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SEED VIGOUR TESTS FOR PREDICTING FIELD EMERGENCE OF SELECTED CROPS IN ZIMBABWE

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ABSTRACT

Seed vigour testing provides valuable information for assessing seed lot quality, under less than optimum or diverse growing conditions, similar to those which occur in the field. A study was done to evaluate the effectiveness of vigour tests in distinguishing seedlots using cold test (CT), heat shock stress test (HSST), accelerated ageing test (AAT) and standard germination test (SGT) to predict field emergence of various crops, with farmer retained seed used as a control for all the crops. Analysis of variance revealed that the twelve genotypes (ZM401, SC403, local maize landrace, Macia, SV4, local sorghum landrace, Okashana, PMV3, local pearlmillet landrace, CBC 2, IT18 and local cowpea landrace) and five vigour test methods showed a significant difference (P<0.05) for germination percentage and days to 50% germination. There were significant interactions between crops × vigour tests and variety × vigour tests, indicating a differential performance of the vigour test in all the crops and varieties evaluated. This suggests that out of the vigour test methods evaluated, no single method can be used in isolation to accurately predict field emergence in the crops and varieties under study.

Key Words: Accelerated ageing, heat shock stress

RÉSUMÉ

Les tests de vigueur des semences fournissent des informations précieuses pour évaluer la qualité des lots de semences, dans des conditions de croissance moins qu'optimales ou diverses, similaires à celles qui se produisent sur le terrain. Une étude a été réalisée pour évaluer l'efficacité des tests de vigueur dans la distinction des lots de semences à l'aide d'un test à froid (CT), d'un test de stress par choc thermique (HSST), d'un test de vieillissement accéléré (AAT) et d'un test de germination standard (SGT) pour prédire l'émergence au champ de diverses cultures, avec l'agriculteur a conservé les semences utilisées comme contrôle pour toutes les cultures. L'analyse de la variance a révélé que les douze génotypes (ZM401, SC403, race locale de maïs local, Macia, SV4, race locale de sorgho, Okashana, PMV3, race locale de filet de perle, CBC 2, IT18 et race locale de niébé) et cinq méthodes de test de vigueur ont montré une importante différence (P <0,05) pour le pourcentage de germination et les jours jusqu'à 50% de germination. Il y avait des interactions significatives entre les cultures × tests de vigueur et les tests variété × vigueur, indiquant une performance différentielle du test de vigueur dans toutes les cultures et variétés évaluées. Cela suggère que parmi les méthodes d'essai de vigueur évaluées, aucune méthode ne peut être utilisée isolément pour prédire avec précision l'émergence au champ dans les cultures et les variétés étudiées.

Mots Clés: Vieillissement accéléré, stress dû au choc thermique

INTRODUCTION

The most critical stages in a plant's life cycle are germination and seedling establishment. Successful crop establishment does not only depend on fast and uniform germination, but also on the ability of the seed itself to germinate, even under low soil moisture (Khazaie *et al.*, 2013). One of the major drawbacks to high yields and production of crops is the lack of synchronised crop establishment, due to drought at crucial times during crop growth period; and also due to poor soil conditions (Eskandari and Kazemi, 2011). In Zimbabwe, like in most African countries, most farmers produce crops in marginal and drought prone areas. In these areas, where drought is prevalent, the moisture required for germination is available only for a short time (Musemwa *et al.*, 2013), and ultimately lowers the germination rate and germination percentage.

Seed vigour testing provides valuable information for assessing seedlot quality under less than optimum or diverse growing conditions, similar to those which occur in the farmers' fields (Marcos-Filho, 2015). In any seedlot, losses of seed vigour are related to reduction in the ability of seeds to carry out all the physiological functions, that allow them to perform well in the field. This process, called physiological ageing, starts before harvest and continues through, processing and storage (Copeland and McDonald, 2001). The associated biochemical changes can occur depending on genetic, production and environmental factors; and the deterioration ultimately results in death of the seed.

Seeds lose vigour before they lose the ability to germinate, which explains why seedlots that have similar high germination values can differ in their physiological ageing. Seed chemical composition, resistance to diseases and hard seededness are genetic factors; and warm temperatures and high humidity are abiotic factors that affect seed vigour. Farmers, seed companies, agro-dealers and seed marketers, information on seed vigour is important for assessing physiological potential, storability and field performance (Fatonah *et al.*, 2017).

