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Comparative Study of Induced-stress Methods on Seedling Vigour and Storability Potential of Bambara Groundnut (*Vigna subterranea* (L.) verdc.) Genotypes

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Post-harvest pests have been a menace that affects agricultural productions across the world, causing significant yield losses, aggravating food insecurity especially in developing countries like Nigeria. This study was carried out to evaluate seedling vigour and storability potential in 15 Bambara groundnut genotypes using artificial ageing and methanol test. The seeds were aged artificially at 45°C and 99% relative humidity for 48 hours and methanol stress test. In methanol test, seeds were placed in a moist chamber at room temperature for 2 days and afterward soaked in 20% aqueous solutions of methanol for two hours followed by soaking in distilled water for 5 minutes. The aged seeds were then evaluated following seed testing traits: rate of seed germination, seed germination, seedling fresh weight, seedling dry weight, seedling length and seedling vigour index. The experiment was carried out in a Completely Randomized Design (CRD) in three replicates. Methanol ageing method gave the best performance in all the, seed quality attributes. The results showed that the various genotype of Bambara groundnut had different qualities after being different ageing treatments. Genotypes TVSU-633, TVSU-455 and TVSU-438

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aged using accelerated and methanol and had better adaptive mechanisms to deal with the physiological damages induced by short-term ageing treatments when compared to the other genotypes. This implies that the afore-mentioned genotypes can with-stand stress and highly storable, thus recommended for use by seed companies, crop breeders and Bambara groundnut improvement programme. Accelerated and methanol can therefore, be used to predict the storability of bambara groundnut seeds.

Keywords: *Bambara groundnut; seedling vigour index; artificial ageing test; storability.*

1. INTRODUCTION

Post-harvest pests have been a menace that affects agricultural productions in Nigeria and across the world, causing significant yield losses, aggravating food insecurity especially in developing countries like Nigeria. In order to substantiate the resilience of African populations to food insecurity, there is need to embrace crops diversification and drastic steps in reducing post-harvest losses [1]. According to Hillocks et al. [2], Bambara groundnut (*Vigna subterranean*) is mainly grown as a subsistence crop, mostly by women and generally on fallow land. It is the third most important grain legume after cowpea (*Vigna unguiculata*) and groundnut [3]. Azam-Ali et al. [4] in their findings reported that although, bambara is deficient in sulphur-containing amino acids when compared with other legumes. Some of the examined genotypes were recorded to contain higher amounts of methionine and lysine than what is obtainable in other legumes [5]. The crop has the ability to fix nitrogen in the soil, which ultimately increase and maintain the soil fertility. The crop is suitable for intercropping with other crops and so conservative that could accommodate any preferred crops considered to be lucrative, without interference. Hillocks, [2] reported that Bambara groundnut when used in revitalization of the soil during crop rotation contributed nitrogen to the soil at a level similar to other legumes (20 – 100 kg ha⁻¹). However, despite all these usages, the crop is not a lucrative cash crop and under-utilized. In some eastern part of Nigeria, the fresh pods are boiled with salt and pepper, and eaten as a snack, while the matured seeds are the once that are hard and require more time of cooking. The flour can also be used to make a stiff porridge while the roasted seeds can be boiled, crushed and eaten as a relish [2].

However, despite the numerous usage of the crop, [6] reported that the most dangerous pest affecting the crop quality and quantity in post-harvest are weevils (*Coleoptera: Bruchidae*). White, (2001) reported that these insects infest

seeds in the field and continue to multiply during storage thereby resulting to huge losses and in some instances can reach 100% destruction especially in the case of a *Callosobruchus maculatus*. Maina et al. [7] in their studies reported that the severity of damage caused by *Callosobruchus subinnotatus* increases as the days of storage increases. He further reported that as early as 90 days after storage, the effect of damage of *C. subinnotatus* was evident and severe on the seed quality of cowpea and this increases as the number of days of storage increases. The reasons were accrued to increase in the rate of spawning and adult emergence of *C. subinnotatus* which ultimately increased the rate of damage. Similarly, Baoua et al. [8] reported losses of about 61.8% caused as a result of *C. maculatus* and 83.9% by *C. subinnotatus* infestation on Bambara groundnut after 200 days of storage. Several, researchers have reported the ineffectiveness of traditional storage methods of Bambara groundnut, as its rate of grain damage can be as high as 100% in some cases, improved storage techniques, especially hermetic methods; allow a significant reduction in storage losses [9]. According to Moussa et al. [10] an estimation of about 61.8% of bambara groundnut grains losses was recorded after 7 months of storage using traditional methods. The above degradation of seed quality tends to extend to the seedling germination that will emanate from such seed lot.

