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SEED GERMINATION AND SEEDLING ESTABLISHMENT OF *SECURIDACA LONGEPEDUNCULATA* (POLYGALACEAE)

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Abstract

Securidaca longepedunculata Fresen. (Polygalaceae) is a multipurpose tree valued for its medicinal uses in Limpopo Province, South Africa. Hence, it is threatened due to human pressure which affects its regeneration potential since it is uncultivated. This poses a challenge in efforts aimed at its conservation. The objective of the present study was to investigate the germination potential of *Securidaca longepedunculata* seeds which have a very low and erratic germination under natural conditions. Seeds were collected in Nylsvley Nature Reserve and a number of pre-treatments, namely seed coat removal, sulfuric acid, gibberellic acid, boiled water, cold water and control were tested for their efficiency to improve germination under field, laboratory and greenhouse conditions. The results showed that some of pre-treatments have a stimulating effects on seed germination and seedling growth. Removal of the seed coat resulted in the highest germination percentage. This pre-treatment gave 90% seed germination under greenhouse conditions, while it was 63.3% for those grown in the laboratory, whereas untreated seeds under field conditions showed 0% germination. Boiled and cold water pre-treatments did not improve seed germination. The average heights of seedlings from different pre-treatments ranged from 4.5 cm to 22 cm with the highest seedling (22 cm) obtained in seed pre-treated with 400mg/l of gibberellic acid. It is concluded that removal of the seed coat is the most effective pre-treatment.

Key words: Regeneration potential, Conservation, Germination, Greenhouse, Pre-treatment.

Introduction

There is growing evidence that the rate of extinction of plant species is increasing across local and regional scales throughout the world (Thomas *et al*., 2004). This is fueled, among others, by human population growth and the associated escalating demands on resources and climate driven changes (Gaston, 2005). Species most at risk of extinction are those that are already limited in abundance and/or distribution (Wilson *et al*., 2004).

Seed germination and seedlings establishment are critical stages in the life-cycle, which determine plant population dynamics and ultimately community development, structure and sustainability in the recruitment of plant populations (Chen & Xie, 2007; Baeten *et al*., 2009). On the other hand, seed germination and seedling establishment are the most vulnerable stages to environmental stress and are characterized by extremely high mortality rate and most intense natural selection of the entire life-cycle (Leck *et al.,* 2008; Kolb & Barsch, 2010). Information on seed germination and seedlings establishment is therefore crucial for predicting a plant's survival capacity and establishing the appropriate measures for its conservation. Such studies may help to identify the factors that reduce seed germination ability and seedlings growth capacity of individual plants and the subsequent maintenance or regeneration of populations.

Securidaca longepedunculata Fresen., commonly known in English as the violet tree, is an important multipurpose species belonging to the family Polygalaceae. The tree is widely distributed in tropical Africa. It has been recorded from Senegal, Sierra Leone, Liberia, Cote d'Ivoire, Ghana, Togo, Benin, Angola, Benin, Burundi, Chad, Cameroon, Botswana, D.R Congo, Rwanda, Sudan, Niger, Zambia, Zimbabwe, South Africa, Mozambique and Tanzania (Irvine, 1961). This tree occurs in various types of woodlands and arid savannas and is common in savanna woodlands in South Africa. The species has been reported from the North West and Limpopo provinces of South Africa (Van Wyk & Van Wyk, 1998).

Securidaca longepedunculata is a semi-deciduous erect shrub species or a small 2-12 m high savanna tree. It can be distinguished from other species co-occurring by its pale-grey smooth bark. Leaves are variable in size and shape, ranging from alternate, clustered, simple and broadly oblong to narrowly elliptic; apex rounded; base narrow; margin entire; petiole slender and up to 5 mm long. Flowers are small racemes of about 10 mm long, bright purple or pink in colour (Tshisikhawe *et al*., 2012). The fruits are one-seeded round samaras with a rigid or membranous wing of up to 4 cm at one end; purplish green when young and pale grey when mature. Seed are enclosed in an indehiscent pericarp which is hard and somewhat resistant to the passage of water.

The use of *S. longepedunculata* varies immensely across different countries. It is commonly used as a traditional medicine in many parts of Africa for the treatment of rheumatic conditions, fever, headache, coughs, chest complaints, toothaches, gout, constipation, microbial infections and various other inflammatory conditions (Iwu, 1993). It is reputed to have over one hundred medicinal uses (Dapar *et al.,* 2007). There is a great demand for its roots, which is the prime cause of its indiscriminate uprooting. Presently, all supplies of *S. longepedunculata* roots are obtained from the natural resource, which is declining due to over-exploitation.

Due to its over-harvesting, *S. longepedunculata* is becoming a threatened species at risk of extinction. It has been listed as a protected tree in the National Forest Act of 1998 of South Africa. Because seed germination and seedling establishment determine the long-term persistence of populations and species; the motivation behind this research was to build the knowledge base on seed germination and seedling establishment of *S. longepedunculata* to understand seeds behaviour of this tree under field, laboratory and greenhouse conditions. Such an understanding could serve to increase the availability of, and also assist in the introduction of this species into the agroecosytem sector which can go a long way in remedying the situation. Despite its poor recruitment rate and economic and ecological importance, almost all existing research has focused on its medicinal properties with little attention on the germination biology of the species. This research therefore attempts to answer this question: Can pre-treatment of seeds help us understand the causes of germination complexities of this species? Therefore, the present study was aiming at enhancing and determining the optimum conditions for seed germination and early seedling establishment of *S. longepedunculata* through the use of several pre-treatments. The hypothesis was that optimum conditions for seed development can be attained thereby establishing a protocol for successful propagation.

