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Germination Studies and Early Seedling Growth of *Sphenostylis stenocarpa* (Hochst. Ex A. Rich.) Harms Following Some Pretreatment Protocol for Enhanced Germination

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Abstract

Germination and early seedling growth of *Sphenostylis stenocarpa* were studied to determine the most effective method of breaking the seed coat-dormancy. *Sphenostylis stenocarpa* seeds were treated with concentrated sulphuric acid (H_2SO_4), nitric acid (HNO_3), hydrochloric acid (HCl), nicking and hot water for 5, 10, 15 and 20 minutes. Chemical scarification with concentrated sulphuric acid at 5 seconds significantly ($P<0.05$) enhanced the germination and growth of *Sphenostylis stenocarpa*. The ratio of occurrence of small size (SS) to large size (LS) seeds *Sphenostylis stenocarpa* was 1:4. The small size seed has an average weight of 0.24 ± 0.05g while the large size seed has an average weight of 0.36 ± 0.08g. This study shows that sulphuric acid scarification is an effective method for breaking seed coat-imposed dormancy in *Sphenostylis stenocarpa*

Keywords: Germination, Growth, Pretreatment, *Sphenostylis stenocarpa*

INTRODUCTION

It is widely accepted that temperature regulates both dormancy and germination and that light regulates germination; however, it is a matter of debate whether light is also a regulator of dormancy (Vleeshouwers *et al.*, 1995; Casal and Sanchez, 1998; Pons, 2000; Baskin and Baskin, 2004; Fenner and Thompson, 2005; Kucera, *et al*, 2005). Light has been considered both to stimulate germination (Vleeshouwers *et al*., 1995) and to terminate dormancy (Benech-Arnold *et al*., 2000; Batlla *et al*., 2004).
Underutilized crops are indigenous, relatively common in specific areas, available, accessible, well-adopted, easy and cheap to produce, and culturally linked to the people who use them traditionally (Oniango *et al*., 2006; Okigbo, 1973; Padulosi *et al*., 2003; Jaenicke and Pasiecznik, 2009). Their cultivation and utilization usually draw on indigenous knowledge. The ecotypes and landraces of these species are cultivated less than in the past. They are rarely found in urban markets. They cannot compete with crops which now dominate the world's food. They are hardly represented in ex situ genebanks, so efforts to characterize them depend on the limited available and loosely representative diversity (Padulosi and Hoeschlie- Zeledon, 2004). They are therefore usually ignored by policy makers probably because their economic value is not apparent (Stifel, 1990) and hence are excluded from the development agenda by research institutions. However, underutilized crops are important as household food and their contribution to food security is unquestionably significant (Oniango *et al*., 2006; Naylor *et al*., 2004).

Grain legumes constitute the main source of protein in the diets of the average Nigerian home. The most important ones are cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogea*) and lima bean (*Phaseolus lunatus*). However, there are other pulses that could help meet dietary needs but are cultivated only in localized areas and used less (Klu *et al.*, 2001). These underexploited legumes include African yam bean (*Sphenostylis stenocarpa*), Bambara groundnut (*Vigna subterranea*) and pigeon pea (*Cajanus cajan*)

Sphenostylis stenocarpa is a leguminous crop belonging to the family Fabaceae, sub-family papilonoidae, tribe Phaseoleae, sub-tribe Phaseolinae and genus *Sphenostylis* (Okigbo, 1973; Allen and Allen, 1987). It is most economically important among the seven species of *Sphenostylis* (Porter, 1992) and it is one of the most important tuberous legumes. The domestication, cultivation and distribution of the crop are very evident in the tropics of Africa (Okigbo, 1973; Porter, 1992; Anochili, 1984; Opara and Omaliko, 1997) where it had been reported to exhibit very high diversity. African yam bean is distributed throughout

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most tropical Africa (Anonymous, 1979; Porter, 1992). It is found in forest, open and wooded grasslands, rocky fields as well as marshy grounds, occurring both as a weed and cultivated crop (Duke *et al.,* 1977; Porter, 1992). It grows on a wide range of soils including acid and highly leached sandy soils at altitudes from sea level to 1,950m (Duke *et al.,* 1977; Anonymous, 1979). This work is therefore aimed at determining a highly effective pretreatment protocol for breaking the seed coat-imposed dormancy in *Sphenostylis stenocarpa*.