Improvements in seedling quality would only occur when both morphological and physiological attributes are considered. The storability potentials of seed lot under varying conditions can be predicted using accelerated ageing test technique. A cursory look at literature revealed that scanty information is available in this aspect as regards to Bambara groundnut grown in Nigeria. This research would be important as it provided much needed information about accelerated ageing test and methanol test on the vigour and storability potential of 10 bambara groundnut. The essential purposes of vigour tests is to indicate whether or not causality may

be expected from a high germinating seed lot if the lot is placed under adverse environmental conditions either in the field, during transportation or even storage. Ouili et al. [11] was of the opinion that vigour tests results of any seed lot should be able to provide reliable information to rank seed lots according to seed quality level and to eliminate those lots that fall below average. If positive ageing effect is found, it would go a long way in increasing of viable and high quality seeds of *Bambara groundnut* to farmers thus improving the average yield produced. Unfortunately, the cultivation of this highly legume (*Bambara groundnut*) is subjected to many constraints, including the deterioration of crops during storage by insects and their contamination by fungi (Ouoba et al. 2016). Sequel, to the above, the objectives of this study is to identify *Bambara groundnut* varieties with superior seedling vigor and high storability potential.

2. MATERIALS AND METHODS

Seeds of 15 *Bambara groundnut* genotypes that were used for this experiment were sourced from Institute of Agricultural Research and Training (IAR&T) Ibadan, Oyo State, Nigeria. The seed samples used were freshly harvested (2 month old) from a seed multiplication trial in 2019. The laboratory experiment was carried out in the Department of Crop Production laboratory, University of Agriculture and Environmental Sciences, Owerri, Imo State, Nigeria. The laboratory experimental design was a Completely Randomized Design (CRD) in three replicates while factor investigated was the genotype in 15 levels. The seedling vigour potential and relative storability of the 15 genotypes of *Bambara groundnut* seeds were stressed tested using accelerated ageing and methanol. Seeds of each genotype were put in net bags in the desiccator containing 40g of NaCl salts dissolved in 100ml of distilled water in the lower chamber. The desiccator was covered and maintain in an incubator at 45+1°C for 48 hours of ageing which also included control. Adebisi, 2004 formulated Methanol Seed Ageing Test methods were used to determine the methanol test. Three replicates each of 15 *Bambara groundnut* seeds per genotype were used; seeds were placed in a moist chamber at room temperature for 2 days and were soaked in 20% aqueous solutions of methanol (i.e. 20% methanol to 80% of distilled water) for two hours follow by soaking in distilled water for 5mins. The *Bambara groundnut* seeds were placed in petri-dishes for germination at

25°C incubator, germination count was taken after 7 days. However, seed samples of the 15 genotypes were evaluated void of any of the ageing treatments. The seed lots (aged and un-aged) were assessed for the following physiological quality traits:

2.1 Seed Germination

Twenty-five seeds in three replicates for each genotype were placed in a petri-dish which was lined with moistened towel paper with 10ml of distilled water. The petri-dishes were placed in germination chamber, germination counts was taken after 7 days and expressed in percentage [12].

2.2 Seedling Shoot Length (cm)

Shoot length of 10 randomly picked normal seedlings from each replicate was measured using ruler.

2.3 Seedling Root Length (cm)

Root lengths of 10 randomly picked normal seedlings from each replicate were measured.

2.4 Rate of Germination

This was determined at the 3rd day of germination as number of seed geminated at the 3rd day of germination count and expressed in percentage of the seed sown.

2.5 Seedling Vigour Index (SVI)

This was determined using the formula modified by Adebisi [13].

$$SVI = \frac{\text{Germination (\%)} \times \text{Shoot length}}{100}$$

$$SVI = \frac{\text{Germination (\%)} \times \text{Root length}}{100}$$

2.6 Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) while means for the variables were compared using Tukey's HSD at 5% level of significance.

