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Physiological Quality of Seeds of Promising African Yam Bean (*Sphenostylis stenocarpa* (Hochst. Ex A. Rich) Harms) and Pigeon Pea (*Cajanus cajan* L. Mill sp.) Landraces

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Abstract: Pigeon pea and African yam bean are underutilized but important crops for household food security that are grown for consumption and rarely for commercial purposes. One of the major problems that have limited the cultivation of these crops is poor field establishment. Scaling up production and consequently the benefit of these crops requires rapid and uniform emergence and establishment. The study was undertaken to assess physiological quality of 6 selected promising landraces each from several collections of African yam bean and pigeon pea germplasm in Nigeria. Traits used to assess quality were 100 seed weight, standard germination test, bulk conductivity, seedling vigour and amount of mineral ion leaked by fully imbibed seeds after a 24 h soak in distilled water. Irrespective of species, selections showed no apparent seed viability problem, overall mean germination percentage being 81.78% for pigeon pea and 79.56% for African yam bean. The corresponding germination rate indices were 3.28 and 4.63 days, respectively. However, the mean bulk conductivity values were 84.76 for pigeon pea and 48.23 μS/cm/g for African yam bean and these values indicate that seeds of all the lines had low vigour and by inference were unsuitable for sowing. Thus, the poor field establishment problems associated with the crops are vigour and not viability problem. Results were discussed in the context of what areas of further research need to be explored in order to address constraints that have made these crops underutilized.

Key words: Dormancy, vigour, conductivity, emergence, underutilized

INTRODUCTION

Increasing population, high prices of staples food items and policy constraints on food importation are worsening the food security in developing countries where protein deficiency malnutrition is predominant (Weaver, 1994; FAO, 1994, 2008). In order to meet the increasing gap in the provision of balanced food for the growing population of developing countries, attention is now being paid to lesser-known crops that have played major roles in the livelihoods of subsistent rural farming families (Ezeagu *et al.*, 2002). Among these crops are African yam bean (*Sphenostylis stenocarpa* Hoechst, Exa. Rich Harms) and pigeon pea

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(*Cajanus cajan* L. Mill sp.). They are grown for household consumption and rarely for commercial purposes in Nigeria (Saka *et al.*, 2004) despite their great potentials to meet adequate nutrition requirements. Such plants have variedly been referred to as under-exploited, under-utilized, orphan or neglected (Jaenicke *et al.*, 2009). One of the major problems that has limited the cultivation of these crops is poor field establishment. Scaling up production and consequently the benefit of these crops requires rapid and uniform emergence and establishment which will also improve the competitiveness of the resultant seedlings against weeds (Qiu *et al.*, 1995).

Rapid germination is a function of inherent seed quality which in turn is the sum total of all seed properties that affect its performance on farmers' fields (Hampton, 2002). Seed viability and vigour describe different aspects of seed quality. While viability is the property of the seed that enables it to germinate under favourable conditions in the absence of dormancy (Basu, 1995) seed vigour is measured as germination or emergence under stress (Gallagher and Fuerst, 2006). Following physiological maturity, seeds deteriorate thereby making the loss of seed vigour inevitable. Deteriorated seed often leak more mineral ions and have high conductivity value during testing (Dornbos, 1995; Powell, 2006). The amount of mineral ions leaked out, most especially of K^+, is a good measure of physiological quality of a seedlot (Miguel and Marcos-Filho, 2002; Ajayi, 2003). Seeds with high conductivity value often exhibit weak germination and reduced growth rate of the emerging seedling (Panobianco *et al.*, 2007). This automatically results in uneven plant stand in the field. High vigour seed lots tend to imbibe moisture slowly and germinate rapidly and establish fast before the seedbed deteriorate with time (Powell, 1988). On the other hand, low vigour seeds imbibe rapidly. Rapid water uptake may lead to embryonic cell damage that can negatively affect germination and growth (Hahalis *et al.*, 1996). This may consequently lead to reduction in seedling emergence as a result of what is known as imbibition damage (Asiedu *et al.*, 2000; Powell, 2006). Therefore, the interaction of seed quality and the seedbed environment is an important factor that influences seedling emergence and subsequent establishment.

