832	IS 2464	PI 182303	AKDARI
PI 152596	ANKOLIB TEQUIL	PI 183149	JUAR
PI 152629	Feterita Fayoumi D.S. 8	PI 189114	MN 2972
PI 152630	FETERITA FAYOUMI D.S. 10	PI 195754	KAOLIANG
PI 152633	FETERITA FAYOUMI D.S. 13	PI 196049	IS 2131
PI 152646	FETERITA GEZIRA	PI 196592	MN 3089
PI 152650	FETERITA FULLI	PI 196598	MN 3095
PI 152651	Feterita Geshaish	PI 197542	SUCRE DROME
PI 152671	GISHISH	PI 198885	SWEET SACCALINE
PI 152675	Heger Taie	PI 201723	FETERITA LA ESTENZUELA
PI 152676	HEGIRI 1	PI 217691	NAGAD EL MUR
PI 152683	HEMAISI RED SHENDI SHERSHER	PI 217770	BARGOWI
PI 152692	KAFIR PINK	PI 218112	IS 2352

PI 152714	LUEL	PI 221560	BALAKA
PI 152725	MALWAL AWEIL	PI 247136	MN 4052
PI 152733	MERISSA (BARI)	PI 247744	U. g. 6. 7.
PI 152751	NYTWAL	PI 247745	Tjolotjo
PI 152755	POTCH 4	PI 250232	MN 4118
PI 152764	QUERY 3	PI 250234	MN 4120
PI 152771	RAHMETALLA GALLABAT	PI 250402	MN 4126
PI 152813	Wad Aker Red	PI 250521	MN 4122
PI 152816	WAD FUR WHITE	PI 250582	MN 4124
PI 152828	U.T. 23	PI 250897	MN 4133
PI 152860	MERASI	PI 250898	MN 4134
PI 152872	FETERITA ABDEL MAGID	PI 251672	MN 4135
PI 152880	LWEL FADIANG	PI 253795	MN 4136
PI 152898	BILICHIGAN	PI 253796	MN 4137
PI 152909	Mahananga	PI 253986	MN 4138
PI 152914	WAXY CLUB	PI 255239	CAXA
PI 152923	Duro El Jack	PI 257599	NO. 5 GAMBELA
PI 152953	CHIKKORI	PI 257600	NO. 6 GAMBELA
PI 152961	MALNAL	PI 257602	NO. 8 GAMBELA
PI 152963	Thok (B)	PI 260210	Darso 28
PI 152966	Ayuak	PI 248298	CHINESE AMBER
PI 152971	AWANLEK	PI 267476	Tseta 27/51
PI 152998	GUMBILU	PI 273955	MN 4566
PI 153871	MUBEYA	PI 273969	MN 4578
PI 154750	Serere	PI 287625	MN 48
PI 154787	MN 1344	PI 287627	MN 12
PI 154796	NKUMBA	PI 302120	MN 4155
PI 154800	Wenabu	PI 302122	IS 13718
PI 154844	GRASSL	PI 302131	MN 4179
PI 154846	KABIRI	PI 302198	MN 4243
PI 154929	J56 Akouangok	PI 302199	MN 4369
PI 154943	L28 Lawere	PI 302252	IS 13726
PI 154944	L31 Emiroit	PI 302264	MN 4330
PI 154962	V3 Nakyeru	PI 303658	Nerum Boer
PI 154980	Wheatland	PI 511355	SMITH
PI 154987	S. A. 1	PI 533998	Brawley
PI 154988	S. A. 2	PI 535783	N98
PI 154990	P 127 (S.A. 5)	PI 535785	N100
PI 155336	MUYO	PI 535792	N107