A standard germination test does not adequately predict crop field emergence, the latter being an important attribute for farmers as it ultimately determines yield (Kolasinska *et al.*, 2000; Wang *et al.*, 2004; Goggi *et al.,* 2007). Several methods have been recommended for seed vigour testing for different crops (ISTA, 2015). Seed vigour testing methods depend on the crop and variety; and there are no studies on their relative performance and accuracy in predicting field emergence for popular varieties of crops. There is need to identify the best, fast and low cost methods that accurately predict field emergence under local conditions, and for available varieties for informed decision making.

Currently, there are several physical, biochemical and performance tests that are used for vigour tests, but no one method is accurate in predicting field emergence due to variations in seed mineral composition and genetic makeup. Furthermore, there is no study in the Eastern and Southern African region that has been done to evaluate the relative effectiveness of common and easy-to-use vigour test methods. This is important, given the unique crop varieties that are prevalent in the region and the need to predict field performance, especially under non-optimal conditions that characterise smallholder farmer environments. Accordingly, the objectives of the study were to identify the most accurate field emergence predictor and relative performance of different vigour tests for the different crops in Zimbabwe.

MATERIALS AND METHODS

Site description. A field and laboratory experiment was carried out at Lupane State University, Department of Crop and Soil Sciences in Lupane, Zimbabwe. Lupane is located in Matabeleland North Province, which lies in Agroecological Region IV at a latitude of 18° 45' 00" S and longitude 28° 10' 00" E. The area is characterised by low and erratic rainfall, ranging from 450 to 550 mm; and is usually affected by mid-season dry spells. Cooler temperatures ranging from 10 to 28 °C are experienced, especially during the months of April to August. The field emergence test was done during this period on deep Kalahari sands. The laboratory experiment was done in a growth room where temperature was regulated at 25 °C with a relative humidity of 75%.

Experimental design. A laboratory experiment was laid in a 4x4x3x2 completely randomised design with seed vigour tests evaluated being accelerated ageing, heat shock stress test, cold test and the standard germination test (control). Crop types evaluated were maize (ZM401 and SC403), sorghum (Macia and SV4), pearl millet (Okashana and PMV3) and cowpeas (CBC 2 and IT18). These crops were evaluated based on whether commercial certified seed was available from registered agrodealer shops. Selection was on the basis of widespread production in Zimbabwe and other semi-arid areas, as well as availability. For all the crops, seed of landraces sold at the market were used as a controls. These seeds were distinguished basing on characteristics e.g., kernel/seed size, colour and heterogeneity. The laboratory experiment was repeated twice (set 1 and set 2) each time replicated twice.

Laboratory procedures

Standard germination test (SGT). A standard germination test using the on-top-of-paper method was conducted according to the method specified by the Association of Official Seed Analysts (AOSA, 1983). The alteration to these procedures was that two replications of sixty seeds were used for each genotype, instead of the hundred seeds per replication recommended by AOSA (1983); due to limited quantities available. Sixty seeds were randomly selected from a 2 kg packet of each variety using a sampling probe, and sown on one sheet of moistened germination paper in petri dishes. Germination and days to 50% germination counts of normal seedlings showing typical crop characteristic features, such as a normal shoot, root, and first leaves, were performed from the fourth to the eighth day (last count) after sowing and the results expressed as percentages.

Accelerated ageing test (AAT). The accelerated ageing vigour test method was conducted in an accelerated chamber, in two replicates of 60 seeds for each crop and genotype as described by AOSA (1983). The seeds were exposed to 45 °C and 100% relative humidity for 72 hours. Following the treatment, they were tested for germination using the on top-of-paper method (ISTA, 2009) and days taken to 50% germination.

Cold test (CT). For the cold test, on-top-of-paper was conducted with sixty seeds for each variety in two replicates, placed in the refrigerator maintained at ±5°C temperature, for five days (Moyo *et al.*, 2015). After that period, the trays were removed and placed in a germinator (KRG-250, Yunboshi, China) at 25°C temperature. Germination counts were recorded starting from day 4; and germination percentages computed by counting the number of normal seedlings as in the germination test and days taken to reach 50% germination.