In this study, the relationship among seed quality and seed germination characteristics in African yam bean and pigeon pea were assessed in order to promote global cultivation and consequently arrest the rapid disappearance of indigenous crops.

MATERIALS AND METHODS

Planting Materials

Six promising cultivars each of African yam bean and pigeon pea were used for the experiment. These were the ones selected from preliminary screening of germplasm collected across the country (Akande, 2007, 2009).

Seed Quality Tests

Seeds of the cultivars were subjected to seed quality tests. Standard germination was used to assess viability while speed of germination, seedling vigour, bulk conductivity test and the type and amount of mineral ion leaked into solution after a 24 h soak was used to assess vigour. Bulk conductivity has been widely reported (Black *et al.*, 2006; Powell, 2006) as a sensitive test for assessing the vigour of legume seeds. The tests were performed as follows:

Standard Germination Test

Twenty-five seeds replicated three times for every cultivar were planted in moistened sharp sand substrate in a germination tray. First germination count was taken on day 3 and final count on day 10 after planting. Germination was assessed as the percentage of seeds producing normal seedlings as defined in the handbook of seedling classification (International Seed Testing Association, 1993). Parameters such as germination percentage (GPCT), Germination Index (GI) and Germination Rate Index (GRI) were calculated from germination data as follows:

$$\text{GPCT} = \frac{\text{Total No. of seedings that emerged on the final count}}{\text{Total No. of seeds planted}} \times 100$$

$$\text{GI} = \frac{\sum (\text{Nx})(\text{DAS})}{\text{Total No. of seedings that emerged on the final count}}$$

where, Nx is the number of seedling that emerge on day x after planting, DAS is day after planting.

$$\text{GRI} = \frac{\text{Germination index}}{\text{Germination percentage } (0-1 \text{ scale})}$$

From the normal seedlings, five samples were taken from each replicate for the measurement of shoot length (SLT) as suggested by Ajayi and Fakorede (2000).

Bulk Conductivity Test

The leakage of electrolytes was monitored by placing a pre-weighed bulk of seeds in 100 mL distilled water for 24 h at 25°C. The conductivity per gram of seed weight for subsample was measured after 24 h with conductivity meter MC 126 (Mettle Toledo, Gmbh Schwerzenbach, Switzerland) and expressed as μS/cm/g as described by Hampton and TeKrony (1995).

$$\text{Conductivity} = \frac{\text{Conductivity for each flask-Conductivity of water}}{\text{Initial weight (g) of seed sample}}$$

At the end of the conductivity test, the seeds were carefully blotted dried without applying pressure and the weight of the seeds was taken in order to determine the amount of water imbibed by the seeds as a percentage of the initial weight of the seeds.

Mineral Ions Determination

After conductivity measurements had been taken, the amounts of K^+ and Na^+ in the aliquot were determined using Digital Flame Analyser Model 2655-00 (Cole-Parmer Instrument Company Chicago, Illinois 60061). Ca^{2+}, Mg^{2+} and Mn^{2+} were determined using Atomic Absorption Spectrophotometer Model Alpha 4 (Chemtech Analytical, United Kingdom).

RESULTS

The pigeon pea cultivars were generally more divergent in hundred seed weight, germination characteristics and shoot length (Table 1) than the African yam bean cultivars (Table 2). Significant differences ($p<0.05$) were observed for 100 seed weight (HSW), germination percentage (GPCT) and shoot length (SLT) among the pigeon pea landraces.

