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Research Article
Physiology of Breaking Seed Dormancy and Increasing Seed Germination in *Senna alata* (L) Roxb Seeds in Nigeria

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Abstract

Background and Objective: Nowadays, most of the medicinal plants can no longer be found in the proximity of rural and urban areas and even in the far away forests as before due to deforestation and progressive desertification. Therefore, the objective of this study was to investigate and find out the best possible methods (hormonal regulator, chemicals and hot water) of breaking dormancy in *Senna alata*. **Materials and Methods:** The seeds were subjected to different methods and durations of exposure to break the dormancy and enhance germination. The seeds were treated with 85 mg of GA3 and 350 mg of GA3, 98% concentrated H_2SO_4, 70.5% HNO_3 and100°C hot water, for 10 and 20 min and were arranged in a completely randomized block design with 3 replications. **Results:** The experimental results demonstrated that hot water and 98% concentrated H_2SO_4 for 10 and 20 min were the most effective treatments for augmenting seed germination (83 and 93; 76.67 and 93.33%, respectively), followed by GA_3 for 10 and 20 min (49.83 and 46.67%). **Conclusion:** Hot water and H_2SO_4 treatments are highly recommended to break seed dormancy in *Senna alata*.

Key words: Medicinal plant, *Senna alata*, dormancy, germination, afforestation, augmenting, completely randomized block design

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants have been essential to human from the creation for health and medical purposes. Most of the people in developing countries continue to bank on traditional medicine as herbal drugs to meet their heath needs. The use of medicinal plants nowadays are not only confined in the remote areas but are also intensively used in the urban areas. In the same vein, the World Health Organization estimated that approximately 75-80% of the world's population uses plant medicines either in part or entirely, this because many especially poor people cannot afford the high costs of pharmaceutical drugs[1,2].

Various parts of *Senna alata* plant (leaves, flowers, roots or the stem) are used in traditional medicines. *Senna alata* medicines have been used as concoctions and decoction to treat many diseases[3-5] such as skin problems, stomach pain during pregnancy, dysentery, haemorrhoids, blood in the urine (schistosomiasis, gonorrhoea), convulsions, heart failure, oedema, jaundice, headache, hernia, intestinal worms, hepatitis, yellow fever, typhoid fever, one-sided weakness or paralysis, syphilis, diabetes, wounds and viral infections and itching[6-9]. Also, in veterinary medicine, leaf decoctions of *Senna alata* is used to treat a wide range of skin problems in livestock. Decoctions are also used against external parasites such as mites and ticks[10]. The young pods are eaten as a vegetable; the seeds have been used as a source of gum. Toasted leaves are sometimes used as a coffee substitute. The bark is used as fish poison and for tanning leather. The roots and the bark are reported to be used for tattooing. *Senna alata* is widely appreciated as a garden ornamental and bee forage[10]. The plant is also popular for its laxative or purgative and wound healing properties and for the treatment of abscesses[8,11].

The therapeutic effectiveness of phytochemical components of *Senna alata* such as steroids, terpenes, alkaloids, flavonoids, anthraquinones, saponins, tannins and carbohydrates have been studied and confirmed by previous study[7]. *Senna alata* leaves have several medicinally important phytochemical constituents including, mineral elements: K, Zn, Cd, Na, Mg, Fe and Ca. The vitamin elements are β-Carotene (IU), Vitamin C (mg L^{-1}), Vitamin E (IU). Vitamin C anthraquinones and anthracene derivatives of rhein, emodol, aloe-emodin, sennosides A and B, 4,5-dihydroxy-1-hydroxymethylanthrone and 4,5-dihydroxy-2-hydroxymethylanthrone, alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosides[12-15]. Amongst the secondary metabolites are steroids, flavonoids, anthraquinones, anthrones and a few less common compounds such as ellagitannin, naphthalene, phenolic acid, purine and xanthone[16]. However, despite being such an important crop species, there are still a number of factors currently limiting its availability.

Senna alata like other medicinal plants are being faced with many challenges. Among these challenges are availability of these plants due to deforestation, excessive harvesting and human activities. There is a need to embark on afforestation asa way out to alleviate these challenges through propagation and domestication of these medicinal plants. The majority of medicinal plants are at their wild stages with most of their seeds dormant. A viable seed (or other germination unit) is said to be dormant when it does not have capacity to germinate in a specified period of time under normal physical environmental factors that otherwise is favourable to its germination[17]. Seeds of most cultivated plant species lose their dormancy before or shortly after being separated from the parent plant, whereas seeds of wildest shrubs have long period of dormancy[18].

In order to domesticate and cultivate any plant species, information is needed on the seed germination and how to overcome the problem of dormancy of such plant species. Therefore, it is of paramount importance to domesticate medicinal plant which cannot be achieved except through breaking their dormancy in order to sort out the problems of deforestation and non-availability of these medicinal plants.