PI 155485	Maila	PI 535796	N111
PI 155516	MASAKA	PI 562716	HONEY NO. 2
PI 155543	Hasesa	PI 563295	RIO
PI 155556	MAILA	PI 566819	DELLA
PI 155571	LONGWE	PI 583832	TOP 76-6
PI 155609	MAPIERA	PI 584989	POPSORGHUM
PI 155721	WAQUEMA	PI 586443	MN 818
PI 155760	Namuse	PI 586541	TRACY
PI 155805	MAPIRA	PI 641806	AMES AMBER
PI 155845	MN 2077	PI 641807	ATLAS
PI 155902	MN 2103	PI 641815	EARLY FOLGER
PI 155924	CHIFUNGO	PI 641817	EARLY SUMAC
PI 156136	MAILA	PI 641821	HONEY DRIP
PI 156203	MN 2089	PI 641834	PLANTER
PI 156217	MN 2109	PI 641835	REX
PI 156252	Nefee	PI 641848	TEXAS SEEDED RIBBON
PI 156352	MN 2238	PI 641862	COLLIER
PI 156356	Sonkwe	PI 641893	DWARF ASHBURN
PI 156393	MN 2277	PI 641904	H.C. 41-13
PI 156890	Dura Huria	PI 641909	Red Losinga
PI 157030	Andiwo III 57	PI 642999	Leoti-Peltier
PI 157033	Ifube No. 18	PI 643008	MN 2751
PI 157035	Nyagwang No. 56	PI 643013	MN 2756
PI 157804	Feterita Abu Derega	PI 643016	MN 2761
PI 167047	AKDARI	PI 643017	MN 2762
PI 167352	AKDARI	PI 643464	IS 3986
PI 170783	AKDARI	PI 651493	RAMADA
PI 170787	MN 2826	PI 651495	DALE
PI 170788	MN 2827	PI 651497	Theis
PI 170799	MN 2838	PI 653616	WRAY
PI 170802	IS 12807	PI 653617	KELLER
PI 170805	IS 12810	PI 655983	SUGAR DRIP
PI 173112	7392	PI 655983	M81 E
PI 173118	8371	GT26056	Cold tolerance check
PI 173120	8493	PI 610727	Cold tolerance check, China
PI 173121	MN 2857	Rio	Sweet sorghum, TX
PI 173808	GILGIL	BTx 623	Grain sorghum, TX

Lab cold germination test: The growth chamber (Percival Scientific, Inc., model GR36L) was set at a constant 12°C and 60% humidity with eight hours of light and 16 hours of dark. Seeds were treated with thiram before testing. Fifty seeds were evenly spread out onto a double layer of wetted germination paper towels, wrapped up, and bound in place with a rubber

band. The bound paper towels were then transferred standing upright into a lattice plastic tray (to allow air circulation around towels) and placed in the growth chamber. Whenever the paper towels needed to be rewetted, 12°C water was used from a carboy stored within the same growth chamber. High humidity (~60%) was maintained by keeping an open plastic basin containing clean tap water at the bottom of the growth chamber at all times. After two weeks, the wrapped paper towels were opened on a clean table to count germinated seeds, which were removed from the towels. Seeds not yet germinated were then wrapped up again and put back into the growth chamber for further germination. Following the same procedure, seeds were recounted at weekly intervals three more times. Germination rates for each accession were calculated cumulatively for each counting time.

Field early-spring planting evaluation: Normal planting time for sorghum in Lubbock, TX is around May 15th, but the planting time for early-spring cold tolerance test in this study was April 1st (45 days earlier than the normal planting time). The same seed lots for the growth chamber test from 212 accessions were also used for the field planting evaluation. Twenty-five seeds were planted in a 6 x 1 meter row, and two replicates were planted for each accession. The seedling emergence was first counted fourteen days after planting. Seedling emergence was recounted at weekly intervals two more times. Similar to lab germination rates, the seed germination rates in the field were also calculated cumulatively.

