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Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] is well known for its great potential to produce grain, feed, and fuel under stressful conditions such as drought and heat. However, its genetic potential to withstand cold, wet environments at germination has not been extensively exploited due to limited sources of early season cold tolerance. The objective of this study was to select novel, not previously characterized, cold-tolerant sorghum accessions that can germinate in a traditional cold test at 10°C in the laboratory and emerge in the field under a wide range of stressful conditions. Fifty six accessions (38 Kaoliangs and 18 non-Kaoliang of different geographic origin) were screened in the laboratory using a 7-d and a 14-d cold test at 10°C with soil, and they were also evaluated under early planting conditions in the field at two locations in the Midwest. The 7-d cold test was the best predictor of seed emergence in the field ($r=0.50-0.61$), while the standard germination test overestimated field performance. Twelve of the top 15 accessions exhibiting cold tolerance in the 7-d cold test were also ranked within the top 15 under field conditions. Eight novel accessions were identified in this experiment with potential superior alleles for cold tolerance. Breeders could perform preliminary screening of germplasm for cold-tolerant alleles in the laboratory using the 7-day, 10°C cold test.

Disciplines

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Novel germplasm and screening methods for early cold tolerance in sorghum
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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Moench] is well known for its great potential to produce grain, feed, and fuel under stressful conditions such as drought and heat. However, its genetic potential to withstand cold, wet environments at germination has not been extensively exploited due to limited sources of early season cold tolerance. The objective of this study was to select novel, not previously characterized, cold-tolerant sorghum accessions that can germinate in a traditional cold test at 10°C in the laboratory and emerge in the field under a wide range of stressful conditions. Fifty six accessions (38 Kaoliangs and 18 non-Kaoliang of different geographic origin) were screened in the laboratory using a 7-d and a 14-d cold test at 10°C with soil, and they were also evaluated under early planting conditions in the field at two locations in the Midwest. The 7-d cold test was the best predictor of seed emergence in the field ($r=0.50-0.61$), while the standard germination test overestimated field performance. Twelve of the top 15 accessions exhibiting cold tolerance in the 7-d cold test were also ranked within the top 15 under field conditions. Eight novel accessions were identified in this experiment with potential superior alleles for cold tolerance. Breeders could perform preliminary screening of germplasm for cold-tolerant alleles in the laboratory using the 7-day, 10°C cold test.

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Sorghum [*Sorghum bicolor* (L.) Moench] can be cultivated in many countries and regions in the world, but it is best adapted to tropical and subtropical areas due to its African origin. Low soil temperature during early season is one of the major limitations for sorghum production in temperate climates. Tolerance to low temperatures at germination is not only desirable to ensure a good stand but also facilitates early planting, which could translate into longer growing seasons and, therefore, higher grain and biomass yields (Dalton, 1967). Tolerance to cold temperatures at germination is also essential to expand sorghum cultivation to more extreme latitudes and is required for no-till or minimum-tillage practices in temperate regions.

Breeding for cold tolerance at germination has a good prospect considering the high heritability of the trait (Cisse and Ejeta, 2003) and that general combining ability is more important than specific combining ability (Yu and Tuinstra, 2001). QTL (quantitative trait loci) have been identified and could be incorporated into a marker-assisted breeding program after validation (Knoll *et al.*, 2008). However, a limited number of sorghum lines have been classified as cold tolerant at germination, most of them being of Russian or Chinese origin (particularly the working group Nervosum-Kaoliang) (Tiryaki and Andrews, 2001; Maulana and Tesso, 2013; Franks *et al.*, 2006). The plant introduction (PI) 610727, a Chinese line, was the best performing accession out of ten evaluated by Franks *et al.* (2006). This accession was later used as the donor of cold tolerant alleles for a second QTL study in which eight genomic regions were associated with superior emergence performance under cold temperatures (Burow *et al.*, 2011).

The limited number of sources of cold tolerant alleles is not the only factor hindering the improvement of the trait. An efficient and reproducible screening method for large sets of germplasm is required to reliably select accessions and develop new inbred lines with better germination under cold conditions.

