Hindawi
International Journal of Forestry Research
Volume 2022, Article ID 6731479, 7 pages
https://doi.org/10.1155/2022/6731479

Research Article

Effect of Seed Pretreatment Methods on Germination and Early Seedling Growth of *Senna spectabilis*

Erick R Zembele and Elisha S Ngulube

Department of Forestry and Environmental Management, Faculty of Environmental Sciences, Mzuzu University, Private Bag 201, Luwinga, Mzuzu, Malawi

Correspondence should be addressed to Elisha S Ngulube; esn12003@yahoo.co.uk

Received 6 June 2022; Accepted 10 August 2022; Published 28 August 2022

Academic Editor: Ahmad A. Omar

The objectives of the study were to evaluate the effect of pretreatment methods on germination of *Senna spectabilis* seed and determine the effect of pretreatment methods on height and root collar diameter growth of *Senna spectabilis* seedlings. The experimental treatments involved were control; seeds soaked in cold water for 12 h, 24 h, and 48 h; seeds soaked in hot water and allowed to cool for 12 h, 24 h, and 48 h; and nicked seeds using secateurs. The experiment was arranged in a completely randomized design (CRD) with four replications. The data variables assessed were seedling height, root collar diameter, and survival rate. Data were subjected to one-way ANOVA using Minitab software. Results showed that there were significant differences ($p \leq 0.05$) among treatment means on germination, seedling height, and root collar diameter, while the survival rate was not statistically significant ($p \geq 0.05$). Nicked seeds and those soaked in hot water and allowed to cool for 12 h had an outstanding performance in terms of germination and seedling growth. It is, therefore, recommended that farmers should be encouraged to use nicked and hot water soaked for 12 h seed in order to achieve high germination and growth efficiencies in their homestead gardens.

1. Introduction

Senna belongs to the family Leguminosae and is a subfamily of Caesalpinioideae [1, 2]. The Caesalpinioideae subfamily comprises over 2800 species which are mostly tree species [3]. The genus *Senna*, previously known as *Cassia* L., is estimated to have more than 600 species distributed across the world [4, 5]. *Senna spectabilis* (DC) H. S. Irwin and R. C. Barneby is a small rounded deciduous tree with a height of about 7–10 m (15 m at maximum) and 30 cm in trunk diameter, with a spreading crown [6]. The bole is generally short and tends to fork near the ground. *S. spectabilis* has wide spreading and drooping leafy branches [7].

The tree has a wide range of applications. It is a source of energy (in form of firewood and charcoal), forage for honeybees, and shade or shelter in homesteads as well as other agroforestry systems. The shade cast helps to improve soil water conservation in some agroforestry system applications [8]. As an ornamental tree, *Senna spectabilis* is aesthetically attractive. Consequently, it is commonly planted along community tertiary roads and in between buildings in order to increase the amenity value of the surroundings. Furthermore, the tree species are also planted for property boundary marking between local community members. In addition, the tree has wide medicinal uses [4, 9, 10] in many parts of the world. However, in Malawi, *S. spectabilis* is mainly used as a source of fuel wood and poles for light construction [11].

Despite being a fast-growing tree species, the seed of *Senna spectabilis* exhibits seed coat-imposed dormancy, causing delayed germination. According to Schmidt [12], seed coat-imposed dormancy is a result of impermeable layer(s) that develops during maturation and drying of the seed or fruit. The presence of an impermeable seed coat in most seeds is considered as the main challenge in establishing forests of this kind of species [13, 14]. Over the years, however, seed presowing treatment has been used to improve and accelerate germination and establishment of the plant species [15, 16]. Seed presowing treatment techniques used include soaking in cold and hot water, soaking in acid solution, and nicking and rubbing seeds on abrasive surfaces [17]. For instance, the boiling of seeds in water helps to remove the cuticle and sometimes part of the palisade layers of the seed coat, thereby effectively breaking the seed dormancy. A range of responses to boiling treatment is illustrated among the acacias by the following examples: *Acacia sieberiana* (an African *Acacia*) yields 60% germination after boiling for 1 h [18]. *A. acuminata* and *A. pycnantha* will withstand 100°C for a maximum of 5 seconds and *A. terminalis* for a maximum of 30 seconds [19]. The study conducted by Mwang'ingo et al. [20] on the effect of different seed presowing treatments on the germination of *Senna grandis* and *Senna spectabilis* showed that hot water treatment at 90°C followed by overnight soaking in cold water is an effective presowing treatment with cumulative germination of 44.25%. The partial removal of the seed coat at the radical end of *Senna spectabilis* attained a cumulative germination percent of 54 and cold water soaking for 24 h gave 10% germination in a research study conducted by Mwang'ingo et al. [20]. Direct seeding of *Senna spectabilis* encounters limited success because of poor seed emergence attributable to dormancy. For example, the untreated seed of *Senna spectabilis* gives germination of 3.7% [21]. Appropriate presowing treatments are required to increase the emergence of *Senna spectabilis* seed. Hence, there is a need to pretreat the seed before sowing to enhance germination and growth.

