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Improving germination and seedling growth to promote the widespread cultivation of the rare *Balanites aegyptiaca* trees

Zienab S. Ghaba, A.F.A. Ebeid, Wagdi S. Soliman and Abd-Allah Gahory

[1] Department of Horticulture, Faculty of Agriculture and Natural Resources, Aswan University, Egypt

Abstract

The current study was carried out in Agricultural Research Station of Al-Marashda, Qena Governorate, A.R.C., Egypt, during 2023 and 2024 seasons to investigate the impact of different pre-germination treatments on seed germination and seedling growth characteristics of *Balanites aegyptiaca* L. The experiment was arranged in a completely randomized block design consisting 9 treatments, three replications for each treatment. The pre-germination treatments were control (untreated seeds); soaking in tap-water for 24 and 48 h; soaking seeds in previously boiled water for 24 h at ambient temperature; soaking in GA_3 2000, 4000 and 6000 ppm for 24 h; mechanical scarification and immersion seeds in concentrated sulphuric acid for 5 min. The obtained results showed that *B. aegyptiaca* seeds appeared to be affected by the different pre-germination treatments for breaking their dormancy. Soaking seeds of *B. aegyptiaca* in tap-water for 24 h, followed by soaking seeds in previously boiled water for 24 h at room temperature and mechanical scarification resulted in the highest germination parameters i.e. germination percentage, daily germination speed, mean germination rate, and vigor index as well as enhanced seedling growth characteristics. The present investigation concluded the possibility of breaking seed dormancy this tree species and promoting its cultivation using the recommended pre-germination treatments in tap-water or previously boiled water for 24 h.

Key words: seed dormancy, pre-germination treatments, germination parameters, early seedling growth, ***Balanites aegyptiaca***.

Introduction

Since ancient times, woody trees are used for several purposes including traditional medicine as the source for the potent and powerful drugs, as well as ornamental trees and for timber production **[1]**. The traditional medical practices become an integral part of the culture even for the developing countries. Woody plants are considered as the primary source of supply of various important drugs and minerals in the orthodox medicine today **[2]**. Plants are being exploited for its active compound

Corresponding author E-mail: wagdi79@agr.aswu.edu.eg

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which is responsible for their biological function. One of the important and promising trees is *Balanites aegyptiaca*, which falls in the category of endangered plants species which requist conversation of these wild growing trees **[3]**. *B. aegyptiaca* belongs to Zygophyllaceae family, and is an evergreen tree widely spread in the arid regions of Africa **[4]**. *B. aegyptiaca* is a multi-purpose tree; roots are used an emetic and boiled into a soup and used to treat edema and stomach pains; the bark is used to deworm cattle, bark extracts are used to kill freshwater snails and copepods; the fruits are processed into beverages and liquor, and seed oil is used as laxative and for the treatment of hemorrhoids, stomach aches, jaundice, yellow fever, syphilis, epilepsy and in the production of biodiesel fuel **[5]**.

Many plant species face difficulty in germination and need pre-sowing treatments for breaking seed dormancy. It is important to understand seed biology and physiology for identifying the nature of specific seed problem and probable pre-germination treatment for overcoming seed dormancy **[6]**. Seed dormancy differs among plant species, stage of maturity and degree of hardness, so pre-germination treatment must be adjusted consequently. Physical seed dormancy may be overcome by mechanical scarification of the seed coat by nicking, piercing, chipping, filing with the help of knife, needle, hot wire burner, abrasion paper **[7]**. Hot water or acid treatments are also other techniques for overcoming physical dormancy **[8, 9]**. Effective propagation and seedlings establishment are the initial and basic requirements for sustainable management for these species. So, maintenance and sustainable development for this rare species has become indispensable. Germination of some species is sometimes difficult due to seed coat and the dormant embryos. However, seeds often fail to germinate even under favorable conditions of moisture, oxygen and soil. To overcome these problems, several methods such as mechanical scarification, soaking in water and acids are used for treating seeds prior to sowing **[10]**. A significant different was shown across such treatments applied on seeds of *B. aegyptiaca* **[11]**. Soaking in cold water for 24 h maintained the highest germination percentage. Therefore, the present investigation was carried out with the aim to enhance germination and seedling growth of *B. aegyptiaca* seeds as rare tree species in Egypt by applying various pre-germination treatments.

