ABSTRACT

Doum palm *Hyphaene thebaica* Mart is a very important natural flora in the Egyptian territory. It is integrated in food, drink and medicine of the Egyptian population. Seeds germination faces difficulties due to hardness and viability. The fruit (a date) is edible, the foliage is also used for local crafts, the stems for construction purposes, and the roots for phytomedicine, mainly for the treatment of bilharziasis. This study tried different priming methods after removal hard kernel. First treatment; non-treated as direct plantation (control). Second; Cold water (tap water for 48 hours), third; freezing storage in Deep-freezer at -18°C for 12 hours then immersed in 60°C water for 1 hour, fourth; hot water at 60°C ± 5 for 3 hours then left till gradually cooled, fifth; soaking seeds in a solution of gibberellic acid 200 ppm for 48 hours, sixth; Soaking seeds in 3% solution of KNO₃ for 48 hours, seventh; soaking seeds in diluted sulfuric acid (H₂SO₄) at 30% for 15 minutes. Data collected were germination percentage %, roots length, root diameter, root fresh and dry weight, root dry weight, seedling length, wide of leaf, Fresh and dry weight of above ground parts. Freezing followed by hot water gave the highest seed germination % (90%) followed by KNO₃ (80%). The lowest germination % was obtained from non-treated control and cold water in a range between 45-65%. Cold water treatment gave significant increase in root fresh weight in both seasons when compared to other treatment. Freezing and cold water treatment gave significant increase in plant height in both seasons when compared to other treatments. Moreover, the effect of various priming treatment demonstrated significant effect on characteristics of young growing seedling. The best performance obtained from seeds treated with cold water and freezing treatment. Thus, treating Doum palm seeds after removal of hard kernel with freezing at -18 for 12 hours followed by immersing seed in hot water at 60°C for one hour.

Keywords: Seed priming, *Hyphaene thebaica*, Seedling, KNO₃, GAs, Freezing.

INTRODUCTION

Dormancy of seed due to inhibitory factors, are either due to hard seed coat (external) or physical of physiological status of interior of the seed. Seed dormancy in Doum palm (*H. thebaica* Mart) belongs to the first type. Some trees that produces seeds with hard seed coat such as Doum which is common component of flora of the southern of Egypt and other arid region are restricted in the distribution in the region because of the prolong dormancy and poor germination rate. The dormancy breaking treatment overcome the dormancy of this plant (Al-Fredan and Ali, 2008).

Zevallos and De La Cruz (1991) reported that, the seed germination rates were increased in *Acacia marcранtha* and *cyathostegia matthewsii* by soaking in cold water for 28h. In contrast, Chris (1994) reported that soaking in cold water not appreciably increase level of germination for some *Acacia* species. Kasera et al (2002) were subjected seeds to tap water for 24h in *Leptadaena reticulate* plant and were found that, the highest germination percentage 100% obtained with this treatment. Also, Çtrak et al (2007) clarified that, the soaked seeds in tap water of *Hypericum aviculavifolium* resulted in better seed germination percentage.
Chris (1994) reported that, hot water treatment for some Acacia species did not appreciably increase germination level as well as the duration of water soaking treatments. Cervantes et al (1996) found that, seed germination of leucaena excelsa and L. macrophylla occurred after soaking in boiling hot water for 1 min. Mechanical Scarification for seed coat can be done by scratch the seed coat by agrail, knife or shaking seeds with some abrasive materials.

Smith (1979) reported that, mechanical seed scarification improved the germination rate of Zamia integripolia and Z. furfuracea. Also, the same results were found for scarified seeds of Albizzia elurozz when compared to non-scarified ones (Gogue and Emino, 1980). Similarly, Scarified seeds of koelreuteria paniculata gave the best result of germination (91.0%) (Garner and Lewis 1980). Gupta et al (2001) subjected seeds of Abutilon indicum to different hot water treatments and found that, the highest germination percentage (82%) occurred with 70°C hot water treatment.

Witte (1977) clarified that, seeds of Zamia plants were collected in 1976 and cleaned, then either germinate or stored one year at 5°C. With fresh seed planted in 30°C media, germination was about equal for cracked and uncracked seed (60 to 65%), but only 43% for shelled seed. After storage, cracked seed germination better than uncracked (71% vs. 38%) and mild bottom heat increased germination of uncracked seed (49% vs. 27%) but not cracked seed. Heavier seed (29 g) germinated better than lighter one (1.69 g).

