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Enhancing propagation and growth of *Balanites aegyptiaca* through seed treatment

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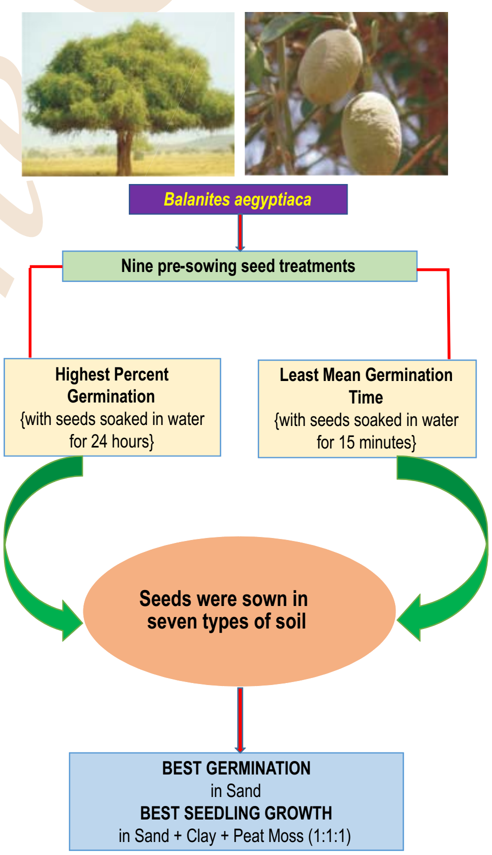
Abstract

Aim: Effect of nine seed pretreatments and seven soil types was studied on seed germination and seedling growth of *Balanites aegyptiaca* (L.) Del., an endangered medicinal plant, under growth chamber and greenhouse conditions, with a view to enhance its propagation and growth.

Methodology: Out of 10 lots of 100 seeds each, nine were soaked separately (1) in concentrated H_2SO_4 for 5 min, 10 min and 15 min, (2) in water for 24 hr, 48 hr, and 72 hr, and (3) in boiling distilled water for 5 min, 10 min and 15 min. One (untreated) lot was used as the control. Pre-treated seeds with the best % germination were sown in 7 types of soil, *viz.* Sand, Clay, Sand + Clay (1:1), Sand + Clay (2:1), Sand + Clay (1:2), Sand + Clay + Peat moss (1:1:1) and Sand + Clay + Peat moss + Perlite (2:2:2:1).

Results: Seed pretreatments had a significant effect on seed germination, the highest germination percentage (82.5%) coming from soaking in water for 24 h. The least mean germination time (14.5 days) was obtained with seeds soaked in water for 15 min, although they showed a low (25%) germination percentage. Among the soil types, pure sand was the best, giving the highest (82%) germination percentage and the least (18 days) mean germination time. With reference to parameters related to seedling growth, combination of sand, clay and peat moss (1:1:1) was the best media for cultivation of this species, resulting in the maximum height (64.1cm), girth (3.6mm) and vigor index (50) of the seedling after a six month growth.

Interpretation: Soaking seeds in water for 24 hr was the best pretreatment for optimal germination performance. Sand was the best germination media, which became highly favourable for seedling growth on being supplemented with clay and peat moss.



Introduction

Effective propagation and seedling establishment are the basic requirements for sustainable management of rare species. However, initial stages of plant life cycle, including seed germination and seedling establishment are most susceptible to environmental disturbances, and hence associated with high mortality rates (Moles and Westby, 2004). Seed germination in tree species is sometimes difficult due to hard seed coat and dormant embryos (Jaiswal and Chaudhary, 2005), and the seeds often fail to germinate even under favorable moisture, oxygen and soil conditions. To overcome this problem, several methods including mechanical scarification, soaking in water and acids (Patane and Gresta, 2006), chilling and heating (Beigh *et al*., 2002; Iavoglou and Radoglou, 2015) and irradiation (Jan *et al*., 2012; Aref *et al*., 2016) are used for treating seeds prior to sowing.

