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Imbibition and Response of Pigeon Pea (*Cajanus cajan* L. Mill sp.) and African Yam Bean (*Sphenostylis stenocarpa* (Hochst. ex A. Rich) Harms) Seeds to Scarification

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Abstract: This study was undertaken with the aim of elucidating the role that imbibition process play in causing poor germination of seeds of two underutilized legumes and whether scarification could improve germination percentage. Twenty individual seeds were placed in numbered positions in a germination tray and used to monitor the imbibition process. Similarly, another set of seeds were used for standard germination tests in moist sand as a control for scarified seeds that were tested both in sand and paper substrata. The phases I and II of imbibition lasted 4 and 18 h in pigeon pea and 6 and 24 h in African yam bean, respectively. Threshold water content for germination was 127.47 and 80.84% respectively for pigeon pea and African yam bean seeds despite that African yam bean seeds were about 2.5 times bigger than pigeon pea seeds. In sand substratum, scarified seeds imbibed significantly ($p<0.05$) more water compared with intact seeds in both species. Increase in weight of scarified seeds as a result of imbibition in paper substratum was significantly less than in sand substratum, by 52.32% in pigeon pea and 20.05% in African yam bean. However, relative to intact seeds, germination percentage of scarified seeds was reduced respectively by 13.86 and 29.70%. Similarly, germination of scarified seeds in sand substratum was reduced by 15.83% in pigeon pea and 27.64% in African yam bean compared with paper substratum. Therefore, rapid imbibition was a cause of reduced germination and scarification is not necessary for both species as it accelerated imbibition.

Key words: Viability, germination, threshold water content, water uptake, underutilized species

INTRODUCTION

African yam bean and pigeon pea are important underutilised crops which are grown in traditional farming systems. They play a significant role in household food security as cheap and available sources of protein for farming communities where balanced diet is a luxury. Although the two species used to be very important in the farming systems of resource-limited farmers (Amoatey *et al*., 2000; Kiu *et al*., 2001; Saka *et al*., 2004), there is now a dwindling interest of farmers in cultivating these crops and this has been attributed to,

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among other factors, poor, slow and non-uniform emergence when compared to other more prominent legumes like cowpea and soybean. Seed germination begins when dry seed takes in water and is completed with protrusion of the radicle from the seed coat (Hilhorst *et al.*, 2006). Germination of seeds can also be viewed as a physiological response of seeds to their environment) involving sequential processes such as imbibition of water, activation of enzyme systems, mobilization of food reserves, radicle emergence and finally seedling emergence. The progress of these different stages depends firstly on the availability of water for imbibition and secondly on the structural nature of the seed coat or embryo coverings and their permeability to water and oxygen which are necessary for the completion of steps one and two in the germination sequence.

Imbibition is the essential, first step towards seed hydration required for initiation of biochemical changes that will lead to germination (Bewley and Black, 1994; Asiedu *et al.*, 2000). But the rate of imbibition is often regulated by the nature of seed coat, the seed coat being important as a barrier to entry of water into seeds and at same time a restriction to loss of intracellular molecules that are indispensable for cell development, seedling growth and establishment (Duke and Kakefuder, 1981). However, the impermeability of seed coat to water uptake has been implicated in the delay of germination in some selected crops species (Bewley and Black, 1994) especially legumes (Powell *et al.*, 1984) even when the conditions necessary for germination are favourable. Scarification is a method often employed to break such barrier in order to enhance water uptake for rapid and successful completion of germination process that leads to seedling growth and establishment. Imbibition has also been implicated as a cause of poor seed quality in legumes (Powell and Mathews, 1978). But when imbibition is too fast seed viability and vigour could be reduced through injury inflicted on the cotyledon cells (Powell, 1989). Therefore, an understanding of the events and processes associated with imbibition is crucial to solving germination-related problems in any species.