The field screening for cold tolerance requires large plantings to evaluate all possible entries at a perfect timing. If climatic conditions in the spring are too wet and planting is delayed, the window of opportunity to evaluate cold tolerance at the seedling stage could be lost. One of the most important causes of physiological damage to seed at planting is imbibitional chilling injury (Cal and Obendorf, 1972; Tully et al., 1980). This damage occurs when dry seeds imbibe cold water in the field (Phillips and Youngman, 1971; Cal and Obendorf, 1972). If planting is delayed due to a wet spring, soil temperatures could be too warm for this phenomenon to occur and the opportunity to evaluate cold tolerance at germination would be lost.

A preliminary screening of germplasm for cold tolerance could be conducted in a controlled environment, similar to those used to evaluate seed vigor. Seed vigor is defined as the strength and speed of germination; or the rapid and uniform seedling emergence under a wide range of field conditions (AOSA, 2009). Seed physiologists have developed laboratory tests to assess seed vigor. One of them is the cold test that imitates the emergence of seedlings in poor field conditions. This stress test relies on exposing seeds to cold temperature (10°C), excess moisture, and non-sterilized soil to introduce soil-borne pathogens, such as *Phythium* spp. (Burriss and Navratil, 1979). It is widely used in the corn seed industry to determine the fitness of a corn seed lot for emergence and survival under stressful conditions in the field (AOSA, 2009). It has also been used for early selection of inbred lines with improved seed vigor in a Midwest corn breeding program (Goggi et al., 2007; Goggi et al., 2008), and to screen subtropical hybrids (Shah et al., 2002). This same test could be used for preliminary screening of sorghum plant introductions for cold tolerance.

In the past, other researchers have attempted to screen sorghum plant introductions in the laboratory (Anda and Pinter, 1994; Abdullahi and Vanderlip, 1972; Yu et al., 2004; Franks et al.,

2006). Differentiating between cold tolerant and susceptible lines is not the only objective in laboratory tests but also to predict field performance. In some reports, the percentage of seedling emergence and rate of shoot development under laboratory conditions correlated well with field emergence (Yu *et al.*, 2004). However, the temperatures used in these experiments (Yu *et al.*, 2004; Maulana and Tesso, 2013; Harris *et al.* 1987) were usually higher (12°C and 15°C) and, at those temperatures, the seedling has a growth competitive advantage over the soil pathogens and could escape infection. Therefore, it is important to use lower temperatures (10°C) and soil in the cold test to slow seedling growth and promote seed-pathogen interaction (Woltz *et al.*, 1998).

The specific objectives of this study were to (i) use the traditional cold test (10°C, excess moisture, and soil) to screen sorghum plant introductions in the laboratory; and to establish the relationship of these values with emergence under stressful field conditions of cold, wet soils; (ii) evaluate a new set of sorghum accessions with potential value as sources of tolerance to low soil temperatures at germination; and (iii) compare this new set of accessions with previously identified cold tolerant sorghum materials.

MATERIALS AND METHODS

Genetic materials and seed production

Sorghum accessions included in this study were obtained from the National Genetic Resources Program and selected based on geographic origin information obtained at the Germplasm Resources Information Network (GRIN). Seeds were multiplied at the ISU Agricultural Engineering and Agronomy Research Farm near Ames, Iowa, in 2012. A total of 56 sorghum accessions were evaluated in this study, including 38 Kaoliangs of different geographic origin (26 from China, five from Korea and seven of unknown origin) and 18 non-Kaoliang accessions

(four from China, 12 from Russian Federation or Former Soviet Union and two elite inbred lines as controls) (Supplementary Table 1). Nine Chinese accessions were previously evaluated for cold tolerance under field and laboratory conditions (Franks et al. 2006), and eight accessions were also previously evaluated but only under field conditions (Maulana and Tesso, 2013) (Supplementary Table 1). These materials of known performance were included as additional checks and as a reference for the novel germplasm tested in this study. The two well characterized inbred lines used as controls were included to represent elite germplasm not adapted to cold conditions.