Materials and Method

Seed collection and study area: Seeds used in the experiments were collected from mature S. *longepedunculata* trees in Nylsvley Nature Reserve (NNR) in September 2013. NNR is located in the Waterberg region between the latitudes of 24º35'S and 24º40'S, and longitudes of 28º35'E and 28º45'E in Limpopo Province, South Africa.

To test the germination capacity of *S. longepedunculata* seeds, three separate experiments were conducted under field, laboratory and greenhouse conditions.

Experiment-1

Germination under field conditions: Seed germination experiment was conducted in the field to estimate the germination and seedlings growth for this species under natural conditions. This experiment was carried out in September 2013 to determine whether the seeds of *S. longepedunculata* were able to germinate or to maintain their germination ability over one entire year under field conditions. Only for this experiment, all seeds were collected on the standing mature tree.

The evaluation of seed germination under field conditions was conducted as follows: fifteen intact seeds were enclosed in 1 mm mesh nylon bags filled with moistened sieved soil collected from around the mature tree. Ten bags were randomly buried at 5 cm deep inside the plot near the parent tree and ten other bags were buried 50 m away from the parent tree. Each bag was tied to a nylon string to facilitate recovery. Two randomly selected bags from each site were taken out every two months. Recovered seeds were examined and split seeds as well as radicles already emerged were described as seeds germinating *in situ*.

Experiment-2

Seed pre-treatment and germination under laboratory conditions: A number of pre-treatments were applied to improve germination of *S. longepedunculata* seeds under laboratory conditions. A total of 900 seeds were treated.

Pre-treatment methods (sulfuric acid, gibberellic acid, boiled water, cold water, seed coat removal and control) were used in this experiment. Each pre-treatment had four replicates. Each replicate used a sample of fifteen seeds which were randomly selected from the total of *S. longepedunculata* seed collected under and on the standing mature trees. Each replicate after pre-treatment was placed in Petri dishes which were lined with three sheets of moistened filter paper and then, the Petri dishes were closed and placed on the table in the laboratory at ambient temperature. Watering was done when necessary with distilled water over a period of 30 days. Germination was monitored daily and recorded. Seeds were considered germinated when the healthy, white radical had emerged through the integument/seed coat. The experiment was done in the School of Mathematical and Natural Sciences, University of Venda, and was conducted between the months of August and September 2014.

Pre-treatment-1

Seed coat removal: This pre-treatment assessed the existence of a physical barrier to germination due to the seed coat. Seed coats were removed and the seeds were placed in distilled water for 24 hours and those which had sunk were considered viable (Rossini-Olive *et al*., 2009). The testing of seeds for viability and germination has been developed to reduce the risk of sowing seed that are non-viable. Sixty seeds divided into four replications were used for this pre-treatment.

Pre-treatment-2

Sulfuric acid (H_2SO_4): This pre-treatment assessed the effect of sulfuric acid (H_2SO_4) and compared different periods of pre-treatment. Two hundred and forty seeds were divided into four groups of 60 seeds. They were soaked in concentrated sulfuric acid (95-97%) for 10, 20, 40 and 60 minutes, respectively. After each period, seeds were washed thoroughly with tap water to remove all the acid residuals. Treated group of 60 seeds corresponding at each period was distributed into four different Petri dishes containing three layers of moistened filter paper.

Pre-treatment-3:

Gibberellic acid (GA_3): This pre-treatment assessed the effect of gibberellic acid on seeds and compared different concentration of GA_3. Seeds were soaked for 24 h in different concentrations (50, 100, 200 and 400 mg/L) of gibberellic acid (GA_3). For each concentration, 60 seeds were immersed in 200 ml flasks containing 100 ml solution of a given gibberellic acid concentration. After 24 h of soaking, the solution was drained off and seeds rinsed thoroughly with distilled water for 2 minutes. Treated seeds were distributed into four different Petri dishes containing three layers of moistened filter paper.

Pre-treatment-4

Boiled water: This pre-treatment assessed the effect of hot water treatment on the seeds. Water was boiled at ca. 100°C. The seeds were then immersed in the hot water in a 100 ml beaker for periods of 1, 5, 10 and 15 minutes respectively at a rate of 60 seeds for each period. Treated seeds for each period were distributed into four Petri dishes containing three layers of moistened filter paper.

Pre-treatment-5

Cold water: For cold water pre-treatment, 60 seeds were soaked in distilled water at room temperature for 48 hours and then distributed into four Petri dishes containing three layers of moistened filter paper.

Pre-treatment-6:

Control: Sixty seeds were washed, divided into four and placed on three layers of moistened filter paper in four Petri dishes.

All these germination experiments in the laboratory were monitored and recorded daily and over a period of 30 days. Germinated seeds were removed during every inspection. Seeds were considered to have germinated when a 1 mm tall white radicle emerges through the seed coat. Thereafter, germination percentage for each pre-treatment was calculated.