Table 1: Mean values for hundred seed weight, germination parameters and shoot length for pigeon pea cultivars

		Germination and seedling characteristics			
Cultivars	HSW	GPCT	GI	GRI	SLT
NSWCC 4	9.70	70.67	3.20	14.59	19.71
NSWCC6	10.88	90.67	3.13	13.04	19.57
NSWCC18	9.96	88.00	3.04	13.25	18.53
NSWCC 27	11.12	81.33	3.72	16.68	20.25
NSWCC29	8.34	76.00	3.37	16.34	18.53
NSWCC32	10.88	84.00	3.23	14.71	21.69
LSD	0.75	12.58	0.45	2.79	1.84
Mean±SE	10.15±0.25	81.78± 2.13	3.28±0.07	14.77±0.46	20.01±0.29

HSW: 100 seed weight (g), GPCT: Germination percentage, GI: Germination index (days), GRI: Germination rate index (days), SLT: Shoot length (cm)

Table 2: Mean values for hundred seed weight, germination parameters and shoot length for African yam bean cultivars

		Germination and seedling characteristics			
Cultivars	HSW	GPCT	GI	GRI	SLT
NSWSS23	25.89	78.67	4.48	20.90	24.79
NSWSS61	26.99	72.00	4.54	20.67	29.35
NSWSS 56	24.47	84.00	4.72	19.98	25.62
NSWSS57	25.88	88.00	4.39	18.31	27.98
NSWSS45	26.24	76.00	5.02	23.89	32.84
NSWSS70	26.61	78.67	4.64	21.07	29.03
LSD	1.03	14.41	0.62	3.71	2.28
Mean±SE	26.01±0.23	79.5± 1.94	4.63±0.08	20.8 ±0.56	28.2±0.69

HSW: 100 seed weight (g), GPCT: Germination percentage, GI: Germination index (days), GRI: Germination rate index (days), SLT: Shoot length (cm)

Table 3: Conductivity and amount of mineral ion leaked after 24 h soak in water for pigeon pea cultivars

		Mineral ions (ppm)			
Cultivars	Conductivity (µS/cm/g)	Na^+	K^+	Mg^{2+}	Ca^{2+}
NSWCC 4	88.44	0.08	33.68	1.05	0.06
NSWCC6	82.88	0.09	25.92	0.58	0.02
NSWCC18	117.20	0.09	46.66	0.72	0.03
NSWCC27	58.43	0.08	22.77	0.32	0.08
NSWCC29	86.95	0.14	30.93	0.19	0.06
NSWCC32	74.67	0.08	41.77	0.22	0.11
LSD	21.81	0.06	15.75	0.30	0.11
Overall mean	84.76	0.09	33.62	0.51	0.06

One hundred seed weight ranged from 8.34 to 11.12 g while germination percentage ranged from 70.67 to 90.67 with an overall mean of 81.78%. While overall mean germination index was 3.28 days, the corresponding mean for growth rate index was almost 15 days. Cultivar NSWCC6 had the highest germination percentage and a hundred seed weight value that was not significantly different from the highest absolute value. Statistically, the corresponding germination and germination rate indices were also the least. Of the six African yam bean cultivars, one hundred seed weight of four cultivars, germination indices of five cultivars and germination percentages of all the six cultivars were not statistically different ($p>0.05$). However, wide variability was observed for shoot length of the cultivars ranging from 24.79 to 32.84 cm with an overall mean of 28.27 cm.

The overall mean conductivity value for pigeon pea cultivars was very high because five of the cultivars had very high values with only one, NSWCC27, having a moderate value of 58.43 $\mu S\ cm^{-1}g^{-1}$ (Table 3). There were no significant differences among all the cultivars in the amount of Na^+ and Ca^{2+} leaked during the conductivity test. Significant differences in

Table 4: Conductivity and amount of mineral ion leaked after 24 h soak in water for African yam bean cultivars