MATERIALS AND METHODS

Sources and Collection of Seeds

The seeds of African yam bean- *Sphenostylis stenocarpa* (Hochst. ex.A. Rich) Harms were collected from local farmers in Use Offot, and Nsukara Offot in Uyo Local Government Area of Akwa Ibom State. The seeds were identified by Prof. (Mrs.) M. E. Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo. The seeds were extracted from the dried pods. Observation showed that two sizes of seeds and two colours were present, a small size (SS) and a large size (LS), brown and white. The weight of each seed was also determined. Premature and infected seeds were discarded, and selected seeds were taken to the laboratory for preservation and germination studies. Seeds from both sources (Use Offot and Nsukara Offot) were pooled together and the brown colour seeds were used for the studies.

Viability, Germination and Growth Studies

Viability of 3 replicates of 26 seeds was assessed using the tetrazolium chloride (TZ) staining technique (ISTA, 2003). Seeds were placed in 1% tetrazolium chloride solution at 30^{o}C and darkness for 24 hours. Seeds were then cut in half and examined. Only uniformly stained red/dark pink embryos were considered 'viable'.

The seeds were surface sterilized by soaking in 5% sodium hypochlorite (NaOcl), solution for 5 minutes and subsequently rinsed thoroughly with sterilized water prior to applying any treatment. Germination and growth experiments were conducted using three replications of 20 seeds per treatment. Seeds were scarified with each of concentrated Nitric acid (HNO_3), Sulphuric acid (H_2S0_4), Hydrochloric acid (HCl), Nicking and Hot water (100^{o}C) for 5, 10, 15 and 20 seconds. Seeds were placed on double layered Whatman No.1 filter paper moistened with 10ml of distilled water in 15cm diameter Petri dishes. Percentage germination at two days interval, seedling height, leaf area, number of roots, length of longest roots and fresh weight were determined four weeks after the treatments. A seed was considered germinated when the tip of the radicle had pierced through and grown free of the seed coat (Wiese and Binning, 1987; Auld *et al*., 1988).

Germination Studies in the Field

In order to find out the responses of *Sphenostylis stenocarpa* seeds in the field, bush was cleared and ridges made in the Postgraduate Research Farm of the University of Uyo, Uyo. Twenty seeds were surface sterilized by steeping in 5% sodium hypochlorite (NaOCl) solution for 5 minutes and subsequently rinsed thoroughly with sterilized water. Seeds scarified with $HN0_3$, H_2SO_4, HCl, Nicked and treated with Hot water (100^{o}C) for 5, 10, 15 and 20 seconds were planted 15cm apart on ridges. Ridges were weeded as need arose. Seed emergence was recorded daily.

RESULTS

Sampling of Collected Seeds

In all the samples investigated, the ratio of occurrence of small size, (SS) to large size (LS) seed was within the range of 1:4 to 1:6 (Table 1). The small size seed has an average weight of 0.24 ± 0.05 g while the large size seed has an average weight of 0.36 ± 0.08g. Both types were brown in colour and the mixed population was used for the study.

Germination and Growth Studies

The results of germination and growth studies are presented in Figures 1 to 6.

In Figure 1, sulphuric acid and hydrochloric acid significantly ($P < 0.05$) enhanced the percentage germination of the seeds at 5 and 20 seconds respectively. In Figure 2, sulphuric acid significantly ($P<0.05$) increased seedlings height at 5 seconds. Figure 3 showed that hydrochloric acid significantly ($P <0.05$) increased leaf area at 20 seconds. Figure 4 show that hydrochloric acid significantly ($P <0.05$) increased number of roots at 20 seconds. Figure 5 show that sulphuric acid significantly ($P< 0.05$) increased the length of longest roots at 20 seconds. Hot water significantly ($P< 0.05$) increased fresh weight at 10 seconds (Figure 6).

