academicJournals

Vol. 6(6), pp. 31-38, July 2015
DOI 10.5897/JCO15. 0139
Article Number: 705C60854401
ISSN 2141-6591

http://www.academicjournals.org/JCO

Journal of Cereals and Oilseeds

Full Length Research Paper

Physiological parameters of seed vigour in *ex situ* stored sorghum germplasm

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Received 5 March, 2015; Accepted 20 May, 2015

Viability and vigour are seed quality parameters that affect the seed chain. Seed vigour is a measure of accumulated damage in seed as viability declines from physiological maturity. This study aimed at determining the seed vigour of various sorghum genotypes stored *ex situ* for 10 years using the Heat Shock Stress Test (HSST), Cold Test (CT), Standard Germination Test (SGT) and field germination. The samples from the regeneration trial were kept in *ex situ* storage at 0-4°C and 40% relative humidity from 2003 to 2014. The experiments were arranged in a completely randomised design with four replication and differences in radicle length, shoot length for the 65 genotypes evaluated at 5% level of significance. An unpaired T- test was used to compare the accuracy of the quality methods in predicting field emergence. All the evaluated genotypes responded differentially to all the vigour test methods. There were significant differences (P<0.01) in the performance of genotypes with respect to radical and shoot length in response to the CT, HSST and the SGT. The results of CT and HSST predicted field emergence better than the standard germination test. None of the quality test methods and a strong and significant correlation with field emergence. Genotypes IS 30260 and IS 34637 were vigourous as they had the highest shoot length of 43.75 and 42.38 mm and IS 30063 had 45.52 mm in root length after 48 h of incubation. Overall, this study showed that field emergence of sorghum cannot be accurately predicted from a standard germination, HSST and CT.

Key words: *Sorghum bicolor*, seed quality, cold test, heat shock stress test, germination test.

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) in Africa is among the leading cereal grains and in Zimbabwe it ranks the third most important cereal crop after maize and wheat (FAO, 2002). It is grown mainly in the semi-arid areas of the tropics as a staple food for poor people (ICRISAT, 2004) and also in subtropics (FAO, 2005). In Zimbabwe, it is a crop of smallholder farmers mostly in Natural Regions III, IV and V characterized by seasonal droughts and severe mid-season dry spells during the rainy season because of its good adaptation to marginal environments and good yield of production (Dicko et al., 2005).

Seed quality is one of the attributes that determines yield potential of any crop. High quality seed lots may improve crop yield in two ways: firstly, seedling

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emergence from the seedbed is rapid and uniform, leading to the production of vigorous plants: secondly, percentage seedling emergence is high, so optimum plant population density could be achieved under a wide range of environmental conditions (Ghassemi-Golezani and Mazloomi-Oskooyi, 2008). The standard germination test, which is considered the universal test for seed quality, evaluates the maximum potential of a particular seed lot under an ideal set of conditions (ISTA, 1985; AOSA, 1986). However, conditions in which the seed is found during standard germination test are often in conflict with the conditions in the field, and therefore seed vigour test is necessary (Morab, 2013).

Seed vigor is defined as "seed properties that determines potential for fast and uniform emergence, and development of seedlings under a wide range of field conditions" (AOSA, 2002). In any seed lot especially agricultural species, losses of seed vigour are related to a reduction in the ability of seeds to carry out all the physiological functions that allow them to perform (ISTA, 2009). Generally, low germination speed, high sensibility to stresses of seeds and seedlings during germination process, and plants with slow, low and irregular growth or with less root development, are typical characteristics of seed with low physiological potential. Each biotic or abiotic factor that affect seed vigour during seed's development, subsequently will affect production especially when seeds produced under stress condition (Zakaria, 2009).

Ex situ storage may influence seed viability, seed germinability and seed vigour depending on the time duration and storage conditions. *Ex situ* techniques rely on the storage of dried seeds of germplasm at low temperatures and the most important factors influencing seed vigour are temperature, relative humidity, nature of the seed, and power failure (Brutting et al., 2013). The vigour intensity decrease in stored seed is different among plant species and within plant species, implying that there is a considerable influence of genetic components on phenotypic expression of traits that determine seed quality (Morenomartinez et al., 1994; Al-Yahya, 1995, 2001). Power failure is the major limitation facing *ex-situ* collections maintained at low temperatures (Borokini, 2013). This has caused losses of conserved germplasm by reducing in the ability of seeds to carry out all the physiological functions that allow them to perform (Guberac et al., 2003; Borokini, 2013).