Heat shock stress test (HSST). The same sources of crops and genotypes used in accelerated ageing, cold test and standard germination, were subjected to HSST. The seeds of each crop and genotype were initially imbibed with distilled water in petri dishes. The imbibition period was 24 hours at a temperature of 28 °C. After the 24-hour imbibition period, the seeds were exposed to 50 °C for 2 hours, according to procedures described by van de Venter and Lock (1992). Seeds were subsequently spread on a germination paper, folded and incubated for 46 hours at 28 °C in darkness. The final germination counts of normal seedlings were taken after 72 hours. The number of days taken to fifty percent germination was also determined.

Field emergence test (FET). Plots measuring 10 m × 10 m for each crop were ploughed, disced, harrowed and ridged. Varieties for the different crops (Table 1) were planted in a field with fine tilth, for maximum seed-soil contact. Sixty seeds for each genotype were planted on ridges and the genotypes randomly assigned to each plot during planting. Inter- and intra-row plant spacing was at 75 cm x 25 cm (maize), 60 cm x 15 cm sorghum and millet, and 50 x 10 cm for cowpeas, respectively. The experimental design was a randomised complete block design, blocked according to slope and replicated twice. Since the experiment was done up to 2 weeks after seedling emergence, no fertilisers or pesticides were added.

Field emergence was assessed by counting the proportion of emerged seedlings and recorded at 24 hour intervals after day four; and this continued until day 14 as recommended by ISTA (ISTA, 2009) guidelines. The number of days taken to fifty percent germination was also recorded for each crop variety.

In both field and laboratory experiments, germinated seedlings were counted and calculation of germination percentage for all the crops and genotypes was done using the formula:

Germination percentage =

$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

The number of days taken to 50% germination was counted, shoot and radicle length measured from randomly selected five germinated seeds per petri dish for both replicates for the two sets of experiments.

Data analyses. The data on germination percentages, days taken to 50% germination and shoot and radicle lengths in the different vigour test were analysed using Analysis of variance (ANOVA) statistical model of GenStat version 13th edition (Payne *et al.*, 2010). Prior to analysis, counts data were transformed to satisfy parametric tests of normality using log transformation. Means were separated using Fisher's protected Least Significant Differences (LSD) at 5% level of probability.

RESULTS

Effectiveness of vigour tests. In all the varieties for a given crop, seed vigour tests were able to differentiate certified seed from retained seed from the local markets. Farmer retained seed took longer to reach 50% germination, compared to purchased certified seed as shown by the different superscripts for the respective varieties (Table 1).

There were consistent significant differences (P< 0.001) for all the sources of variance, i.e. crop, variety, vigour test and crop x vigour and variety x vigour test interactions for shoot and radicle length. The standard germination test had the greatest mean shoot length of 14.15 mm, which was 4.69 mm greater than the least length recorded from

TABLE 1. Number of days taken to 50% germination of different crops and varieties in the laboratory for twelve crop varieties as influenced by five seed vigour test methods

Crop	Variety	Set 1 Days to 50% germination	Set 2 Days to 50% germination
Cowpeas	CBC2	3.53[e]	4.17[d]
	IT18	3.63[de]	3.79[e]
	RT cowpeas seed	**4.00[c]**	**4.54[c]**
Maize	SC403	5.30[b]	5.54[b]
	ZM401	5.20[b]	5.42[b]
	RT maize seed	**6.27[a]**	**6.70[a]**
Pearl millet	PMV3	3.00[g]	3.00[g]
	Okashana	3.00[g]	3.04[g]
	RT pearl millet seed	**3.16[f]**	**3.00[g]**
Sorghum	Macia	3.00[g]	3.00[g]
	SV4	3.00[g]	3.00[g]
	RT sorghum seed	**3.73[d]**	**3.42[f]**
Mean		3.95	3.95
CV (%)		5.5	5.3
P<0.05		0.41	0.96
LSD (0.05)		0.12	0.12

Means were separated using LSD. Different superscript letters against means indicate significant differences at P<0.05. RT = retained. Bold means represent RT for each crop

accelerated ageing test. Similarly, for radicle length, the SGT had the longest primary root length and AAT had the least (Fig. 1).

