

The Influence of Seed Pre-Treatments on Seed Germination and Seedling Vigour in *Acacia senegal* in the Nurs

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Abstract

Viable seeds of *A. senegal* were subjected to varied scarification treatments to determine their early germinability and subsequent development of vigour in the nursery. The treatments included micropylar, distal and circumference cuts, soaking in hot water at varying degrees and soaking time, H₂SO₄ and HCl treatments at varying concentrations and soaking time. The design of the experiments in the laboratory and in the nursery was completely randomized design (CRD). In the laboratory, the percentage germination of the seeds soaking in 50% concentrated H₂SO₄ and those cut at the circumference were significantly high ($P < 0.05$) having 89.0% and 92.0% respectively with the least mean germination time (4.0) and the highest seedling vigour index (1.94). Mechanical scarification on seeds of appropriate forest trees by the circumference cut should be used for breaking seed dormancy for quick and optimum germination.

Key Words: Scarification, Germinability, Mean-germination-time, dormancy, seedling-vigour-index

1. Introduction

Acacia senegal (L) wild commonly known as Gum Arabic is a deciduous shrub and is usually branched from the ground. This plant grows well on two types of soils, the sandy soil and dark loamy – clay soil and it tolerates pH of 5 - 8. It survives on a rainfall as low as 200mm and up to 800mm per year. *A. senegal* is sensitive to frost while it is heat tolerant (El-fadi, 1997). *A. senegal* produces high quality gum-Arabic used in the food, pharmaceuticals, paper, and cosmetics and in the production of ink. The leaf fall is mineralized to build up the fertility of sandy soil, and it is effective in erosion control (ICRAF, 1992). The bark, leaves and gum are used to treat gastritis disorders, hemorrhage, ophthalmia, colds and diarrhoea (Bello and Ambursa, 2006) while the flowers are relished by honeybees. *A. senegal* stabilizes sand dunes, fixes atmospheric nitrogen and it is a source of firewood and fodder (Abdullahi, 2002). The poor yield from *A. senegal* for its multipurpose economic importance and for ecological utilities in the semi-arid region of Nigeria may be attributed to inadequate information regarding its physiological and silvicultural requirements. Therefore, the present study investigated the influence of seed pre-treatments and other silvicultural manipulations on the germination and seedling performance of *A. senegal* in a semi-arid environment with the aim to enhance the germination and seedling vigour. In northern Nigeria, the international development agencies prioritized *A. senegal* for conservation through ex-site cultivation (Nautiyal *et al.*, 2002). The seeds of *A. senegal* have hard seed coat that impedes germination (Ovcharov, 1977) and thus the scarification of the seeds, either by mechanical or chemical means may be needed to influence seed germination (Duguma *et al.*, 1988). However, the influence of pre-germination treatments on germination percentage, mean germination and seedling vigour of the seedlings of *A. senegal* both in the nursery and on the field is yet to be assessed.

The objective of this study is to examine the influence of pre-treatments on seed germination and seedling vigour on *A. senegal* in the nursery of a semi-arid environment of Nigeria.

2. Materials and Methods

2.1 Experimental site: The seed germination tests were carried out in the ecology laboratory of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto – Nigeria. The nursery experiment was conducted in a screened house in the botanical garden of Usmanu Danfodiyo University, Sokoto –Nigeria. Sokoto is in the North- western Nigeria in the Sudan Savannah vegetation zone. It is located between latitude 11^o 30 to 13^o 50N and longitude 4^o - 6^oE (Federal surveys, 1964, cited in Ogigirigi, 1993).

2.2 Source of seeds.

The seeds of *A. senegal* were obtained from Institute for Agricultural Research (I.A.R), Ahmadu Bello University, Samaru, Zaria- Nigeria, and stored in sample closed containers at about 4^oC before experimentation.

