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Research Article

Response of groundnut (*Arachis hypogaea* L.) genotypes to accelerated ageing treatment

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Abstract

Reduction in germination of crop seeds due to depletion of food reserves and decline in synthetic activity due to ageing has become a serious concern to groundnut growers who need adequate, high quality seeds to sustain groundnut production. Therefore, to stimulate farmers' interest in groundnut production, an experiment was conducted to evaluate some groundnut varieties for their tolerance to seed ageing stress, with a view to recommending varieties that can be considered for production in tropical countries. Seeds of nine elite groundnut genotypes, sourced from The International Crops Research Institute of Semi-Arid Tropics (ICRISAT), Kano and three other genotypes sourced from local seed dealer in Ibadan, Nigeria were subjected to seed quality assessments in the seed testing laboratory of Institute of Agricultural Research and Training, Ibadan. The seed lots were subjected to accelerated ageing procedures of 42 °C temperature and 100% relative humidity for 24 hours. Twenty-five seeds of each genotype were drawn from each genotype in three replicates at 3, 6, 12 and 24 hours of ageing. The drawn samples were reassessed to determine their tolerance ability to ageing stress. Percentage germination was transformed using arc-sine before the data on preliminary seed germination and seedling vigour data and seed ageing data were subjected to analysis of variance (ANOVA) using SAS™ Means were separated using Duncan Multiple Range Test (DMRT) at 5% level of significance while k-means non-hierarchical clustering analysis was used to group the genotypes based on their response to the ageing. Result showed that seeds of the groundnut genotypes differ in their response to ageing stress factors. Seeds of 'Samnut-24', 'Samnut-25' and 'Ex-Dakar' (R) were found to be more tolerant to ageing stress while 'Samnut 22' and 'Boro White' were susceptible to ageing stress. Optimum ageing for 24 hours is recommended for testing seeds of groundnut varieties for storage tolerance

Keywords: accelerated ageing; groundnut genotypes; seed germination; seedling vigour; storage tolerance

Introduction

Groundnut (*Arachis hypogaea* L.) is ranked the thirteenth among the important food crops and sixth most important oilseed crop in the world. It is a rich source of dietary fibre, minerals, and vitamins, containing

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48-50% oil and 26-28% protein (Ntare *et al.*, 2008). Groundnut production in West Africa averaged about 4.832 million tonnes, which represents about 60% of Africa's production and about 15% of world production (is grown on 26.4 million hectares worldwide with a total production of 37.1 million metric tonnes and an average productivity of 1.4 metric t/ha (FAO, 2011). The productivity in Asia and America is relatively better with an average yield of 2.4 metric tonnes /ha and over 3.3 metric tonnes/ha respectively. Nigeria is the fourth largest producer of groundnut in the world and the largest groundnut producing country in West Africa, accounting for 51% of production in the region (Ajeigbe *et al.*, 2015). Despite the numerous benefits and roles groundnut play at individual and national level in many developing countries, pod yield from farmers' field have remain low (Ndjeunga *et al.*, 2010). Thus, effort has been concentrated on increasing land cultivated to meet up national demand. Increase in area of production has resulted to corresponding increase in seed requirement. Groundnut farmers often store their seeds under ambient condition where temperature and humidity can be very high, particularly in countries with warmer climate like Nigeria. There have been several studies on storability of groundnut seed. Dvssr *et al.* (2007) reported that groundnut seeds dried up to 4% mc using sorption type drier with secondary refrigeration (15 °C and 15% RH) can retain viability considerably for longer periods. Similarly, Rao *et al.* (2002) reported that groundnut seeds that is hermetically stored at room temperature (23-25 °C) with low moisture content (below 4%) can retain high germination (>85%) for up to 8 years. Other studies have argued that groundnut varieties react differently to environmental field conditions, like reaction to water stress, disease, and pest infection, with less information about genetic variability of groundnut seeds to storage stress. Most reports have stressed reduction in germination of groundnut seed during storage (Dvssr *et al.*, 2007; Ameer *et al.*, 2013) The reduction in germination of such crop seeds has been attributed to depletion of food reserves and decline in synthetic activity due to ageing, as well as bio-chemical changes of seed during storage (Ameer *et al.*, 2013) This has become a serious concern to groundnut growers who need adequate, high quality seeds to sustain groundnut production, most especially in South Western Nigeria. In stimulating farmers' interest in groundnut production, there is a need to develop a sustainable seed availability strategy that will involve cultivation of varieties with moderate level of tolerance to storage stress under ambient condition in tropical climate. Therefore, this study is set to evaluate some groundnut varieties for their tolerance to seed ageing stress, with a view to recommending varieties that can be considered for production in Tropical countries.