This study focused on assessing the effect of the seed pretreatment methods on germination and early seedling growth of *Senna spectabilis.* The specific objectives of this study were to assess the effect of pretreatment methods on the germination period, percentage, value, and survival of *S. spectabilis* seed; determine the effect of pretreatment methods on height and root collar diameter growth of *S. spectabilis* seedlings, and assess the effect of the pretreatment methods on seedling survival rate of *S. spectabilis* over time.

2. Materials and Methods

2.1. Experimental Site. The experiment was conducted at Mzuzu University (Mzuni) nursery in Malawi. Mzuni is located at an altitude of 1 270 m above sea level and on coordinates 11° 25′ 19.2″ S and 33° 59′ 34.8″ E. The area receives a mean annual rainfall of approximately 1 150 mm from December to March. The dry season is from May to November. The mean annual temperature of the area ranges from 13.5°C to 20.9°C with the coldest months being June and July and the hottest being November.

2.2. Seed Source. *Senna spectabilis* seeds were acquired from the Forestry Research Institute of Malawi (FRIM) in Zomba, Malawi. The seed lot was sourced from FRIM to ensure that it was of high quality.

2.3. Experimental Design and Treatments. Due to homogeneity of the experimental units, a completely randomized design (CRD) was used in this study. The seeds were exposed to various pretreatment methods (Table 1), such as soaking in hot water at 100°C (boiling point of water) and allowed to cool for 12, 24, and 48 h, soaking in cold water for 12, 24 and 48 h, nicking, and a control (seeds were left untreated).

Each treatment consisted of 25 seeds which were replicated four times. Thus, each treatment had a total of 100 seeds. The seeds were directly sown in small-sized polyethylene tubes (9 cm × 15 cm) filled with a mixture of natural woodland clay and sand soil. One seed was sown per tube. Tubes were labeled according to the replicates and treatments assigned. The tubes/seedlings were watered early in the morning and late in the afternoon to provide enough moisture.

2.4. Data Collection Procedure and Variables. Data collection involved counting and recording all the seeds which had germinated. The seeds were observed each day to have germinated after the emergence of a radicle or plumule ≥2 mm from the seed coat as outlined by the International Standards Association, ISTA [22]. All seeds that had failed to emerge were evaluated for rot, viability, or other condition using a cutting test [23, 24] on the 28^{th} day. The diameter of the root collar was measured in millimeters using a vernier caliper, while shoot height was measured in centimeters using a metric ruler. The number of seedlings that survived was recorded against the number that had germinated. Data collection proceeded for 60 days for seedling growth before the closure of the experiment.

2.5. Data Management and Analysis. Germination percentage was calculated based on the number of seeds germinated relative to seeds sown using William [25] formula as follows:

$$\text{Germination \%}\,(G) = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100. \tag{1}$$

Germination value was calculated as a function of daily germination speed obtained by dividing the cumulative germination percent by the number of days since sowing relative to the number of daily germinants and germination percentage [26] as follows:

$$\text{Germination value} = \left(\frac{\sum S_g}{n}\right) \times\ 10\,G, \tag{2}$$

where S_g is the daily germination speed, n is the number of daily counts from the date of first germination, G is the germination percentage, and 10 is a constant generated through many germination tests.

Seedling survival was calculated by dividing the number of survivors by the number of emerged seedlings from the tubes.