Crops and varietal vigour tests. Analysis of variance for the four crops, twelve varieties and five vigour test methods showed a significant difference (P<0.001) for germination percentage in the two sets of experiments perfomed (Table 1). The varieties contributed the greatest source of variation for germination percentage (mean squares ranging from 56-62%, respectively), for the two sets of experiments (Table 2). Crops x vigour test interaction was consistently significant, suggesting that crop performance responded differentially to the vigour test methods. However, crop and varietal vigour tests were inconsistent as they had significant influence in Set 1, but not significant in Set 2 (Table 2).

Relationship between FET and vigour tests. All the vigour test methods evaluated could not consistently predict field emergence (Table 3). In the first set of means for germination percentage, SGT was not significantly (P>0.05) different from FET (Table 1); however, in the second set AAT and CT were not different from FET. No vigour test method was a predictor of days to 50% germination in both sets of experiments (Table 3).

DISCUSSION

Effectiveness of vigour tests. From the results of the experiment, seed vigour test methods distinguished highly retained seeds from commercial certified (Table 1), suggesting the usefulness of this attribute in determining planting value of seed. Commercially certified seed undergoes a

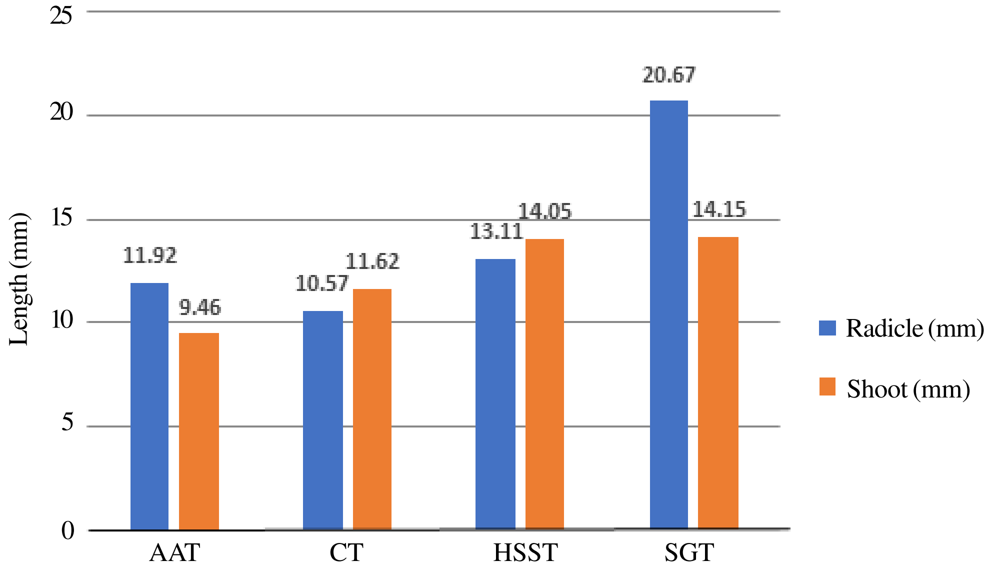

Figure 1. Relationship between seed vigour test methods and shoot length for all the evaluated selected crops. The Figure represents the mean root lengths across all the crops that were evaluated from each vigour test method.

AAT = Accelerated Ageing Test, CT = Cold Test**,** HSST = Heat Shock Stress Test, SGT = Standard Germination Test.

TABLE 2. ANOVA results for germination percentage in four crops, each with three varieties and five vigour tests

Source	Degrees of freedom	Set 1		Set 2	
		Mean squares	P	Mean squares	P
Crop	3	1405.3	<0.001	1354.4	<0.001
Variety	11	10280.0	<0.001	11729.1	<0.001
Vigour test	4	3326.8	<0.001	3638.6	<0.001
Crop × vigour test	12	2624.6	<0.001	1966.4	<0.001
Variety × vigour test	44	637.6	<0.001	209.5	0.082
Residual	285	144.0		149.7	
Total	359				

system of quality control for sanitary and physiological attributes prior to being sold on the market; while farmers' retained seed does not undergo such treatments. Since seed vigour depends on the sum total of those properties that allow for rapid, uniform and wholesome development of a seed into a normal seedling, the inclusion of several vigour tests was meant to capture all the facets of seed performance, irrespective of the crop (Marcos-Filho, 2015). Thus, from Table 1, all retained seed regardless of variety and crop took longer to germinate than certified seed. This is attributed to lack of quality control in the handling of