Materials and methods

This investigation was conducted at the Agricultural Research Station of Al-Marashda, Qena Governorate, ARC, Egypt (26° 9' N, 32° 42' E.) during two successive seasons of 2023 and 2024. The aim of study was to evaluate various pre-germination treatments on *Balanites aegyptiaca* L. seeds and their effect on seedlings growth. The seeds of *B. aegyptiaca* were obtained from Aswan Medicinal plant Market. The seeds were subjected to one of the following treatments: control (untreated seeds) (T1); soaking seeds in tap water for 24 h (T2); soaking seeds in tap water for 48 h (T3); soaking seeds in previously boiled water for 24 h at ambient temperature (T4); soaking in GA_3 concentration 2000 ppm for 24 h (T5); soaking in GA_3 concentration 4000 ppm for 24 h (T6); soaking in GA_3 concentration 6000 ppm for 24 h (T7); immersing seeds in concentrated sulphuric acid (98%) for five minutes with the quick washing with tap water (T8), and mechanical scarification (abrasion seed coat with sand paper) (T9).

Experimental design:

This experiment was done from 20th February to 20th May during the two growing seasons. Seeds were sown in 20 × 30 poly pots after pre-germination treatments. Poly pots were filled with clay loamy soil. The chemical and physical properties of soil were shown in Table (1). Ten seeds were sown per pot, about 2 cm depth and irrigation was done every two days until full germination. Observations for germination traits were recorded every three days. This experiment was layout as a randomized complete blocks design system including nine pre-germination treatments with three replicates, each replicate contained three pots.

Table 1: Physical and chemical analysis of the used growing media.

<table>
<tr><td rowspan="2">Physical properties</td><td>Soil texture</td><td colspan="2">Sand (%)</td><td>Silt (%)</td><td colspan="2">Clay (%)</td></tr>
<tr><td>Clay loam</td><td colspan="2">17.00</td><td>36.35</td><td colspan="2">46.65</td></tr>
<tr><td rowspan="2">Chemical properties</td><td>Organic Matter %</td><td>HCO_3 (meq/ 100 g soil)</td><td>SO_4^{--} (meq/ 100g soil)</td><td>Soil pH</td><td>E.C. (mmhose/cm)</td><td>Ca CO_3%</td></tr>
<tr><td>1.85</td><td>0.82</td><td>3.86</td><td>7.81</td><td>1.42</td><td>2.31</td></tr>
</table>

Collected data:

The data were recorded after three months from sowing seeds, at the end of the experiment, on 20th May and germination and seedlings were measured as follow: Germination percentage (G %), at the end of the germination period the germination percentage was calculated using the following equation by [12]:

$$G\ \% = (Ng \div Nt) \times 100,$$

where G % is germination percentage, Ng is the number of germinated seeds and Nt is the total number of sowing seeds.

Vigor index was calculated using the formulas proposed by [13]: VI= Seedling length × Germination %. Mean germination rate (MGR) was recorded according to [14] as number of days to attain 50% of total germination. Daily germination speed (DGS) was obtained by dividing the cumulative germination percentage by the number of days since sowing.

Stem length (cm), root length (cm), leaves number, stem diameter (cm), shoot fresh and dry weight (g), and root fresh and dry weight (g) were recoded three months after sowing seeds.

Statistical analysis:

The data of the two seasons were statistically analyzed according to the procedure outlined by [15]. The least significant difference (L.S.D) at 5% and 1% was used for comparing the means of treatments. Statistical analysis was performed using Microsoft Office 2010 Excel program.