3. RESULTS

The summary analysis of variance (ANOVA) for the effect of ageing treatment on seedling vigour and storability potential in *Bambara groundnut* genotypes is presented in Table 1. The result revealed that replicate effect was not significant

($P > 0.01$) on all the six parameters (i.e. rate of germination, seedling fresh weight, seedling dry weight, seedling length, seedling vigour index and seed germination). The genotype effect was highly significant ($P < 0.01$) on all the six parameters and the interaction of genotype and ageing treatment was also highly significant on all the parameters.

The effect of genotype on seed quality evaluated across ageing treatments is presented in Table 2. From the table, TVSU-454 and TVSU-455, recorded the highest rate of seed germination (RSD) of 73.01 and 70.09%, respectively. This was closely followed by TVSU-194 with 68% rate of seed germination (13day) while TVSU-1520 recorded the lowest rate of seed germination of 22.31%/3day. In terms of seed germination TVSU-1392, TVSU-455 and TVSU-633 recorded the highest seed germination of 73.01, 76.31 and 70.87%, respectively while TVSU-1520 recorded the lowest seed germination of 24.65% for seedling fresh weight. TVSU-633 and TVSU-438 recorded the highest seedling fresh weight of 2.27 and 1.87g, respectively, while TVSU-939 recorded the lowest seedling fresh weight of 0.37g. Seedling dry weight of TVSU-454 recorded the highest seedling dry weight of 1.54g, closely followed by TVSU-1392 with 0.92g seedling dry weight while TVSU-1520 recorded the lowest dry weight of 0.16g. In seedling length, TVSU-633 recorded the highest seedling length of 11.16cm, closely followed by TVSU-1392 (8.60cm) while TVSU-643 recorded the lowest seedling length of 3.38cm. In seedling vigour index, TVSU-633 recorded the highest seedling vigour index of 9.02, closely followed by TVSU-455(8.07) and TVSU-392 (8.00) while TVSU-1611 had the lowest seedling vigour index of 2.25.

The effect of ageing treatments on seed quality parameters of Bambara groundnut across genotypes is presented in Table 3. From the table, the result showed that the rate of germination was highest in seeds under methanol ageing treatment (61.79%) followed by artificial ageing (48.13%) while the control had the least value of (47.60%). In seed germination, methanol ageing treatment had the highest value of 77.06% followed by artificial ageing (66.62%) and control which had statistically similar values of (66.42%). For seedling fresh weight, methanol ageing gave the highest seedling fresh weight of 1.56g, followed by control (1.20) while artificial ageing gave the least seedling fresh weight

(1.10g). In term of seedling dry weight, methanol ageing and control recorded the highest seedling dry weight of 0.61 and 0.60g followed by artificial ageing (0.51g). For seedling length, methanol ageing gave the highest seedling length of 8.49cm, followed by control which had 6.89cm while artificial ageing had the lowest seedling length of 5.89cm. In term of seedling vigour index, methanol ageing had the highest vigour index of 5.68 followed by control which had 4.32 while artificial ageing had the lowest seedling vigour index of 3.15.

The summarized interaction effect of genotype and ageing treatment on rate of seed germination (%/3day) in Bambara groundnut is presented in Table 4. For seeds under control, TVSU-1392 TVSU-513 and TVSU-438 recorded the highest rate of seed germination of 93.31%, 76.66%, and 71.66%, respectively while TVSU-1520 and TVSU-1611, recorded the lowest rate of seed germination of 31.67 and 43.32, respectively. For artificial ageing, TVSU-455 TVSU-194 and TVSU-633 recorded the highest rate of seed germination of 86.66%, 71.66%, and 68.32%, respectively while TVSU-1520 and TVSU-454, recorded the lowest rate of seed germination of 31.66% and 35.00%, respectively. For methanol ageing, TVSU-633, TVSU-1392 and TVSU-454 recorded the highest rate of seed germination of 98.33%, 95.00%, and 95.00%, respectively while TVSU-1520 recorded the lowest rate of seed germination of 46.67%.