Experiment-3

Seed pre-treatment and germination under greenhouse conditions: In late August 2014, the commercial growth medium, hygromix produced by FertAgChem a division of Hygrotech in South Africa, were filled into a 200 cavity polystyrene seeds trays of 30 ml cavity volume. Hygromix is a high quality peat based growing medium with the correct macro- and micro-nutrients ensures healthy growth of seedlings. The trays were placed on a metal bench in the greenhouse situated at the School of Agriculture, University of Venda. The greenhouse was equipped with a wet wall for humidity control and a fan for temperature control. Four hundred and fifty seeds were used for all the pre-treatments methods in the greenhouse.

After the same pre-treatment done in the laboratory as described above with sulfuric acid (10, 20, 40 and 60 minutes), gibberellic acid (50, 100, 200 and 400 mg/l), boiled water (1; 5; 10 and 15 minutes), cold water for 48 hours and control, 30 seeds were used for each pre-treatment and grown in the greenhouse.

One seed from each pre-treatment was sown in each cavity to about the same depth as the size of the seed. Watering was done when necessary to maintain the moisture content for better seed germination conditions throughout the duration of the experiments. Germination was monitored daily and recorded. Seeds were considered germinated when the healthy, white radical had emerged through the integument/seed coat. Germinated seeds were manually removed from the cavity of seed trays and transferred into 300 ml black plastic bags filled with “hygromix" growth medium.

Calculation of germination percentages: Average percentages of all germination experiment under field, laboratory and greenhouse conditions were calculated. Germination percentage (GP) was calculated as follows:

$$GP = (\text{germinated seeds}/\text{total tested seeds}) \times 100$$

Production of seedlings: Germinated seeds were used to produce seedlings. They were transferred directly into black plastic bags filled with "hygromix" growth medium and monitored in the greenhouse. The height of each seedling was measured and the numbers of leaves counted and recorded after every five days over a period of 70 days. After this period, seedlings were taken out of the greenhouse and placed in the shade house for seven days for acclimatization. Of the seedlings that survived acclimatization, 105 seedlings were randomly selected and transported to NNR for transplanting in their natural habitat (*in situ*). Thirty five seedlings were transplanted to shaded sites beneath the canopy of a parent tree; another 35 seedlings were transplanted to shaded sites but away from the parent tree and the last 35 seedlings were transplanted to open sites fully exposed to the sunlight throughout the day. Once planted the seedlings in the field received only natural rainfall. The height of each seedling at the moment of planting on the field was measured and the number of leaves were counted.

Calculation of survival percentages: Survival percentage (SP) of seedlings which survived over a period of 70 days under greenhouse conditions was calculated based on the germinated seeds.

$$SP = (\text{Survived seedlings}/\text{Germinated seeds}) \times 100$$

Statistical methods of data analysis: All data were recorded on Excel 2010 spreadsheets and then subjected to Kolmogorov and Smirnov test and after that the data were subjected to One Way Analysis of Variance (ANOVA) at the 0.05 level of significance using the Graghpad Instat 3 Computer Program. Tukey-Kramer Multiple Comparisons Test was used to compare mean. The data presentation was done using tables and line graph.

Results and Discussion

Germination under field conditions: The germination under field conditions was carried out from September 2013 to September 2014 in the Nylsvley Nature Reserve to determine the germination ability of *S. longepedunculata* seed over one entire year. The results showed the inability of these seeds to emerge successfully from 5 cm depth where they were sown.

No germinating seeds of *S. longepedunculata* were found in the 1-mm mesh nylon bags taken out after every two months during an entire year of the study. From the first two months, recovered seeds from two randomly selected bags for each site were examined. The inspection of these seeds revealed that none of the seeds had germinated and they were opened and rotted, containing grains of sand inside the coat, suggesting that the germination process had been interrupted and the embryo

had died as it has, for example, also been reported for *Pistacia* species (Crane & Forde, 1974). Probably this interruption has been done due to the fact that seeds were buried at 5 cm deep; whereas, the general rule is to plant a seed at a depth equal to the size of the seed itself (Amponsah *et al*., 2002). Seed of *S. longepedunculata* is approximately 1 cm in size and should therefore have been buried at a depth of about 1 cm. In this case, the experiment did not benefit germination for these seeds when buried at a depth of 5 cm and did not allow qualifying seeds of *S. longepedunculata* as a transient or persistent seed. Besides, seeds in field soil banks, experience stresses of drought and frost, pathogen infection and animal feeding which makes the species lost its seed resources further.

Germination under laboratory conditions: No germination was detected during 30 days of observation in the petri dishes with intact seeds treated with sulfuric acid, gibberellic acid, boiled water and cold water (48 hours). Seeds used as a control also did not show any germination during 30 days, whereas the germination when the coat was removed started 2 days after sowing and on the fifth day which was the last day of counting and because the remaining seeds were rotten, the germination percentage was 63.3%.