Materials and Methods

Germplasm collection

Seeds of nine elite groundnut genotypes were sourced from The International Crops Research Institute of Semi-Arid Tropics (ICRISAT), Kano, while three genotypes were sourced from local seed dealer in Ibadan, Nigeria. Length and breadth of 10 seeds of each genotype were measured, using Vernier-calliper to calculate the surface area of the seeds (mm^2). The area of the seeds was used to classify the seed to sizes. Genotypes with seed area above 100 mm^2 (>100) were classified as big, while genotypes with seed area between 70 to 99 mm^2 were regarded as medium and genotypes with seed area less than 70 mm^2 (<70) were regarded as small. The list of the genotypes and their seed characteristics are presented in Table 1, while the pictorial view of the seeds of the genotypes is presented in Figure 1.

Table 1. Quantitative characteristics of the genotypes

Genotypes	Source	Seed size	Seed coat texture	Seed colour
'Samnut-21'	ICRISAT, Kano	Big	Smooth	Whitish brown
'Samnut-22'	ICRISAT, Kano	Big	Smooth	Dark brown
'Samnut-23'	ICRISAT, Kano	Medium	Smooth	Light brown
'Samnut-24'	ICRISAT, Kano	Small	Smooth	Light brown
'Samnut-25'	ICRISAT, Kano	Small	Smooth	Light brown
'Samnut-26'	ICRISAT, Kano	Medium	Smooth	Light brown
'Ex-Dakar-Red'	ICRISAT, Kano	Medium	Smooth	Deep brown
'Ex-Dakar-W'	ICRISAT, Kano	Medium	Smooth	Brown
'Kuta-Red'	Local seed dealer	Big	Shrivel	Dark brown
'Boro-White'	Local seed dealer	Medium	Smooth	Light brown
'Boro-Red'	Local seed dealer	Medium	Smooth	Deep brown
'Kwankwaso'	ICRISAT, Kano	Medium	Shrivel	Brown