Tilki and Dirik (2007) studied the effect of cold stratification for 0 or 45 days at 4±1°C in three provenances of Pinus brutia and found that, stratification significantly increased germination % at all potentials under study regardless germination temperature.

Thus, we conducted this research to conduct effect of seed priming on germination of Doum palm (Hyphaene thebaica) seeds and growing young seedling.

**MATERIAL AND METHODS**

**Plant Material**

Seeds of Hyphaene thebaica plants (Doum Palm) were bought from El Obour Market as a source of plant material during two seasons of the study. The average fresh weight of the seeds were about 18 g and 10 cm in diameter.

**Media used & preparation**

The culture media used were 1:1 sand: peat-moss (v/v). Sand was washed by water and mixed with peatmoss then treated with 1 g/l Rizolex as disinfectant for soil spores, then media were mixed with calcium nitrate to adapt pH of the soil. Plastic bags used were about (18×25 cm) in diameter as a container filled with the above mentioned medium.

**Seed treatments**

Seeds of Doum Palm were treated after removal of the hard kernel with different priming treatments as follows:

1. Control treatment without soaking. (Direct plantation).
2. Soaking in tap water for 48 hours (cold water).
3. Freezing storage in Deep-freezer at -18°C for 12 hours then soaked in Hot water at 60°C for 1 hour.
4. Soaking in Hot water (60°C ± 5) for 3 hours then left till gradually cooled.
5. Soaking in a solution of gibberellic acid (GA3) 200 ppm for 48 hours.
6. Soaking in a solution of potassium nitrate (KNO3) 3% for 48 hours.
7. Soaking in sulfuric acid (H2SO4) 30% for 15 minutes.

**Sowing date & farming procedure**

The sowing date was in 12 April for the two seasons under study (2017/2018). All treatments were cultured in the plastic bags about 3 cm in depth under the surface of media then coated with sand. All treatments were then shaded with serran sheets and irrigated immediately after sowing. Irrigation of all treatments was performed every week by tap water.

**Data Recorded & Statistical analysis**

The data were recorded in October of both seasons as follow.

1- Germination percentage % = \( \frac{\text{Number of germinated seeds}}{\text{total number of seeds}} \) \times 100 %
2- Length of roots.
3- Root diameter diameter.
4- Root fresh weight.
5- Root dry weight.
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Collected data were subjected to one-way analysis of variance was carried out, as described by Snedecor and Cochran (1989). The means were compared by determining for the germination experiment. Each treatment of the seed presoaking experiments consisted of 3 replicates and 5 seeds in each replicate. Duncan test (P≤0) was used for analysis of the data, to evaluate differences among means (Waller and Duncan, 1969).

**RESULTS**

**Germination percentage**

Data presented in Fig. (1) indicate that seed germination percentage of *H. thebaica* was significantly affected by different treatments. The highest germination % was obtained from seeds treated with Freezing treatment (86.66 and 90%) and followed by soaked seeds in KNO$_3$ (80 and 76%) in respect order by season. Meanwhile, the lowest germination % was observed when seeds were not treated with any priming methods as control (46.66 and 50%).

**Fig. 1.** Effect of various priming treatments on germination percentage of Doum palm *Hyphaene thebaica* seeds

**Root length**

Data presented in Fig. (2) showed that H$_2$SO$_4$, gibberellic acid and cold water treatments gave significant increase in root length in both seasons when compared to other treatment. In the 1$^{st}$ season H$_2$SO$_4$, gibberellic acid and cold water gave the longest root length (83.17, 79.67 and 79.00 cm) and in the 2$^{nd}$ season (89.00, 74.80 and 74.47 cm) in respect order. Whereas, the lowest root length was obtained when seeds were treated in the 1$^{st}$ season with hot water and freezing (38.83 and 38.00 cm) and in the 2$^{nd}$ season gave (34.67, and 47.33 cm) in respect order.

**Root diameter**

Data presented in Fig. (3) showed that cold water treatment gave significant increase in root diameter in both seasons when compared to other treatment. Whereas, the smallest root diameter was obtained when seeds were treated in the 1$^{st}$ with KNO$_3$ gave the smallest root diameter (0.6 cm) and in the 2$^{nd}$ season H$_2$SO$_4$ gave the smallest root diameter (0.63cm).

