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Full Length Research Paper

Genetic variability in seed quality of African yam beans (*Sphenostylis stenocarpa* Hochst. Ex A. Rich Harms)

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African yam bean (AYB) (*Sphenostylis stenocarpa* (Hochst ex. Rich) Harms) is an important food in most tropical African countries where it is consumed as either dry cooked seeds or as tuber. Seed quality properties play a major role in genetic improvement and conservation of AYB, if it will contribute to national food security, and prevention of the looming food crisis. The genetic variability in seeds of some African Yam Beans accessions were selected from southwestern Nigeria, and were estimated by studying the physiological quality, traits using germination test, accelerated ageing test and conducting test. Cluster analysis was conducted on similarity estimates using single linkage method (nearest neighbor). Application of cluster analysis resulted in a dendogram representing the genetic relationship among the accessions. A wide genetic variation was observed among the accessions in seed quality traits such as hundred seed weight, germination percentage, accelerated ageing germination percentage as well as bulk conductivity readings. Overall, standard germination percentage was 60.53±27.56%, while standard germination index was 5.74±0.74%, accelerated ageing germination was 28.65±16.81 days and bulk conductivity recorded 62.24±36.25 $\mu Scm^{-1}g^{-1}$. These results indicate high level of significance ($p<0.01$) for variability especially in viability and vigor of the tested seeds. African yam beans accessions were classified into 4 subgroups with hundred seed weight, germination percentage and seedling quality traits contributing significantly to the grouping underlying the broad genetic base of the accessions. Results showed a high genetic variability among the AYB accessions such genetic variability is useful in facilitating the development of a large number of new genotypes through hybridization by transfer of useful genes, thus maximizing the use of such available genetic potentials in boasting food production for sustainable food security.

Key words: Accession, genetic variability, *Sphenostylis stenocarpa,* cluster group.

INTRODUCTION

African yam bean (*Sphenostylis stenocarpa* (Hochst ex. Rich) Harms) is an important grain legume in tropical Africa especially in countries such as Nigeria, Ivory Coast, Ghana, Togo, Gabon, Congo, Ethiopia and some parts of East Africa, where it is used as food or food components. African yam bean provides two consumable products: the tuber which grows as the root source and the actual yam beans which develop in pods above ground. The crop also helps agriculturally to enrich the soil by its ability to fix nitrogen from the atmosphere. Dry bean is an important source of protein participating in human diet all over the world (Singh, 2001). Although the vast genetic and economic potentials of AYB have been recognized, especially in reducing malnutrition among Africans, however the crop has not received adequate research attention. Till date, it is classified as a neglected underutilized species (NUS) (Biodiversity, 2009). Devos et al. (1980) stressed that the danger of losing essential germplasm hangs over all cultivated food crop species in tropical Africa, especially those that are not receiving research attention. The quality and availability of AYB germplasm is decreasing with time. At one time, Klu et al. (2001) had speculated that the crop was nearing extinction; its inherent ability to adapt to diverse environment (Anochili, 1984; Schippers, 2000) may have been responsible for its continual existence and survival.

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Nevertheless, scientists believe that the genetic resources of AYB may have been undergoing gradual erosion. Analysis of genetic relationships in crop species is an important component of crop improvement programs, as it serves to provide information about genetic diversity. It is also a platform for stratified sampling of breeding populations. For optimal crop production and marketing, it is important to know the genetic identity, uniformity, and purity of the cultivar to be used. Morphological tests, growth chamber tests chemical varietal identification tests, electrophoresis techniques (AOSA, 1991) and DNA fingerprint are among the tests used to distinguish cultivars, and to ensure their genetic integrity. Traditionally, genetic variability analysis in AYB has been done with easily distinguishable phenotypic traits. However, the limitation of physiological and seed quality characters for estimating genetic diversity in African Yam Bean has been faintly demonstrated. There is very little information available on the nature and extent of genetic diversity of Nigerian accessions of African Yam Bean, particularly those that are based on seed quality characters. This information would be valuable for the rationalization of AYB germplasm conservation and utilization in a breeding programme. Therefore, the study was undertaken to evaluate the genetic variability in seed quality of 10 African Yam Bean accessions using laboratory vigor tests.