Vigour testing is important because it often gives a better prediction of field performance and is a more sensitive indicator of seed quality than the standard germination test (Younis et al., 1990). Various tests have been presented to identify seed vigour of different plants like accelerated aging test for peas (Hampton and TeKrony, 1995), Seed Conductivity test for safflower (Khavari et al., 2009), Cold Test (CT) for maize (Noli et al., 2008), Deterioration Test (DT) and CT for sugar beet (Hampton and TeKrony, 1995), some of which are now accepted internationally. However, there is no universally accepted test for measuring seed vigour in all the plant species.

Some methods such as the CT and the Heat Shock Stress Test (HSST) are simple, and easy to conduct in laboratories with limited infrastructure. CT provides data on seed viability even in very adverse germination conditions, which gives better insight into seed behavior during field emergence (Milošević and Malešević, 2004). Furthermore, a brief high-temperature stress (heat shock) is used to create a greater strain on less vigourous seeds and reduces their performance more than it does for more vigourous seeds. Therefore this study sought to determine the seed vigour of sorghum genotypes in *ex-situ* medium-term storage and compare sorghum seed vigour between HSST and CT in predicting actual field emergence.

MATERIALS AND METHODS

Germplasm

The 60 samples of sorghum germplasm for this study were harvested from an off season regeneration trial carried out at Muzarabani in 2003. An additional five genotypes were harvested in 2013 and served as a control. Regeneration of the germplasm was necessitated mainly for increasing the original sample of the accessions for conservation, maintenance, distribution and seed multiplication of accessions with low seed stocks and/or with viabilities below a critical level (FAO/IPGRI, 1994). The sorghum germplasm has been kept in an *ex situ* storage at 0 to 4°C and 40% relative humidity from 2003 up to 2014.

Seed quality analyses

Standard germination test

A standard germination test using the between the paper method for 65 genotypes was conducted according to the method specified by the Association of Official Seed Analysts (AOSA, 1983). The only deviation from these procedures was that four replications of fifty seeds were used for each genotype, instead of the hundred seeds per replication recommended by AOSA due to limitations in seed samples. Fifty seeds from randomly selected hundred samples were sown on two sheets of moistened germination towels. The rolls were then be wrapped and placed in an upright position inside a plastic container and kept in an incubator at a temperature ranging between 25 to 30°C for 48 h after which the final germination counts of normal seedlings were done.

Seed vigour tests

Sixty samples plus five control samples randomly selected from one hundred samples with average germination rate of 90% and above were utilized in this study. The sixty five samples were subjected to a CT, HSST and a field emergences test using a completely randomized design with four replications.

Cold test

The CT was conducted using rolled paper without soil (RPTWS) according to the Association of Official Seed Analyst specifications (AOSA, 1983). Fifty seeds from each genotype were sown on a germination paper towel moistened with distilled water to 2.5 times its dry weight. The rolls were then wrapped and placed in upright position inside a plastic container and kept in a cold chamber at ±5°C for five days. The rolls were then transferred to an incubator with temperature ranging between 25 and 30°C for four days (Loeffler et al., 1985). The final germination counts of normal seedlings were done after nine days.

Heat shock stress test

The same sources of genotypes used in the cold test were subjected to a HSST. The seeds of each genotype were initially imbibed with 7 ml of distilled water in a 9 cm Petri dish on germination paper. The imbibition period in the Petri dish was for 24 h at temperatures ranging between 25 and 30°C. After the 24 h imbibition period in distilled water in a Petri dish, the seeds were exposed to 50°C for 2 h according to procedures explained by Van de Venter and Lock (1992). The seeds were then spread on germination toweling moistened with 10 ml of distilled water. The toweling was then folded and rolled into a ragdolls. The ragdolls were then placed in zip-lock plastic bags to retain their moisture, and the seeds were incubated for another 46 h at temperature ranging 25 to 30°C in darkness. The final germination counts of normal seedlings were done after 72 h.