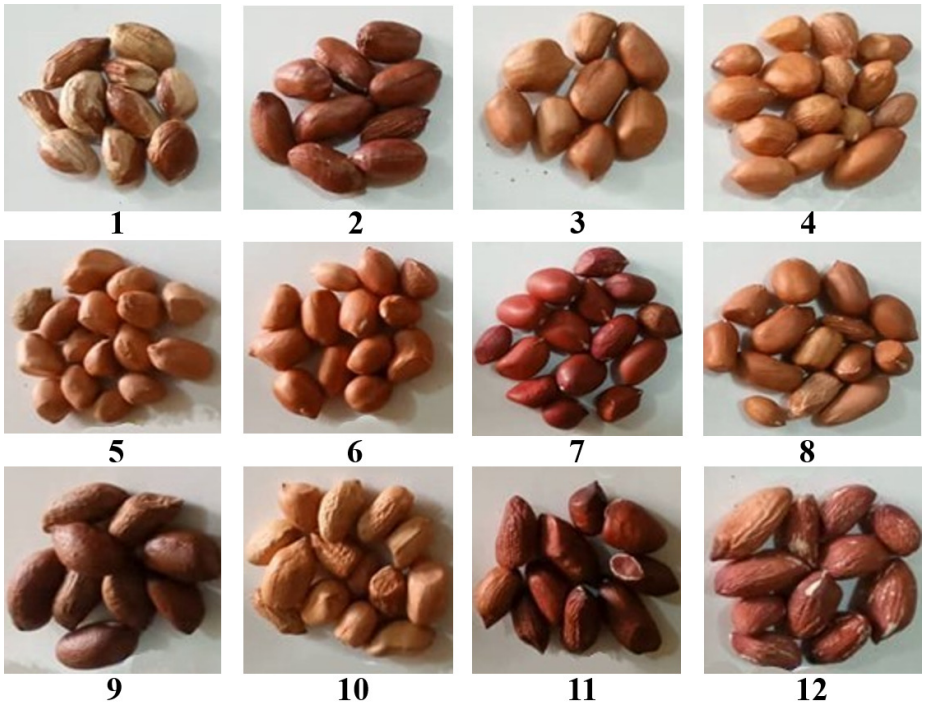

Figure 1. Pictorial view of groundnut genotypes
Keys: 1: 'Samnut-21', 2: 'Samnut-22'; 3: 'Samnut-23'; 4: 'Samnut-24'; 5: 'Samnut-25'; 6: 'Samnut-26'; 7: 'Ex-Dakar-R', 8: 'Ex-Dakar-W', 9: 'Kuta-Red'; 10: 'Boro-White'; 11: 'Boro-Red'; 12: 'Kwankwaso'

Experimental location and procedure

The experiment was carried out at the Seed Testing Laboratory of Institute of Agricultural Research and Training, Moor Plantation, Ibadan. The seeds of the collected genotypes were subjected to preliminary seed quality assessment as follows:

Germination test

50 seeds of each genotype were randomly picked in three replicates and planted in seed bowls filled with adequately moistened sterilized river sand. Seedlings were counted at 4, 6, 8, 10, 12 and 14 days after sowing. Germination percentage was calculated by finding the ratio of normal germinated seeds at 14 days after sowing to total number of seeds planted using the formula:

$$G\ (\%) = \frac{No\ of\ normal\ seedlings\ that\ germinated}{Total\ number\ of\ seeds\ planted} x\ 100$$ (ISTA, 2020)

Seed vigour assessment

The vigour of the seeds of each genotype was assessed to determine the quality of life in the seeds. Germinating seedlings were counted on 4, 6, 8, 10, 12 and 14 days after sowing. Also, seedling length of 5 randomly selected seedlings per pot were tagged and measured every two days, until the 14th day of planting, when the seedling weight of the tagged 5 seedlings were measured. Seedling vigour parameters were then estimated using the following procedures:

Mean Germination Time (MGT)

This is determined to estimate mean time required by each seed lot to initiate and end germination. It was estimated as:

$$MGT = \frac{\Sigma(nT)}{\Sigma n}$$ (Mavi *et al.*, 2010)

Where n is number of seeds newly germinated at time T, while T is days from the beginning of the germination test.

Germination Index (GI)

This parameter emphasizes the germination percentage and the speed of germination. A higher germination index value implies a high germination percentage as well as the rate of germination (Al-Mudaris, 1998). It is estimated as:

$$GI = \frac{\Sigma\ (Nx)(DAP)}{Total\ number\ of\ normal\ seedlings\ that\ emerged\ on\ final\ day}$$ (Akande *et al.*, 2012)

where Nx is the number of normal seedlings that emerged on day x after seeding and DAP is days after planting

Germination Rate Index (GRI)

This reflects the percentage of germination on each day of the germination period and was calculated as follows:

$$GRI = = \frac{G1}{x} + \frac{G2}{x} + \frac{G3}{x} + \cdots \frac{Gx}{x}$$ (Esechi, 1994)

where, G = germination on each day after seed placement 1, 2, x = corresponding day of germination