MATERIALS AND METHODS

Laboratory analysis of seed quality was carried out on 10 selected African yam bean accessions. These were selected from preliminary screening of germplasm collected across Nigeria (Akande, 2007; Akande, 2009). The seed quality test was carried out in the Seed Testing Laboratory of the Institute of Agricultural Research and Training, Obafemi Awolowo University, Ile-Ife, Moor Plantation, Ibadan in 2011. The following seed quality tests were performed.

Standard germination test

Standard germination test was carried out in three replications of 25 seeds in each replicate. Plastic germination bowls were filled with moistened sand and seeds were evenly spaced on the sand and thereafter thinly covered with moistened sand. The bowls were covered with nylon sheets to conserve moisture and kept at ambient temperature (27 to 32°C). Germination counts were made from the 7th to 9th day after planting. At the 9th day, seedling analysis was carried out and the numbers of normal and abnormal seedlings were recorded. Germination was interpreted as the percentage (%) of seeds producing normal seedlings (International Seed Testing Association, 1993).

$$Ger\min ation\ percentage = \frac{Number\ of\ normal\ seedlings}{Total\ number\ of\ seeds\ pl} \quad (1)$$

From the germination data aforementioned, germination index (GI) was calculated for each replicate according to Ajayi and Fakorede (2000) as follows:

$$Ger\min ation\ Index = \frac{\sum (Nx)(DAP)}{Total\ number\ of\ seedlings\ that\ emerged\ on\ the\ final\ day} \quad (2)$$

Where Nx is the number of seedlings that emerged on day x after seeding, DAP is the days after planting.

$$Ger\min ation\ Rate\ Index = \frac{Ger\min ation\ Index}{Ger\min ation\ Percentage\ (0-1\ scale)} \quad (3)$$

Seedling vigor index (SVI): Seedling vigor Index of each accession was calculated by multiplying percent normal germination by the average of the plumule length for each genotype after nine days of germination (Kim et al., 1994) and divided by 1000.

100- Seed weight: Three 100- seed replicates were weighed for each genotype in gram.

Accelerated ageing test

Twenty five seeds were placed on top of the screen inside Accelerated Aging (AA) boxes and 40 ml of distil water was added. Boxes were covered and placed in an AA chamber at 42°C for 72 h. Seeds were weighed before and after being placed in the chamber to calculate seed moisture increase during ageing. Seeds were allowed to pass through germination test as stated earlier. Seedlings were evaluated after 9 days according to AOSA (2002).

Electrical conductivity test

Twenty five clean intact seeds in three replicates were counted, weighed, and placed in a glass flask containing 100 ml of distilled water. The flasks were covered with aluminum foil to prevent contamination and the flasks were gently shaken intermittently. Conductivity measurements were taken after 24 h at 25°C reference temperature using Mettler Toledo MC126 conductivity meter. All measurements were expressed as $\mu Scm^{-1}g^{-1}$ and the results were interpreted, as suggested by Hampton and Tekrony (1995).

RESULTS AND DISCUSSION

All the 10 accessions of AYB studied revealed genetic variability and similarity. The statistical analysis of mean values of all the accessions confirmed variations in all the seed quality characters. Laboratory germination percentages ranged from 52.00 to 86.67% ($P<0.01$) with overall means of 72.00%. The lowest germination percentages were found in NSWSS 50 and NSWSS 57 accessions with 52.00 and 58.67% germination respectively. Germination percentage of the other genotypes were 60.00% and above (Table 1). This finding was in agreement with the earlier work of Olisa et al. (2010) who reported no seed viability problem with AYB. From the results in Table 1, significant differences were observed among some of these accessions for all determined traits, except for accelerated ageing germination index (AAGI). Overall mean germination index was 6 days, the corresponding mean for germination rate index was almost 8.73 days. Some of the accessions

Table 1. Seed standard germination test (SG) and germination after accelerated ageing test (AA) for African Yam Beans.