2.3 Experimentation

2.3.1 Experimentation. Laboratory test on the germination response of *A. senegal* seeds to pre-germination treatments.

Viable seeds were subjected to four different pre-germination treatments with the aim of identifying and selecting the promising pre-treatment methods for quick and efficient germination compared to untreated seeds (control).

2.3.2 Mechanical scarification included clipping off seed coat from the micropylar region, the distal end and clipping round the seed circumference, each of ten viable seeds.

2.3.3 Soaking ten viable seeds in hot water at 70°C, 80°C and 90°C, each for 5 min, 10min and 15min. Ten viable seeds were also soaked in sulphuric acid, and hydrochloric acid at concentrations of 10%, 50% and 98% each for 5min, 10min and 15min. All the treated seeds were rinsed thoroughly in distilled water and were placed in moist cotton wool in covered 9cm petridishes. The experiment was laid out in a completely randomized arrangement with five replications per treatment at room temperature in the laboratory. Seeds were considered germinated upon plumule emergence. The number of seeds that germinated was recorded while the percentage seed germination was calculated.

2.3.4 *Experiment* : Influence of mechanical scarification and treatment with 50% acid (chemical scarification) on germination and seedling vigour of *A. senegal*

Ten viable seeds were subjected to pre-treatments viz. Micropylar cut (MD), distal cut (DL), circumference cut (CC), soaking in 50% H₂SO₄ for 15min and 50% HCL for 15min. The treated seeds were sown at 5cm depth and a distance of 4.0cm between seeds in polypots (25cm³); filled with a mixture of farmyard manure and sandy loam (50:50% v/v). The experiment was laid out in a completely randomized design with five replicates per treatment. The experiment was conducted in a netted house, and watered every alternate day. The mean germination time (MGT) was calculated using the relation:

$$MGT = \frac{\sum(xf)}{\sum f}$$

Where 'x' is the number of newly germinated seeds on each day and 'f' is the number of days after seeds were set to germinate.

After four weeks of germination, seedlings were thinned to three seedlings per pot to mitigate competition. After twelve weeks, the seedlings were harvested per treatment. Different growth parameters such as plant height, root length, shoot fresh weights; root fresh weight and collar diameter were measured. The oven-dried weight of the shoot and root were also obtained by drying the seedlings at 70°C to constant weight. The seedling vigour index (SVI) was determined from dry matter accumulation (dry weight) of the seedlings and mean germination time (Nicholas and Heydeckes, 1968). This is given as SVI = dry weight per seedling per MGT.

Data were subjected to analysis of variance (ANOVA) correlation coefficient was determined. Significant difference in the treatments was further subjected to Duncan Multiple Range test (DMRT) for the separation of treatment means.

3.0 Results

Under the laboratory condition, the percentage germination of all the scarified seeds was significantly higher ($P < 0.05$) than the control (5.8%). The percentage germination of the distal scarified seeds (48.2%) was significantly higher ($P < .05$) than those of the circumference (40.2%) and the micropylar (8.9%) scarified seeds (Table 1). The percentage germination of the seeds with sulphuric acid treatments for all the concentrations were significantly higher ($P < .05$) than the untreated seeds (control; 0.5%) Table 2. The percentage germination of seeds treated with 50% concentration of sulphuric acid for 15mins was significantly higher (89%) than those for 10mins (83%) and 5 mins (70%). The seeds treated with 10% sulphuric acid concentration for 15mins also gave significantly higher ($P < 0.05$) germination (81%) than those for 10mins (70%) and 5mins (55%). The seeds treated with 98% sulphuric acid concentration have significantly lower ($P < 0.05$) percentage germination which were 13%, 21% and 19% for 5mins, 10mins and 15mins soaking period respectively. The percentage germination of the seeds soaked in 70°C of hot water for 5mins was significantly higher (36.0%) ($P < 0.05$) than those of all other hot water treatments. The seeds treated with hot water at 70°C for 10mins and 80°C for 5mins gave germination percentages of 16.8% and 11% respectively which were significantly higher than the control (5.2%). The seeds treated with hot water at 90°C did not germinate at all (Table 3). As shown in Table 4, seeds treated with 50% concentration of hydrochloric acid for 15mins gave a significantly higher germination percentage (65%) than for 10mins (45%) and 5mins (28%). Also, the germination percentage was significantly higher ($P < 0.05$) when the same acid concentration was used in soaking the seeds for 10mins than for 5mins. The seeds treated with 10% and 98% concentration of HCL gave significantly lower ($P < 0.05$) germination percentages than that for 50% aid concentration although significantly higher than the control.