Radicle and coleoptile measurement

Radicle and coleoptile length measurements were done for the Standard germination, CT and HSST tests with seedling randomly selected from each replicate. Seedlings with a coleoptiles length of 3 mm and radicle length of 2 mm were considered normal germinated seedlings (ISTA, 2012). The seedling length was measured using a graduated ruler and average radicle and coleoptile length were calculated by dividing the total radicle and coleoptile length by the number of selected normal seedlings.

Field emergence test

The soils used in the field emergence tests were mixtures of 3:1:1 black clay soil: sand: manure. The soils were placed in a standard plastic planting pot with a diameter of 2000 cm^3 (2 L). The same genotypes which were used in the CT and HSST experienced the field emergence tests evaluation. Four replications of 50 seeds from each treatment were planted in individual 2 L plastic pot and placed inside an even span type structure. Field emergence was assessed 15 days after planting by counting the proportion of emerged seedlings.

Data analyses

Data on proportion of germinated seeds, coleoptiles and radicle length in the standard germination Test, CT and HSST, and field emergence across all the genotypes were subjected to ANOVA using GENSTAT 13^{th} Edition (Payne et al., 2010). Significant means were separated using Fisher's protected least significant difference (LSD) at 5% probability to rank the quality of genotype seed lots. An unpaired T-test was done for CT, HSST and field germination percentage to ascertain whether these methods were significantly different from each other at 5% probability. Correlation coefficients were calculated between the laboratory tests and field emergence across the 65 genotypes.

RESULTS

Seed vigour of sorghum genotypes

Based on the growth characteristics of the sorghum seedlings, the evaluated genotypes responded differentially to all the vigour test methods and the standard germination test. For example genotype IS 30260 had the highest shoot (coleoptiles) length of 43.75 mm in the CT, which was followed by the SGT and HSST with lengths of genotypes IS 34637, 42.38 mm and IS 30086, 20.50 mm respectively. A different set of genotypes also had variable root length responses. Similarly, the CT had the highest mean root length of genotype IS 30063, the HSST with a length of 45.52 mm for IS 30108 and 43.90 mm for SGT in genotype IS 14387. However, control genotypes IS 29751 and IS 29747 which were stored in 2013 had the least germination capacity in the CT and HSST with 74% and 72% respectively.

Comparison of HSST and CT in predicting field emergence

The result of variance analysis indicated there were significant differences (P<0.01) in radicle and shoot (coleoptiles) length for all the genotypes for the three seed quality assessment methods (Table 1).

Based on the actual parameters that determine seed vigour, the HSST had the greatest effect on seedling roots and shoots as it produced the least values across all the genotypes compared to CT and SGT. All the germination results were an overestimate of field emergence. However, CT and HSST germination estimate were similar and better estimates of field emerge compared to the SGT (Table 2).

The HSST test for germination percentage had the least minimum of 32 compared to 50% of CT and field emergence test (Figures 1, 2, 3, 4). Genotypes responded more variably in the HSST with a variance of 95 compared to the CT with a variance of 52. Both these vigour tests were far much less that the field emergence variance of 145.

The unpaired T-test for germination percentage for the CT and HSST indicated that there were no significant differences (P=0.713) between these two vigour testing methods. However, there were highly significant differences (P<0.001) between these vigour testing methods and field emergence. Across the 65 genotypes there was no strong positive correlation between the various vigour tests and field emergence. Cold test and standard germination test has weak significant correlation

Table 1. The analysis of variance for seed quality attributes.

Source of variation	df	CT		SGT		HSST	
		Radicle length (cm)	Shoot length (cm)	Radicle length (cm)	Shoot length (cm)	Radicle length (cm)	Shoot length (cm)
Treatment	64	56.71***	37.38***	12.81**	20.10***	119.63***	24.87***
Error	195						
Total	259						
CV%		5.60	6.20	6.80	7.80	19.40	16.20
LSD		3.19	2.92	3.92	4.03	9.15	3.72
SED		1.62	1.48	1.99	2.04	4.64	1.89

*0.05, **0.01, ***0.001, CT -Cold test, HSST -Heat Shock Stress Test, SGT- Standard Germination Test, CV-Coefficient of Variation, LSD-Least Significant Difference, SED-Standard error of the differences.

Table 2. The mean comparison of sorghum genotype after standard germination test, cold test, heat shock stress test and field emergence test.