Germination under greenhouse conditions: In the greenhouse, seed treatments were similar to the ones under laboratory conditions. After the treatment, seeds were sown in the growth medium. Uncoated seeds were sown in the growth medium as well. Under greenhouse conditions, uncoated seeds produced the best germination, the germination occurred after seven days and the germination percentage for uncoated seeds was 90% whereas none of the seeds treated with boiled water have germinated during a period of 30 days. Average germination percentage for all treated seeds except the boiled water ones in the greenhouse was 48.12%.

Effect of different pre-treatment under laboratory and greenhouse conditions

Effects of coat removal and control (untreated seeds) on seed germination and seedlings growth: Seeds used as a control under laboratory conditions did not germinate at all. In the greenhouse, the germination percentage of *S. longepedunculata* seeds under control treatment was 26.7%. The results show that the complete removal of the seed coat positively affected the germination of *S. longepedunculata* seeds under both laboratory and greenhouse conditions. The highest germination percentage (laboratory: 63.3% and greenhouse: 90%) was recorded for this pre-treatment, indicating that it was the most efficient treatment. This treatment was the best to overcome seed dormancy. Within 15 days, seeds exhibited high germination percentages. The removal of the seed coat enhanced seed germination of *S. longepedunculata*. Similar results were found in *Olea europaea* that the removal of the stony endocarp improved seed germination (Rugini, 1986). Removing the seed coat in *Securidaca longepedunculata* improved its germination percentage. The comparison between control and coat removal showed that there was a very significant differences ($p<0.01$) in all the parameters studied (Table 2). The results of the present study revealed that removal of stony endocarp significantly improved the chances of seed germination when compared with untreated seeds. In the control experiment, no effect on germination was observed under laboratory conditions. Seventy days after transferring the germinated seeds to black plastic bags containing a growth medium (hygromix) and maintained in a greenhouse, the survival percentage of seedlings produced from the uncoated seeds was 85.1% whereas control treatment presented 62.5%. The average height was 17 cm with 22 leaves for uncoated seeds and 11.2 cm with 17 leaves for control.

Effects of sulfuric acid (H_2SO_4) on seed germination and seedlings growth: Under greenhouse conditions, *S. longepedunculata* seeds soaked in 95-97% concentrated sulfuric acid showed differences between various durations of sulfuric acid on germination (Table 1). Seeds treated with H_2SO_4, for 10, 20, 40 and 60 minutes have 56.7%, 43.3%, 10% and 13.3% germination percentages respectively. However, difference in germination percentage observed between seeds treated with 95-97% concentrated H_2SO_4 at 10 and 20 minutes did not exhibit significant differences similar with 40 and 60 minutes where their germination percentage was low but without significant differences. Significant differences ($p<0.05$) were detected between seeds soaked in H_2SO_4 for 10 minutes and seeds soaked in H_2SO_4 for 40 minutes and between seeds soaked in H_2SO_4 for 10 minutes and seeds soaked in H_2SO_4 for 60 minutes.

From these results, after an appropriate time of H_2SO_4 treatments, H_2SO_4 could start to have a negative effect on the seeds germination. Thus, germination decreases when seeds were left in sulfuric acid for more than 20 minutes, suggesting that the embryo may get killed due to the penetration of concentrated sulfuric acid into the seed for a prolonged period (Table 1). Exposure of seeds to a longer duration in H_2SO_4 reduced seed germination. For instance, seeds soaked in H_2SO_4, for 40 and 60 min gave a significantly lower seed germination percentage (10% and 12%) compared to other pre-treatments. This has also been reported by Wang *et al*. (2007), who showed that too long seed treatment within the H_2SO_4 will damage the embryo of a seed, resulting in a lower germination rate or the loss of seed vigor. Herron & Clemens (2001) noted a similar reduction in seed germination following acid scarification of *Melicytus ramiflorus* seeds. In the present experiment, long seed treatment within the H_2SO_4 reduced the germination percentage of *S. longepedunculata* (Table 1). Therefore, prolonged submersion in 95-97% concentrated H_2SO_4 was rather harmful for the germination; since the concentration of the acid was more and the time of exposure of the seeds to the acid was long. Hence this prolonged submersion time should be reduced in seeds of *S. longepedunculata*.

Seventy days after transferring the germinated seeds to black plastic bags containing a hygromix growth medium and maintained in a greenhouse, the survival percentage of the seedlings produced from the seeds that were soaked in sulfuric acid for 10, 20, 40, and 60 minutes, were 82.3%, 61.5%, 33.3% and 75% respectively. The survival percentage showed a significant difference ($p<0.01$) between seeds soaked in H_2SO_4 for 10 minutes and seeds soaked in H_2SO_4 for 40 minutes and another significant difference

($p<0.05$) between seeds soaked in H_2SO_4 for 10 minutes and seeds soaked in H_2SO_4 for 60 minutes (Table 1). However, there were no significant differences among other pre-treatments. The average heights and number of leaves of the seedlings produced from the seeds that were soaked in sulfuric acid for 10, 20, 40, and 60 minutes, were 10.9 cm and 19 leaves, 8.9 cm and 16 leaves, 11.8 cm and 18 leaves and 9.2 and 16 leaves respectively. The shortest height (4.5 cm) was observed in seed treated with H_2SO_4 for 60 minutes. Seedlings height did not show significant differences between pre-treatments. Number of leaves for different pre-treatment methods showed a significant difference between seeds soaked in H_2SO_4 for 10 minutes and seeds soaked in H_2SO_4 for 20 minutes and significant difference between seeds soaked in H_2SO_4 for 10 minutes and seeds soaked in H_2SO_4 for 60 minutes (Table 1). Overall for this experiment, the differences may arise due to the time of exposure of the seeds to the acid, since seeds exposed in acid for a long time get damaged easily.