Results and discussion

Germination measurements:

The effect of pre-germination treatments on germination percentage (G %), daily germination speed (DGS), mean germination rate (MGR) and vigor index (VI) of *B. aegyptiaca* seeds were measured (Table 2). The results showed significant differences among pre-germination treatments for the studied traits in the two seasons. The obtained results showed that the most effective treatments for G%, DGS, MGR and VI were recorded with soaking seeds in tap water for 24 h, followed by soaking in boiled water and then mechanical scarification treatment in the two studied seasons, respectively. On the other side, the least effective treatment for G%, DGS, MGR and VI were produced with untreated seeds, followed by immersion seeds in concentrated sulphuric acid for 5 min, and then soaking seeds in 2000 ppm GA_3 in the mean of the two seasons, respectively.

Table 2. Influence of pre-germination treatments on germination percentage (G %), daily germination speed (DGS), mean germination rate (MGR) and vigor index (VI) of *Balanites aegyptiaca* L. seeds during the 1^{st} season (2023) and 2^{nd} season (2024).

Pre-germination treatments	G %		DGS		MGR		VI	
	1^{st}	2^{nd}	1^{st}	2^{nd}	1^{st}	2^{nd}	1^{st}	2^{nd}
Control	18.5^{d}	22.2^{d}	0.032^{e}	0.041^{e}	26.3^{a}	24.5^{a}	747.8^{e}	983.3^{e}
Tap water 24 h.	85.2^{a}	92.6^{a}	0.247^{a}	0.317^{a}	15.7^{e}	13.2^{e}	5748.2^{a}	6498.5^{a}
Tap water 48 h.	48.2^{bc}	55.6^{b}	0.095^{bc}	0.124^{bc}	22.8^{c}	20.2^{bc}	2588.5^{bc}	3201.1^{bc}
Boiled water 24 h.	51.9^{b}	59.3^{b}	0.118^{b}	0.154^{b}	19.7^{d}	17.3^{d}	3243.0^{b}	3830.4^{b}
GA_3 2000 ppm	29.6^{cd}	37.0^{cd}	0.055^{cde}	0.076^{de}	24.7^{b}	21.8^{ab}	1505.2^{de}	1918.2^{de}
GA_3 4000 ppm	40.7^{bc}	44.4^{bc}	0.079^{bcd}	0.098^{cd}	23.0c	20.3^{bc}	2145.2^{cd}	2479.3^{cd}
GA_3 6000 ppm	51.9^{b}	55.6^{b}	0.106^{b}	0.135^{bc}	22.0^{d}	18.7^{c}	2994.4^{bc}	3320.0^{bc}
H_2SO_4 5 min.	29.6^{cd}	33.3^{cd}	0.052^{de}	0.064^{de}	25.7^{a}	23.3^{a}	1403.7^{de}	1676.3^{de}
Scarification	51.9^{b}	55.6^{b}	0.110^{b}	0.138^{b}	21.2^{d}	18.0^{cd}	3102.6^{bc}	3406.0^{bc}
LSD 5%	18.9	17.9	0.041	0.040	2.0	1.4	1030.2	1002.2
LSD 1%	26.1	24.6	0.057	0.055	2.8	1.9	1419.0	1380.5

Our results pointed out that the highest values of germination parameters were recorded with soaking seeds in tap water for 24 h, followed by soaking in boiled water for 24 h, and mechanical scarification. The least improvement in germination traits were attained by immersion in concentrated H_2SO_4, followed by soaking in GA_3 in the two seasons. These results were in accordance with previous studies **[11, 16]**. The enhancement of germination of soaking seeds in tap water for 24 h. treatment may be returned to the softening seed coats and allowed for the imbibition of water by its ability to create tension which subsequently causes cracking of the macroscleroid layer without hurt the embryos. Also, it was reported that soaking *B. aegyptiaca* fruits in cold water for 18 and 24 h resulted in higher germination than other treatments **[3]**. Our result also was in agreement with previous study who pointed out that soaking of *Afzelia africana* in cold water for 48 hours increased germination rate **[17]**. The result also corroborates the finding of authors who asserted that soaking seeds of *Azadirachta indica* for a long period in water increase the germination percentage **[18]**. Mechanical scarification tends to increase germination percentage and different germination parameters compared to the control. Similar findings were obtained for hard seed coat [16, 19]. Also, treatment with concentrated sulphuric acid tends to increase germination measurements compared to untreated seeds. It was reclaimed that chemical scarification with H_2SO_4 was effective in decreasing the hardness of the seed-coat [20].