Seedling dry weight and emergence index: At 28 days after counting, the above-ground seedling tissues from five seedlings for each accession were cut, harvested, and then were dried in an oven at 80°C for 72 hours. After drying, the seedling tissues were weighed and the dry weight recorded (g/5 seedlings). Emergence index (EI), a measurement of rate of emergence, was calculated using the following formula: EI=∑ (Ej x Dj)/E where Ej=emergence on day j, Dj=days after planting, and E=final stand. The final stand counts were taken at 28 days and 35 days for field test and lab test, respectively [8].

Statistical analysis: An analysis of variance was performed on the data and means were using minimum significant difference (MSD) comparison procedure (SAS, 2008, Online Doc® 9.2. Cary, NC: SAS Institute Inc.). Significant correlations between investigated traits were determined using Pearson correlation coefficients.

Results and Discussion

Variation in germination rates from lab and field, emergence index, and dry weight of field seedlings: The germination rates from lab and field conditions counted at different times plus dry weight of five field seedlings are listed in (Table 2) and shown in (Figure 1). Overall, at the beginning the seeds germinated more slowly in the field (2.01% at 14 days) than the lab (18.71% at 14 days). After 21 days, the germination rate was higher in the field (35.69% at 21 days, 43.20% at 28 days) than in the lab (28.86% at 21 days, 36.92% at 28 days) (Table 2 and Figure 1). Some accessions (e.g. PI 610727, a very cold tolerant check in replicate 2) reached 100% germination in the field at 21 days; while in the lab 100% germination was reached by some accessions (e.g. PI 154800 in replicate 1) at 28 days. Sorghum seeds appear to germinate more quickly in the field than in the lab. This observation is supported by the emergence index (EI). The average emergence index was lower in the field test (19.45) than in the lab test (20.02). Temperature fluctuation (between day-time high and night-time low) may be required for a better germination. In addition to lab/field environmental conditions, differences in accessions (genotypes) also significantly affected the germination rate. Significant differences in germination rates for both lab (0–100%) and field conditions (0–100%) were identified among sweet sorghum accessions. The results from this study are consistent with the results from a previous study in which different cultivars differed significantly between lab germination and field emergence9. For example in the lab, the germination rate of the sweet sorghum cultivar Rio was only 42% at 14 days (Figure 2a), while the germination rate of GT26056 reached 94% at 14 days (Figure 2b). In the field, the germination rate of sweet sorghum accession PI 653617 at 28 days was only 68% (Figure 3a), while the rate of PI 152751 at 28 days reached 89% (Figure 3b).

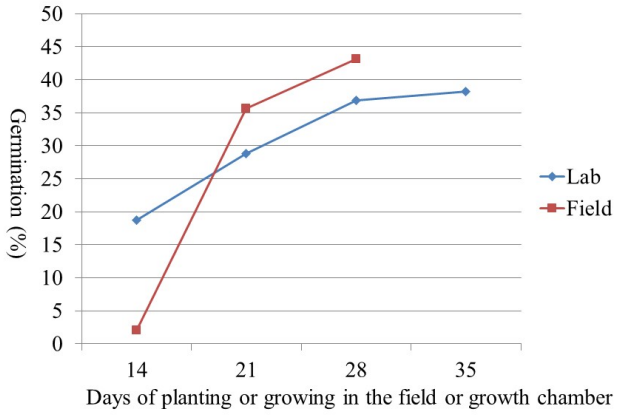

Figure 1. *Comparison of germination rates between field and lab at different time-counting intervals. The x-axis indicates days after planting or growing in the field or growth chamber, the y-axis is the germination rate (%). Blue curve represents the lab experiment and red curve represents the field experiment.*

In sweet sorghum growing regions where early spring cold may be an issue, accessions with good tolerance to early spring cold should be selected and used as parental materials to make crosses for developing new cultivars. The average dry weight of five seedlings in the field was 0.72 g, ranging from 0.23 to 1.74 g. PI 201723 (1.74 g/5 seedlings) had a significant higher ($P<0.05$) dry weight than PI 155516 (0.23 g/5 seedlings). Seedling dry weight may relate to early spring cold tolerance. This issue will be discussed in the following section of unique germplasm accessions identified.