Effects of gibberellic acid (GA_3) on seed germination and seedlings growth: Gibberellic acid (GA_3) treatment was ineffective under laboratory conditions; whereas in the greenhouse, GA_3 promoted germination percentage and this response was dependent on the concentration of applied GA_3 (Table 1). Difference in germination percentage and other parameters was observed among seeds treated with various concentration of GA_3. As GA_3 concentration increased from 50 mg/l to 400 mg/l, the germination percentage increased from 30% to 70%. At lower concentration of 50 mg/l, germination percentage was low at about 30% and at higher concentrations 400 mg/l, germination percentage was increased by about 70%. There were differences among various applied concentrations of gibberellic acid on different parameters related to seed germination and seedling growth (Table 1). The application of gibberellic acid treatments had enhancing effects on germination. The results of the experiment revealed that soaking of *S. longepedunculata* seeds in gibberellic acid at 400 mg/l had the highest germination percentage of 70%; whereas, 56.7%, 20% and 30% seed germination were achieved at 200 mg/l, 100 mg/l and 50 mg/l of GA_3 respectively.

It was found that 400 mg/l of GA_3 treatment resulted in a higher seed germination with difference in all the parameters studied compared to other concentrations (Table 1). All the parameters studied with 400 mg/l of GA_3 produced the highest value and the second effective method to improve germination and seedlings growth was 200 mg/l of GA_3 treatment. This indicates that the regulation of endogenous GA_3 levels after seed imbibition is a crucial factor in determining seed germination. The results of the current study are in agreement with the findings of other researchers (Rahnama & Tavakkol-Aafshari, 2007) who showed that germination of *Ferula gummosa* and *Teucrium polium* seeds increased at higher concentrations of GA_3. However, the results of this current research are in contrast to the work reported in Zulu *et al*. (2011) which showed that germination rates of *S. longepedunculata* seeds declined with increasing concentrations of GA_3 in the growing medium with compost manure. Germination percentages for these species obtained in the present study are higher than for the same study reported in Zulu *et al*. (2011). The experiment conducted in that study showed that germination rates of *S. longepedunculata* seed were very low (<45%) in all treatments with gibberellic acid. Nevertheless, the results in our study indicated that there were no significant differences ($p>0.05$) between different pre-treatments methods for the germination percentage except between 400mg/l and 100 mg/l where the difference in germination percentage was observed ($p<0.05$).

Table 1. Effects of sulfuric acid (H_2SO_4) and Gibberellic acid (GA_3) pre-treatments on seed germination, survival and height *Securidaca longepedunculata* seedlings, with means in parenthesis.

	Treatments	Germination (%)	Survival (%)	Height (cm)	No of leaves
Sulfuric acid	H_2SO_4 10min	56.7 (1.2)^{a}	82.3 (0.8)^{a}	10.9 (0.5)	19 (1)^{a}
	H_2SO_4 20min	43.3 (0.6)^{ab}	61.5 (0.8)^{ab}	8.9 (0.3)	16 (0.5)^{b}
	H_2SO_4 40min	10 (0.5)^{b}	33.3 (0.3)^{b}	11.8 (1)	18 (0)^{ab}
	H_2SO_4 60min	13.3 (0.3)^{b}	75 (0)^{b}	9.5 (0.7)	16 (0.5)^{b}
Gibberellic acid	GA_3 50mg/l	30 (1.5)^{ab}	77.7 (2)^{a}	11.6 (0.9)^{a}	17 (1)^{a}
	GA_3 100mg/l	20 (1)^{a}	66.7 (1.5)^{a}	11(0.5)^{a}	18 (1.7)^{ab}
	GA_3 200mg/l	56.7 (0.8)^{ab}	88.2 (1.1)^{ab}	11.7 (0.6)^{a}	18 (1)^{ab}
	GA_3 400mg/l	70 (0.5)^{b}	90 (1.1)^{b}	17.4 (1.1)^{b}	21(1)^{b}

In each column, means with the similar letters are not significantly different at $p<0.05$

Table 2. Summary of pre-treatments effects on various parameters related to seed germination and growth of *Securidaca longepedunculata* seedlings in greenhouse with means in parenthesis.