Table 5 presents the interaction effect of genotypes and ageing treatment on seed germination (%) in Bambara groundnut. For seeds under control, TVSU-1392, TVSU-513 and TVSU-455 recorded the highest rate of seed germination of 95.67%, 81.67%, and 80.00%, respectively while TVSU-1520 and TVSU-939, recorded the lowest rate of seed germination of 22.33 and 35.00, respectively. For seeds artificial ageing, TVSU-1392, TVSU-455 and TVSU-633 recorded the highest rate of seed germination of 94.00 %, 92.33%, and 90.00 %, respectively while TVSU-1520 and TVSU-939, recorded the lowest rate of seed germination of 32.33 and 35.67, respectively. In methanol, ageing, TVSU-455 and TVSU-194 jointly had the highest with 90.67% while TVSU-454 closely followed and TVSU-1392 with 90.66 % and 90.00, respectively while TVSU-1520 and TVSU-939, recorded the lowest rate of seed germination of 42.33 and 55.67%, respectively.

Table 1. Summary of analysis of variance for the effect of ageing treatment on seedling vigour and storability potential of several Bambara groundnut genotypes

Source of variation	Degree of freedom	Rate of germination (%)	Seed germination (%)	Seedling fresh weight	Seedling dry weight	Seedling length	Seedling vigour index
Replicate	2	83.66 ^{ns}	147.28 ^{ns}	0.30 ^{ns}	0.06 ^{ns}	1.63 ^{ns}	3.22 ^{ns}
Genotype (G)	15	2062.15 ^{**}	2464.61 ^{**}	2.31 ^{**}	0.31 ^{**}	45.38 ^{**}	47.18 ^{**}
Treatment (T)	2	3051.86 ^{**}	2442.50 ^{**}	4.69 ^{**}	0.20 ^{**}	286.20 ^{**}	113.28 ^{**}
G x T	45	311.16 ^{**}	302.84 ^{**}	0.53 ^{**}	0.06 ^{**}	15.82 ^{**}	11.12 ^{**}
Error	156	146.65	137.87	0.13	0.02	4.34	2.14

^{**}Significant at 1% probability level ^{ns} not significant

Table 2. Effect of several bambara genotype on seed quality parameters evaluated across artificial ageing treatments

Genotype	Rate of seed germination (%)	Seed germination (%)	Seedling fresh weight (g)	Seedling dry weight (g)	Seedling length (cm)	Seedling vigour index
TVSU-454	46.76 ^{e-i}	55.20 ^{b-h}	1.35 ^{b-g}	1.54 ^a	3.84 ^f	3.23 ^{e-i}
TVSU-158	46.20 ^{e-i}	49.09 ^d	0.74 ^{fg}	0.29 ^{fg}	3.85 ^f	2.61 ^{f-i}
TVSU-438	54.54 ^{b-g}	62.42 ^{b-g}	1.87 ^{ab}	0.75 ^{abc}	7.91 ^{a-d}	5.92 ^{b-e}
TVSU-633	63.42 ^{b-f}	70.87 ^{bc}	2.27 ^a	0.75 ^{abc}	11.16 ^a	9.02 ^a
TVSU-1520	22.31 ^j	24.65 ⁱ	0.45 ^h	0.16 ^g	4.56 ^{def}	2.56 ^{f-i}
TVSU-939	27.87 ^{ij}	30.76 ^{ij}	0.37 ^h	0.13 ^g	4.45 ^{def}	1.82 ⁱ
TVSU-513	52.54 ^{b-h}	62.65 ^{b-g}	1.29 ^{b-g}	0.47 ^{b-g}	5.35 ^{c-f}	4.29 ^{d-i}
TVSU-455	70.09 ^{b-c}	76.31 ^b	1.69 ^{abc}	0.65 ^{a-e}	9.55 ^{ab}	8.07 ^{abc}
TVSU-643	47.31 ^{d-i}	49.09 ^{d-i}	0.82 ^{e-h}	0.36 ^{efg}	3.38 ^f	2.41 ^{ghi}
TVSU-2096	53.08 ^{b-g}	56.87 ^{b-h}	1.66 ^{a-d}	0.63 ^{a-f}	7.94 ^{a-d}	5.43 ^{d-h}
TVSU-194	68.42 ^{b-c}	63.54 ^{b-f}	1.64 ^{a-d}	0.64 ^{a-e}	6.29 ^{b-f}	5.13 ^{c-h}
TVSU-1611	37.31 ^{g-j}	40.76 ^{hij}	0.93 ^{d-h}	0.39 ^{d-g}	4.00 ^f	2.25 ^{hi}
TVSU-1920	47.31 ^{d-i}	52.42 ^{c-h}	1.05 ^{c-h}	0.43 ^{d-h}	3.71 ^f	2.63 ^{f-i}
TVSU-1531	39.02 ^{g-j}	47.42 ^{e-i}	0.96 ^{d-h}	0.43 ^{d-h}	5.71 ^{c-f}	3.24 ^{e-i}
TVSU-1392	73.01 ^a	81.87 ^a	1.66 ^{a-d}	0.92 ^{a-f}	8.60 ^{abc}	8.00 ^{abc}