Various pretreatment methods have been used to overcome seed dormancy. There are heating, soaking, leaching, potassium nitrate (KNO_3) and Gibberellic acid (GA_3); heating at 40°C for one to five days was the most effective method with up to 90% germination capacity reported. In addition, (i) Leaching by washing seed under running water at room temperature for a few minutes); (ii) Soaking in tap water for a few hours before the germination test and (iii) Pre-chilling by moistening and maintaining at cold temperature for a number of days before the germination, were observed to increase germination up to 74%. Good seed germination is very important for crop production. Uneven or poor germination and subsequently uneven seedling growth can lead to great financial losses by reducing crop yield[19]. Therefore, to achieve the aim of any regeneration program, seed collection and germination must be taken into consideration. Thus, the specific objectives were to (1) Investigate different method(s) of breaking seed dormancy in *S. alata*, (2) Compare the effects of different treatments on the germination of *S. alata* and (3) Propose the best methods of breaking seed dormancy to farmers.

MATERIALS AND METHODS

Experimental location: The experiment was conducted at the Plant biology Laboratory, Bowen University Iwo, Osun State, Nigeria. The study was carried out for 2 months from March, 2018 to May, 2018.

Plant material, viability test and seed disinfection: *Senna alata* seeds were collected from the parent plant in its natural habitat near Gbadamosi Hospital, Olomo area, Feesuiwo, Osun state, Nigeria with latitude of N 7°39'15,318" (7.654255) and a longitude of E 4°10'27 599" (4.174333).

These seeds were removed manually from the mature and dry pods and the good, healthy and viable ones were used for the germination study. The seed viability test was carried out by soaking them in a container of water; the floating seeds were discarded while those settled at the bottom were considered as viable.

The viable seeds were disinfected in 50% alcohol for 5 min. Afterwards, the seeds were rinsed 3 times in distilled water before applying the various dormancy-breaking treatments.

Seed treatments with different methods of breaking dormancy: The disinfected seeds were treated with H_2SO_4, distilled water, hot water, GA_3 and HNO_3 for 10 and 20 min. Then they rinsed with distilled water before the germination experiments in plastic Petri dishes. Untreated seed served as control.

Treatment with H_2SO_4: The disinfected seeds were soaked in two beakers containing 98% concentrated H_2SO_4 for 10 and 20 min according to a modified method of Arowosegbe[20] as treatments 1 and 2, respectively. With the aid of forceps, the seeds were removed from the beakers after the time of treatment and rinsed with 4 changes of distilled water to be sure that no trace of H_2SO_4 remained. Untreated seed served as control.

Treatment with HNO_3: The disinfected were soaked in beakers containing 70.5% concentrated HNO_3 for 10 and 20 min as treatments 1 and 2. With the aid of forceps, the seeds were removed from the beakers after the time of treatment and rinsed with 4 changes of distilled water to be sure that no trace of HNO_3 remained. Untreated seed served as control.

Treatment with 85 g gibberellic acid: About 85 g of gibberellic acid was dissolved in 1000 mL of distilled water. One hundred disinfected seeds were soaked in the solution of gibberellic acid for 10 and 20 min, respectively. The seeds were then rinsed with distilled water before plating. Untreated seed served as control.

Treatment with 350 g gibberellic acid: About 350 g of gibberellic acid was dissolved in 1000 mL of distilled water. Disinfected seeds were soaked in the solution of gibberellic acid for 10 and 20 min, respectively. The seeds were then rinsed with distilled water. Untreated seed served as control.

Treatment with 100°C distilled water: About 500 mL distilled water was boiled to100°C, disinfected seeds were soaked in the water for 10 and 20 min according to a modified method of Arowosegbe[20] respectively; after which the seeds rinse with distilled water and waited for the seeds to cool down. Untreated seed served as control.

Germination test, experimental design and data collection: Ten seeds from each of the seed lots in the treatments described above were placed in petri dishes containing two filter papers. The papers were moistened with 4 mL distilled water and covered up. The level of water in each petri dish was regularly checked and water was added as needed for proper germination.

The experimental design was a complete randomized block consisting of a factorial combination of the two treatment durations and the five different methods of breaking dormancy, with three replicates of 10 seeds each per treatment. All the Petri dishes were placed on the germination table in the laboratory at room temperature (29±2°C). Seed germination was counted and recorded daily until no further germination occurred. Shoot length and root length were measured on the 13th day. Other measurements taken were germination percentage, index of velocity of germination and seed vigor index.

Data analyses: All data recorded were subjected to statistical analysis to identify significant differences among the treatments. ANOVA was performed for the assessment of the variation at 0.05 level using LSD and Multiple Comparison-*post hoc* test.