The seeds were harvested in the panicle at physiological maturity or the point of maximum seed vigor (Bewley and Black, 1994; Bewley et al., 2013) and air-dried in small-scale experimental dryers (Navratil and Burris, 1982) with forced air at temperatures below 35°C until the seed was at 120 g H₂O kg⁻¹ fw. A total of six panicles per accession were harvested and separated in two groups to be used as the two biological replications for each test. Panicles were threshed and seeds stored for six months in a cold room at 10°C and 50% relative humidity before screening in the laboratory. Seeds were sorted by hand before testing to remove broken or damaged seeds and inert matter. Only healthy, intact seed were used in the tests.

Seed viability and cold tolerance screening tests

Seed viability

The standard germination test was used to evaluate seed viability and was conducted according to the Association of Official Seed Analysts Rules for Testing Seeds (AOSA, 2010). AOSA (2013) defines seed germination as the development of a root and shoot sufficient to constitute a normal seedling under ideal growing conditions. Normal seedlings are “seedlings possessing the essential structures that are indicative of their ability to produce plants under favorable

conditions” (AOSA, 2013). Each replication consisted of 100 seeds per entry, and two replications per entry were planted over time. Entries were randomized within trays and germination carts, and four entries were planted in each tray. Seeds were placed on top of two sheets of crepe cellulose paper media (Kimberly Clark Corp., Neenah, WI) moistened with 800 mL of tap water on fiberglass trays (45 cm x 66cm x 2.54 cm), and lightly pressed into the media to create good seed-media contact. The trays were placed inside germination carts after planting, and the carts were placed inside an alternate 20-30°C (16-h at 20°C and 8 h at 30°C) walk-in germination chamber with alternating 8-h of light and 16-h of darkness d⁻¹. First seedling count was done at 4 d and the final evaluation was made at 10 d according to AOSA Rules for Testing Seeds (2010).

Cold tolerance

A 7-d and 14-d cold tests (AOSA, 2009) were used to evaluate seedling cold tolerance. The protocols used in these two tests were similar, except for the length of time seeds were exposed to cold temperature (7-d or 14-d, respectively). Entries were randomized within trays and germination carts, and four entries were planted in each tray. Fifty seed replicates per entry were planted on one sheet of Kimpak (Kimberly Clark Corp., Neenah, WI) watered with 1100 mL of water pre-chilled 24-h at 10°C on fiberglass trays (45 cm x 66cm x 2.54 cm). One cm of dry 80% sand:20% soil mixture was used to cover the seed and media. The trays were placed inside enclosed germination carts after planting, and the carts were placed inside a dark walk-in chamber at constant 10°C for either 7-d or 14-d, respectively; and then moved to an alternate 20-30°C (16-h at 20°C and 8-h at 30°C) walk-in germination chamber with alternating 8-h of light and 16-h of darkness d⁻¹. Normal seedlings (AOSA, 2010) were evaluated and recorded at 4- and 10-d after placing in the alternate 20-30°C walk-in germination chamber.

Field Emergence

Field plots were planted using a three point mounted experimental cone planter and seeded at 140,000 seeds/hectare in two locations, Agricultural Engineering and Agronomy Research Farm near Ames, Iowa (Lat. 42° 1' 20.31" N, Long. 93° 46' 36.42" W, and Webster soil type), and Neely-Kinyon Farm in Greenfield, Iowa (Lat. 41° 16' 23.25" N, Long. 94° 26' 36.72" W, and Sharpsburg soil type). The 56 sorghum accessions were planted in single rows using a randomized complete block design with two replications per location.

Both locations were planted at the end of April, approximately three weeks earlier than normal planting time for sorghum in the Midwest. A summary of the field emergence conditions is in Table 1. Number of emerged seedlings were counted every other day until no changes in seedling emergence were observed (day 22 after planting). Emergence index was calculated according to Smith and Millet (1964) as $\sum(E_j \times D_j)/E$, where E_j indicated emergence on day j , D_j indicated days after planting and E was final emergence. Finally, a sample of 10 seedlings per entry and rep were harvested 30 days after planting, fresh weight was recorded and samples were subsequently dried to constant weight to determine final dry weight. These parameters (emergence index and seedling dry weight) are indicators of field seedling vigor.