Table 1: The ratio of small and large size seeds of *Sphenostylis stenocarpa*

Total of five collections	Small seeds (SS)	Large seeds (LS)	Ratio of SS:LS
69	10	59	1:6
61	13	48	1:4
46	7	39	1:6
63	13	50	1:4
60	12	48	1:4

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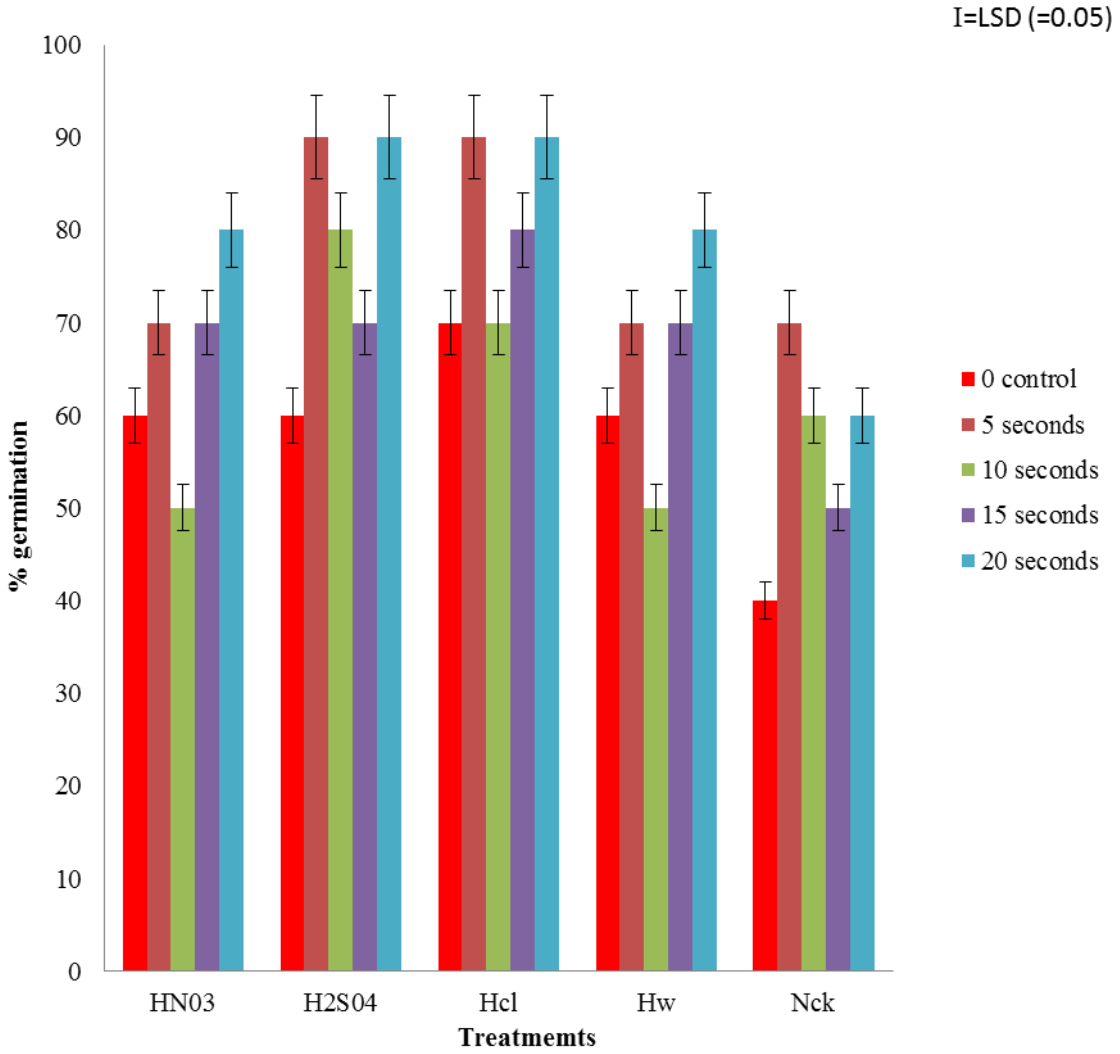