Table 1: Details of pretreatment methods used.

Treatment	Description of presowing treatment
T1	Control
T2	Seed soaked in cold water for 12 h
T3	Seed soaked in cold water for 24 h
T4	Seed soaked in cold water for 48 h
T5	Seed soaked in boiled water and allowed to cool for 12 h
T6	Seed soaked in boiled water and allowed to cool for 24 h
T7	Seed soaked in boiled water and allowed to cool for 48 h
T8	Nicked seed using secateurs

An arcsine transformation was conducted to normalize the germination datasets. A one-way analysis of variance (ANOVA) using Minitab Version 16 was used to compare treatment mean values at $p = 0.05$. Fischer's least significant difference test ($LSD_{0.05}$) was conducted to determine the significantly different treatment means.

3. Results

3.1. Trend of Seed Germination. The results in Figure 1 show a germination trend of *Senna spectabilis* from the first emergence till 28 days after sowing. Nicked seeds (T8) germinated more rapidly than the other treatments, with germination commencing 5 days after sowing and completing on the 13th day. In the other treatments (T1, T2, T3, and T4), the germination was rather sporadic. For example, in T2 where seeds were soaked in cold water for 12 h, germination started 8 days after seed sowing and was completed on the 26th day.

3.2. Germination Percentage and Value of Senna spectabilis Seeds. Table 2 shows germination percentages of the eight treatments, 28 days after seed sowing. Nicked seeds had the highest germination percentage (95 ± 2%), followed by seeds that were soaked in hot water for 12 h (67 ± 1%), which were not statistically different from seeds soaked in hot water for 24 h and 48 h; whereas, treatment 3 which represented seeds soaked in cold water for 24 h was the least (12 ± 0.3%). Control (no pretreatment applied) attained 23 ± 0.5% germination. The results showed that there were highly significant ($p \leq 0.001$) differences in germination percentage among the treatments.

The results further showed that the germination value was highest in nicked seeds (T8) with an observed value of 105.9. The control exhibited even a higher value (0.8) than T2 which was at 0.4. Treatments involving hot water (T5—T7) had higher germination values than those with cold water treatments.

3.3. The Cutting Test for Senna spectabilis Seeds. Figure 2 shows the results of the cutting test at termination (28 days) of the experiment. Viability ranged from 3 to 84%. The highest number of viable seeds (84%) was observed in seeds soaked in cold water for 24 h, followed by seeds soaked in cold water for 12 h (82%); whereas, control registered (71%) viable seeds. The least number of viable seeds (3%) was observed in nicked seeds. Generally, rotten seeds were few in number in all the treatments.

3.4. Seedling Height Growth. Table 3 shows the effect of the pretreatment methods on seedling mean height (cm). There were significant differences ($p \leq 0.05$) in seedling height between treatments for the whole period (2 months). The tallest seedlings (5.8 cm) after 60 days were observed in treatment 8, which were nicked seeds; although, T8 was not statistically different ($p \geq 0.05$) from those seeds soaked in hot water for 48, 24, and 12 h and seeds soaked in cold water for 48 h. The same trend continued in the 2nd month, except for seeds soaked in cold water for 12 and 48 h, as well as control which were not significantly different ($p \geq 0.05$). The shortest shoot height growth (2.7 cm) was observed in seeds soaked in cold water for 24 h during the whole period of experiment.

3.5. Root Collar Diameter Growth of Seedlings. Further, Table 3 shows significant differences ($p \leq 0.05$) in mean root collar diameter between treatments for the whole period (2 months). The largest root collar diameter (1.8 mm) was attained by treatment 8, which represented nicked seeds, although it was not significantly different ($p \geq 0.05$) from the seeds soaked in hot water for 48, 24, and 12 h in the 1st month. The control was not significantly different ($p \geq 0.05$) from seeds soaked in cold water for 12 and 48 h. The trend differed in the 2nd month where nicked seeds recorded the largest root collar diameter growth similar to those soaked in hot water for 48, 24, and 12 hours as well as control. Seeds soaked in cold water for 24 hours attained the smallest root collar diameter (1.0 mm) growth.