The factors that directly influence seed germination include soil type, soil moisture and soil temperature. Seed-soil interface is important for initial water uptake required for seed germination, and during the emergence phase, when seeds continue to require water and oxygen and utilize their nutrition reserves (Baker, 2006). Therefore, degree of aeration, temperature and water content of soil strongly affects seed germination and seedling emergence via the seed-soil contact. Influence of soil factors on seed germination is often based on transmittance of light through soil, which in turn is determined by particle size and color, moisture content and presence of organic matter; in general, light transmittance decreases with decrease in the size of soil particles. According to Hartmann *et al.* (2007), seed germination is influenced by the type of substrate used, and environmental factors such as oxygen, water, temperature and for some species light.

Of late, combinations of soil types with different ratios of nutrients have been tested for their suitability towards seed germination (Selivanovskaya and Latypova, 2006). Use of poor planting stock can reduce plantation survival and growth, and increase the site-maintenance costs (Oliet *et al*., 2009). Generally, growth medium, the most critical factor determining the seedling quality in nursery, acts as a reservoir of nutrients and moisture (Baiyeri and Mbah, 2006). Studies on seed germination become especially important when they refer to threatened plant species.

Balanites aegyptiaca (L.) Del. of Zygophyllaceae is well known for its multifarious medicinal uses. The plant is rich in steroidal saponins, which yield diosgenin, a source of steroidal drugs such as corticosteroids, contraceptives and sex hormones (Farid *et al.,* 2002). The fruit pulp, especially contains carbohydrates and mono-unsaturated fat (Chothani and Vaghasiya, 2011). Because of its excessive exploitation for a variety of purposes, this species falls in the category of endangered plant species (Elfeel, 2012) which has necessitated conservation of this wild growing tree, and hence action plan for its maintenance and sustainable development has become indispensable. Elfeel (2012) studied the effect of water soaking and sowing orientation of seeds on germination rate of this species but any extensive study is yet to be conducted. Given this, the present study attempts to investigate the effects of various seed pretreatments and different soil media on seed germination and seedling growth of this tree species, growing wild in the arid regions of Saudi Arabia.

Materials and Methods

Seed collection: Mature seeds of *Balanites aegyptiaca (L.)* Del. of Zygophyllaceae were collected direct from tree stands around Alarda in Jazan region, Saudi Arabia, where this species grows naturally. Average monthly temperature of this area normally hovers around 25°C (January) to 35°C (July) and the annual mean rainfall over the last five years has been about 106 mm. Intact seeds extracted manually were air-dried and kept at room temperature.

Pretreatment of seeds: Nine pretreatments were given to separate seed lots to enhance their potential to germinate. For each pretreatment, a total of 100 seeds were used. Seeds were soaked in concentrated H_2SO_4 for 5 min (A1), 10 min (A2) and 15 min (A3), then rinsed thoroughly in distilled water, and finally air-dried. In another set, seeds were soaked in water for 24 hrs (W1), 48 hrs (W2) and 72 hrs (W3). Similarly, seeds were soaked in boiling distilled water at 100°C for 5 min (BW1), 10 min (BW2) and 15 min (BW3) in a 100 ml flask. The untreated seeds were used as control. A total of 25 seeds were placed on moistened cotton layer in plastic container (20×15 cm) for germination, and four replicates were used for each treatment in addition to control. The plastic containers were kept in a growth chamber in completely randomized way at a constant temperature (25±1°C) under 12/12 hr of light and darkness. Germination was monitored and recorded daily. Seeds were considered germinated on emergence of healthy, white radicle through the integument.