Scaling up production and consequently the benefit of African yam bean and pigeon pea as food and industrial crops require rapid and uniform germination and establishment which in turn will also improve the competitiveness of the resultant seedlings against weeds (Qiu *et al.*, 1995). However, poor field emergence is a major problem that has constrained the two crops to the category of underutilized species (Van der Maesen, 2006; Akande, 2009). Under laboratory conditions, it was established that some of the landraces of these two crops that were considered to be promising for further varietal development had high viability but low vigour (Olisa *et al.*, 2010). Usually, seeds with low vigour produce weak and unproductive mature plants if at all they are able to produce one (Powell, 2006). Therefore, the problem of inherent low seed vigour need to be solved and this can only be possible if the primary cause is known. It was not clear whether the low vigour was a genetic problem or a physiological one traceable to germinative events like imbibition damage to which many legumes are susceptible (Powell *et al.*, 1984; Powell, 2006). Given the significance of these events to seedling growth and emergence and in order to clearly identify the cause of low vigour in the promising pigeon pea and African yam bean cultivars, this study was designed to investigate the imbibition process as it relates to seed germination in the two species.

MATERIALS AND METHODS

Plant Material

Six cultivars each of pigeon pea and African yam bean were used for this study carried out in 2008. These were the promising ones selected from preliminary screening of germplasm collected across Nigeria (Akande, 2007, 2009).

Water Imbibition Test

Germination trays were filled with uniformly distilled water-wetted sharp sand. Twenty seeds were individually weighed and placed in pre-numbered positions in the tray. The seeds were extracted from the trays and blotted dry without applying pressure at 0.25, 0.5, 1, 2 h and thereafter at 2 h interval up to 24 h then at 48 and 72 h of imbibition. The seeds were re-weighed to determine weight gained as a result of water imbibition and this was expressed as a percentage of the initial seed weight using the following equation below:

$$\text{Percent seed weight increase} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$$

Influence of Scarification on Viability

Following the procedure of Powell (1989), a small area of the coat (approximately 1 mm^2 for pigeon pea and 2 mm^2 for African yam bean) was carefully nicked behind the hilum at the end of radicle from a set of carefully selected seeds of each cultivar that showed no apparent damage and therefore had intact seed coats. Twenty individually pre-weighed seeds for each cultivar were set to imbibe water in moist sand substratum as described above. Simultaneously, another twenty individually pre-weighed seeds were placed on germination papers with the exposed cotyledon in contact with the paper. Germination of intact seed in sand was also carried out as a control for the preceding two treatments and germination percentage for each of the three treatments were calculated as described by Olisa *et al.* (2010). For the three treatments, weight gain as a result of water imbition after 6 h was calculated as described above. The 6th hour was chosen to determine imbibition level because it was the point at which the stage I of the imbibition process had been estimated to be completed in both species.

Statistical Analysis

Analysis of variance was carried out using SAS version 9.1 (SAS, 2003) on the data collected but only means were reported. The means were separated using Tukey's honestly significant difference test. The amount of water imbibed was expressed as percent of original seed weight and plotted against duration of imbibition. In order to identify the inflexion points, that is, maxima and minima points (Clarke, 1970) on the imbibition curve and by inference the different stages of imbibition, differences in the amount of water at each measurement point and the preceding one were also plotted against time on the same graph.

RESULTS

Seed Imbibition

Imbibition of water during the germination of pigeon pea seeds was noticeably triphasic and each phase ended with an inflexion point, first a maximum point at 4 h and then a minimum point at 18 h (Fig. 1). Averaged over the six cultivars investigated, the first phase of imbibition was characterized by an initial rapid and progressively increasing rate of water imbibition for the first 4 h during which time seed weight increased by an across-cultivars average of 72.90% of the initial seed weight. Subsequently and in the second phase, the amount of water imbibed progressively decreased up to the 18th h after the commencement of imbibition. Again, seed weight increased by an additional 54.57%, to a total of 127.47% of the initial weight at the end of phase II. The third phase generally regarded as marking the beginning of growth commenced from the 18th h during which water uptake rapidly increased