Statistical Analysis

Analysis of variance was performed using SAS 9.3 (SAS Institute Inc., Cary, NC). Proc mixed was used to fit phenotypic traits (% emergence, emergence index, seedling fresh weight and dry weight) to a linear model and estimate variance components. The linear model included the random effects location (Loc), replications nested within location (Rep (Loc)) and genotype by location interaction (Genotype*Loc). Genotype was considered a fixed effect. For laboratory

tests (14-d cold, 7-d cold and germination tests), replication (Rep) was considered random and genotype a fixed effect.

Pearson correlation coefficients between all tests and between 4d and final count for laboratory tests were also estimated.

Multiple comparisons for % emergence in each field and laboratory tests were performed using Tukey-Kramer test and the commands proc PLM and Adjust=Tukey.

RESULTS

Laboratory screening tests

The standard germination test confirmed that viability of all accessions was very high, with an average germination of 92.8% (Table 2). As expected, the percentage germination in the 14-d cold test was, on average, lower (77.5%) than in the 7-d cold test (85.9%) (Table 2). Some accessions had 100% germination in the cold test after 10 d at 20/30°C but, in general, all of them had manifested their potential for cold tolerance after 4 d at 20/30°C. The average germination percentage difference between data collected after 4 and 10 d at 20/30°C was only 3.04% for the 14-d cold test and 2.88% for the 7-d cold test.

The analysis of variance indicated that the effect of genotypes was significant in all laboratory tests (germination, 14-d cold and 7-d cold) while the replication effect was not (Table 3). The identification of superior accessions was consistent between laboratory tests since the same eleven genotypes were within the 15 best performing accessions in both tests (Supplementary table 2). PI 408822, 76407 and 68003 were the best performing materials.

Field emergence

Normal planting dates for sorghum in Iowa are at the end of May, when soil temperatures stabilize around 15-20°C for several days. Early planting dates for this study were established at the end of April. When the experiment was initiated, soil temperatures were approximately 12°C but an unusual cold front affected the Midwest during the first week of May. Temperatures decreased and snow fell on May 3, 2013 (approximately 96 hours after planting). Therefore, field conditions over the 30-d period were very similar to the lab screening tests with soil temperatures ranging from below zero to 23.3°C and average soil temperatures of 11.8°C and 14.9°C for Greenfield and Ames, respectively (Table 1).

Emergence was lower in Greenfield than in Ames due to lower temperatures over the 30-d evaluation period (Table 2). Genotypes differed for all variables evaluated: % emergence, emergence index, seedling fresh, and dry weight. Emergence varied from 0% to 85.71% averaged over locations, with emergence index being very similar in Ames and Greenfield (Table 2). The analysis of variance indicated that genotype was a significant effect for % emergence and seedling dry weight while genotype by location interaction was significant ($p < 0.5$) only for % emergence. The correlation analysis performed between % emergence and all other parameters averaged over locations demonstrated a positive and significant correlation with fresh weight ($r = 0.18$, $p < 0.01$) and dry weight ($r = 0.62$, $p < 0.0001$).

Comparison between laboratory tests and field emergence

As expected, germination was lower in the 14-d than in the 7-d cold test. However, the general performance was consistent between both tests ($r = 0.72$, $p < 0.0001$) (Table 5).