Means followed by the same alphabet along the column are not different from one another according to the Tukey's HSD at 5% probability level

Table 3. Effect of ageing treatment on seed quality of Bambara groundnut across varieties

Treatment	Rate of germination @3 rd day (%)	Seed germination @7 th day (%)	Seedling fresh weight (g)	Seedling dry weight(g)	Seedling length(cm)	Seedling vigour index
Control	47.60 ^b	66.42 ^b	1.20 ^b	0.60 ^a	7.87 ^b	4.32 ^b
Artificial ageing	48.13 ^b	66.62 ^b	1.10 ^c	0.51 ^b	5.89 ^c	3.15 ^c
Methanol ageing	61.79 ^a	77.06 ^a	1.56 ^a	0.61 ^a	8.49 ^a	5.68 ^a
standard error	3.51	3.41	0.06	0.06	0.04	0.16

Means followed by the same alphabet along the column are different from one another according to Tukey's HSD at 5% probability level

Table 4. Interaction effect of genotype and ageing treatment on rate of seed germination (%/3day) in Bambara groundnut

Genotypes	Control	Artificial ageing	Methanol ageing
TVSU-454	53.32 ^c	35.00 ^d	95.00 ^a
TVSU-158	58.32 ^c	56.66 ^c	66.67 ^c
TVSU-438	71.66 ^b	56.66 ^c	78.33 ^c
TVSU-633	66.66 ^{bc}	68.32 ^b	98.33 ^a
TVSU-1520	31.67 ^d	31.66 ^d	46.67 ^e
TVSU-939	35.00 ^d	36.66 ^d	55.00 ^d
TVSU-513	76.66 ^b	58.34 ^c	65.67 ^c
TVSU-455	71.66 ^b	86.66 ^a	95.00 ^a
TVSU-643	53.32 ^c	53.32 ^c	78.33 ^c
TVSU-2096	56.66 ^c	56.66 ^c	91.67 ^{ab}
TVSU-194	59.00 ^{bc}	71.66 ^{ab}	95.67 ^a
TVSU-1611	43.32 ^d	36.66 ^d	75.00 ^c
TVSU-1920	63.33 ^{bc}	51.67 ^c	70.00 ^c
TVSU-1531	51.66 ^c	48.32 ^c	63.33 ^d
TVSU-1392	93.31 ^a	71.66 ^{ab}	95.00 ^a
Standard error	7.00	6.98	6.70

Means followed by the same alphabet along the column are different from one another according to Tukey's HSD at 5% probability level

Table 5. Interaction effect of genotype and ageing treatment on rate of seed germination (%/7day) in Bambara groundnut

Genotype	Control	Artificial ageing	Methanol ageing
TVSU-454	60.00 ^d	50.00 ^d	90.66 ^a
TVSU-158	65.00 ^d	58.33 ^d	60.00 ^d
TVSU-438	73.33 ^c	67.33 ^{cd}	80.67 ^{bc}
TVSU-633	71.67 ^c	90.00 ^a	89.00 ^b
TVSU-1520	33.33 ^f	22.33 ^e	42.33 ^e
TVSU-939	35.00 ^f	35.67 ^e	55.67 ^d
TVSU-513	81.67 ^b	55.67 ^d	84.67 ^{bc}
TVSU-455	80.00 ^c	92.33 ^a	90.67 ^a
TVSU-643	52.33 ^d	57.33 ^d	70.67 ^c
TVSU-2096	62.33 ^d	60.00 ^d	82.33 ^{bc}
TVSU-194	60.67 ^d	72.33 ^c	90.67 ^a
TVSU-1611	42.33 ^e	42.33 ^e	70.67 ^c
TVSU-1920	65.67 ^d	60.67 ^d	64.00 ^d
TVSU-1531	55.67 ^d	67.33 ^{cd}	52.44 ^d
TVSU-1392	95.67 ^a	94.00 ^a	90.00 ^a
Standard error	6.46	6.95	6.88