Variables	SGT	CT	HSST	FT
Root length (cm)	41.52	40.52	33.86	_
Shoot length (cm)	37.21	34.09	16.49	_
Percent germination	96.10	91.09	91.37	82
Comparison with field test[1]	+14	+9.09	+9.37	0

CT: Cold test, HSST: Heat Shock Stress Test, SGT: Standard Germination Test, [1]The 82% field emergence was used a reference point.

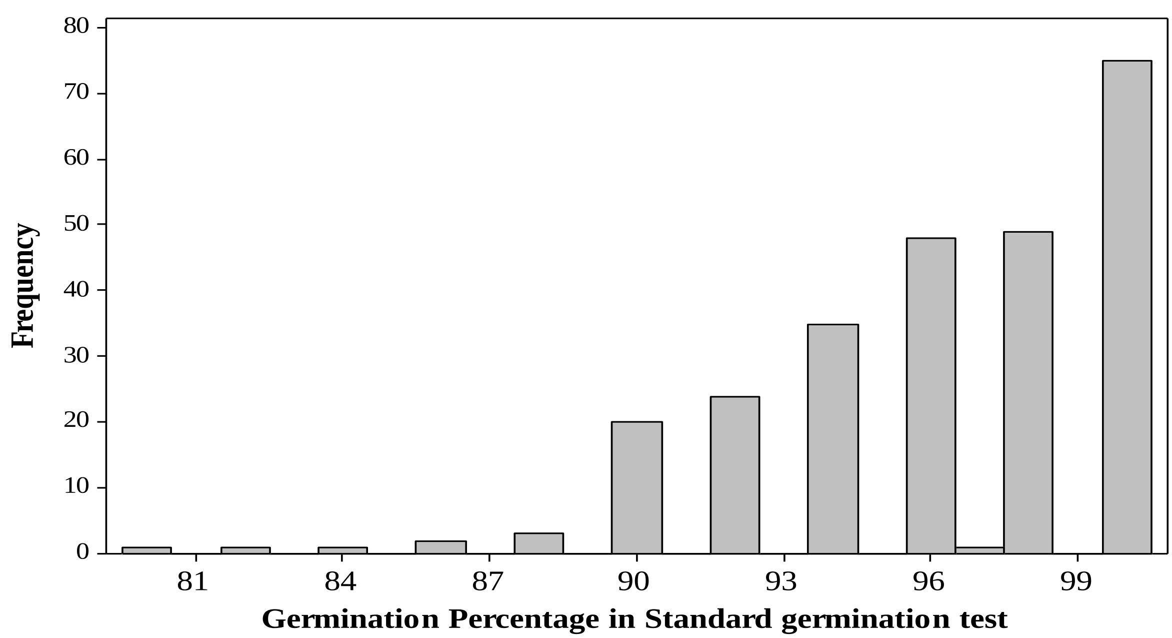

Figure 1. Histogram of germination percentage for the 65 sorghum genotypes evaluated in standard germination test.

whilst in cold test and the field emergence test have the strong significant correlation (Table 3).