3.6. Seedling Survival Rate of S. spectabilis. Table 4 displays the effect of pretreatment methods on the mean survival percentage of seedlings at the end of the experiment. There were no significant differences ($p \geq 0.05$) in survival percentages between treatments. In general, mean survival was high in all the treatments, although some seedlings perished within the treatments (Table 4). The maximum number of dead seedlings was 5 in seeds soaked in cold water for 12 h.

4. Discussion

4.1. Germination Percentage and Value of Senna spectabilis Seeds. Significant differences observed between the pretreatments in germination percentage (Table 2) may indicate that germination of *S. spectabilis* seed is influenced by pretreatment methods. This assertion is confirmed by the highest germination in seeds that were nicked as opposed to those of other presowing treatments (Table 2 and Figure 1). The results in the present study are in agreement with [27] who reported that the pretreatment methods such as hot water, cold water, mechanical scarification, and chemical

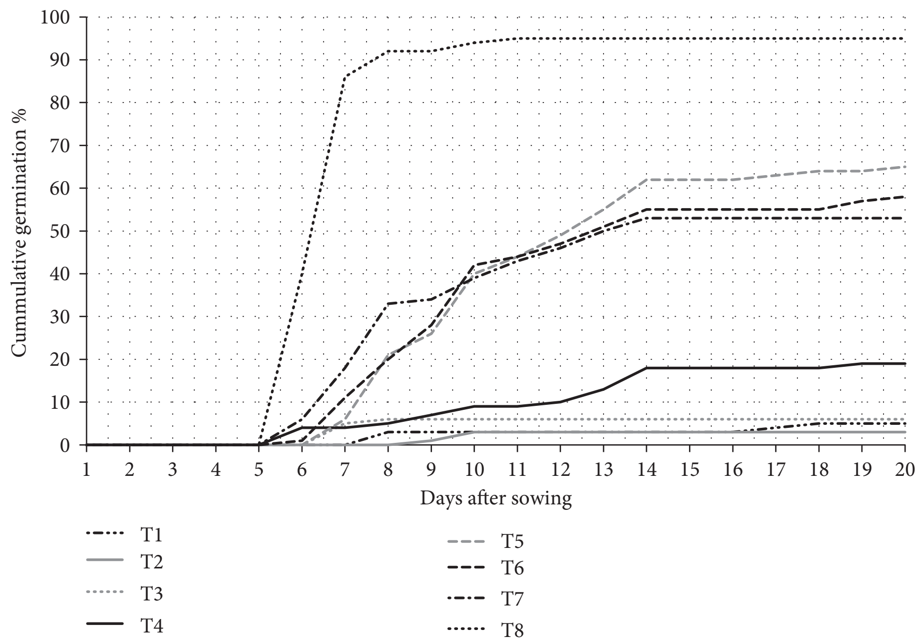

Figure 1: Germination trend of *Senna spectabilis* seeds, 28 days after seed sowing.

Table 2: Treatment method, germination percentage, and germination value of *S. spectabilis*, 28 days after seed sowing.

Treatment	Treatment description	Germination % (mean ± SD)	Germination value
T1	Control: no pretreatment applied	23 ± 0.5^{c}	0.8
T2	Seed soaked in cold water for 12 h	16 ± 0.4^{c}	0.4
T3	Seed soaked in cold water for 24 h	12 ± 0.3^{c}	0.6
T4	Seed soaked in cold water for 48 h	23 ± 0.4^{c}	2.3
T5	Seed soaked in hot water for 12 h	67 ± 1^{b}	25.4
T6	Seed soaked in hot water for 24 h	58 ± 1^{b}	19.6
T7	Seed soaked in hot water for 48 h	53 ± 1^{b}	20.2
T8	Nicked seeds	95 ± 2^{a}	105.9
P value		<0.001	
$LSD_{0.05}$		0.143	

SD denotes standard deviation. Treatment means within each column followed by the same letters are not significantly different from each other at $p \leq 0.05$; $LSD_{0.05}$, least significant differences of means (5% level).