Table 1: Effect of treatments on seed germination

	Germination (%)	
Treatment	10 min	20 min
H_2SO_4	76.67[a]	93.33[a]
Hot water	83.33[a]	93.33[a]
GA_3 (85mg)	49.83[b]	43.33[b]
GA_3 (350 mg)	27.75[c]	46.67[b]
HNO_3	23.33[c]	30.00[c]
Distill water	3.33[d]	10.00[d]

Same letters in each column are not significantly different at 0.05 level

Table 2: Effect of treatments on index of velocity of germination

	Index of velocity of germination (min)	
Treatment	10	20
H_2SO_4	1.80[a]	2.70[a]
Hot water	1.71[a]	2.05[a]
GA_3 (85 mg)	1.02[b]	1.22[b]
GA_3 (350 mg)	0.71[b]	1.05[b]
HNO_3	0.46[c]	0.60[c]
Distill water	0.17[d]	0.31[c]

Same letters in each column are not significantly different at 0.05 level

Table 3: Effect of treatments on seed vigor index

	Index of velocity of germination (min)	
Treatments	10	20
H_2SO_4	81.47[a]	93.17[a]
Hot water	85.47[a]	99.33[a]
GA_3 (85 mg)	35.17[b]	40.63[b]
GA_3 (350 mg)	26.97[c]	44.17[c]
HNO_3	19.00[d]	26.01[d]
Distill water	11.00[e]	15.67[e]

Same letters in each column are not significantly different at 0.05 level

RESULTS

The results obtained from the experiment have shown how dormancy can be broken using different treatments and also to observe which treatments is best suitable for breaking seed dormancy in *Senna alata*. The durations for the treatments were 10 and 20 min, the essence of the time for treatments was to know which time interval or how long the seeds needs to be treated and also to see which treatment time interval germinates best.

The effects of acids (H_2SO_4, HNO_3), hormone (GA_3) and hot water on seed germination percentage showed significant differences at $p<0.05$ (Table 1). At 10 min treatment duration the highest germination percentage (83.33%) was recorded with hot water followed by H_2SO_4 (76.67%) while the lowest was recorded in distilled water (3.33%) used as control. The second treatment duration (20 min) demonstrated the highest germination percentage when compared to 10 min treatment duration in all treatments except 35 mg GA_3. For the control, the highest germination percentage was 93.33% while the lowest was 10%. Seeds treated with hot water and concentrated H_2SO_4 (98%) for 10 and 20 min significantly enhanced germination percentage.

The rate of the total germination per day was further recorded and was calculated as index of velocity of germination (Table 2). The highest indexes (1.8 and 1.71 for 10 min; 2.7 and 2.05 for 20 min) were observed in H_2SO_4 and hot water, respectively whereas the lowest index (0.31) was recorded in the control (distilled water).

The seed vigor index is presented in Table 3. There were significant differences amongst treatments and for treatment duration. The seed vigor index ranged from 11-85.47 for 10 min and from 15-99.33 for 20 min. The result showed that vigor index increased with treatment duration. The highest (99.33) was obtained from hot water treatment followed by H_2SO_4 (93.17) which was significantly different from the control (15.67) at 20 min treatment.

DISCUSSION

Seed germination has been a vital point in crop production and nutrition in the world. Understanding the physiological process of seed is crucial in germination and seedling establishment. This study evaluated the different dormancy breaking methods (hot water, gibberellic acid, sulfuric acid and nitric acid) in *Senna alata* for its domestication and production. It has been observed that most of wild medicinal seeds including *Senna alata* are dormant due to hard seed integuments which prevent the exchange of gases and water[20-23]. A seed is said to be dormant if no or only few seeds germinate under suitable conditions however, if the majority (80-100%) of the seeds germinate, they are said to be non-dormant[18]. It has also been reported that seed coat can harbor inhibitors or may prevent the leaching of inhibitors from the embryo[18]. This study revealed the presence of hard coat and possibly the presence of inhibitors in the coat which were broken by hot water, sulfuric acid, nitric acid, GA_3 confirming that *S. alata* seeds were dormant because the germination percentage of the control seeds were very low compared to all the treatments. The results obtained from this study substantiate the previous work[21] that wild species exhibit dormancy when compared to cultivated species.

Pre-treatment of seeds by hot water (100°C) and 98% H_2SO_4 significantly broke seed dormancy in *S. alata* and also fastens the seed germination. Similar results were obtained by Arowosegbe[20] who reported that seeds scarified with 100%

H_2SO_4 for 4 and 6 min were the most effective treatments for enhancing seed germination (both gave 100% germination), followed by 100°C wet heat for 6 min (77.50%) and 80% H_2SO_4 for 6 min (70%). According to another study[24], pre-treatment of seeds by 10% HNO_3 and warm water (30°C) fastens the seed germination in *Centella asiatica*.

CONCLUSION

Freshly harvested seeds of *S. alata* exhibited dormancy of seed coats. Hot water treatment and 98% concentrated H_2SO_4 are the best methods of breaking dormancy in *S. alata* seeds because they significantly increased seed germination percentage, velocity and seed vigor though the use of HNO_3 and GA_3 are not bad as methods of breaking dormancy when compared to the untreated seeds (control). Therefore, hot water treatment and 98% concentrated H_2SO_4 treatment are highly recommended to break seed dormancy.

SIGNIFICANCE STATEMENT

This study discovered hot water treatment and 98% concentrated H_2SO_4 treatment are highly recommended to break seed dormancy that can be beneficial for the farmers and the scientific world. This study will help the researchers to uncover the critical areas of seed physiology and dormancy that many researchers were not able to explore. Thus a new theory on physiology of seed dormancy may be arrived at.

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