Seedling vigour Index (SVI)

The estimation of the seedling vigour index, involved the measurement of seedling length of five randomly selected seedlings of each replicate from the soil level at 8 and 14 days after planting. The SVI was then calculated as follows:

$$SVI = \frac{(Germination\ \%\ x\ Seedling\ length)}{100}$$ (Adetumbi *et al.*, 2019)

Accelerated ageing procedure with corresponding germination and vigor test

Clean seeds of the twelve genotypes were selected and subjected to accelerated ageing using a modified procedure of Ghosh *et al.* (2013) and Okunlola *et al.* (2020). A hundred seeds in three replicates of each genotype was counted and placed in an improvised plastic ageing box (11.0 × 11.0 × 3.5 cm), ensuring the seeds form a single layer. The ageing boxes has false bottom created through placement of wire mesh screen, which was suspended over 50 ml distilled water to create 100% relative humidity during the ageing. The ageing boxes containing the seeds were arranged in the laboratory oven set at 42 °C. Twenty-five seeds of each genotype were drawn at 3, 6, 12 and 24 hours of ageing and placed on soft tissue paper to absorb excess moisture on the surface

of the seed. Thereafter, the procedure for conducting preliminary assessment of the germination and vigour of the seed samples was used to evaluate each replicate of the aged seed lots of the genotypes to determine their tolerance ability of the seeds to ageing stress.

Germination loss (G loss)

This is the measure of the effect of the ageing on the germination percentage of the genotypes. It is measured by deducting the G% of each genotype after the ageing at 3, 6, 12 & 24 hours from the G% before accelerated ageing (Initial G%).

Data analysis

All percentage germination data was transformed using arc-sine formula. The preliminary seed germination and seedling vigour data, as well as the data obtained after the seed ageing were subjected to analysis of variance (ANOVA) using SAS™ software. Significant means were separated using Duncan Multiple Range Test (DMRT) at 5% level of significance while cluster analysis was conducted to group the genotypes based on their response to the ageing, using k-means non-hierarchical clustering analysis.

Results

Mean square for seed germination and seedling vigour parameters for groundnut genotypes subjected to different ageing duration.

Seed ageing period significantly affected the seed quality variables except Mean Germination Time (MGT) at 5% significance level (Table 2). Also, there were significant differences in the response of genotypes to artificial ageing at 5% significant probability. The interaction between ageing duration and genotypes was significant for seedling vigour index and seed germination parameters except germination loss. The result also showed that the coefficient of variance was highest in the germination loss (52.8), followed by germination index (17.6).

Table 2. Mean square for seed germination and seedling vigour parameters for groundnut genotypes subjected to different ageing duration

SV	Df	G Loss (%)	MGT (Days)	GRI	SVI	GI
Ageing duration (A)	4	2650.7*	0.79	13.6*	264.1*	733.1
Variety (V)	11	444.82*	1.42*	2.42*	129.1*	4117.1*
A x V	44	71.9	0.8*	0.9*	10.7*	542.6*
Error	120	34.1	0.93	0.3	2.3	208.3
CV%		52.8	10.3	13.9	12.1	17.6

*Significant at P<0.05: SV = Source of variation, Df = Degree of freedom, GRI = Germination Rate Index; GI = Germination index; SVI = Seedling Vigour Index; CV% = Coefficient of Variation; Gloss = Germination loss MGT = Mean germination time

Effect of accelerated ageing duration on seed germination and seedling vigour parameters of groundnut seeds

The result of the effect of accelerated ageing duration on germination and seedling vigour of groundnut showed that increase in ageing duration significantly reduces the germination of groundnut seeds. Also, there was significant increase in germination loss as the ageing period increases, while SVI, GI and GRI decreases. Although the mean of MGT recorded before ageing (4.9) and the mean recorded at 3, 6, 12 and 24 hours of ageing were close, there was no significant difference between the MGT of the seeds before ageing (4.9) and ageing at 3 (5.1) and 6 hours (5.0) of ageing. Most of the seeds germinated in about 5 days after planting (Table 3).