Treatments	Germination (%)	Survival (%)	Height (cm)	No of leaves
Control	26.7 9 (0.66)^{c}	62.5 (0.57)^{c}	11.2 (0.34)^{c}	17 (1)^{c}
Coat removal	90 (0.57)^{a}	85.1 (1.1)^{a}	17 (1.2)^{a}	21 (2)^{a}
H_2SO_4 10min	56.7 (1.2)^{abc}	88.3 (1.5)^{a}	10.9 (2.7)^{c}	19 (1.7)^{bc}
GA_3 400mg/l	70 (0.57)^{ab}	90 (1.1)^{ab}	17.4 (1.9)^{a}	21 (1)^{ab}
Cold water	46.7 (0.88)^{bc}	71.4 (1.5)^{bc}	12 (1.2)^{bc}	19 (1)^{bc}

In each column, means with similar letters are not significantly different at $p<0.05$

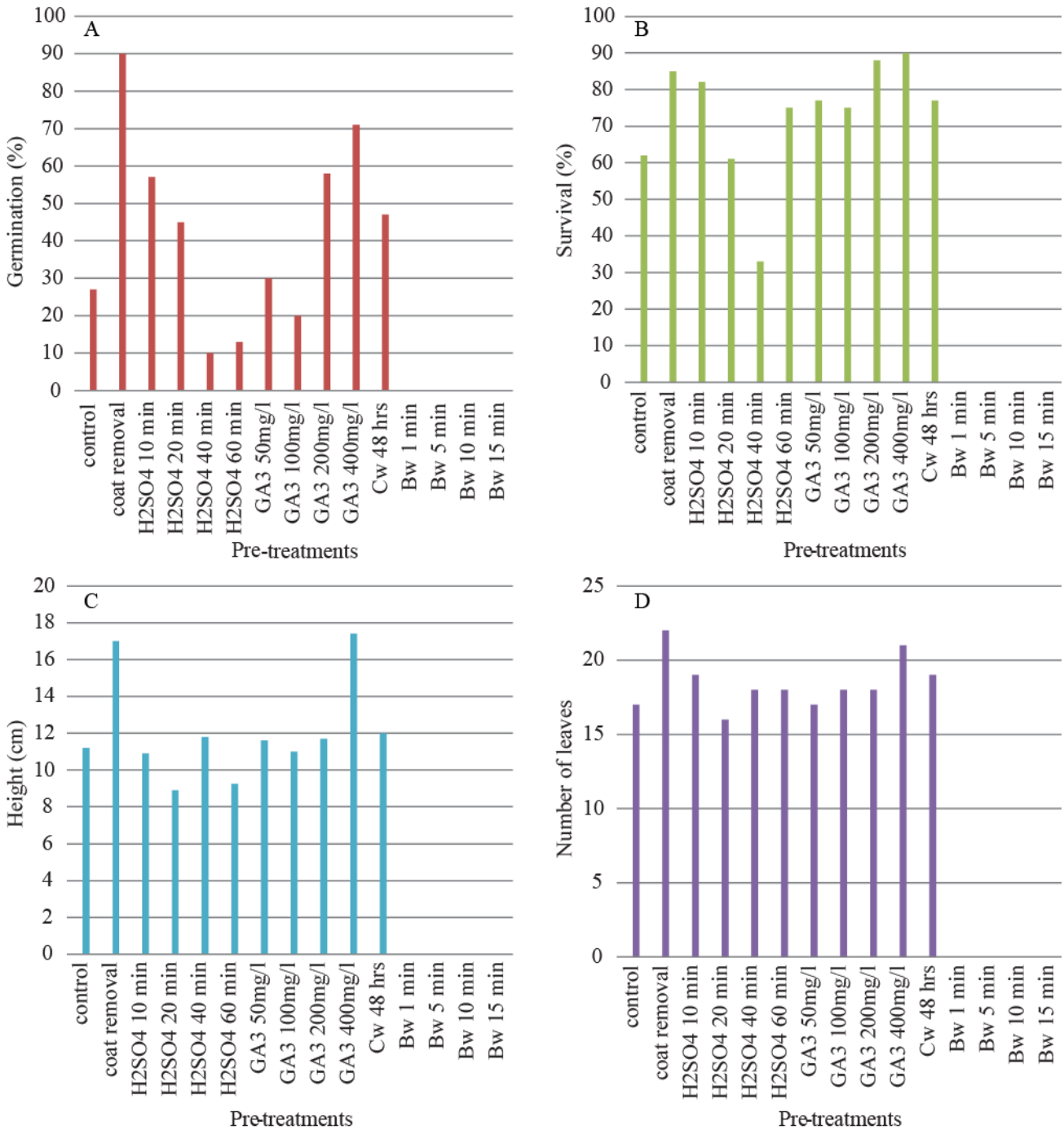

Fig. 1. Effect of different pre-treatments on (a) seed germination, (b) seedlings survival, (c) seedling height, and (d) number of seedling leaves of *S. longepedunculata.*

Seventy days after transferring the germinated seeds in black plastic bags containing a hygromix growth medium and maintained in a greenhouse, the survival percentage of the seedlings produced from the seeds that were soaked in different concentration of GA_3 at 50, 100, 200, and 400 mg/l, were 77.7%, 66.7%, 88.2% and 90% respectively. For survival percentage, significant differences were observed between 400mg/l and 50 mg/l and between 400mg/l and 100 mg/l. An amount of 400 mg/l of GA_3 gave the highest seedling height growth and the results showed a significant difference ($p<0.05$) between 400 mg/l of GA_3 and other pre-treatments. The highest seedling (22 cm) with the average of 17.4 cm with 21 leaves was observed in seeds soaked in 400 mg/l followed by 200 mg/l (11.7 cm with 18 leaves), 50 mg/l (11.6 cm and 17 leaves) and 100 mg/l (11 cm and 18 leaves) No significant differences in the number of leaves were detected between all the pre-treatments.