Figure 1: Effects of scarification, hot water and nicking on germination of *Sphenostylis stenocarpa*

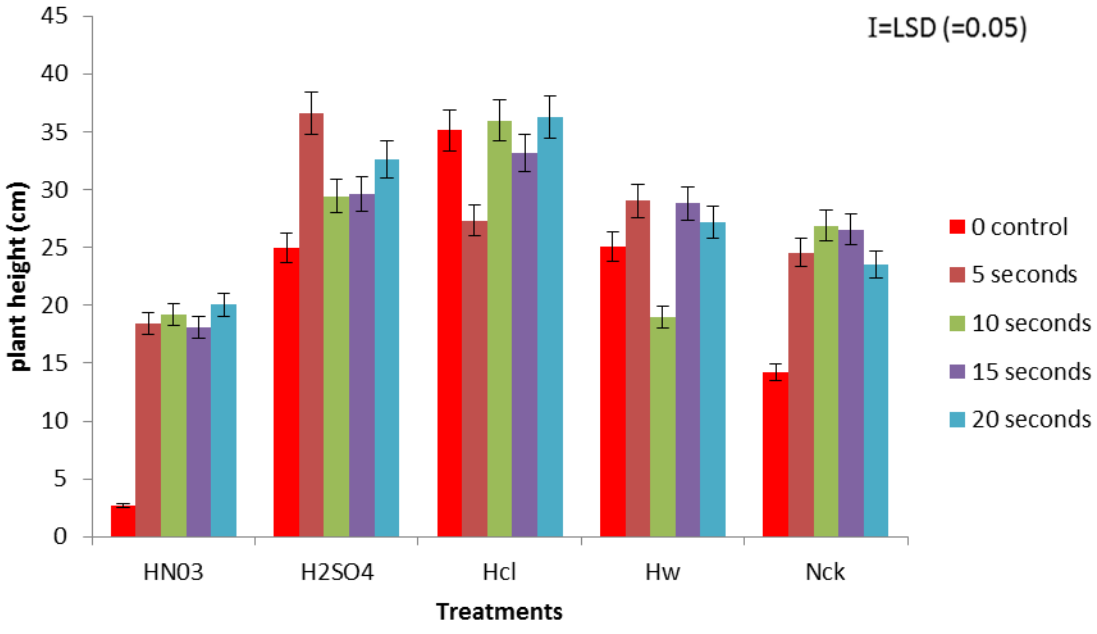

Figure 2: Effects of scarification, hot water and nicking on seedlings height of *Sphenostylis stenocarpa*