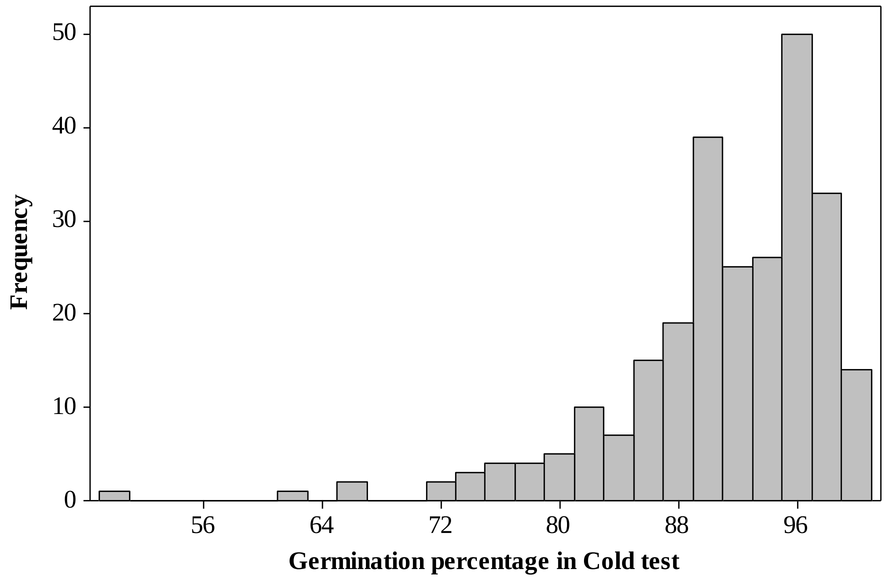

Figure 2. Germination percentage histogram of 65 sorghum genotypes evaluated in a cold test.

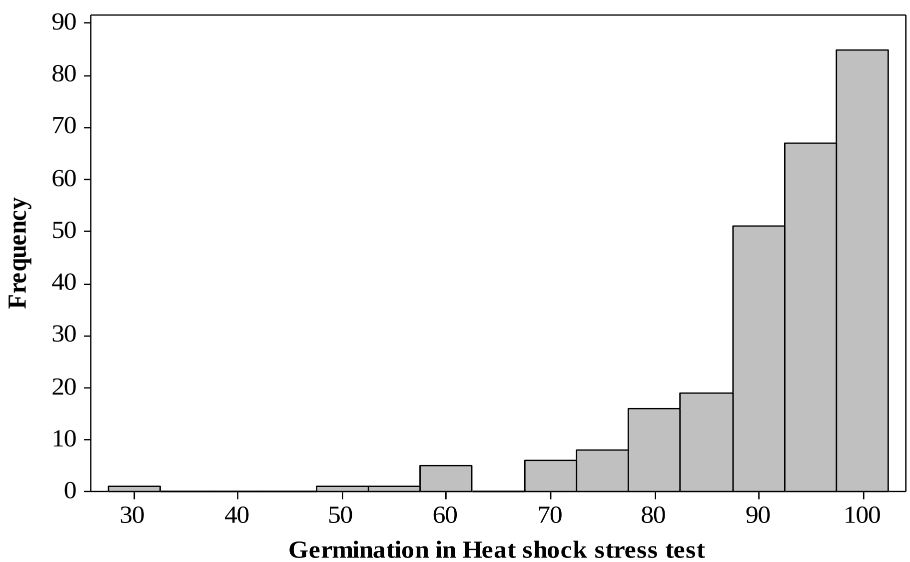

Figure 3. Histogram of germination percentage of 65 sorghum genotypes evaluated in heat shock stress test.

DISCUSSION

Seed vigour of sorghum genotypes

The evaluated genotypes responded differentially to all vigour test methods and the standard germination test because genetically seed perform differently when exposed to different conditions. According to Noli et al. (2008), two seed lots with similar germination percentage, but differing vigour, could show significant variation in stand and yield when planted under various stress conditions. Superiority amongst genotypes in terms of growth was shown by variation in radicle length and shoot (coleoptile) length which was genotype and vigour method dependent. The control genotypes IS 29751 and IS 29747 had the least germination capacityafter being kept for one year in ex situ storage. This could be attributed to the drought period that was