Under nursery condition, all the five pre-germination treatments improved seed germination and reduced mean germination time than the untreated seeds (Table 5). Untreated seeds (control) had late germination when other treatments had already completed germination. The control had significantly higher mean germination time (24.68) than all the scarified seeds. The circumference cut treatment has the least mean germination time (MGT) (4.02) whereas the MGT between the micropylar cut (4.7), distal cut (4.82) and sulphuric acid treatment (4.72) were not significantly different ($P < 0.05$). The percentage germination of seeds scarified at the circumference was significantly higher (92.0%) than all the other treatments. This was followed by the distal cut treatment (67.09%), sulphuric acid treatment (63.74%) and hydrochloric acid treatment (53.12%). The percentage germination for the micropylar cut treatment was much lower (23.9%) than the other treatments but was

significantly higher ($P < 0.05$) than the control treatment (13.41%). The effect of scarification on seedling growth and seedling vigour of *A. senegal* after 12 weeks of growth in the nursery showed that all the pre-treatment methods showed significantly higher growth parameters than the control. The 50% sulphuric acid treated seedlings have significantly higher ($P < 0.05$) growth parameters with plant height (64.1cm); root length (40.7cm); shoot fresh weight (11.5g); root fresh weight (5.78g); shoot dry weight (6.78g); root dry weight (3.32g); collar diameter (4.86mm), than all the other pre-treatment methods (Table 6). With respect to seedling vigour index (SVI), the circumference cut treatment has significantly higher value (1.94) than all the other pre-treatment methods while the control had significantly low value (0.15) compared to all the pre-treatment methods. The correlation analysis of the growth parameters measured showed that the shoot dry weight showed negative correlation ($P < 0.01$) with days taken for first germination ($r = -0.963$) and mean germination time ($r = -0.969$). Plant height showed positive correlation ($P < 0.01$) with collar diameter ($r = 0.982$), shoot dry weight ($r = 0.964$), root dry weight ($r = 0.964$) and seedling vigour index (SVI) ($r = 0.968$). Shoot dry weight ($r = 0.960$) and root dry weight ($r = 0.964$) were significantly correlated ($P < 0.01$) with seedling vigour index (Table 7)