Seedling measurements:

The effect of pre-germination treatments on the stem length, root length, stem diameter and levees number of *B. aegyptiaca* three months after sowing seeds were highly significant as indicated in Table (3). The longest stem and roots was obtained from soaking seed in tap water for 24 h, soaking seeds in boiled water for 24 h followed by mechanical scarification, while the shortest ones were produced from untreated seeds, followed by immersion seeds in concentrated sulphuric acid for 5 min. in the mean values of both seasons. The thickest stem was obtained with soaking seed in tap water for 24 h., followed by soaking seeds in boiled water for 24 h., and mechanical scarification, while the least stem diameter was with untreated seeds, followed by immersion seeds in concentrated sulphuric acid for 5 min. The highest values of leaves number were due to soaking seed in tap water for 24 h, soaking seeds in boiled water for 24 h followed by mechanical scarification, while the lowest ones were due to untreated seeds, followed by immersion seeds in concentrated sulphuric acid for 5 min.

Table 3. Influence of pre-germination treatments on stem length (cm), root length (cm), stem diameter (mm) and leaves number of *Balanites aegyptiaca* L. during the 1st season (2023) and 2nd season (2024).

Pre-germination treatments	Stem length (cm)		Root length (cm)		Stem diameter (mm)		Leaves number	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	22.6^{f}	26.1^{f}	18.1^{g}	18.7^{e}	0.24^{e}	0.26^{e}	15.3^{f}	16.7^{e}
Tap water 24 h.	34.3^{a}	36.4^{a}	33.2^{a}	33.8^{a}	0.37^{a}	0.35^{a}	27.7^{a}	28.0^{a}
Tap water 48 h.	26.2^{d}	29.4^{de}	27.5^{cde}	28.3^{c}	0.33^{bc}	0.31^{c}	22.3^{cd}	23.7^{bc}
Boiled water 24 h.	32.4^{b}	34.1^{b}	30.2^{b}	30.5^{b}	0.35^{ab}	0.34^{ab}	26.3^{ab}	26.0^{ab}
GA_3 2000 ppm	25.6^{de}	27.7^{ef}	25.6^{e}	24.2^{d}	0.26^{d}	0.29^{d}	19.7^{e}	20.3^{d}
GA_3 4000 ppm	25.9^{de}	28.8^{e}	27.1^{de}	27.1^{c}	0.32^{c}	0.31^{c}	21.3^{de}	22.3^{cd}
GA_3 6000 ppm	29.1^{c}	30.9^{cd}	28.5^{bcd}	28.8^{bc}	0.33^{bc}	0.33^{bc}	24.3^{bc}	25.0^{abc}
H_2SO_4 5 min.	24.1^{ef}	26.3^{f}	23.3^{f}	23.8^{d}	0.25^{de}	0.27^{de}	19.7^{e}	20.3^{d}
Scarification	30.4^{c}	32.0^{c}	29.4^{bc}	29.2^{bc}	0.33^{bc}	0.33^{b}	25.3^{b}	25.0^{abc}
LSD 5%	1.9	1.7	2.2	2.1	0.03	0.01	2.1	3.0
LSD 1%	2.6	2.4	3.0	2.9	0.04	0.03	2.9	4.1

The effect of pre-germination treatments on the fresh shoot weight (g), dry shoot weight (g), fresh root weight (g) and dry root weight (g) on three months *B. aegyptiaca* seedlings were highly significant as indicated in Table (4). The heaviest fresh and dry shoot weight (g) was obtained from soaking seed in tap water for 24 h, followed by soaking seeds in boiled water for 24 h and then mechanical scarification, while the lightest ones were produced from untreated seeds, followed by immersion seeds in concentrated sulphuric acid for 5 min in the mean values of two seasons.