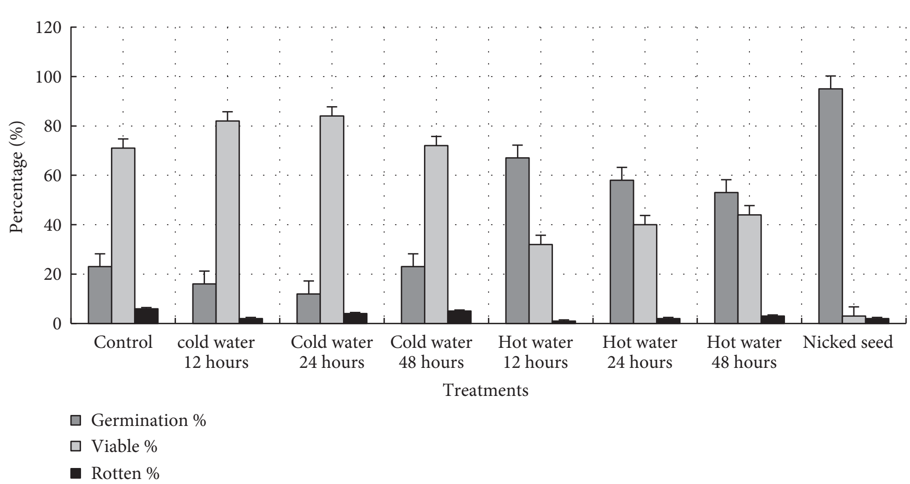

Figure 2: Germination and viability (cutting) test of *Senna spectabilis*, 28 days after seed sowing.

Table 3: Effect of the pretreatment methods on seedling mean height and diameter growth (cm) at 30 and 60 days after seed sowing.

Treatment	Treatment description	Mean height (cm) at 30 days	Mean height (cm) at 60 days	Mean diameter (mm) at 30 days	Mean diameter (mm) at 60 days
T1	Control (no pretreatment applied)	2.470^{c}	4.348^{b}	0.922^{cd}	1.603^{abc}
T2	Seeds soaked in cold water for 12 h	2.498^{c}	4.305^{b}	0.849^{cd}	1.525^{bc}
T3	Seeds soaked in cold water for 24 h	1.545^{d}	2.663^{c}	0.739^{d}	1.03^{d}
T4	Seeds soaked in cold water for 48 h	2.665^{bc}	4.585^{b}	0.986^{c}	1.468^{c}
T5	Seeds soaked in hot water for 12 h	3.075^{abc}	5.040^{ab}	1.002^{bc}	1.639^{abc}
T6	Seeds soaked in hot water for 24 h	3.215^{abc}	4.908^{ab}	1.176^{ab}	1.698^{abc}
T7	Seeds soaked in hot water for 48 h	3.375^{ab}	5.273^{ab}	1.171^{ab}	1.764^{ab}
T8	Nicked seeds	3.803^{a}	5.835^{a}	1.227^{a}	1.846^{a}
	P value	<0.001	<0.001	<0.001	<0.001
	$LSD_{0.05}$	0.536	0.536	0.417	0.536

Treatment means within each column followed by the same letters are not significantly different from each other at $p \leq 0.05$; $LSD_{0.05}$, least significant differences of means (5% level).

Table 4: Mean survival and survival rate of *Senna spectabilis* seedlings, 60 days after sowing.

Treatment	Treatment description	Mean survival (%)	Survival rate (%) (mean ± SD)
T1	Control: no pretreatment applied	24.3	97 ± 0.1
T2	Seed soaked in cold water for 12 h	23.8	95 ± 0.2
T3	Seed soaked in cold water for 24 h	23.5	96 ± 0.1
T4	Seed soaked in cold water for 48 h	24.0	97 ± 0.1
T5	Seed soaked in hot water for 12 h	24.8	99 ± 0.1
T6	Seed soaked in hot water for 24 h	24.3	97 ± 0.3
T7	Seed soaked in hot water for 48 h	24.5	98 ± 0.1
T8	Nicked seeds	24.8	99 ± 0.1
P value		>0.05	

treatment of seeds enhance germination percentage. According to Mwase and Mvula [28], nicking is known to break the physical dormancy of seeds with hard coats which inhibit water uptake and gaseous exchange. Thus, the highest germination percentage (95 ± 2%) of nicked seeds may be attributed to cracks or cuts made on the seed coat which might have made it easier for entry of water and exchange of gases, transforming the embryo into a seedling [29].