Table 3. Effect of accelerated ageing duration on groundnut germination and seedling vigour of groundnut genotypes

Ageing duration (Hours)	Germination (%)	G Loss (%)	MGT (Days)	GRI	SVI	GI
0	76.2a	0.0e	4.9b	3.9a	15.6a	8.9a
3	69.5b	6.7d	5.1ab	3.6b	14.3b	8.3ab
6	65.5c	10.7c	5.0ab	3.5bc	13.2c	8.1b
12	61.1d	15.1b	5.2a	3.3cd	10.9d	8.0b
24	53.4e	22.8a	5.3a	3.2d	8.8e	7.7b

Means followed by same alphabet along the column are not significantly different at P<0.05 according to DMRT
GRI = Germination Rate Index; GI = Germination index; SVI = Seedling Vigour Index;
Gloss = Germination loss MGT = Mean germination time

Effect of accelerated ageing on seed viability and seedling vigour characteristics of groundnut genotypes.

There was significant difference in the germination loss and seedling vigour characteristics of the groundnut genotypes (Table 4). 'Samnut 24' recorded the lowest germination loss (3%) after ageing. This was followed by 'Samnut 25' that recorded the mean value of 6% germination loss. 'Boro Red' recorded the highest germination loss (21%), followed by 'Samnut 21' (20.5%). Although the MGT recorded by the groundnut genotypes after ageing were very close, there were significant differences in the MGT recorded among the genotypes. 'Samnut-24' germinated earlier than all the genotypes at 4 days after planting while 'Kwankwanso' significantly germinated later at 6 days after planting. Most of the genotypes germinated at 5 days after at planting. The GRI of 'Samnut-24' (5.5) was the highest, followed by 'Samnut-25' and 'Samnut-26' that recorded 4.9 and 4.1 respectively while 'Samnut-21' and 'Kwankwaso' recorded the lowest GRI of 2.4 and 2.5 respectively (Table 4). The Seedling Vigour Index indicated no significant difference between 'Samnut 24' and 'Samnut 25'. Also, there was no significant difference between 'Samnut 26', 'Ex-dakar Red', 'Ex-dakar white' and 'Kuta Red' while 'Samnut 23' and 'Samnut 21' recorded the lowest SVI of 8.6 and 8.3 respectively. 'Ex-dakar Red' and 'Samnut-25' recorded the highest GI (10.7) followed by 'Samnut 24' (10.0) while 'Samnut 21' recorded the lowest GI (5.8).

Table 4. Mean value of seed germination loss and seedling vigour parameters of groundnut genotypes after artificial ageing

Groundnut genotypes	G Loss (%)	MGT (Days)	GRI	SVI	GI
'Samnut-21'	20.5a	5.0bc	2.4f	8.3f	5.8g
'Samnut-22'	12.9b	5.1bc	3.1de	11.7cd	7.6de
'Samnut-23'	8.1c	4.7cd	3.0de	8.6ef	6.8efg
'Samnut-24'	2.9d	4.4d	5.5a	17.3ab	10.0ab
'Samnut-25'	6.1cd	4.8cd	4.9b	16.5a	10.7a
'Samnut-26'	9.9bc	5.1bc	4.1c	14.6b	9.3bc
'Ex-Dakar-R'	9.6bc	5.2bc	4.0c	14.3b	10.7a
'Ex-Dakar-W'	10.1bc	5.1bc	3.8c	13.5b	8.7cd
'Kuta-Red'	13.3b	5.4ab	3.2d	13.8b	7.8de
'Boro-White'	7.6c	5.4ab	3.0de	12.0e	7.5def
'Boro-Red'	21.0a	5.2bc	2.8ef	10.7d	6.4fg
'Kwankwaso'	9.9bc	5.6a	2.5f	9.6e	7.0ef