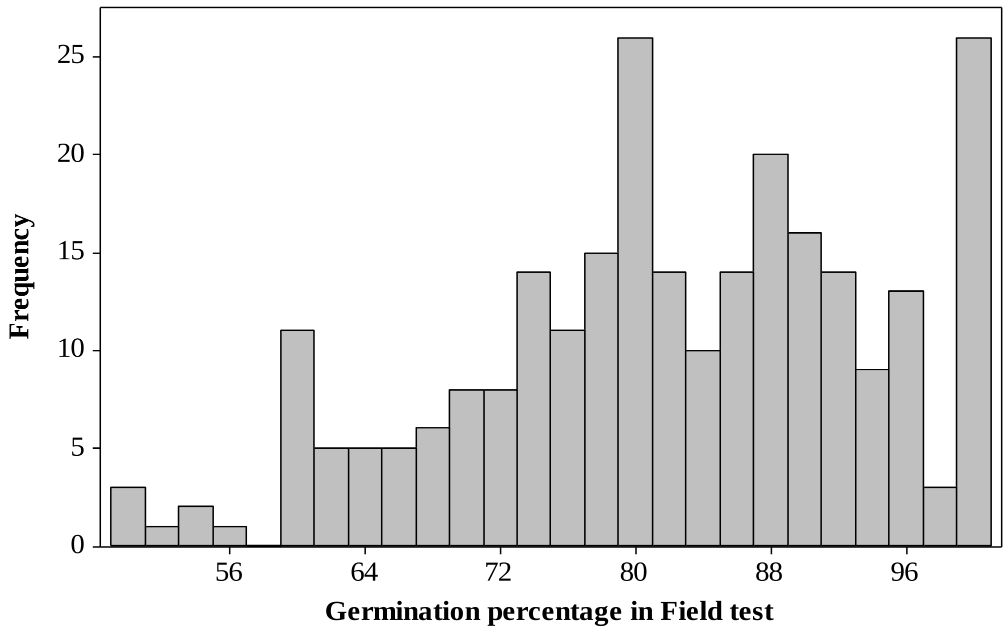

Figure 4. Histogram on germination percentage in field emergence test.

Table 3. Correlation between the SGT, CT, HSST and field emergence test.

Test		1	2	3	4
CT	1	-			
FT	2	0.21***	0.03		
HSST	3	0.02*	0.19**	0.11	-
ST	4	0.25***			

*0.05, **0.01, ***0.001, CT- Cold test, HSST -Heat Shock Stress Test, SGT- Standard Germination Test, Germination percentage.

experienced in the season that prior to harvest. Proper seed maturity is one of the factors that influence the seed longevity (Hay et al., 1997). Furthermore Elias (2006) noted that weather conditions during seed germination such as drought and extreme temperature reduce seed vigour.

Comparison of HSST and CT in predicting field emergence

The variation in germination percentage under standard germination test, CT, HSST and field emergences test showed the variable sensitivity of sorghum genotypes to *ex situ* storage period of 10 years. The significant differences in radicle and shoot length for the three seed quality assessment methods were attributed the genetic constitution of the germplasm which interacted with the environments (stressful and ideal conditions).

Exposing genotypes to high temperature under HSST resulted in some genotypes succumbing thereby having reduced seedling root and shoot (coleoptile) length. Similarly, Brar and Stewart (1994) reported that temperature strongly influenced the establishment of dryland sorghum (*Sorghum bicolor*) and as temperature increased from 15.5 to 37.5°C, the average time to germination decreased.

Kolasinska et al. (2000) observed that common bean with a standard germination above 80% had vigour that varied from 0 to 90%. Similar results were observed in the standard germination test having an average of above 90% with CT and HSST having a range of 30 to 98%. Wongvarodom and Rangsikansong (2007) reported the sweet corn seeds with 79.50 to 91.00% germination of two varieties which had field emergence of 61 to 79%. The lower quality seeds had the field emergence of lower than 60%. Comparable results were obtained from under field emergence test showed a variation from 50 to 98%

due to that seed in the field is under various stress conditions such as variation in temperature.

Standard germination test is unable to predict the field emergences due to high germination percentage obtained under standard germination test. Kulik and Yaklich (1982) and Wang et al. (2004) were unable to predict field emergence using laboratory tests. Egli and TeKrony (1995) found that field emergence was lower than standard germination test results and the predicting precision of germination and vigour tests was lower for field emergence.

The study showed that there was no significant difference between the CT and HSST as these vigour tests can predict field emergence better than standard germination test. This study found no strong correlation between SGT, FT, CT and HSST. Similarly, Tavakoli et al. (2006), found no significant correlation between the standard seed germination and field emergence in alfalfa. Considering the results of this study in sorghum, HSST and CT vigour tests can better predict the seedling emergence in field and germination percentages do not always correlate with field emergence.

Conclusion

A standard germination is not an accurate predictor of field emergence. Vigour tests methods are better than the standard germination test in predicting field emergence. Genotypes IS 30260, IS 34637 and IS 30063 were identified to have more vigour.

RECOMMENDATION AND FUTURE PERSPECTIVES

Other low cost vigour test methods need to be evaluated for their predictability of field performance. High vigour genotypes need to be evaluated for other agronomic traits before they are recommended to farmers.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

The authors extend their gratitude to ICRISAT, Bulawayo for the laboratory and germplasm assistance.

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