On the other hand, seeds that were soaked in hot water for 12 h had a germination percentage of 67 ± 1%, as being the second from nicking pretreatment. Msanga [30] rates germination of 60–79% as good. Hence, soaking *S. spectabilis* seeds in hot water for a period of 12 h may be regarded as a good presowing treatment for *S. spectabilis*. According to Mwase and Mvula [28], soaking in hot water makes the seed coats permeable to water, and the seeds imbibe and swell as the water cools. Probably, hot water treatment for 12 hours effectively softened the hard coat of *S. spectabilis* seeds in this study. The results are in agreement with Padma et al. [31] who reported that treating the seeds of *Senna siamea* with hot water produced the highest germination percentage.

Significantly lower germination percentage observed in cold water and the other pretreatment methods (Table 2) could be a result of the hard coat of the seeds. The hard seed coat hindered water imbibition and exchange of gases for the embryo to be transformed into a seedling, hence low germination. The results of the cutting test at 28 days of the experiment revealed high percentages of viable seeds in cold water and other pretreatment methods (Figure 1). The inability of the *S. spectabilis* seeds to germinate could, therefore, be attributed to the coat-imposed dormancy mechanism. In support, Dalling et al. [32] reported that if a large proportion of seeds do not germinate but appear fresh at the end of a germination test, it is likely that the seeds are dormant.

The fast germination displayed by nicked seeds (Table 3) may be due to the effectiveness of nicking in breaking the hard coat dormancy of the seeds through the cuts made on the seeds. This allowed the seeds to imbibe water and exchange gases for germination to be triggered and vice versa for the other treatments where germination was sporadic. Speed of germination is a measure of fast seedling establishment in the field [33]. Thus, extrapolating these results, nicked seeds have shown the potential of high field survival, hence the best presowing treatment for *S. spectabilis* seeds (Table 2).

4.2. Seedling Height and Root Collar Diameter Growth. Substantial significant differences observed in mean height and root collar diameter growth (Table 3) between the treatments could imply that pretreatments influenced seedling growth of *S. spectabilis* in this study. The effectiveness of nicking as a pretreatment method in breaking the hard seed coat dormancy produced high vigour seedlings, hence rapid growth. The results in the present study are in conformity with other researchers [27, 34]. According to Tian et al. [33], seed germination is the most crucial stage that affects earlier seedling growth and establishment. Seeds that were soaked in hot water for 12 h were equally good as they also showed the highest increment in mean height and root collar diameter growth.

4.3. Seedling Survival. The lack of significant differences in mean survival between the treatments (Table 4) may mean that the pretreatment method did not necessarily have any influence on the survival of *S. spectabilis* seedlings. However, the high survival rate in seedlings developed from nicked seed (99 ± 0.1%) and seed soaked in hot water for 12 h (99 ± 0.1%) could be due to the effectiveness of the presowing methods.

5. Conclusion

The findings of the study revealed that pretreatment methods significantly enhance the germination process and seedling growth of *Senna spectabilis* seed. With hard seed coat, *Senna spectabilis,* takes more time to germinate but with a lower germination percentage in nursery establishment. However, effective pretreatment methods can ensure the successful germination and growth of this agroforestry tree species among farmers. Among the treatments applied in the experiment, the best effective treatment found for *Senna spectabilis* was nicking and hot water soaking for 24 h method in respect to faster germination and fast seedling growth. Since seed germination and seedling growth under soaking in hot water for 24 hours is quite simple and inexpensive, it is also recommended for raising *Senna spectabilis* seedlings on a large scale to enable acceleration of domestication and commercialization drives among farmers in subtropical regions. Research related to the growth performance of *Senna spectabilis* after the seedling stage is relatively rare. It has been well recognized from literature that the growth performance of many species is also associated with presowing seed treatments. So, further studies are highly recommended on biomass production of *Senna spectabilis* seedlings and their volume production performance under different pretreatment methods of their seeds.

Data Availability

The data used to support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors thank Messrs Elton Pwele and Clement Msamale who made great contributions towards the daily management of the experiments in the Departmental Nursery at Mzuzu University, Malawi. The entire Department of Forestry and Environmental Management at Mzuzu University deserve their gratitude for our constructive criticisms.

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