Gibberellic acid is known to play an essential role in seed germination, leaf expansion, stem elongation, flowering and flower development. Our results are in agreement with those of Olmez *et al.* (2004) for *Capparis spinosa*, who found that seed dormancy is mainly due to the seed coat and that GA_3 has a positive effect on germination.

Effects of soaking in water on seed germination and seedlings growth: Boiled water: In our results, soaking of seeds in boiled water (100°C) for a period of time 1, 5, 10 and 15 minutes did not stimulate seed germination either in the laboratory or in the greenhouse (Fig. 1a). This shows that boiled water pre-treatment is not effective in the seeds of *S. longepedunculata*, suggesting that the embryos may get killed on contact with boiled water for a prolonged period of time. The results confirmed the earlier reports in Sudan by El Nour *et al*. (1993), where they found that treated seeds with boiled water resulted in reduced germination, although it is in contrast with another study by Schmidt & Joker (2000).

Cold water: Soaking seeds in cold water for 48 hours promoted the germination percentage of *S. longepedunculata* and gave 46.7% germination. Matias *et al*. (1973) reported that soaking in water for 2 to 48 hours improved seed germination of many tropical tree species such as *Acacia mearnsii*, *Acacia melanoxylon*, *Acacia nilotica*, *Adenanthere mirosperma* and *Albizia amara*. In the present study, soaking seeds in cold water was found to be effective in promoting germination (Table 2) as compared with soaking seeds in hot water. There were very significant differences ($p<0.001$) between the two treatments. Survival percentage in cold water soaking was 78.5%, and the average height was 12 cm with average number of leaves of 19 leaves.

High germination, seedling growth, and survival from seeds with coats removed (Table 2) suggested that, this is the best method to be applied before planting seeds of this multipurpose tree. While uncoated seeds started germinating seven days after sowing, coated seeds started germinating ten days after sowing. Results indicated that seeds of *S. longepedunculata* had an impermeable seed coat, which was hard to overcome. Breaking down impermeability of the seed coat by means of numerous experimental treatments above, resulted in considerable improvement of germination percentages in relatively short time and growth of seedlings. Therefore, seed germination occurs as a result of seed coat rapture due to pre-treating of *S. longepedunculata* seeds.

Seventy days after transferring the germinated seeds in black plastic bags containing a growth medium (hygromix) and maintained in a greenhouse, mean height growth of seedlings varied between a minimum of 4.5 cm and a maximum of 22 cm. Overall, seedlings height, number of leaves, and survival percentage was not very significantly variable in all the pre-treatments. The effect of different pre-treatment on germination percentage, survival, height and number of leaves of *S. longepedunculata* for the period of seventy days in greenhouse are presented in Figure 1. The tallest seedlings (22 cm) were produced by seeds treated with 400mg/l of gibberellic acid and the shortest (4.5 cm) was observed in seeds treated with sulfuric acid for 60 minutes. Height growth measurements indicated that most seeds need to be sown and kept in the greenhouse at least seventy days and more, before field planting. Therefore, the results indicated that hygromix medium used in greenhouse conditions can be preferably used to improve germination and seedling development of *S. longepedunculata*.

Comparison of laboratory and greenhouse environments on different parameters related to seed germination and seedlings establishment: Laboratory and greenhouse studies were conducted to develop techniques which could be used to characterise seed germination and seedling survival of *S. longepedunculata* when subjected to different environmental conditions. Results showed no consistent relationships between various parameters related to seed germination and growth of seedlings derived from filter paper experiments (laboratory) and obtained from the seed trays containing hygromix in the greenhouse. Statistical analyses showed that the pre-treatments used in this study affected seed germination percentage in the laboratory and in the greenhouse conditions significantly ($p<0.001$).

The differences in seed germination percentage between the laboratory and greenhouse could be attributed to differences between water-holding capacity of the "hygromix" used as the growing medium in the greenhouse and the filter paper used in the laboratory. Greenhouse conditions were well aerated than laboratory conditions, so the differences could have resulted from aeration variation of the two conditions. These effects are believed to be the reason for the differences in the computed seed germination percentage and seedling establishment in the greenhouse. Average percentage germination under greenhouse conditions amounted 48.12% in all the treatments 30 days after sowing. To reduce the time necessary to achieve consistent results, laboratory/ greenhouse studies are used to control or limit the number of environmental variables.

Conclusion

Field, laboratory and greenhouse studies were conducted to find techniques which could be used to promote the seed germination and early seedling establishment of this tree species when subjected to various pre-treatment protocols that could be used in the development of a standard germination protocol. It is evident that the seeds of this tree have a hard seed coat and would not germinate early and easily under natural conditions. Hence, this study investigated the effect of different pre-treatment methods and a growing media (hygromix) on germination and seedlings establishment of *Securidaca longepedunculata*. The study showed that pre-treatment of seeds have a positive effect on seeds germination and seedling growth. Removal of the seed coat showed the highest germination percentage whereas treatment with boiled water was not useful. It is hoped that the results of this study could provide useful information for enhancing the germination and seedling growth of *Securidaca longepedunculata* for large scale propagation practices. Therefore, the cultivation of these plants is needed to ensure a consistent and continued supply, since *in situ* conservation of these resources alone cannot meet the growing demand for material from this multipurpose tree. The development of cultural practices and propagation methods for these plants in suitable agroecosystem are necessary in order to prevent its eventual extinction through over exploitation.