Cultivars	Conductivity (µS/cm/g)	Mineral ions (ppm) Na$^+$	K$^+$	Mg^{2+}	Ca^{2+}
NSWSS23	57.99	0.04	21.51	0.25	0.02
NSWSS61	56.28	0.05	29.73	0.33	Negligible
NSWSS 56	58.26	0.05	18.04	0.56	Negligible
NSWSS57	27.17	0.03	11.18	0.17	Negligible
NSWSS45	41.78	0.04	17.17	0.22	0.11
NSWSS70	47.89	0.04	18.45	0.33	0.14
LSD	10.95	0.03	10.37	0.13	0.03
Overall mean	48.23	0.04	19.35	0.31	0.0009

Table 5: Percentage of the total sum of squares associated with the different sources of variation for 100 seed weight, conductivity, mineral ions and germination parameters and shoot length

Source of variation	GPCT	GI	GRI	HSW	COND	SLT	K$^+$	Mg^{2+}	Ca^{2+}	Na$^+$
Pigeon pea										
Replication	5.97	3.68	9.62	6.11	1.72	2.09	4.43	6.60	22.59	5.88
Cultivar	58.26	56.35	53.67	85.26	78.23	60.28	59.96	80.66	23.79	35.26
Error	35.77	40.01	36.71	8.63	20.05	37.63	35.61	13.21	52.70	58.18
African yam bean										
Replication	2.86	3.94	4.39	11.94	0.86	4.26	20.91	8.33	31.51	7.56
Cultivar	42.70	36.89	52.01	68.59	85.33	85.06	50.00	77.78	32.36	11.43
Error	54.43	59.07	43.59	19.47	13.81	10.68	29.09	13.89	35.99	81.26

the amount of K$^+$ and Mg^{2+} were detected among the cultivars and the pattern of the amount of K$^+$ was similar to that of the conductivity test values. In absolute terms, the cultivars with the least and the highest conductivity values also had the corresponding least and highest amount of leaked K$^+$. The conductivity values for all the African yam bean cultivars were moderately high and some cultivar-to-cultivar differences were significant ($p<0.05$), mainly between of NSWSS57 and the other 5 cultivars on the one hand and between NSWSS45 and each of NSWSS23, 61 and 56 (Table 4). While mean differences for Na$^+$ and Ca^{2+}were negligible, differences among the cultivars for K and Mg^{2+} were significant. The partitioning of the observed variability in the analysis of variance revealed that cultivar differences accounted for more than 65% of observed variabilities in one Hundred Seed Weight (HSW), conductivity and the amount of Mg^{2+} across species (Table 5).

DISCUSSION

Selection for high seed quality should be an integral part of varietal breeding in order to guarantee intrinsic ability of seeds of the variety to withstand non-uniform field conditions and germinate uniformly, rapidly, timely and produce a vigorous seedling that will ultimately become a productive mature plant. However, it is rarely the case in practice. High quality, particularly physiological quality that deals with viability and vigour is often taken for granted once seeds germinate. The growing global interest in promoting the cultivation and widespread utilization of underutilized but nonetheless useful plants, among which are many African indigenous food crops and majority of which are still growing uncultivated in the wild or at best in homesteads, needs to be supported with research on the agronomy of the species. This is expected to facilitate the integration of such plants into existing farming systems.

The evaluated cultivars were not only promising in terms of their yield potential which was the basis for their selection, but also showed no apparent problem with seed viability and speed of germination. The possibility of enhancing the germination characteristics of pigeon pea cultivars through selection was also demonstrated because seed weight was a cultivar characteristic and cultivars with higher seed weight tended to have higher germination percentage. Therefore, in varietal breeding of pigeon pea, seed weight may be used as a direct selection criterion for both viability and speed of germination. Unlike cultivars and forms characterized with some form of dormancy (Anonymous, 2008) the results of this study suggest that the landraces had no seed dormancy and that rapid and uniform germination considered to be constraints would not be a critically limiting factor if the cultivars are eventually released as noted varieties or as parental lines for further breeding. However, the considerably higher conductivity values indicate that the seeds were suitable for sowing. Conductivity values in the range 30-43 μS/cm/g indicate that the seed is unsuitable for sowing under adverse conditions and when it is more than 43, it indicate that seed in unsuitable for sowing at all (Hampton and TeKrony, 1995). Unlike the viability characteristics of the cultivars, the low vigour potentials all the cultivars clearly indicates that whatever seedlings result from this seeds will be weak and that if at all yield is produced it will be low. This may explain why the plants have remained minor and subsistent crops to date. Viability as measured by standard germination test in this study was carried out under ideal conditions which is practically unachievable in the field where the interdependence of the triad of inheritance, physiological quality and environment moderate seed performance (Delouche, 2004). Therefore, the slowness in field emergence of only a small percentage of sown seeds that is typically observed in field situations is a vigour problem as Powell *et al.* (1984) had earlier noted.