4.0 Discussion

The mechanical scarification on the seeds of *A. senegal* caused early germination, breaking their normal period of dormancy. This is in agreement with earlier findings of Iyamabo (1967), Duguma *et al.*, 1988 and Shinkafi (2006) who observed that mechanical scarification is an efficient way of improving seed coat permeability of *Pterocarpus angolensis* and *Leucaenia leucocephala* seeds. Opening into the cotyledon of *A. senegal* especially through the distal end and circumference cut allowed ready imbibition of water, which enhanced early germination. Mechanical scarification of seeds elicits high rate of respiration. Higher germination in the seeds of *Tamarindus indica* (Awodola, 1988) and in the seeds of *Leucaenia leucocephala* (Duguma *et al.*, 1998) was argued to be due to enhanced rate of respiration. Soaking of the seeds of *A. senegal* in 50% or 10% concentration of H_2SO_4 or HCL for 15min or 10min which produced high percentage germination of the seeds, lend further support for need of the seed scarification. This finding corroborates the earlier reports of Nwoboshi (1982), Duguma *et al.*, (1988), Awodola (1993) and Shinkafi (2006) who reported that soaking *A. senegal* and *A. nilotica* seeds in concentrated H_2SO_4 for some minutes modified the seed coat. The scarification with H_2SO_4 or HCL modified the seed Coat that enhanced the germination process. It is evident from the present study that for optimum seed germination of *A. senegal*, the concentration of the acid should not exceed 50% and the soaking period should be limited to 15min. Optimal temperature of $70^{\circ}C$ hot water for soaking the seeds of *A. senegal* either for 5min or 10min, which enhanced the germination though slightly more than untreated seeds, is also a method of possible scarification. This is in agreement with the report of earlier investigations (Duguma *et al.*, 1988; Awodola and Abdullahi, 1990) who observed higher germination percentage in the seeds of *Leucaenia leucocephala* and *A. nilotica* soaked in hot water. In the nursery, the germination of the seeds of *A. senegal* also responded to scarification treatments. Circumference cut seeds had the highest germination percentage; perhaps it provided the largest surface area for absorption of water. In view of good germination percentage with distal end scarification, it can be deduced that the seeds required variable pre-germination treatment methods. This method has an advantage over the chemical methods of scarification, which may be lethal on the seeds. As observed by Mapongmetsem *et al.*, (1999), who conducted pre-germination treatment experiments on ten indigenous species, for all the species, manual scarification which involved puncturing of the seed coat at the micropyle and distal ends were the best. Harrie and Erick (1998) have reported that soaking of certain forest seeds in cold water before sowing, hot water treatment, short exposure to a high temperature; scarification or stratification would produce the best germination result. Sieverding (1981) also observed that heating of *Gmelina arborea* seeds at $80^{\circ}C$ for hours increase both germination percentage and germination speed substantially. Similarly, Abdullahi (2002) noted that seed priming which involves soaking in water for 48h and 12h in ± 500 mm potassium plus zinc mixture and later drying to storage moisture content enhanced germination and seedling growth of barley. Furthermore, Mustapha (2001) observed that seed immersed in methanol, ethanol and sulphuric acid for 10min produced seedlings with high vigour. The findings of the present study corroborate the earlier report by Kattima *et al.*, (1999) who indicated differential seed germination percentage in untreated seeds of *Withamnia sommifera*. Also Pandey *et al.*, (2000) reported similar observation in chemical stimulation of seed germination in *Aconitum heterophyllum*. In *A. senegal* the seedling parameters of growth developed better with seeds that were scarified with the chemicals, especially sulphuric acid. Plant height appeared to be the strongest morphological trait and it was convenient to identify vigorous seedlings of *A. senegal*. Plant height showed positive correlation with collar diameter, shoot dry weight and root dry weight. Negative correlation also occurred between seedling dry weight and number of days taken for germination to occur. The implication of the negative correlation is that early germinated seedlings should be healthier and may be retained during thinning to mitigate competition.

CONCLUSION

It is evident that the seeds of *A. senegal* have dormancy and would not germinate early and easily. Therefore, soaking the seeds in 50% concentrated H_2SO_4 for 15min or circumference scarification of the seeds before

sowing reduced the time taken for the seed to germinate and gave high percentage germination. It can also be concluded from the study that adequate growth of seedlings in the nursery is dependent on the ease of seed germination while the vigour of the seedlings is dependent on the seedling growth rate and biomass accumulation.

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Table 1: Influence of point of mechanical scarification on germination of the seeds of *Acacia senegal* under laboratory condition

Mechanical scarification	Germination percent (%)
Control	5.8d
Micropylar	8.9c
Circumference	48.2b
Distal	40.2a
S.E	0.16

Means in a column followed by the same letter (S) are not significantly using DMRT at 5% level.