The correlation between lab cold tests and field performance was also positive and highly significant in all cases (for individual locations or averaged over locations) (Table 5). Both lab

screening tests (7-d and 14-d cold) were effective in differentiating the best and poorest performing materials but the 7-d test was a better predictor of field performance under cold stress. Out of the top 15 accessions exhibiting cold tolerance in the 7-d cold test, 12 of them also ranked within the top 15 with superior cold tolerance under field conditions (Ames and Greenfield individually or averaged over locations) (Supplementary Table 2). These accessions included eight Kaoliangs from China (PI 76407, 408822, 90769, 568015, 567929, 90271, 563923 and 408824), one Kaoliang from Korea (PI 88000), two accessions from Russian Federation (PI 619672, 550608), and one non-Kaoliang accessions from China (PI 542764). The better prediction capacity of the 7-d cold test was also demonstrated by a higher correlation coefficient with field tests, both as individual locations or average over environments ($r=0.58$, $r=0.50$, and $r=0.61$ vs $r=0.50$, $r=0.36$, and $r=0.49$) (Table 5).

In general, seed viability as determined by the standard germination test was not significantly correlated with emergence under cold conditions, in spite of the good seed quality of all materials (Table 2 and 5).

Performance of sorghum accessions

The performance of accessions under cold stress conditions showed large variation with the lowest emergence (average over reps) being 34% (PI 619678) for the 14-d cold test, 55% (PI 550600) for the 7-d cold test and 7.9% (PI 560824) for field emergence. The best germination was 98% (PI 76407, 408822) for the 7-d cold test, 96% (PI 68003, 408822) for the 14-d cold test and 60.4% (PI 408816) for field emergence averaged over locations (Supplementary Table 2).

In general, sorghum accessions from the former Soviet Union had a poor performance under cold conditions in the field, similar to the inbred lines used as controls (Fig. 1), but specific

Russian accessions with very good performance (PI 619672 and 550608) were identified. Accessions of other origin, including Kaoliangs and non-Kaoliangs, had higher and similar emergence percentage over time (Fig. 1).

Several sorghum materials were clearly identified as good potential sources of alleles for early cold tolerance. Considering the best performing 15 genotypes on the 7-d cold test and field experiments, eight previously characterized accessions (PI 76407, 88000, 90769, 542764, 567929, 567974, 567939 and 568015) were confirmed in this study as cold tolerant (Supplementary Table 1 and 2). Using similar criteria, eight novel cold tolerant accessions were identified in this study: PI 76408, 90271, 408822, 408824, 408816, 550608, 563923 and 619672 (Supplementary table 2).

DISCUSSION

Several materials with cold tolerance at germination were identified in this study. Eight of them had been previously reported as cold tolerant (Franks et al., 2006; Maulana and Tesso, 2013), confirming the efficacy of our field evaluations and prediction capacity of our laboratory tests as screening methods. The predictor capacity of the 7-d cold test was demonstrated by its high correlation coefficient with field tests, which is consistent with results obtained for maize inbred lines grown in the Midwest (Martin et al., 1988).

No relation was found between the standard germination test and field emergence under cold conditions. These results were expected, as the standard germination test is performed under ideal growing conditions (AOSA, 2010), but field conditions were less than ideal. Similar results have been reported in other species such as soybean [*Glycine max* (L.) Merr.] (TeKrony and Egli, 1977; Johnson and Wax, 1978; Yaklich and Kulik, 1979), maize (*Zea mays* L.) (Woltz and TeKrony, 2001; DeVries et al., 2007), and Italian ryegrass (*Lolium multiflorum* Lam.) (Naylor,

1981). Seeds used in this experiment were harvested at physiological maturity, also known as the point of maximum seed vigor (Copeland and McDonald, 2009), and dried at ambient temperature with forced air to preserve this good seed vigor.

In spite of a significant genotype by location interaction effect, nine of the top 15 accessions with cold tolerance under field conditions were consistently superior in both locations (PI 408816, 567974, 90769, 567939, 90271, 88000, 567929, 76408 and 408824). Five of those accessions (PI 90769, 90271, 88000, 567929 and 408824) were also identified among the top in the 7-d cold test. PI 76408, 90271, 408822, 408824, 408816, 550608, 563923 and 619672 are new accessions identified in this study with potential as donors of cold tolerance alleles and with superior performance than the broadly used PI 610727.