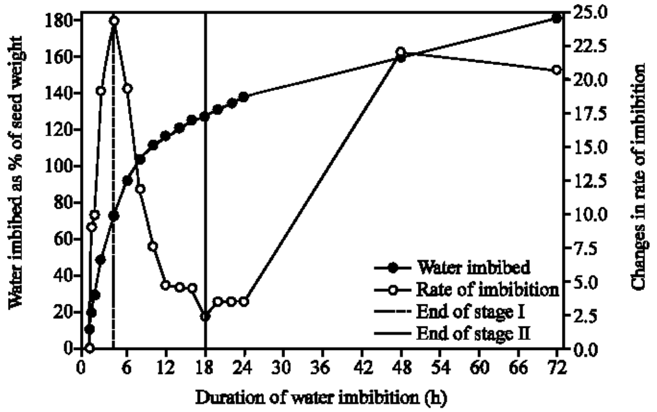

Fig. 1: Pattern and stages of water imbibition in sand substratum by intact seeds of promising pigeon pea cultivars (each point is the mean of 6 cultivars and 20 seeds per cultivar)

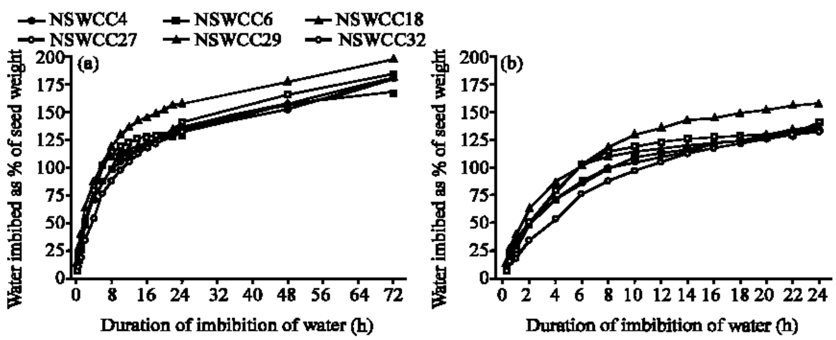

Fig. 2: (a, b) Weight gain during imbibition of intact pigeon pea seeds. Each point is mean of 20 seeds

again. Although the pattern was similar for all the cultivars (Fig. 2a, b), there were differences in the magnitude of seed weight increases as well as in the time each cultivar passed the first two stages of imbibition identified above. While the first phase of imbibition was attained at the 4th h for five of the six cultivars, it was attained at the 6th h for NSWCC 27.

The pattern of imbibition for African yam bean seeds was similar to that observed for pigeon pea seeds (Fig. 3). However, the maximum point occurred at the 6th h and the minimum point at the 24th h after which imbibition increased again. Seed weight increase as a result of water imbibition was 35.75 and 80.84% at the end of the first (maximum point) and second phases (minimum point), respectively. The cultivars exhibited wide divergence for the amount of water imbibed and the duration of each phase (Fig. 4a, b). For NSWSS23, phase I ended and the second one commenced at the 4th h while for NSWSS57 and NSWSS70, it occurred at the 8th and 10th h, respectively.

Germination was first observed and recorded for pigeon pea after 48 h and for African yam bean it was after 72 h (Fig. 5). From the cumulative germination curve, majority of pigeon pea seeds germinated between 48 and 72 h and African yam bean seeds between 72 and 144 h.

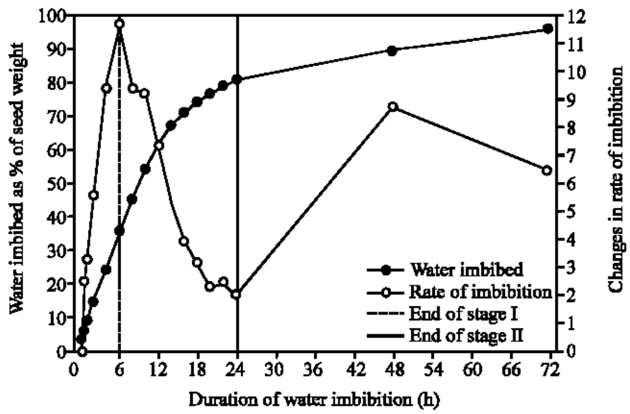

Fig. 3: Pattern and stages of water imbibition in sand substratum by intact seeds of promising African yam bean cultivars (each point is the mean of six cultivars and 20 seeds per cultivar)