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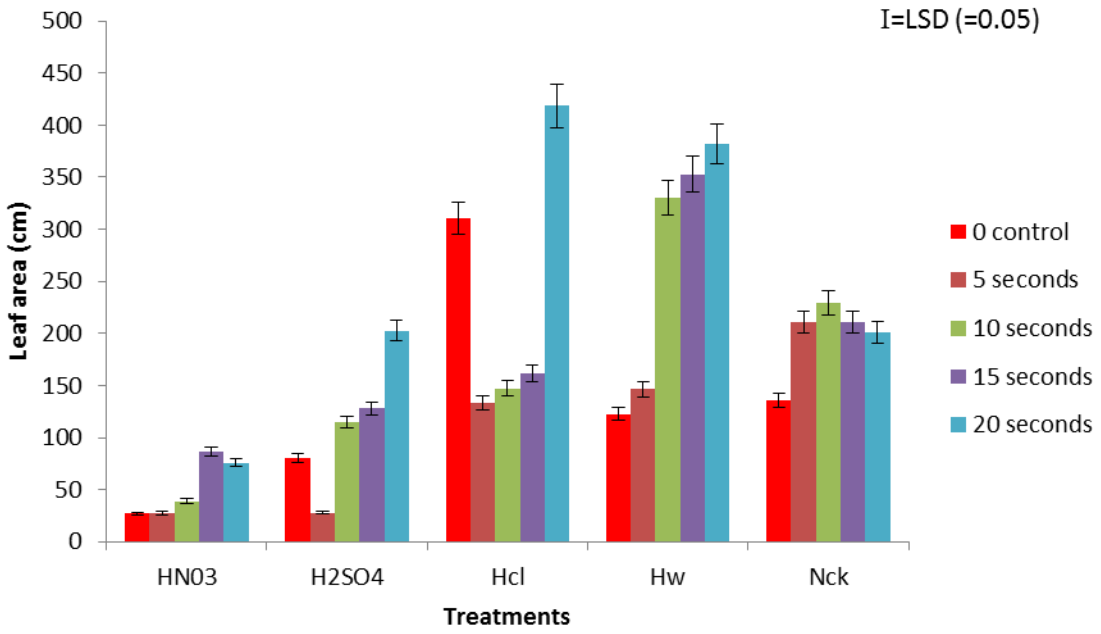

Figure 3: Effects of scarification, hot water and nicking on leaf area of *Sphenostylis stenocarpa*

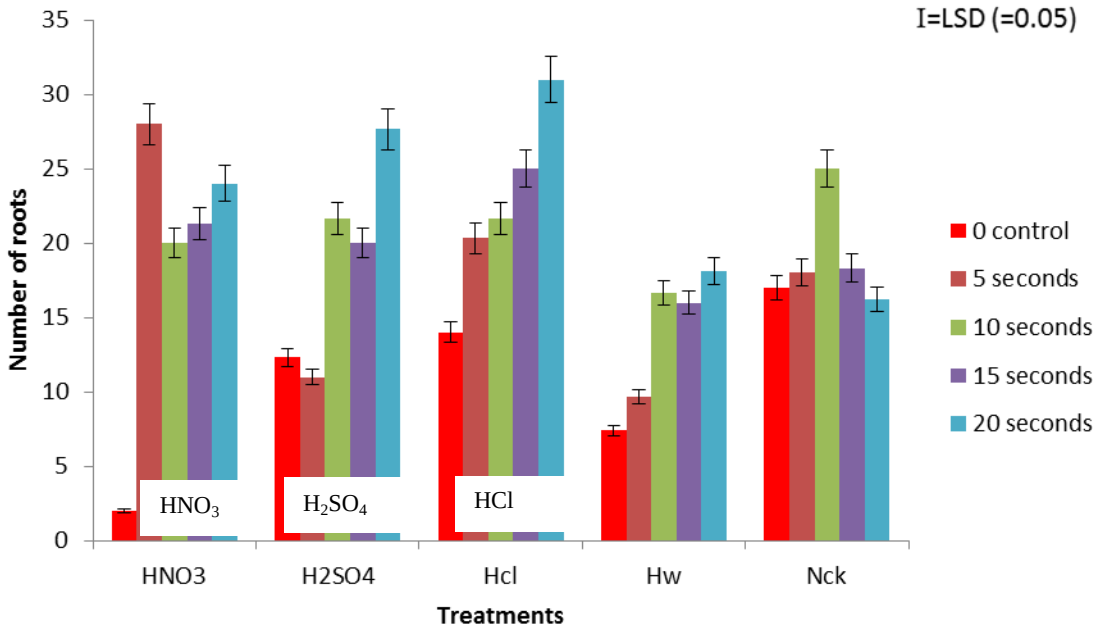

Figure 4: Effects of scarification, hot water and nicking on number of roots of *Sphenostylis stenocarpa*