Seed germination and seedling growth: To determine the effect of different soil types on seed germination and seedling growth, 20 seeds (soaked in water for 24 hrs) were placed per plastic pot (30×25cm), and five replicates were used for each treatment. Seven treatments (T1-T7), consisting of different soil combinations as shown in Table 1, were used. All sets of soil were analyzed for particle size, water-holding capacity and organic matter content in the laboratory of the Soil Department at King Saud University, following the procedures described by Miller and Miller (1987), Topp *et al.* (1993) and Nelson and Sommers (1982), respectively. The pots were kept in green house at a constant temperature (30±1°C) under 12/12 hrs of light and darkness, and irrigated whenever required during the experiment. The experiments were carried out in a completely randomized design.

Seed germination was monitored and recorded daily. Seeds were considered as germinated on emergence of plumule through the media. Germination percentage was calculated by the following formula : germinated seeds/total seeds used × 100, where mean germination time (in days) was determined as ΣTi Ni/S, where Ti is the number of days from the beginning of experiment, Ni is the number of seeds germinated per day, and S is the total number of seeds germinated (Scott *et al.*, 1984). The data obtained on seed-germination parameters were subjected to analysis of variance (ANOVA) to determine the level of significance (at 5%).

The growth of seedlings was monitored continuously for six months. Seedlings were measured for total shoot height (using a 30 cm ruler) and seedling stem diameter (using a microcalliper up to near 0.01mm). Relative growth rate of stem height was estimated as follows : $RGR(H) = (LnH_2\text{-}LnH_1)/\ t_2\text{-}t_1;$ relative growth rate of stem girth as $RGR(G) = (LnD_2\text{-}LnD_1)/\ t_2\text{-}t_1,$ using Ln is natural logarithm, H_2 and H_1 as seedling height (cm), D_2 and D_1 as stem diameter (mm) and t_2 - t_1 as time periods (days) on the last and the first sampling dates, respectively; whereas seedling vigor index was calculated as SVI = Germination (%) × total length of seedling (Iqbal *et al.,* 2007; Ostos *et al.,* 2008)**.** The data obtained were analyzed by applying LSD test.

Results and Discussion

Pretreatment of seeds significantly affected ($P < 0.0010$) germination percentage. Fig. 1 demonstrates that seeds soaked in water for 24 hr (W1) gave the maximum (82.50%) germination percentage, followed by those soaked in normal water for 48 hrs (W2). On the contrary, seeds treated with concentrated sulphuric acid for 10 and 15 min (A2 and A3) showed minimum (28.57 and 25.00%) germination percentage, respectively (Fig. 1). Pre-treatments also affected mean germination time, which was least for seeds soaked in H_2SO_4 for 15 min (A3). The longest mean germination time, on the other hand, was recorded for control (Fig. 2). Soaking seeds in water for 24 and 48 hrs shortened the germination time by 2 to 3 days, which was statistically in significant.

Seed viability (as expressed by germination) and vigor are indicators of physiological potential of seeds, which touches its maximum when seeds mature. Beyond this stage, seeds become prone to deterioration, as is often evident by low seed germination, reduced seedling size, and increased incidence of abnormal seedlings. Whereas low germination rate is associated with early membrane disorganization, seedling anomaly is attributed to the death of seed tissues particularly in the meristematic regions (Bewley, 1997; Marcos-Filho, 2015).

The observations of the present study indicate that the best treatment to increase germination percentage and reduce mean germination time in *B. aegyptiaca* is seed soaking in distilled water (for 24 to 48 hrs) or boiled water (for 5 min); however, increasing the soaking time in boiling water reduced the germination percentage. These results agree with the observation of Elfeel (2012) and proved that increasing the