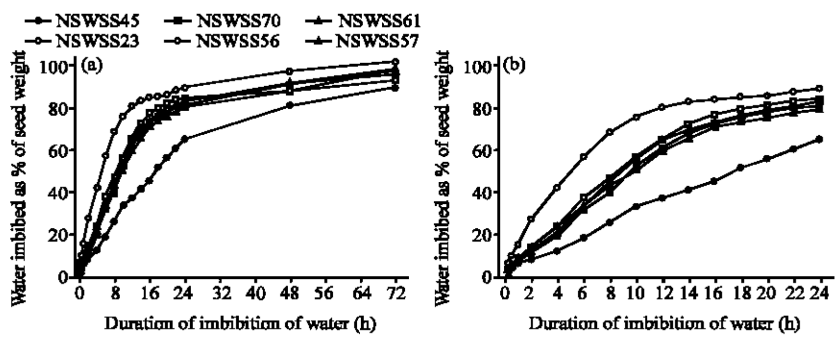

Fig. 4: (a, b) Weight gain during imbibition of intact African yam bean seeds. Each point is the mean of 20 seeds

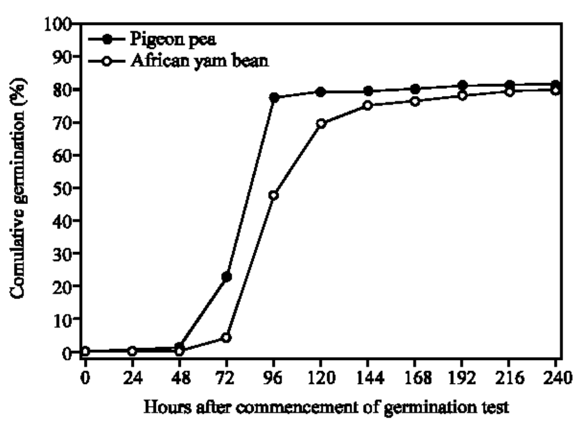

Fig. 5: Cumulative germination percentage overtime averaged over six cultivars of each species

Influence of Scarification on the Seeds Viability

Whether combined for all cultivars or analysed by cultivars of each of the two species investigated, scarification significantly ($p<0.05$) enhanced imbibition of water in sand substratum by an overall mean of 10.20% over intact pigeon pea and 10.54% over intact African yam seeds (Table 1). Increase in weight of scarified seeds as a result of imbibition in paper substratum was significantly less than in sand substratum, 52.32% in pigeon pea and 20.05% in African yam bean.

Across cultivars, germination of scarified seeds in sand substratum was 67.92%, being 13.86% significantly ($p<0.05$) lower than the germination of intact seeds also on sand substratum (Table 2). For scarified seeds, germination on paper substratum was 15.83% higher than on sand substratum. There was also cultivar interaction with the substrata. While germination of NSWCC4 and 6 on sand, with or without scarification, was comparable but significantly lower than germination of scarified seeds on paper substratum, germination of NSWCC18 was significantly different in the order intact seed on sand >scarified on paper> scarified on sand. Germination of NSWSCC32 was comparable in all the three treatments.

Table 1: Weight increase of pigeon pea and African yam bean seeds (intact and scarified) after 6 h of imbibition

	Treatments			
Cultivars	Intact (sand)	Scarified (sand)	Scarified (paper)	Mean
Pigeon pea				
NSWCC4	85.73b	108.67a	33.61c	76.00
NSWCC6	87.26b	96.35a	41.86c	75.16
NSWCC18	101.74b	108.22a	65.23c	91.73
NSWCC27	75.24b	102.19a	54.68c	77.37
NSWCC29	101.66b	109.63a	80.49c	97.26
NSWCC32	101.66a	89.40b	24.72c	71.93
Overall mean	92.21b	102.41a	50.09c	81.57
African yam bean				
NSWSS23	57.08b	62.09a	40.14c	53.10
NSWSS61	34.03b	53.87a	8.69c	32.20
NSWSS56	38.35b	46.56a	27.59c	37.50
NSWSS57	33.43b	37.31a	23.73c	31.49
NSWSS45	18.85c	26.80a	25.43b	23.69
NSWSS70	32.09b	50.46a	31.23c	37.93
Overall mean	35.64b	46.18a	26.13c	35.98