The germination and survival of sorghum seed under cold stress have been previously evaluated under controlled environment conditions (Anda and Pinter, 1994; Abdullahi and Vanderlip, 1972; Yu et al., 2004; Franks et al., 2006). Soil based and plate based tests have been used to evaluate commercial hybrids or diverse lines with variable rates of success but the temperatures used were higher than those in the present study. Twelve and 15°C were the most common temperatures implemented in laboratory cold tests on the premise that the basal temperature for germination in sorghum is 10°C. However, that generalization does not apply to all sorghum germplasm sources as reported decades ago (Thomas and Miller, 1979; Miller 1982). Therefore, we evaluated the potential value of the cold and extended cold test, widely used in the maize seed industry, to determine vigor and identify sorghum cold tolerant germplasm.

This is the first study evaluating sorghum emergence and vigor at 10°C for two duration periods (7-d and 14-d). Our results indicate that these tests were not only successful in

identifying germplasm with potentially useful alleles for cold tolerance but also in predicting field performance under low soil temperature conditions. Other authors have reported correlation values of 0.5 to 0.7 between the cold test and field emergence in other crops; e.g. soybean (Egli and Tekrony, 1995; Egli and Burris, 1971; Tekrony and Egli, 1977) and corn (Burris and Navratil, 1979; Martin et al., 1988; Goggi et al., 2007). Similar correlations ($r=0.5-0.7$) were found between the cold test and field emergence of frost-damaged corn seed (DeVries et al., 2007) and corn seed treated with an insecticide (Goggi et al., 2009). Our correlation results are in agreement with reports for other species and indicate that the cold test was capable of estimating cold tolerance of sorghum accessions planted at two locations in a very cold Spring ($r=0.5-0.61$). In both locations, soil temperature at planting was 1 to 2°C lower than the 10-year average, and remained 2 to 4°C below the 10-year average for 10 days after planting (data not shown). Based on these correlation values, and on the fact that previously known cold tolerant materials had a good performance in our trials, and that the non-tolerant inbred lines (controls) performed poorly, we proposed that the cold test be used for laboratory screening of cold tolerance in sorghum.

The need to develop more stringent germination and vigor tests in sorghum is not only predicated on the knowledge of variable basal germination temperatures that should be exploited but also on two additional important considerations. Testing seed vigor under higher temperatures may reduce the detrimental effects of cold water imbibition by dry seed which causes imbibitional chilling injury (Cal and Obendorf, 1972; Zheng, 1991) and could favor the seedling over the soil pathogens that normally infect seeds in the soil (e.g. *Pythium* spp.) (Woltz et al., 1998). Therefore, from the breeding point of view, it is important to select donor lines of cold tolerant alleles under lower, harsher temperatures in the presence of non-sterilized soil. The

expansion of the sorghum growing areas to more northern latitudes and the yield maximization through longer growing seasons will require early planting under extremely cold conditions in the soil. Other factors, such as reduced tillage or no-tillage and climate change with unpredictable patterns of temperatures and rain in the spring, will make planting conditions even more unfavorable for sorghum. Normal sorghum planting in the Midwest (a non-traditional sorghum growing area) is at the end of May or early June, when average soil temperatures are above 15°C for several days. Average soil temperatures from April 1 to May 31 over the last 10 years are shown in Fig. 2 for three locations in central-south Iowa. As indicated in Fig. 2, soil temperatures during April fluctuate from 7.1 to 13.0°C. These data confirm the need to develop sorghum germplasm adapted to soil temperatures below 15°C in order to move forward planting dates at least 30 days from normal planting in Midwest. This strategy of using more stringent selection conditions will facilitate the identification of donor accessions and the development of elite cold tolerant germplasm to cope with lower and unpredictable soil temperature patterns.

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Figure Captions

Figure 1. Percentage emergence over time for sorghum accessions grouped by geographic origin. The number of accessions for each origin is indicated in the legend.

Figure 2. Ten-year average soil temperatures for three locations in Iowa: Ames, Lewis and Crawfordsville from April 1 to May 31. These locations were selected as representative soil temperature conditions of central-south Iowa.

Tables

Table 1: Summary of field emergence conditions for the 30 day growing period.