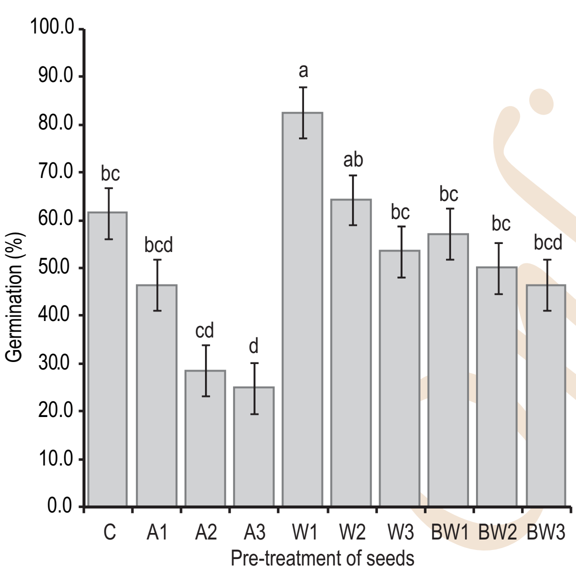


Fig. 1: Effect of seed pretreatments on germination percentage. Seeds were soaked in concentrated H_2SO_4 for 5 min (A1), 10 min (A2) and 15 min (A3); in water for 24 hrs (W1), 48 hrs (W2) and 72 hrs (W3); and in boiling distilled water for 5 min (BW1), 10 min (BW2) and 15 min (BW3). The untreated seeds were used as the control (C)

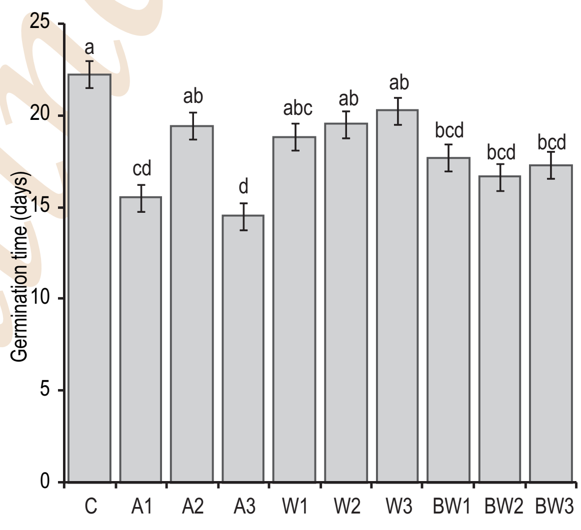


Fig. 2: Effect of seed pretreatments on mean germination time. Seeds were soaked in concentrated H_2SO_4 for 5 min (A1), 10 min (A2) and 15 min (A3); in water for 24 hrs (W1), 48 hrs (W2) and 72 hrs (W3); and in boiling distilled water for 5 min (BW1), 10 min (BW2), and 15 min (BW3). The untreated seeds were used as the control (C)

soaking time in normal or boiling water decreases the germination percentage in this species. Prolonged seed soaking in sulfuric acid also caused similar effect, conforming to Aref *et al.* (2011) who found a decrease in germination percentage in *Acacia ehrenbergiana* with increase in the duration of seed immersion in sulfuric acid. However, the effect may be just reverse in other species, as evident from the study of Olmez *et al.* (2008).

As evident from Table 2, soil media significantly affected the germination percentage, the highest (82% and 78%) appearing in pure sand (T1) and in the combination of sand, clay and peat moss in equal proportions (T6) respectively. Clay (T2) or a mixture (1:2) of sand and clay (T5) gave 74% germination, while the lowest (56%) was recorded with T4, *i.e.,* on sand and clay (2:1). Soil media also had a significant effect ($p < 0.0344$) on mean germination time; the shortest (18.05 days) time was obtained on sand (T1), whereas the longest (29.83 days) on media consisting of sand, clay and peat moss (T6), as shown in Table 2.

The nature of substratum has a significant effect on seed germination and seedling growth (Gairola *et al*., 2011). An ideal planting substratum should be sufficiently porous and capable of retaining moisture, which is important for both seed germination and seedling growth (Jaiswal and Chaudhary, 2005). Germination rate in *Salix variegata* increased with increase in soil moisture but decreased in over-saturated soil (Chen *et al*., 2013). The results of the present study on seed germination in soil media established that sand as a germination substratum is preferable for tree species having large seeds, because aeration is best in the sand medium. Neelam and Ishtiaq (2001) also observed that media with improved soil aeration led to superior plant growth. Mean values given in Table 3 indicate that sandy clay soil with organic matter 'peat moss' (T6) was more effective soil media than others for the growth of *B. aegyptiaca*.