Table 2. *Simple statistics for the lab germination rate, field germination rate, and seedling dry weight. (N=Number of samples; Std Dev=Standard Deviation).*

Variable	N	Mean	Std Dev	Minimum	Maximum
Field 1st count germination rate (%)	425	2.012	7.67	0	80
Field 2nd count germination rate (%)	425	35.69	25.565	0	100
Field 3rd count germination rate (%)	425	43.2	26.177	0	100
Dry weight (g/5 seedlings)	324	0.72	0.32	0.23	1.74
Lab 1st count germination rate (%)	430	18.71	21.057	0	94
Lab 2nd count germination rate (%)	430	28.86	24.239	0	94
Lab 3rd count germination rate (%)	430	36.92	26.808	0	100
Lab 4th count Germination rate (%)	430	38.26	27.088	0	100

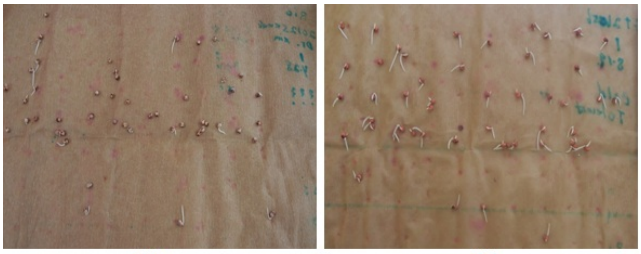

Figure 2. *Comparison of lab germination rates between sweet sorghum cultivar Rio and cold tolerance line GT26056. 2A) Seeds from sweet sorghum Rio in the growth chamber at 12°C for 14 days with a low germination rate of 42% and 2B) seeds from cold tolerance line GT26056 in the growth chamber at 12ºC for 14 days with a high germination rate of 94%.*

Correlation coefficients among investigated traits: The results of Pearson correlation coefficients, probability, and number of observations among lab germination rates, field germination rates, and seedling dry weight are listed in (Table 3). In the field test, all the correlation coefficients among different countings were significant ($p<0.0001$). But the correlation coefficient values between the first counting and the second and third countings were low ($R2=0.287$ and $R2=0.266$, respectively), while the correlation coefficient value between the second counting and the third counting was very high ($R2=0.944$). In the lab test, all correlation coefficients among the different countings were significant ($p<0.0001$), and all correlation coefficient values were also high (all $R2>0.84$). The germination conditions in the lab are very controlled compared to the field. This may partly explain why there was some inconsistency in the correlation coefficient values among the different countings in the field. A high correlation coefficient between lab germination rate and field germination rate ($R2=0.503$, $p<0.0001$) was also observed. The correlation coefficient of seedling dry weight with the field germination rate was much higher ($R2=0.257$, $p<0.0001$) than with the lab germination rate ($R2=0.109$, $p<0.05$).