Mean with same letters in the same column are not significantly different at p<0.05 according to DMRT
GRI = Germination Rate Index; GI = Germination index; SVI = Seedling Vigour Index;
Gloss = Germination loss MGT = Mean germination time

Interaction between groundnut genotypes and ageing duration on germination loss

The response of the groundnut genotypes to accelerated ageing duration as recorded by the germination loss showed that, there was a sharp increase in germination loss for 'Samnut-21' at the ageing duration of 0 to 3 hours, while 'Boro-white' has a steady germination loss till 6 hours of ageing. There was no significant germination loss after 3 hours of ageing for 'Samnut-24', after which, there was a steady low germination loss till 24 hours ageing. All the genotypes recorded varied level of germination loss as the ageing period increases to 24 hours (Figure 2).

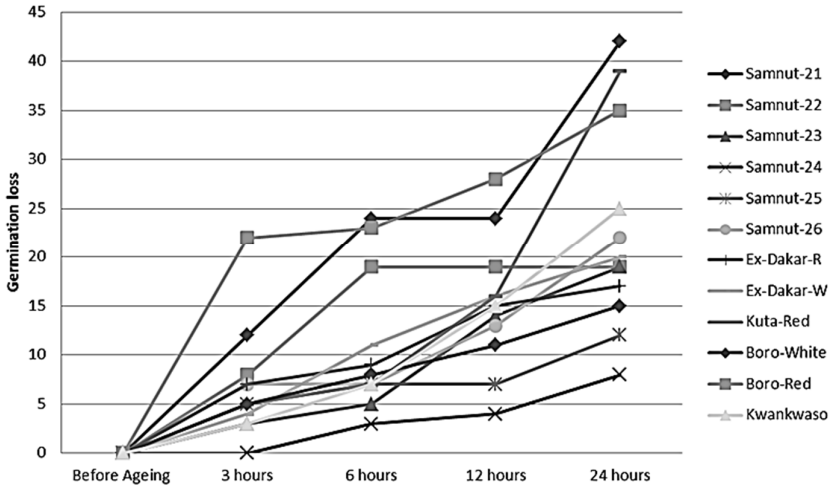

Figure 2. Interaction between groundnut genotypes and accelerated ageing duration on germination loss

Degree of relationship among groundnut varieties based on seed germination loss and seedling vigour characters after 24 hours of accelerated ageing:

The dendrogram drawn from the Single Linkage Cluster Analysis (SLCA) illustrating the relationship between the 12 genotypes based on seed germination loss and seedling vigour characters after 24 hours of accelerated ageing shows that seed of all the groundnut genotypes were different from each other at minimum distance of 0.00% level based on their reaction to ageing stress. Two main clusters (A and B) were identified among the groundnut genotypes at 16% distance level while four groups are distinguishable between 8% and 12% distance level. Group 1 comprises of five genotypes ('Boro white', 'Samnut 22', 'Kuta Red', 'Samnut 23' and 'Kwankwaso') which are less tolerant to ageing stress. Both group 2 ('Boro Red', 'Samnut 21') and 3 ('Samnut 26', 'Ex-Dakar White') has 2 genotypes each that are moderately tolerant to ageing stress. Group 4 has 3 genotypes ('Samnut 24', 'Samnut 25', 'Ex-Dakar Red') that are more tolerant to ageing stress (Figure 3).

Discussion

High temperature and relative humidity are among the major storage stresses that confronts seed during storage. Accelerated ageing technique has been widely used to access the ability of seeds to resist storage stress. In this study, accelerated ageing duration affected the seed quality of groundnut genotypes. The germination loss recorded in groundnut seed increases as the ageing duration increases. This implied that groundnut seeds are sensitive to changes in storage temperature and relative humidity. Germination percentage and mean germination time of groundnut seed were affected at high RH irrespective of the species.