Growth characteristics of seedlings were significantly affected ($p < 0.0130$) by different soil media. Mean values in Table 3 exhibited maximum seedling height (64.10 cm) on soil media consisting of sand, clay and peat moss in equal ratio (T6), followed by a height of 61.04 cm on media consisting of sand, clay , peat moss and perlite (T7). The minimum seedling height (45.80 cm), on the other hand, was recorded on pure sand (T1). Similarly, the largest girth (3.56 mm) of seedling stem was recorded on soil media T6, consisting of sand, clay and peat moss in equal ratio, and on pure sand (T1). On the contrary, the least seedling girth (2.83 mm) was recorded on pure clay media (T2), followed by one (2.94 mm) on sand and clay in 1:2 (T5) ratio.

It is known that organic matter enhances soil fertility (Martinez *et al.,* 2003), thus facilitating the seedling survival and growth (Larchevêque *et al*., 2006), biomass production (Moreno-Peñaranda *et al*., 2004) and seedling quality (Mañas *et al*., 2009). A good growing medium improves the quality of seedlings (Agbo and Omaliko, 2006) by providing sufficient anchorage or support

Table 1 : Soil combinations with different physico-chemical properties used as growth media (T1-T7) for *B. aegyptiaca* seeds

Media	Soil combination	Property			
		Particle size (mm)	WHC (mm m^{-2})	OM (%)	Color
T_1	Sand	0.1 2.0	63 110	0.10	Reddish yellow
T_2	Clay	< 0.01	125 192	1.20	Light grey
T_3	Sand + Clay (1:1)	0.07 0.10	104 146	0.52	Light reddish brown
T_4	Sand + Clay (2:1)	0.05 0.10	90 130	0.42	Light brown
T_5	Sand + Clay (1:2)	0.02 0.10	145 186	2.01	Light yellowish brown
T_6	Sand + Clay + Peat moss (1:1:1)	0.09 0.14	120 180	21.50	Darkish brown
T_7	Sand + Clay + Peat moss + Perlite (2:2:2:1)	0.07 0.10	110 185	14.00	Dark brown

WHC = water-holding capacity; OM = organic matter

Table 2 : Effect of different soil media on seed-germination parameters

Treatment	Germination percentage	Mean germination time
T_1	82.00±5.70^{a}	18.05±2.97^{c}
T_2	74.00±5.48^{ab}	26.49±1.53^{ab}
T_3	60.00±3.54^{bc}	27.02±1.96^{ab}
T_4	56.00±4.47^{c}	21.72±2.21^{bc}
T_5	74.00±4.18^{ab}	21.75±1.84^{bc}
T_6	78.00±2.74^{a}	29.83±1.44^{a}
T_7	70.00±3.54^{abc}	20.85±1.04^{bc}
LSD	**16.09**	**7.22**

Means followed by similar letters are not significantly different at 5% level of significance