Location	Planting date	Harvest date	Air temperature (°C)		Soil temperature (°C) [‡]		Precipitation (mm)	
			Max. [†]	Min. [†]	Average	Range	Total	Average daily
Greenfield	April 29, 2013	May 30, 2013	20.7	10.5	11.8	2.2-17.7	143	5.5
Ames	April 30, 2013	May 31, 2013	20.4	10.1	14.9	-0.5-23.3	224	7.1

[†]Daily mean minimum and maximum temperature for the 30 day growing period.

[‡]Measured at 10cm by an automatic weather station equipped with a Campbell CS655 reflectometer.

Table 2: Summary of emergence and vigor parameters for laboratory and field tests.

Test	Emerg. (%)			Emerg. index (d)			Fresh weight (g)			Dry weight (g)		
	Mean	St. Dev.	Range	Mean	St. Dev.	Range	Mean	St. Dev.	Range	Mean	St. Dev.	Range
(4d) 14-d cold [†]	74.4	17.4	8.0-100.0	-	-	-	-	-	-	-	-	-
14-d cold	77.5	16.1	24.0-100.0	-	-	-	-	-	-	-	-	-
(4d) 7-d cold [†]	82.6	14.2	36.6-100.0	-	-	-	-	-	-	-	-	-
7-d cold	85.9	11.7	46.0-100.0	-	-	-	-	-	-	-	-	-
(4d) Germ ^{†‡}	35.4	23.6	1.0-94.0	-	-	-	-	-	-	-	-	-
Germ [‡]	92.8	6.7	65.0-100.0	-	-	-	-	-	-	-	-	-
Ames	51.2	18.9	10.0-85.7	17.2	1.2	10.0-20.2	3.2	1.1	0.99-6.39	0.49	0.17	0.20-1.08
Greenfield	19.0	11.3	0.0-51.4	18.0	0.9	16.0-20.7	3.1	1.1	1.14-7.71	0.29	0.15	0.08-1.50

[†] First counting at 4 days after planting for Standard Germination test and 4 days after placing seeds at 20-30°C for cold test.

[‡] Standard germination test

Table 3: Analysis of variance for laboratory tests using final data after 10 days at 20/30°C.

Variance component	14-d cold		7-d cold		Germ [†]	
	F ratio	Prob (F)	F ratio	Prob (F)	F ratio	Prob (F)
Rep	1.79	0.186	0.69	0.408	0.05	0.809
Genotype	4.88	<0.0001	2.37	<0.001	3.90	<0.0001

[†] Standard germination test

Table 4: Analysis of variance for field emergence tests including final emergence count (%), emergence index (days), fresh weight (g) and dry weight (g).

Variance component	Emerg. (%)		Emerg. Index (d)		Fresh weight (g)		Dry weight (g)	
	F ratio	Prob (F)	F ratio	Prob (F)	F ratio	Prob (F)	F ratio	Prob (F)
Loc	233.0	<0.001	5.04	0.206	0.03	0.882	22.06	0.051
Rep (Loc)	1.66	0.193	1.22	0.300	8.04	<0.001	4.77	0.010
Genotype	3.30	<0.0001	1.43	0.094	1.00	0.494	2.14	0.002
Genotype*Loc	1.67	0.011	0.81	0.802	1.25	0.159	0.77	0.859

Table 5: Pearson correlation coefficients between all tests.

	14-d cold	7-d cold	Germ [†]	Ames	Greenfield	Both loc.
14-d cold	-					
7-d cold	0.72***	-				
Germ	0.05 ^{NS}	0.27*	-			
Ames	0.50***	0.58***	0.15 ^{NS}	-		
Greenfield	0.36**	0.50***	0.03 ^{NS}	0.60***	-	
Both loc.	0.49***	0.61***	0.12 ^{NS}	0.94***	0.82***	-

***Significant at $P < 0.001$ level of probability.

** Significant at $P < 0.01$ level of probability.

* Significant at $P < 0.05$ level of probability.

NS= nonsignificant at $P < 0.05$ level of probability.

[†] Standard germination test