Cultivar	GCPT	GI (days)	GRI (days)	AAWI	AAGCPT	AAGI (days)	AAGRI (days)
NSWSS 23	60.00^{cd}	6.06^{cd}	10.27^{b}	1.98^{a}	25.00^{b}	5.17	16.94^{bc}
NSWSS70	78.67^{ab}	5.41^{de}	7.02^{d}	1.70^{abc}	48.33^{a}	5.13	10.71^{c}
NSWSS 61	76.00^{abc}	5.09^{e}	6.70^{d}	1.00^{d}	25.00^{b}	5.44	24.45^{b}
NSWSS 1	80.00^{ab}	6.53^{b}	8.17^{a}	1.82^{ab}	43.33^{a}	5.33	13.33^{a}
NSWSS 56	74.67^{abc}	5.49^{cd}	7.35^{cd}	1.23^{dc}	53.33^{a}	5.15	10.22^{c}
NSWSS 45	86.67^{a}	5.88^{cd}	6.19^{bc}	1.63^{abc}	41.67^{a}	5.11	12.96^{c}
NSWSS 57	58.67^{c}	6.00^{bc}	10.53^{b}	1.39^{bdc}	18.33^{b}	5.72	31.76^{a}
NSWSS 96	66.67^{bcd}	6.03^{bc}	9.12^{bc}	1.80^{ab}	15.00^{b}	5.22	37.59^{a}
NSWSS 4	86.67^{a}	6.27^{bc}	7.24^{d}	0.91^{de}	43.33^{a}	5.32	12.43^{c}
NSWSS 50	52.00^{c}	7.28^{a}	14.06^{a}	0.47^{e}	13.33^{b}	5.00	38.89^{a}
Overall Means	72.00	6.00	8.73	1.39	32.67	5.26	20.93
F- Test	**	**	**	**	**	ns	**

Mean in the same column followed by the same letter(s) are not significantly different from each other at $p<0.05$. GCPT-Germination percentage; GI- germination index; GRI-germination rate index; AAWI- accelerated ageing water Imbibed; AAGCPT-accelerated ageing germination percentage; AAGI- accelerated ageing germination index; AAGRI-accelerated ageing germination rate index.

Table 2. Means values for hundred seed weight and seedling vigor traits for African Yam Beans.

Cultivar	HSW	SLT	SDW	SVI
NSWSS 23	27.39^{d}	22.11^{d}	0.07^{bcd}	0.32^{e}
NSWSS70	28.84^{b}	30.84^{a}	0.09^{ab}	2.43^{ab}
NSWSS 61	28.56^{bc}	30.51^{a}	0.09^{ab}	2.32^{ab}
NSWSS 1	26.21^{e}	22.31^{d}	0.08^{abc}	0.25^{e}
NSWSS 56	28.45^{bc}	24.95^{cd}	0.08^{abc}	1.87^{bc}
NSWSS 45	25.21^{f}	27.80^{abc}	0.09^{ab}	2.41^{ab}
NSWSS 57	31.82^{a}	25.79^{bcd}	0.11^{a}	1.52^{cd}
NSWSS 96	23.03^{g}	26.03^{bcd}	0.09^{ab}	1.74^{c}
NSWSS 4	28.04^{c}	29.62^{ab}	0.06^{cd}	2.60^{c}
NSWSS 50	23.24^{g}	22.26^{d}	0.05^{d}	1.16^{d}
Means ±SE	27.07±2.62	26.22±3.79	0.08±0.02	1.65±0.86
F-Test	**	**	**	**