The superior fresh and dry root weight values were attained from soaking seed in tap water for 24 h, followed by soaking seeds in boiled water for 24 h and then mechanical scarification, whereas the least ones were registered from untreated seeds, followed by immersion seeds in concentrated sulphuric acid for 5 min in the mean of two seasons. These results were agreed with previous study **[11]**. In this concern, it was found that the higher germination parameters and growth performance of *Tamarindus indica* were recorded with mechanical scarification, followed by concentrated H_2SO_4 and soaking in tap water compared to control **[21]**. Also, it was pointed out that the partially cracked seeds of *B. aegyptiaca* recorded highest germination parameters and seedling growth **[22]**. There were significant differences in the seed germination and seedling growth of *B. aegyptiaca* of different pre-germination treatments compared with the control which indicated physical and physiological dormancy of *B. aegyptiaca* seeds. The germination and growth of seedlings response to soaking in tap-water water in our study was similar to that reported by previous authors **[16, 23, 24, 25]**.

In the present study, soaking seeds in water was found to be as effective in promoting germination as soaking the seeds in GA_3. These results were confirmed by study which revealed that the break of seed dormancy in *Capparis spinosa* using mechanical, chemical and physical treatments under laboratory, greenhouse and field conditions **[26]**. The highest germination was obtained in seeds soaked in 400 ppm GA_3 for 120 min after treatment with sulfuric acid for 20 minutes. Significant enhancing in the germination responses to GA_3 treatments was attained in the present study. The results obtained by study revealed that the application of gibberellic acid on seeds of amaranth and *Cleome gynandra* can reduce seed dormancy **[27]**.

Table 4. Influence of pre-germination treatments on fresh shoot weight (g), dry shoot weight (g), fresh root weight (g) and dry root weight (g) of *Balanites aegyptiaca* L. during the 1st season (2023) and 2nd season (2024).

Pre-germination treatments	Fresh shoot weight (g)		Dry shoot weight (g)		Fresh root weight (g)		Dry root weight (g)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	1.38^{f}	1.37^{g}	0.68^{g}	0.64^{d}	0.74^{g}	0.74^{g}	0.37^{g}	0.37^{f}
Tap water 24 h.	2.50^{a}	2.32^{a}	1.16^{a}	1.13^{a}	1.70^{a}	1.79^{a}	0.90^{a}	0.84^{a}
Tap water 48 h.	1.67^{cd}	1.61^{de}	0.80^{de}	0.77^{bc}	1.38^{cd}	1.40^{cd}	0.70^{cd}	0.66^{bc}
Boiled water 24 h.	2.12^{b}	2.00^{b}	1.00^{b}	1.05^{a}	1.64^{ab}	1.65^{b}	0.83^{b}	0.80^{a}
GA_3 2000 ppm	1.54^{de}	1.45^{efg}	0.72^{efg}	0.69^{cd}	1.23^{ef}	1.26^{e}	0.63^{e}	0.57^{de}
GA_3 4000 ppm	1.57^{de}	1.57^{def}	0.78^{def}	0.75^{bcd}	1.29^{de}	1.32^{de}	0.66^{de}	0.62^{cd}
GA_3 6000 ppm	1.73^{c}	1.71^{cd}	0.85^{cd}	0.84^{b}	1.42^{c}	1.45^{cd}	0.72^{cd}	0.67^{bc}
H_2SO_4 5 min.	1.47^{ef}	1.41^{fg}	0.71^{fg}	0.66^{d}	1.16^{f}	1.10^{f}	0.55^{f}	0.51^{e}
Scarification	1.79^{c}	1.79^{c}	0.90^{c}	0.86^{b}	1.56^{b}	1.50^{c}	0.75^{c}	0.70^{b}
LSD 5%	0.15	0.17	0.09	0.11	0.11	0.13	0.07	0.07
	0.21	0.24	0.12	0.15	0.15	0.18	0.09	0.10

Conclusion

Softening the seed coat of *B. aegyptiaca* by tap-water for 24 h, followed by soaking seeds in previously boiled water for 24 h at ambient temperature and mechanical scarification treatments were found the most effective for improving germination and early seedlings growth. On the other side, the better performance of germination and early growth of seedlings was attained when mechanical scarification over all other treatments in case of *B. aegyptiaca.* So, the present result recommends the nursery owners and other organizations for seedling producer in Egypt to apply tap water treatment as an easy way for maximum seed germination and better early growth of this tree species.

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