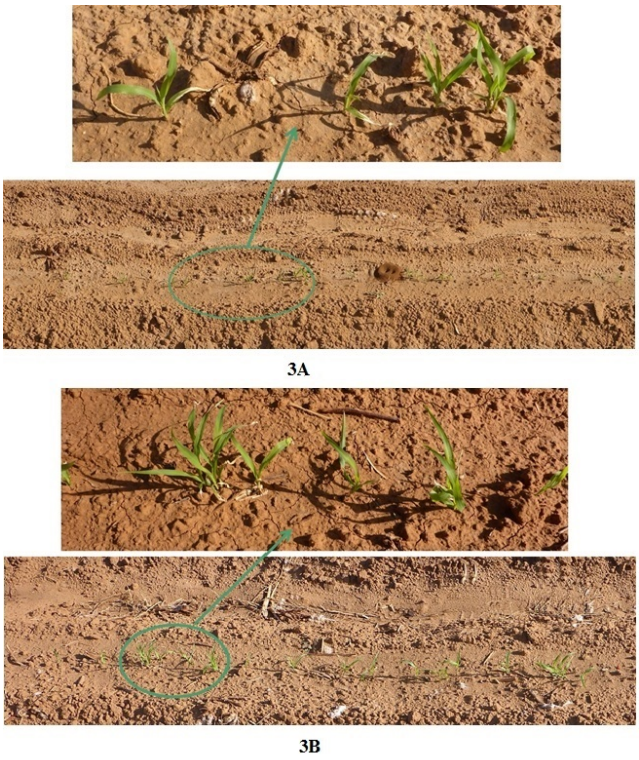

Figure 3. *Comparison of field germination rates between sweet sorghum PI 653617 and PI 152751. Seeds from sweet sorghum PI 653617 28 days after field planting with a moderately high emergence rate (68%). 3A) Number of seeds emerged is shown in the lower panel and a closer image of some seedlings is shown in the upper panel. 3B) Seeds from sweet sorghum PI 152751 28 days after field planting with a high emergence rate (89%). Number of seeds emerged is shown in the lower panel and a closer image of some seedlings is shown in the upper panel (B).*

Consistency and discrepancy in germination rates between lab and field conditions: In general, the lab germination rate can reflect or predict the field germination rate. The results from this study were consistent with the results from an earlier report on sorghum hybrids. But some discrepancies between the field and lab tests were observed. For example, the lab germination rates (84%, 90%, 96%, and 94%) of four checks (Rio, BTx623, GT26056, and PI 610727) were consistent with their field germination rates (80%, 80%, 91%, and 94%), respectively (Table 4).

Thus, the lab germination rates predict well the field germination rates for these four checks. We also observed that the germination rate for some accessions was high in the lab but low in the field. For example, the lab germination rate for PI 247745 was 91%, but its field germination rate was only 48%. The field conditions are more variable (e.g. lower night-time temperature in the field) than the controlled lab conditions. Emergence of the seedlings from soil in the field can be adversely affected by soil type and level of moisture, whereas moistened germination papers in the lab offer no resistance to emergence. Some accessions may be cold tolerant to the lab conditions (12°C) but less tolerant to the field conditions where the temperature can dip much lower than 12°C. This may explain the difference in cold tolerance

between the lab and field tests. Conversely, the lab germination rates for some accessions (PI 146890 and PI 653617) were low (25% and 35%) while their field germination rates were high (92% and 82%). These big differences between lab and field germination rates are difficult to interpret. One possible explanation could be that these accessions may require a lower temperature for breaking seed dormancy. Additionally, since fresh seeds were used, these accessions may have not completely overcome their seed dormancy yet before the start of the germination experiment. The lab experimental temperature of 12°C may not be low enough to break dormancy for some accessions, but the field low temperature can easily break its dormancy resulting in better germination rates. This explanation is supported by the second year lab germination test. After receiving the fresh sweet sorghum seeds from Puerto Rico, the seeds were stored at room temperature for less than two and half months before the beginning of the lab cold germination test. Overall the germination for most accessions from the second year was postponed by about two weeks. The postponed lab germination can only be explained by seed dormancy. To draw a final conclusion, the lab germination test for these accessions needs to be repeated.

Table 3. *Pearson correlation coefficients, probability, and number of observations for lab germination rate, field germination rate, and seedling dry weight.*

Trait		Field^{2nd}	Field^{3rd}	Dry wt.	Lab^{1st}	Lab^{2nd}	Lab^{3rd}	Lab^{4th}
Field count	1st	0.287 <.0001 422	0.266 <.0001 422	0.005 0.9354 321	0.306 <.0001 422	0.217 <.0001 422	0.189 <.0001 422	0.184 0.0001 422
Field count	2nd		0.944 <.0001 425	0.337 <.0001 324	0.393 <.0001 425	0.417 <.0001 425	0.471 <.0001 425	0.462 <.0001 425
Field count	3rd			0.257 <.0001 324	0.408 <.0001 425	0.443 <.0001 425	0.504 <.0001 425	0.503 <.0001 425
Dry weight					0.119 0.032 324	0.104 0.0617 324	0.115 0.039 324	0.109 0.0499 324
Lab count	1st					0.913 <.0001 430	0.842 <.0001 430	0.829 <.0001 430
Lab count	2nd						0.934 <.0001 430	0.929 <.0001 430
Lab count	3rd							0.992 <.0001 430