Mean in the same column followed by the same letter(s) are not significantly different from each other at $p<0.05$. HSW- Hundred seed weight; SLT- shoot length; SDW- seeding dry weight; SVI- seedling vigor index.

had 5 to 6 days germination index indicating that it took the seeds of these genotypes five to six days to emerge. It would take the other genotypes above 6 days for their seeds to emerge. All the accessions with the exception of NSWSS 56 recorded accelerated ageing germination percentage of less than 50% which is significantly lower than laboratory standard germination. The results showed that the seed lots were of medium or low vigor and have low storage potentials (ISTA, 1995). Overall mean for accelerated ageing germination rate index was almost 21 days. The implication of this was that for 100% germination it will take all the AYB accessions 21 days to germinate.

Significant differences ($p<0$-01) were observed for 100-seed weight (HSW), shoot length (SLT), seedling dry weight (SDW), seedling vigor index among all the genotypes (Table 2). One hundred seed weight ranged from 23.03 g to 31.82 g. NSWSS 57 had the highest 100-seed weight while NSWSS 96 and NSWSS 50 recorded the lowest 100-seed weight of 23.03 and 23.24 g, respectively. Similar value for hundred seed weight were reported by Togun and Egunjobi (1997) and Togun and Olatunde (1998). Significant seedling vigor index was shown by NSWSS 4 due to its longer shoot length. Seedling vigor index ranged from 1.16 to 2.58 with overall mean of 1.65. The dendogram drawn from single linkage

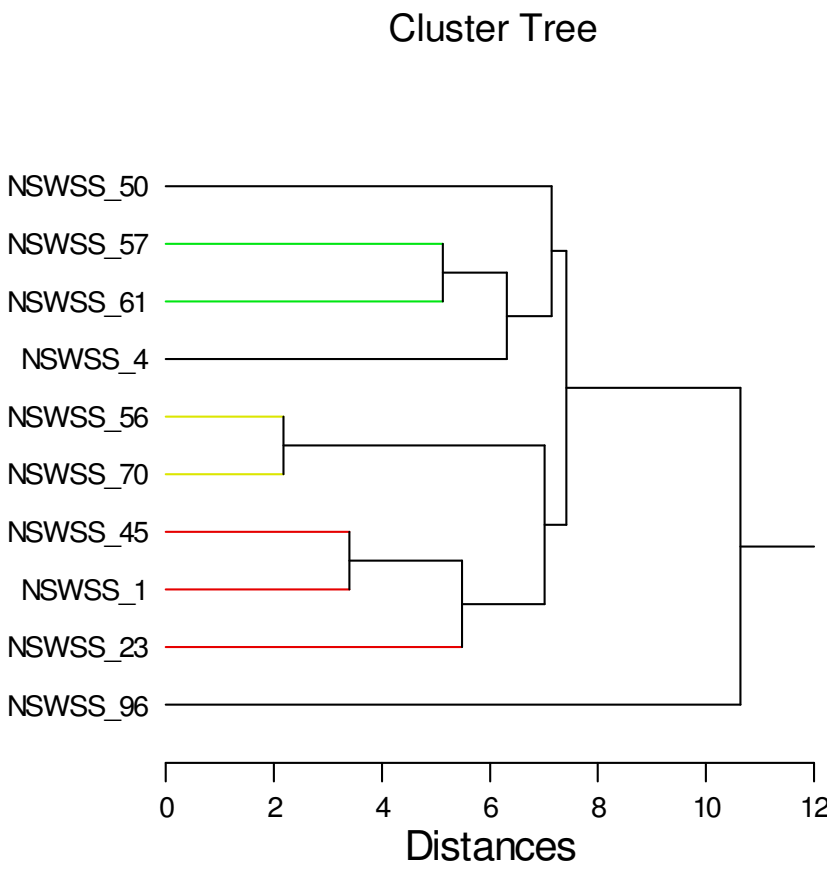

Figure 1. Dendogram showing cluster analysis for the similarity rate of 10 African Yam Bean accessions.