Table 3 : Seedling-growth parameters under the effect of different growth media

Treatment	Seedling height (cm)	Seedling girth (mm)	RGR(H) (cm cm^{-1} month^{-1})	RGR(G) (mm mm^{-1} month^{-1})	SVI
T_1	45.80 ± 4.16^{d}	3.55 ± 0.34^{a}	0.30 ± 0.07^{abcd}	0.89 ± 0.06^{ab}	37.56 ± 3.32^{bc}
T_2	49.46 ± 1.43^{cd}	2.83 ± 0.28^{c}	0.25 ± 0.01^{bcd}	0.69 ± 0.10^{c}	36.60 ± 1.06^{bc}
T_3	51.92 ± 3.60^{cd}	3.33 ± 0.18^{abc}	0.31 ± 0.11^{abc}	0.90 ± 0.08^{a}	31.15 ± 2.92^{c}
T_4	56.20 ± 3.07^{abc}	3.39 ± 0.35^{ab}	0.22 ± 0.09^{cd}	0.90 ± 0.11^{a}	31.47 ± 2.95^{c}
T_5	52.88 ± 3.20^{bcd}	2.94 ± 0.29^{bc}	0.21 ± 0.06^{d}	0.74 ± 0.10^{bc}	39.13 ± 2.17^{b}
T_6	64.10 ± 3.32^{a}	3.56 ± 0.15^{a}	0.36 ± 0.05^{a}	0.96 ± 0.10^{a}	50.00 ± 2.03^{a}
T_7	61.04 ± 2.48^{ab}	3.05 ± 0.19^{abc}	0.33 ± 0.04^{ab}	0.82 ± 0.04^{abc}	42.73 ± 1.55^{b}
LSD	**10.18**	**0.53**	**0.09**	**0.16**	**6.95**

Means followed by similar letters are not significantly different at 5% level of significance

to growing plants, serving as a reservoir for nutrients and water, permitting oxygen diffusion to roots, and facilitating gaseous exchange between roots and the atmosphere outside the substratum (Abad *et al*., 2002). The vigorous and fast growth of seedlings on mixture of sand, clay and peat moss in equal ratio may be attributed to the higher nutrient level and better water-holding capacity of this media, which facilitate plant performance leading to a higher relative growth rate (RGR); the poor seedling growth in sand might be due to low availability of nutrients in this media.

The relative growth rate in height was significantly affected (p < 0.0181) by soil media. The mixture of sand, clay and peat moss in equal ratio (T6) gave maximum (0.36 cm cm^{-1} month^{-1}) RGR(H), which was followed closely (0.33 cm cm^{-1} month^{-1}) by the value obtained on sand + clay + peat moss + perlite (T7). Sand + clay (2:1 or 1:2), *i.e.,* T4 and T5, gave the lowest RGR(H) values (Table 3). A similar trend was recorded for relative growth rate in girth, where maximum (0.96 mm mm^{-1} month^{-1}) RGR(G) was found on media consisting of sand, clay and peat moss (T6), and minimum (0.69 mm mm^{-1} month^{-1}) on clay (T2). Seedling vigor index was significantly (p<0.0001) affected by the soil media. Generally, seedlings grown on soils consisting of sand, clay and organic matter (peat moss), without or with perlite, had the maximum SVI (50.00 and 42.73 respectively), whereas media consisting of sand + clay (1:1 or 2:1), i.e. T3 and T4, gave the least seeding vigour index (SVI) (Table 3).

In the present study, the effect of soil factors was more prominent in RGR(H) than in RGR(G), and this is a normal response, because seedlings in early growth stages grow more in height than in diameter (Selivanovskaya and Latypova, 2006). The highest relative growth rate, as obtained by various treatments, indicates that the organic matters affect the physiological potential of growing plants. Correlation of RGR with nutrient supply was same, as reported earlier by Ostos *et al.* (2008). Larger seedlings generally have a larger photosynthetically-active green surface in terms of foliage. The maximum SVI value was obtained in sand, clay and organic matter (T6) combination, implying that the plant experiences rapid development on this media (Oliet *et al*., 2009). Plants developed under organic matter treatments normally have the maximal values of SVI, as observed in some other species also (Mañas *et al*., 2009). On the whole, *B. aegyptiaca* seedlings have a good potential for survival and growth on media containing sand, clay and organic matter in equal ratio.

In conclusion, the best seed pretreatment for obtaining *B. aegyptiaca* plants with maximum germination percentage and seedling vigor is seed soaking in water for 24 hrs, whereas the best germination media is sand or, alternatively, a mixture of sand, clay and peat moss. In fact, pure sand media improved the germination percentage and reduced the mean germination time, but addition of organic matter to sand and clay enhanced the seedling growth.

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