Table 4. *Difference in seed germination rates between field and lab from some selected accessions.*

Classified type	PI or cultivar name	Field germination (%)	Lab germination (%)
Field rate high Lab rate low	PI 302199	78	28
	PI 653617	82	35
	PI 146890	92	25
Lab rate high Field rate low	PI 247745	47.5	91
	PI 154800	58	93
	PI 154750	60	91
Field and lab rate similar	PI 173112	78	65
	PI 195754	80	70
	PI 152751	74	92
Field and lab rates for Check (selected controls) very consistent	Rio (sweet sorghum)	80	84
	BTx623 (grain sorghum)	80	90
	GT26056 (check for cold tolerance)	91	96
	PI 610727 (check for cold tolerance)	94	94

Unique germplasm accessions identified: Besides the four checks (Rio, BTx623, GT26056, and PI 610727) which had high germination rates under the field cold conditions, three other accessions (PI 146890, PI 653617, and PI 195754) were identified with high field germination rates of 92%, 82%, and 80%, respectively. These three accessions also had lower emergence indexes (EI) (18.29, 17.63, and 18.52 respectively) than the average EI (19.45). The lower the EI value is, the earlier the seeds germinate. This means that these three accessions not only had high germination rates but also had earlier emergence. Compared with other accessions, the seedlings from these three accessions could establish more robustly than other lines within the same time window. For seedling dry weight, PI 146890 and PI 653617 averaged 0.838 g and 0.826 g, respectively, which is similar to the average (0.72 g). PI 195754, however, was 1.363 g, significantly higher

than the average. Seedling dry weight (obtained from the early spring planting) may be an important indicator for early spring cold tolerance. PI 195754 was originally curated in China (GRIN database). The Chinese sorghum germplasm collection is known to contain accessions with good tolerance to early spring cold temperature [9-11].

Conclusion

Early spring cold tolerance is an important and complex trait. When freshly harvested seeds are to be used for screening early spring cold tolerance, seed dormancy should be considered. If the tested seeds have not completely overcome dormancy, the seed germination time and rate can be significantly postponed and reduced. Significant variability in the early spring cold tolerance exists among sweet sorghum accessions. There are over 2,100 sweet sorghum accessions in the USDA germplasm collection. Although some accessions with good tolerance to early spring cold were identified, we only tested 212 accessions (10%). In order to fully characterize the sweet sorghum collection for early spring cold tolerance, all accessions need to be first tested under lab conditions. Then the superior accessions selected from the lab test will be further evaluated in the field. The best sweet sorghum accessions will be selected and recommended for use by sweet sorghum breeders. At the same time, based on the published information of cold tolerance genetics (such as genetic heredity, QTLs, and existing genetic markers) and multiple harvests, crosses will be made between accessions with contrasting cold tolerance to establish bi-parental mapping populations to map the cold tolerance traits to specific chromosome regions for eventually cloning and identifying genes for early spring cold tolerance.

Conflict of Interest

The authors have declared that no competing interest exists.

Significance Statement

This study discovered the relationship between lab cold germination test and field seedling cold tolerance performance that can be beneficial for sweet sorghum breeders. This study will help sweet sorghum breeders to select parents from the unique accessions to make crosses for developing new cultivars in their breeding programs.

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***Correspondence to:**

Wang ML
USDA-ARS, Plant Genetic Resources Conservation Unit,
Griffin, Georgia 30223, United States
E-mail: mingli.wang@ars.usda.gov