cluster analysis (SLCA) to illustrate the relationship among the 10 genotypes was presented in Figure 1. At a minimum distance of 0.00 level of similarity, all the genotypes were distinct from one another while at a distance 11.0 all the genotypes had formed a single cluster indicating that the genotypes had at least one neighbor with more than 11.0 similarity level. At a distance of 6.0, genotypes NSWSS 56, NSWSS 45 NSWSS 70, NSWSS 57, NSWSS 61, NSWSS 1, NSWSS 4 and NSWSS 23 were most similar to one another forming two different mega groups. At a minimum distance of 11.0 level of similarity, NSWSS 96 which was most distinct from all other genotypes had joined to the other 2 groups forming a total of 3 groups. Member At 0.00 level of similarity some of the traits were distinct while shoot dry weight (SDW), and all the ions leached from the seeds of the genotypes such as potassium (K^+), phosphorus($P_20_5^-$), calcium (Ca^{2+}), sodium (Na^+) and magnesium (Mg^{2+}) were similar (Figure 2). At a level of 4.0, accelerated ageing water imbibed (AAWI) and seedling vigor index (SVI) formed a cluster and linked with the previous one. At the same level, accelerated ageing index (AAEI) and germination index (IDEX) formed a group and joined with the earlier one. At 4.0, seed weight (SEDWT) and seedling length (SLT) formed a group and joined to Accelerated ageing and AAERI at 14.00 level of similarity. At 16.0, GRI joined to the previous group at 20.0

The conductivity values for all the accessions were high (Table 3). NSWSS 50 recorded the lowest conductivity value of 11.24 $\mu Scm^{-1}g^{-1}$. This value could be as a result of hard seed coat that reduced seed exudates due to the impermeable nature of the coat. There were significant differences ($p<0.01$) in conductivity values among the accessions. The conductivity values ranged from 11.24 to 119.45 $\mu Scm^{-1}g^{-1}$ with overall mean of 62.24 $\mu Scm^{-1}g^{-1}$. Seed lots that show high levels of solute leakage are low vigor seeds. Conductivity values in the range of 30 to 43 $\mu Scm^{-1}g^{-1}$ indicates that the seed is unsuitable for sowing under adverse condition and when it is more than 43, it is not good at all for planting (Hampton and TeKrony, 1995). From the result, only 4 of the accessions are moderately good for planting. From the study, only 4 out of the 10 accessions were in agreement with the aforementioned findings. With respect to mineral ions leakage, NSWSS 70 had the highest K^+ ion concentration percentage with moderate conductivity value. NSWSS 57 had the lowest K^+ ion concentration percentage with