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References

Amponsah, K., O.R. Crensil, G.T. Odamtten and W. Ofusohene-Djan. 2002. Manual for the propagation and cultivation of medicinal plants of Ghana.

Baeten L., H. Jacquemyn, H. van Calster, E. van Beek, R. Devlaeminck, K. Verheyen and M. Hermy. 2009. Low Recruitment across Life Stages Partly Accounts for the Slow Colonization of Forest Herbs. *J. Ecol.*, 97: 109-117.

Chen, F.Q. and Z.Q. Xie. 2007. Reproductive Allocation, Seed Dispersal and Germination of *Myricaria laxiflora*, an Endangered Species in the Three Gorges Reservoir Area. *Plant Ecol.*, 191: 67-75.

Crane, J.C. and H.I. Forde. 1974. Improved *Pistacia* seed germination. *California Agriculture*, 28(9): 8-9.

Dapar, L.P.M., C.J. Aguiyi, N.N. Wannang, S.S. Gyang and M.N. Tanko. 2007. The histopathologic effects of *Securidaca longepedunculata* on heart, liver, kidney and lungs of rats. *Afr. J. Biotech.*, 6(5): 591-595.

El Nour, M., K. El Khalifa, K. Massimo and B. Hassan. 1993. Preliminary study on seed germination treatment and vegetative propagation of *Balanites aegyptiaca* (L) Del. *Physiologie des Arbres et Arbustes en Zones Arides.* 413-416.

Gaston, K.J. 2005. Biodiversity and extinction: species and people. *Progress in Physical Geography*, 29: 239-247.

Herron, H. and J. Clemens. 2001. Seed Dormancy and Germination in *Melicytus ramiflorus* (Violaceae). *New Zea. J. Bot*., 39: 245-249.

Irvine, F.R. 1961. *Woody Plants of Ghana*. Oxford University. Press, London, United Kingdom.

Iwu, M.M. 1993. Handbook of African Medicinal Plants, CRC Press, Boca Raton.

Kolb, A. and K. Barsch. 2010. Environmental factors and seed abundance influence seedling emergence of a perennial forest herb. *Acta Oecologica*, 36: 507-513.

Leck, M.A., V.T. Parker and R.L. Simpson. 2008. *Seedling Ecology and Evolution*. Cambridge University Press, Cambridge, Unted Kingdom.

Matias, A.R., A. Betancourt, A, Zayas, A. Pena and A.Y. Rivero. 1973. Forest seed in Cuba. En "Seed Processing" Proc. Symposium IUFRO Working Group on seed problems. Bergen, Norway.

Olmez, Z., Z. Yahyaoglu and A.O. Üçler. 2004. Effects of H_2SO_4, KNO_3 and GA_3 treatments on germination of caper (*Capparis ovata* Desf.) seeds. *Pak. Biol. Sci.*, 7(6): 879-882.

Rahnama-Ghahfarokhi, A. and R. Tavakkol-Afshari. 2007. Methods for dormancy breaking and germination of galbanum seeds (*Ferula gummosa*). *Asian J. Pl. Sci*., 6(4): 611-616.

Rossini-Olive, S., E.O. Leidi and B. Valdes. 2009. Germination responses of *Erica andevalensis* to different chemical and physical treatments. *Ecol. Res.*, 24: 655-661.

Rugini, E. 1986. Olive *(Olea europaea* L). In: (Ed.): Bajaj, Y.P.S. Biotechnology in Agriculture and Forestry. 1: Trees. Springer, Berlin, Germany.

Schmidt, L.H. and D. Joker. 2000. *Balanites aegyptiaca* (L) Dell. Danida Forest Seed Centre seed leaflet No. 21. DFSC, Denmark.

Thomas, C.D., A. Cameron and R.E. Green. 2004. Extinction risk from climate change. *Nature*, 427: 145-148.

Tshisikhawe, M.P., O. Baloyi, M.H. Ligavha-Mbelengwa and R.B. Bhat. 2012. The population ecology of *Securidaca longepedunculata* Fresen. in the Nylsvley Nature Reserve, Limpopo Province, South Africa. *Phyton*., 81: 107-112.

Van Wyk, A.E. and P. Van Wyk. 1998. Field guide to trees of Southern Africa. Struik Publishers. Cape Town, South Africa.

Wang, Y.R., J. Hanson and Y.W. Mariam. 2007. Effect of sulphuric acid pre-treatment on breaking hard seed dormancy in diverse accessions of five wild *Vigna* species. *Seed Sci. & Tech*., 35: 550-559.

Wilson, R.J., C.D. Thomas, D.B. Fox and W.E. Kunin. 2004. Spatial patterns in species distributions reveal biodiversity change. *Nature*, 432: 393-396.

Zulu, D., B.L.K. Thokozani, G.W. Sileshi, Z. Teklehaimanot, D.S.B. Gondwe, V. Sarasan and P.C. Stevenson. 2011. Propagation of the Afri41can medicinal and pesticidal plant, *Securidaca longepedunculata*. *Afr. J. Biotech*., 10(32): 5988-5992.

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