Values in a row with different letters are significantly different at $p<0.05$

Table 2: Germination of pigeon pea and African yam bean in different substrata following scarification

	Germination (%)			
Cultivar	Intact (sand)	Scarified (sand)	Scarified (paper)	Mean across treatment
Pigeon pea				
NSWCC4	70.67b	67.50b	82.50a	73.56
NSWCC6	90.67b	87.50b	95.00a	91.06
NSWCC18	88.00a	5.00c	60.00b	51.00
NSWCC27	81.33b	95.00a	97.50a	91.28
NSWCC29	76.00b	82.59a	90.00a	82.86
NSWCC32	84.00a	70.00a	77.50a	77.17
Overall mean	81.78a	67.92b	83.75a	77.82
African yam bean				
NSWSS23	78.67a	45.00b	63.33ab	62.33
NSWSS61	72.00a	40.00b	83.33a	65.11
NSWSS56	84.00a	72.50b	90.00a	82.17
NSWSS57	88.00a	80.00b	81.67b	83.22
NSWSS45	76.00a	51.67a	66.67a	64.78
NSWSS70	78.67a	10.00b	80.00a	56.22
Overall mean	79.56a	49.86b	77.50a	68.97

Values in a row with different letters are significantly different at $p<0.05$

Both for individual cultivars and across the cultivars, the trends for African yam bean seeds were similar to those of pigeon pea.

DISCUSSION

Seed germination is a key developmental process in the life cycle of plants (Gallardo *et al.*, 2001), a sequential series of events that begin with imbibition of water and culminate in the emergence of radicle from the seed coat. Therefore the success of the entire germination process and of seedling establishment is dependent on the success of the first step, the rate of water uptake (Powell *et al.*, 1984). Furthermore, because legumes generally are characterized with variable seed quality and imbibition of water is a major determinant of vigour (Powell and Mathews, 1978; Powel *et al.*, 1984), an understanding of the viability and vigour problems on any leguminous seed will necessitate an elucidation of the imbibition process. The additional information from these studies will complement the earlier report (Olisa *et al.*, 2010) on the physiological quality of pigeon pea and African yam bean seeds and therefore provide a good basis for developing a breeding programme for the improvement of the species.

Strictly, germination refers to phases I and II of the imbibition process (Bewley and Black, 1994; Bewley, 1997; Bradford, 1990). The duration of each phase depends on seed properties such as size, content of hydratable substances, seed coat permeability and oxygen uptake. In addition to these, the conditions during hydration of the seed such as temperature, moisture levels, substrate availability and composition are also important (Arteca, 1995). Tungate *et al.* (2002) suggested that seed coat may restrict entrance of water and respiratory gases during imbibition and that this may be a major cause of poor germination.