The elucidation of the factors underlying the vigour problem was beyond the scope of the experimental objectives of this study. The results were only sufficient to note that vigour was cultivar specific as indicated by relatively high proportions of the variability explained by this factor. The amount of K^+ leaked after 24 h soak in water is a good indicator of seed vigour because it is a measure of the integrity and permeability of cellular membranes (Webes and Karssen, 1990; Custódio and Filho, 1997). Marcos-Filho (1998) succinctly demonstrated that the amount of K^+ leaked by seeds was independent of the mineral composition of seeds prior to the test. It is also a plausible explanation for the frequently observed slow and low field emergence because rapid leakage of K^+ prior to the completion of germination may impair the successful completion of the germination process a consequence of which will be no emergence at all. The trend association of the amount of K^+ leaked with conductivity values further validates the use of conductivity as a vigour test and the associated values as a basis for the comparative assessment of the cultivars investigated. The ranking of the seed and seedling vigour of the cultivars by the other indicators namely, leaked amount of Na^+, Mg^{2+} and Ca^{2+} for seed vigour and shoot length for seedling vigour was different from the ranking by the magnitude of both the conductivity values and the amount of K^+ of leaked.

The viability of African yam bean cultivars and the speed of germination, though worse than in pigeon pea, could also be considered as encouraging for a much lesser known and researched crop. There was no apparent trend association between seed weight and viability in African yam bean cultivars because cultivars with significant differences in seed weight had statistically comparable germination percentage. However, seedling shoot length followed the same trend with seed weight and the tendency of bigger seeds to produce more vigorously growing seedlings has been associated with the availability of additional nutrients from the seeds to the seedling even after the completion of the germination process (Hawkins and Cooper, 1979; Bockstaller and Girardin, 1994; Martinelli and de Carvalho, 1999; Ajayi *et al.*, 2005).

The lack of trend relationship between viability and vigour traits in cultivars of both species further reinforces the established fact that seed viability and vigour are under different physiological mechanisms (Dornbos, 1995) and that viability measurements alone are not sufficient indicators of seed vigour. This has noteworthy implications for further improvement of these crops. The similarity of agronomic and botanical classifications of these crops as legumes on the one hand and underutilized on the other do not suggest the possibility of subjecting them to the same selection criteria for any traits that may be targeted for improvement. While viability could easily be improved by selection for higher seed weight in pigeon pea it cannot be done for African yam bean. Since viability and vigour cannot be separated and treated discretely, there is need for a thorough understanding of the underlying determinants of seed vigour on the one hand and of the expression of vigour potential on the other because environment, inheritance and physiological quality are the determinants of seed performance (Delouche, 2004). Plant breeders will avoid a substantial waste of time and efforts that could result in varieties with low vigour seeds if selection incorporates relevant seed quality components early in varietal development programmes. That a seed germinates on time and normally as it was observed for pigeon pea is not enough evidence to assure that the resultant seedling will be established nor eventually grow into a mature productive plant. Therefore, seed vigour assessment prior to sowing is highly imperative and should be included in seed quality standards for commerce in Nigeria in order to protect and assure confidence in the Nigerian seed sector. Sowing of low vigour seeds has been implicated in the substantial loss of yield by peasant farmers (Jan and Randy, 2004; Finch-Savage, 1995).

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