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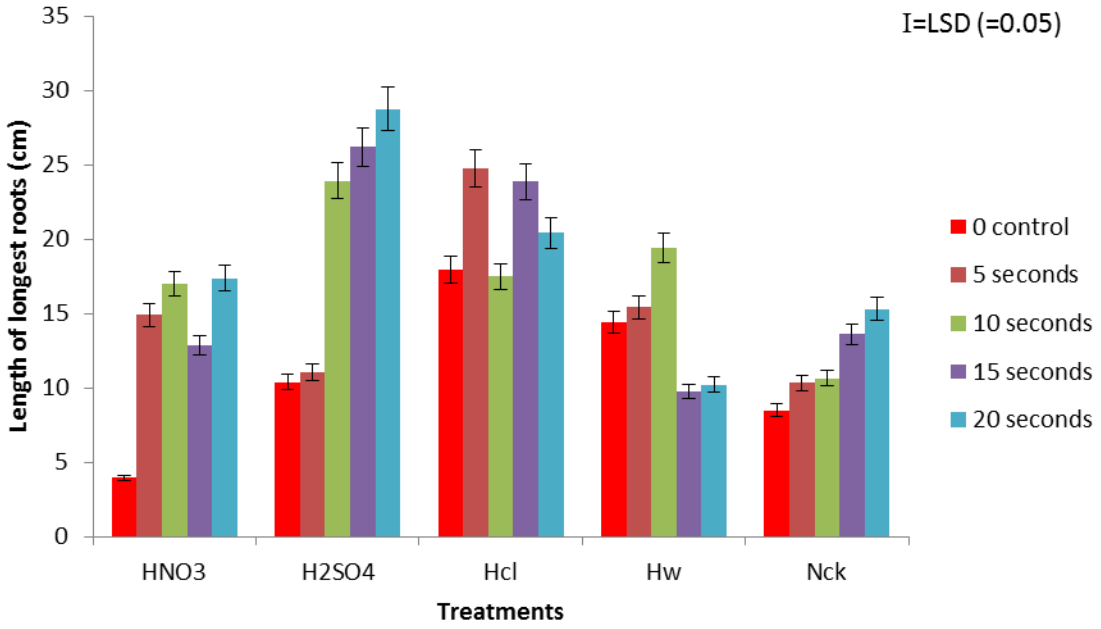

Figure 5: Effects of scarification, hot water and nicking on length of longest roots of *Sphenostylis stenocarpa*