TABLE 3. Germination percentage and days to 50% germination of the vigour tests for the four crops with three varieties each

Vigour test	Germination percentage		Days to 50% germination	
	Set 1	Set 2	Set 1	Set 2
AAT	60.21[c]	60.95[b]	4.00[b]	4.06[b]
CT	62.48[c]	59.72[b]	4.33[a]	4.39[a]
FET	68.55[b]	68.55[b]	3.58[e]	3.58[e]
HSST	77.75[a]	73.61[a]	3.71[d]	4.03[d]
SGT	66.43[bc]	71.43[a]	3.88[c]	3.73[c]
Mean	67.08	66.43	3.95	3.95
CV (%)	17.9	18.4	5.5	5.3
P-value	< 0.001	< 0.001	<0.001	<0.001
LSD (0.05)	3.94	4.02	0.035	0.035

Different superscript letters indicate significant differences at P<0.05. AAT = accelerated ageing test, CT = cold test, FET = field emergence test, HSST = heat shock stress test, SGT = standard germination test

retained seed by farmers that ensures physiological quality associated with rapid crop germination is maintained. This compromises the yield that farmers get even before other field losses due to pests and diseases.

The Cold and Accelerated Ageing Tests adversely affected all the crops, as evidenced by the lower root and shoot (coleoptile) lengths than the standard germination test (Fig. 1). However, Accelerated Ageing seemed to affect elongation of the radicle more than the shoot, which was the opposite of the Cold Test conditions. This could mean that roots are more sensitive to heat and humid conditions than shoots that are prone to cold regardless of crop. This corroborates the findings by Giri *et al.* (2017) in tomato, who observed an adverse effect of high temperature on roots more than shoots. Exposing genotypes to high temperature (45 °C) and humidity (100%) under Accelerated Ageing and Cold temperature (5°C) resulted in some crops and varieties reducing growth rates due to the ageing and low temperature respectively; thereby impeding cell division and subsequently reducing seedling root and shoot lengths. These could be methods of choice for vigour test procedures and parameters for use by local seed companies, agro-dealers and other seed stakeholders interested in assessing seed quality.

Relative performance of crops and varieties to vigour tests. Both crop x vigour test and variety x vigour test were significant (Table 2), suggesting a differential performance of crops with vigour test and variety with vigour test. Individual vigour test methods may, therefore, be of limited use to specific crops and varieties. This concurs with Kumar *et al.* (2018), who evaluated 28 wheat varieties using 13 seed vigour parameter assessments and noted significant variations in the performance, which is attributed to differences in genetic makeup. Similarly, Kapoor *et al.* (2010) noted the effect of the variety on chickpeas that were evaluated using the Accelerated Ageing Test on seed physiological parameters. Table 1 is a summation of all the methods evaluated which gave a better prediction than when the methods were used separately for the crop and varieties evaluated in the study.

Relationship between FET and vigour tests. The vigour test methods were inconsistent in predicting field emergence for germination and days to 50% germination (Table 3). This complicates the usability of these evaluated vigour test methods since there is a lot of variation in the result for germination and days to 50% germination. These findings are contrary to those of Khan *et al*. (2007), who observed that Accelerated Aging highly correlated with field mergence when they tested wheat seedlots in Pakistan. This disparity could be attributed to Khan *et al.* (2007) use of temperate varieties, while in the present study, tropical genotypes were used. van de Venter (2000) noted that vigour tests predict field emergence better under stressful drought conditions compared to ideal conditions. In the present study, the field emergence test plot was irrigated prior to and after sowing, and had a fine tilth. This was done so that the results could be reproducible.

CONCLUSION

No single vigour test method was effective in predicting field emergence in all crops and varieties. Seed vigour tests were, however, able to differentiate the low performance of retained seed from that of high germinating commercial seed.

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