The pattern of imbibition in the two underutilized legumes had two major points, first a maximum point and then a minimum point. At a maximum point, seed weight increase is larger than at points immediately on either side of it and vice versa for minimum point (Clarke, 1970). The maximum point marked the end of the initial rapid water uptake. The second point coincided with the time when germination began (Bewley, 1997). According to Chon *et al.* (2004) the capacity of any seed to germinate depends on what is regarded as the critical water content of the seed at the second point of inflexion and below this water content threshold, germination will not occur. In phase III, the root is exposed and water uptake increases rapidly. However, whether this will be observed as an indefinite progression or a drop, as observed in this study, depends on the amount of water in the substratum. The drop observed between 48 and 72 h does not reflect a declining water requirement by the emerging seedling but a progressive non-availability of water as a result of depletion without replacement of the initial water with which the substratum was wetted. At the third stage, radicle emerged from the seed coat resulting in an accelerated root growth which, in physiological term, marked seedling growth rather than germination *per se* (Bradford, 1990, 1995). With respect to the foregoing therefore, the threshold water content for the germination of pigeon pea seeds was 127.47 and for 80.84% for African yam bean. These thresholds were attained after 18 h of imbibition in pigeon pea and after 24 h for African yam bean. Given that the average 100 seed weight for African yam bean was about 2.5 times that of pigeon pea seeds (Olisa *et al.*, 2010) the amount of water imbibed by pigeon pea seeds over a relatively shorter duration of period of six h as well as at the end of stage II clearly suggest the susceptibility of the seeds to imbibition-related seed quality problems because, with or without scarification, seeds that imbibe very fast as was observed in this study tend to have low vigour as a result of imbibition-induced damages, including death, at the cellular level (Legese and Powell, 1992; Powell, 1988; Hahalis *et al.*, 1996; Asiedu *et al.*, 2000; Powell, 2006). Indeed, Powell and Mathews (1978) asserted that when water enters the cotyledon rapidly during imbibition, it leads to cell death and high solute leakage from the seeds. This then explains the exceptionally high conductivity of pigeon pea seeds reported earlier (Olisa *et al.*, 2010) and it also corroborates similar results on snap bean cultivars (*Phaseolus vulgaris* L.) in which a very high and significant positive correlation was observed between imbibition rate and conductivity values (Balkaya and Odabas, 2002). The germination results for pigeon pea seeds in this study is a further unequivocal evidence of loss of viability in legume seeds as a result of imbibition damage as it has been previously reported by many authors (Powell and Mathews, 1978; Powell, 1988; Legese and Powell, 1992; Hahalis *et al.*, 1996; Asiedu *et al.*, 2000; Powell, 2006). Seeds in sand substratum, whether scarified or not, imbibed more water as imbibition occurred on the entire surface area because seeds were buried in the substratum. That scarified seeds on paper substratum imbibed 52% less water and was able to attain statistically comparable level of germination percentage as intact seeds in sand substratum suggests that pigeon pea seeds require far more less water for seed germination than what was available and than the seeds imbibed in sand substratum. This may explain why the species does well in marginal conditions and is regarded as a one of the most drought resistant crop found in Africa (Rowland, 1993; Van Der Maesen, 2006). Under field situation, the seeds could be expected to imbibe far more water since field soils will hold more water than pure sand used for the test.

African yam bean is a much less researched crop compared with pigeon pea which, nonetheless its underutilized status in Africa, has been subjected to intense breeding elsewhere in the world particularly in India (Van Der Maesen, 2006). It is therefore not surprising that the six African yam bean cultivars exhibited more divergence in imbibition characteristics thereby confirming the results of Moyib *et al.* (2008) that there exist high genetic variability among Nigerian African yam bean accessions. African yam bean seeds imbibed a considerably lower amount of water relative to their weight suggesting a much lower water requirement for seed germination. On average across cultivars, germination appeared impaired when seed weight increase due to water imbibition was above either 35% or below 25% although, cultivar differences were also observed.

The germination of intact seeds of both species was significantly better than that of scarified seeds under similar testing conditions suggesting therefore that even though there is an apparent need for improvement of seed germination percentage above the observed levels in both species, scarification is not necessary. Like many other legumes (Powell *et al.*, 1984), pigeon pea seeds clearly showed sensitivity to imbibition damage and any effort targeted at reducing the rate and speed of water uptake may lead to improvement of germination percentages. In the case of African yam bean it was not clear whether the reduced germination percentage was as a result of low quantity of imbibed water or of the relative slowness of the imbibition process in intact seeds. It is an established fact that even at boiling temperatures African yam bean seeds do not imbibe water easily which therefore prolongs cooking to about 6 h (Onyeike *et al.*, 2005; Moyib *et al.*, 2008). Another factor that needs to be considered in further investigation on the relationship between the imbibition process and germination of African yam bean seeds is the influence of seed coat pigmentation. Powell and associates (Powell, 2006) have severally demonstrated that testa pigmentation is a significant determinant of the speed of water uptake during imbibition in cultivars of common bean, cowpea, chickpea and long bean.

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