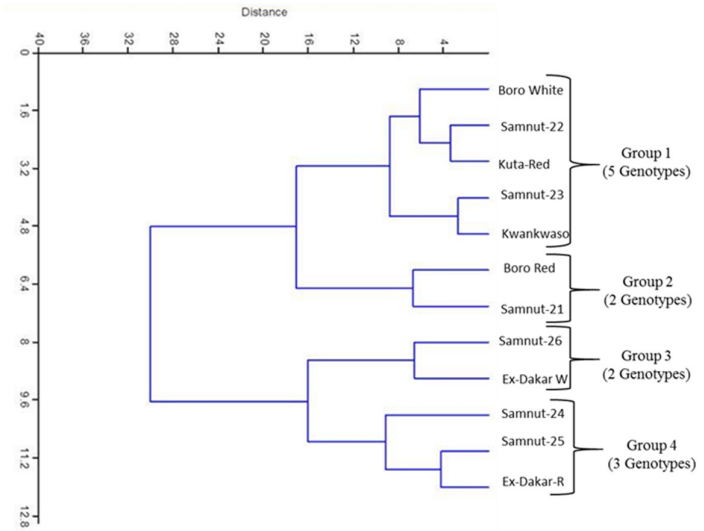

Figure 3. Dendrogram grouping of groundnut genotypes based on germination loss and all seed vigour characteristics after accelerated ageing

A similar report on some oil seed crops *Brassica* spp (Suma *et al.*, 2013) and *Glycine max* (Jagadish *et al.*, 2012) revealed that storage at intermediate relative humidity of about 32% causes minimal decline in viability. The significant variations in the response of genotypes to accelerated ageing in terms of percentage germination loss, seedling vigour index and other seedling germination parameters confirms that seed deterioration in groundnut is inevitable, but responses of the genotypes to ageing factor differs. The variation can be attributed to genetic make-up of the genotypes. Kapoor *et al.* (2010) has reported that varieties of chickpea responded differently with reference to physiological and biochemical changes occurring due to seed ageing. Similarly, Okunlola *et al.*, (2020) attributed significant variation in germination and other seedling vigor parameters recorded under artificial aging treatments to different genetic makeup of the soybean varieties.

Groundnut genotypes with small seed sizes were significantly low in germination loss with high seedling vigour characteristics. This implies that genotypes with small seed size are more tolerant to ageing stress than the other varieties with bigger kernels. Peksen *et al.* (2004) has reported that cultivars of pea (*Pisum sativum* L.) with low 100 seed weight had higher germination percentage than seeds with larger 100 seed weight. Similarly, Rastegar and Kandi (2011) observed that small seeds had better and faster germination than larger ones in soybean. Cluster analysis has been described to have the ability to identify crop genotypes with the highest level of similarity (Aliyu and Fawole, 2000). The cluster analysis identified four groups among the groundnut genotypes with varied level of tolerance to ageing stress. The tolerant genotypes ('Samnut 24', 'Samnut 25', 'Ex-Darka R') clustered together could be linked to their inherent genetic potential to withstand storage stress, while the susceptible genotypes; 'Samnut 22', 'Boro White', 'Kuta Red' might require improvement in their genetic makeup for cultivation among resource poor farmers that might need to preserve seeds under ambient condition.

Conclusions

Groundnut genotypes differ in their response to ageing stress factors. Seeds of 'Samnut-24', 'Samnut-25' and 'Ex-Dakar-Red' were found to be more tolerance to ageing stress and are likely to be easily adopted for production among farmers. Accelerated ageing of 24 hours significantly affected groundnut seed germination and seedling vigour. Therefore, an optimum ageing of about 24 hours is recommended for testing seeds of groundnut varieties for storage tolerance.

Authors' Contributions

Conceptualization: KOSA, TOK and JAA, Laboratory work and data curation: KOSA and JAA, Technical Supervision TOK and MAA, Data analysis and interpretation KOSA, DJO and JAA, Manuscript writing and review: TOK, KOSA, DJO and JAA. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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