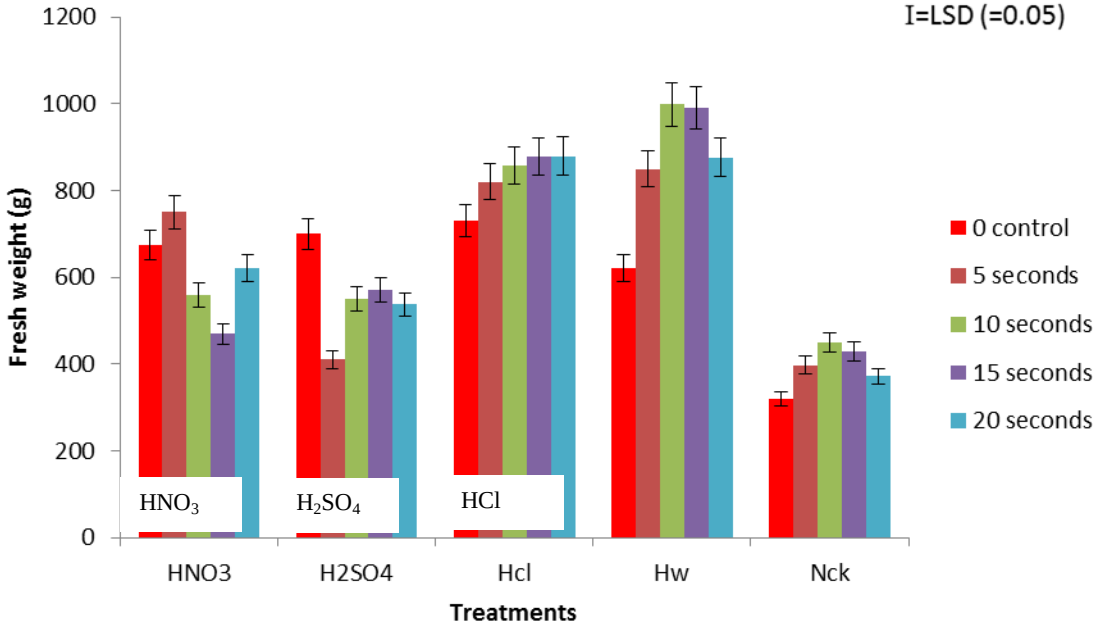

Figure 6: Effects of scarification, hot water and nicking on fresh weight of *Sphenostylis stenocarpa*

Legend:
HNO_3 = Nitric acid
H_2SO_4 = Conc. Sulphuric acid
HCl = Hydrochloric acid
HW = Hot water
NCK = Nicking

DISCUSSION

Proper seed germination and growth are indispensable for the continued existence of any plant. Germination, *sensu stricto* includes those events commencing with imbibition or uptake of water by the quiescent dry seed and culminates with the elongation of the radicle (Bewley and Black, 1994). Visible evidence of the completion of germination is usually protrusion of the radicle through the seed structures surrounding the embryo (such as the testa and endosperm, or megagametophyte). However, some seeds fail to complete germination under seemingly favorable conditions, even though they are viable. Such seeds are said to be dormant. Dormancy in seeds has to be broken, irrespective of the type, for effective germination and vigorous growth.

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Chemical scarification with concentrated sulphuric acid (H_2SO_4) at 5 seconds significantly ($P<0.05$) enhanced the germination and growth of *Sphenostylis stenocarpa* seeds followed by hydrochloric acid at 20 seconds. The strong inhibitory effect of the seed coat on seed germination may be caused by several possible mechanisms, including mechanical constraint, prevention of water and oxygen uptake, and retention or production of chemical inhibitors (Taiz and Zeiger, 2002). The integument breaking or softening, for instance, is needed to remove dormancy imposed by seed coat hardness or impermeability. Therefore, chemical scarification (softening the hard seed coat) with concentrated H_2SO_4 was used to remove exogenous dormancy. In the present study, a significant number of *S. stenocarpa* seeds that had been treated with H_2SO_4 germinated. The response of *S. stenocarpa* seeds to H_2SO_4 as a method for enhancing germination and growth was consistent with other studies (Keshtkar *et al*., 2008; Hermansen *et al*., 2000; Nadjati *et al.*, 2006; Rahnama-Gahfarokhi and Tavakol-Afshar, 2007).

This study indicated that chemical scarification methods are effective in rendering seeds of *S. stenocarpa* permeable, leading to germination up to 90% after 6 days. This confirms the earlier reports of Onyekwelu (1990). A previous study by Lemos-Filho *et al.* (1997) showed that mechanical and chemical scarifications are more effective in breaking the hard seed coat of *Senna multijuga*. It was equally observed in this study that within the scarification methods, chemical scarification with sulphuric and nitric acids, as well as mechanical scarification were more effective, confirming earlier reports by Ayisire *et al.* (2008) in *Piliostigma thonningii,* Lacerna *et al* (2004) in *Senna multijuga* (*Caesalpinoideae*) and *Plathymenia reticulata* (*Mimosoideae*). Sulphuric acid had the highest germination percentage (90%), followed by nitric acid scarified seeds with 80% .

CONCLUSION

These results revealed that seed dormancy in *S. stenocarpa* is mainly due to the hard seed coat, which can be broken effectively by chemical scarification, especially by sulphuric acid preatreatment. It is hoped that the results of this study will provide useful information for domestication and large scale plantation development, and in environmental conservation efforts.

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