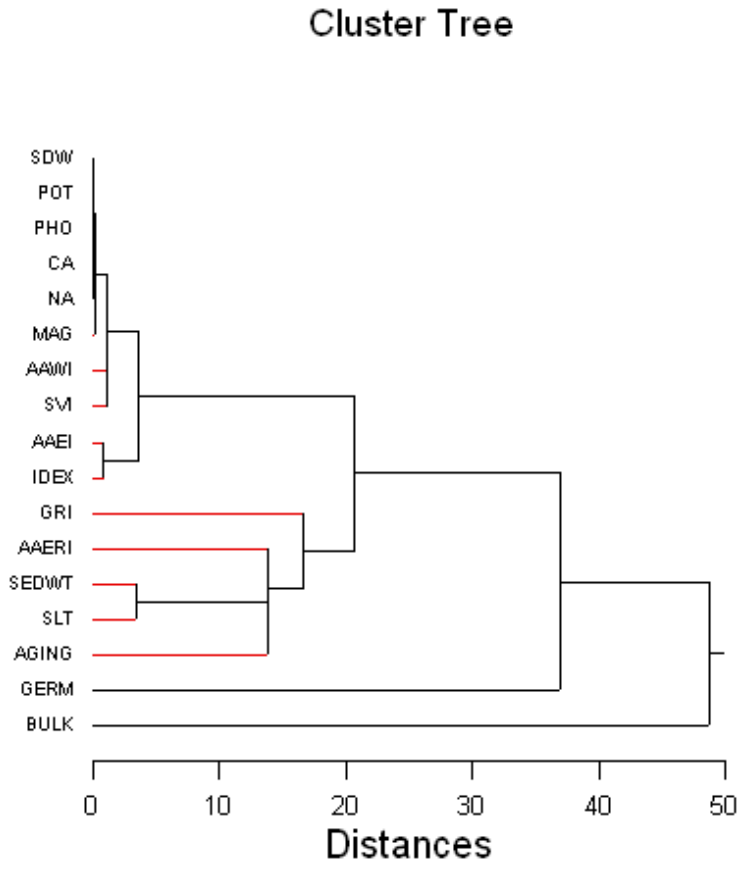

Figure 2. Dendogram showing cluster analysis for the similarity in seed quality traits of 10 African Yam Beans accessions.

Table 3. Conductivity and amount of mineral ions leaked in after 24 h soaked in water for African yam beans.

Cultivar	Conductivity (µS/cm/g)	Mineral ions (%)				
		Na^+	Mg^+	Pho	Ca^{2+}	K^+
NSWSS 23	103.22^{ab}	0.04^{d}	0.20^{e}	0.08^{b}	0.09^{ab}	0.08^{bcd}
NSWSS70	56.65^{c}	0.15^{a}	0.38^{ab}	0.12^{a}	0.13^{a}	0.12^{a}
NSWSS 61	36.71^{cd}	0.12^{ab}	0.21^{e}	0.10^{ab}	0.10^{ab}	0.08^{abcd}
NSWSS 1	93.00^{b}	0.13^{ab}	0.31^{bcd}	0.10^{ab}	0.11^{a}	0.10^{abc}
NSWSS 56	54.72^{c}	0.11^{b}	0.25^{de}	0.09^{ab}	0.07^{bc}	0.05^{d}
NSWSS 45	83.09^{b}	0.09^{bc}	0.34^{abc}	0.10^{ab}	0.12^{a}	0.08^{abcd}
NSWSS 57	27.69^{de}	0.09^{bc}	0.27^{cde}	0.03^{c}	0.04^{c}	0.02^{e}
NSWSS 96	119.45^{a}	0.07^{c}	0.41^{a}	0.10^{ab}	0.12^{a}	0.09^{abc}
NSWSS 4	36.68^{cd}	0.13^{ab}	0.20^{e}	0.11^{ab}	0.11^{a}	0.10^{abc}
NSWSS 50	11.24^{e}	0.04^{d}	0.35^{abc}	0.08^{ab}	0.11^{a}	0.07^{cd}
Overall Means	62.24	0.10	0.29	0.09	0.10	0.08
F-Test	**	**	**	**	**	**

Mean in the same column followed by the same letters are not significantly different from each other at P<0.05.

corresponding lower conductivity reading forming a cluster. At 38.0, GERM joined to the other group while at 50.0, BULK formed a single cluster with the other groups. Each cluster contained AYB accessions that were highly significant. Members of cluster 1 had the lowest values for germination index and high germination rate index.

The amount of water imbibed during artificial ageing was also the highest as compared to other clusters. Hundred seed weight, shoot length, seedling dry weight and seedling vigor were similar except for NSWSS 23 and NSWSS 1 that recorded seedling vigor index of 0.32 and 0.25, respectively.

The only member of cluster 2 (NSWSS 45) had the highest germination percentage with accelerated ageing germination percentage of 41.67. The conductivity value was also high. Members of cluster 3 had all the measured characters not statistically different from one another. The results showed that members of each cluster had features distinct from the others which indicate variability among clusters. This knowledge of existing variation of quality characters in the germplasm is essential for developing high yielding genotypes (Natarajan et al., 1990).

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