Table 2: Germination of the seeds of *Acacia senegal* treated with varying concentrations of sulphuric acid and soaking duration under laboratory condition

Sulphuric acid concentration (%)	Soaking duration (min)	Germination percent (%)
Control	-	0.5g
10	5	55.0d
10	10	70.0c
10	15	81.0b
50	5	70.0c
50	10	83.0b
50	15	89.0a
98	5	13.0f
98	10	21.0e
98	15	19.0e
S.E		0.22

Means in a column followed by the same letter (S) are not significantly different using DMRT at 5% level.

Table 3: Germination of the seeds of *Acacia senegal* subjected to varying hot water temperatures and soaking duration under laboratory condition.

Hot water temperature (°C)	Soaking duration (min)	Germination percent (%)
Control	-	5.2d
70	5	36.0d
70	10	16.8b
70	15	4.0e
80	5	11.0c
80	10	4.2c
80	15	1.2f
90	5	0
90	10	0
90	15	0
S.E		0.18

Means is a column followed by the same letter (s) are not significantly different using DMRT at 5% level.

Table 4: Influence of varying concentrations of hydrochloric acid and soaking durations on seed germination of *Acacia senegal* under laboratory condition.

Hydrochloric acid concentration (%)	Soaking duration (min)	Germination percent (%)
Control	-	5.2g
10	5	8.5f
10	10	8.0f
10	15	15.0d
50	5	28.0c
50	10	45.0b
50	15	65.0a
98	5	7.0f
98	10	15.0d
98	15	11.0e
S.E.		0.24.

Means in a column followed by the same letter (S) are not significantly different using DMRT at 5% level.

Table 5: Germination time and germination percentage of the seeds of *Acacia senegal* subjected to selected pre-treatments in the nursery.

Treatment	Mean germination time (days)	Germination percent (%)
Control	24.69a	13.4f
Micropylar cut	4.74c	23.90e
Distal cut	4.82c	67.09b
Circumference	4.02e	92.00a
Conc. 50% H ₂ SO ₄ (15min)	4.72c	63.74c
Conc. 50% HCL (15min)	5.12b	53.12d
S.E.	1.01	1.03.

Means in a column followed by the same letter (S) are not significantly different using DMRT at 5% level.

Table 6: Effect of pre-treatment on seedling growth of *Acacia senegal* under nursery condition after 12 weeks of sowing

Treatment	Plant Height (cm)	Root Length (cm)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Shoot Dry Weight (g)	Root Dry Weight (g)	Collar Diameter (mm)	Seedling Vigour Index
Control	20.05	9.12e	7.08c	2.1c	2.58d	1.26e	1.94c	0.15e
Micropylar	62.14c	37.02c	10.68b	5.32b	5.98c	2.96d	4.72b	1.62c
Distal cut	62.54c	37.18c	10.92b	5.40b	6.14b	3.04c	4.76b	1.61c
Circumference cut	61.82d	36.80d	10.88b	5.40b	6.18b	3.08c	4.76b	1.94a
50% H ₂ SO ₄ for 15min	64.16a	40.76a	11.58a	5.78a	6.78a	3.32a	4.86a	1.73b
50% HCL for 15min	63.18b	39.46b	11.30a	5.66a	6.20b	3.14b	4.70b	1.54d
S.E.	0.49	0.34	0.32	0.24	0.26	0.13	0.19	0.12

Means in a column followed by the same letter (S) are not significantly different using DMRT at 5% level.

Table 7: Correlation coefficient (r) of associating growth parameters of *Acacia senegal*.

Seedling growth parameters	Correlation coefficient (r)
SWD VS MGT	- 0.969**
NDG VS SDW	- 0.963**
PH VS CD	0.982**
PH VS SDW	0.964**
PH VS RDW	0.964**
SVI VS PH	0.968**
SVI VS SDW	0.960**
SVI VS RDW	0.964**

** = Significant (P < 0.01); SDW = seedling dry weight

MGT = Mean germination time;

NDG = Number of days for first seed germination

PH = Plant height; CD = Collar diameter

SDW = Shoot dry weight; RDW = Root dry weight

SVI = Seedling Vigour Index

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