Means followed by the same alphabet along the column are different from one another according to Tukey's HSD at 5% probability level

4. DISCUSSION

The results showed that there were considerable differences in seed quality parameters among the genotypes after different ageing treatments. As seen in this study, the seed deterioration varies greatly not only between genotypes, but also as the ageing process progresses. Methanol ageing method gave the best performance in all the seed quality attributes evaluated. Based on the evidence gathered in this study, a wide

variation is seen among the genotypes. This could be attributed to the fact that some genotypes e.g. TVSU-633, TVSU-455 and TVSU-438 had better adaptive mechanisms to deal with the physiological damages induced by short-term artificial aging when compared to the other genotypes. Also, the interaction effect of the genotype, artificial ageing and methanol revealed that, TVSU-633, TVSU-455 and TVSU-438 had the best adaptive mechanisms to withstand stress as the genotypes recorded range of

seed germination after ageing treatment of between 67 to 95%. According to Clercx et al. [14], an array of differences recorded between cultivar of the same species for each measured attributes, could be as a result of multiple factors involved in seed longevity.

Generally, deterioration is a mandatory and irreversible physiological process in seeds, the longer seeds were aged, the more physiologically impaired they were. Longer aged seeds have the tendencies to suffered more physiological damage as recorded in seed germination at 3 days (48%) and at 7 days (66%). The result recorded was in agreement with Angelo et al. [15] in stored beans, where alterations were noticed in the arrangement of proteins, that led to the rupture of many cell walls and ultimate decrease in acid phosphatase activities after accelerated ageing treatment was carried out. Also, the seed deterioration recorded in most of the physiological attributes after the ageing test are partly in line with previous report by Sattler et al. [16] where a rapid decrease was noticed in the GSH and vitamin E content in aged crop seeds of watermelon.

A cursor looks at the wide difference between aged and unaged seeds, further re-established and improved the general understanding of seed behavior especially during aging. The physiology and internal adaption to stress by aging is unique. In this study, the germination rates of control Bambara groundnut decreased from an average of 95 to 33%, but even as low as 22% (aged seeds) after artificial ageing treatment. This finding was in support of the work of Das et al. [17] where ageing treatment in species of rice (*Oryza sativa*) seeds was reported to profoundly inhibit seed germination and caused severe decrease (more than 50%) in the phenolics content and antioxidant capacity. According to Ravikumar et al. [18]; Sattler et al. [16] and Oliveira et al. [19] such afore-mentioned aged seeds are unable to germinate and produce healthy seedlings due to reserve shortages thus, they recommended these seeds to be used as animal fodder due to their low nutrients. Song et al. [20] reported melatonin treatment efficiently converted aged seed reserve residues into antioxidant nutrients, providing an alternative use for deteriorated seeds in food production in yellow soybean (*Glycine max* (Linn.) Merr.), black soybean (*Glycine max* var.), mung bean (*Vigna radiata* (Linn.) Wilczek.) and red bean (*Vigna angularis* (Willd).

5. CONCLUSION

This study has further showed that both artificial and methanol ageing methods have tendencies of causing physiological damage of seeds as evidenced by sporadic decrease in seed germination %, seed viability, germination rate over time, seedling fresh and dry weight. The Overall results revealed that accelerated and methanol can be used to predict the storability of bambara groundnut seeds. Methanol ageing method gave the best performance in all the seed quality attributes evaluated. Genotypes TVSU-633, TVSU-455 and TVSU-438 aged using accelerated and methanol had better adaptive mechanisms to deal with the physiological damages induced by short-term artificial aging and methanol, recorded outstanding performance in the induced stress, seedling vigor and storability potential, thus recommended for use in Bambara groundnut improvement programme.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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