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Root length (cm)

Control Cold water Freezing Hot water GA3 KNO3 H2SO4

Fig. 2. Effect of various priming treatments on root length of young growing seedling of Doum palm *Hyphaene thebaica*

Root diameter (cm)

Control Cold water Freezing Hot water GA3 KNO3 H2SO4

Fig. 3. Effect of various priming treatments on root diameter of young growing seedling of Doum palm *Hyphaene thebaica*

Root fresh weight

Data presented in Fig. (4) showed that cold water treatment gave significant increase in root fresh weight in both seasons when compared to other treatment. In the 1st and 2nd seasons, cold water root fresh weight was (22.32 and 23.02 g/plant) in respect order. Whereas, the lowest root fresh weight was obtained when seeds were treated in the 1st season with hot water (10.29 g/plant) and with hot water and freezing (10.78 and 10.19 g/plant, respectively) in the 2nd season.
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**Fig. 4.** Effect of various priming treatments on root fresh weight of young growing seedling of Doum palm *Hyphaene thebaica*

**Root dry weight**

Data presented in **Fig. (5)** indicated that cold water treatment gave significant increase in root dry weight in both seasons when compared to other treatment. Cold water treatment gave the highest value of root dry weight (7.51 and 7.92 g/plant) in respect order by seasons. Whereas, the lowest root dry weight was obtained when seeds were non-treated (control) or hot water in the 1st season (3.97 and 3.00 g/plant) and in the 2nd season (3.77 and 3.01 g/plant) in respect order.

**Fig. 5.** Effect of various priming treatments on root dry weight of young growing seedling of Doum palm *Hyphaene thebaica*
4.2.5. Plant height

Data presented in Fig. (6) showed that freezing, and cold water treatment gave significant increase in plant height in both seasons when compared to other treatments. In the 1st and 2nd seasons, freezing and cold water treatments gave significant increase in plant height in the 1st season gave (45.33 and 39.97 cm) and in the 2nd season (44.6 and 26.17 cm) in respect order. Whereas, the shortest plants was obtained when seeds were treated with gibberellic acid (28.50 and 26.17 cm) in respect order by season.

![Graph showing effect of various priming treatments on length of young growing seedling of Doum palm Hyphaene thebaica](image)

**Fig. 6.** Effect of various priming treatments on length of young growing seedling of Doum palm *Hyphaene thebaica*

Leaf wide

Data presented in Fig. (7) showed that cold water, control and H₂SO₄ treatment gave significant increase in leaf wide in both seasons when compared to other treatments. In the 1st season cold water, control and H₂SO₄ gave larger width of leaves (2.64, 2.27 and 2.00 cm) and in the 2nd season cold water and hot water gave larger width of leaves (2.55 and 2.50 cm) in respect order. Whereas, the smallest leaf wide was obtained when seeds were treated with gibberellic acid (1.52 cm) in the 1st and with H₂SO₄ and gibberellic acid gave the (1.85 and 1.70 cm) in the 2nd season, respectively.
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**Fig. 7.** Effect of various priming treatments on leaf wide of growing seedling of Doum palm *Hyphaene thebaica*

**Length of green part**

Data presented in **Fig. (8)** showed that freezing and H$_2$SO$_4$ treatments gave significant increase in leaf length in both seasons when compared to other treatments. In the 1st season freezing gave the longest length of leaf (28.03 cm) and in the 2nd season freezing and H$_2$SO$_4$ gave the same trend (30.00 and 21.67 cm) in respect order. Whereas, the shortest of leaves were obtained when seeds were treated in the 1st season with hot water and gibberellic acid (13.67 and 12.50 cm) and in the 2nd season gibberellic acid showed the lowest value (12.85 cm).

**Fig. 8.** Effect of various priming treatments on leaf length growing seedling of Doum palm *Hyphaene thebaica*
Fresh weight of aboveground part

Data presented in Fig. (9) showed that freezing treatment gave significant increase in fresh weight of aboveground part in both seasons when compared to other treatment. Freezing gave the highest value of fresh weight for aboveground part in both seasons (14.26 and 13.09 g/plant) in respect order. Whereas, the lowest aboveground part fresh weight was obtained when seeds were treated in the 1st with hot water, KNO₃ and gibberellic acid (6.56, 6.25 and 5.56 g/plant) and in the 2nd season gibberellic acid gave (3.92 g/plant).

![Graph](image_url)

**Fig. 9.** Effect of various priming treatments on aboveground fresh weight of growing seedling of Doum palm *Hyphaene thebaica*

**DISCUSSION**

Pre-germination treatments were carried out to investigate the effect of different seed treatments on germination percentage and growth rate of young seedling of Doum palm *Hyphaene thebaica* Mart.

Results obtained here, showed that the highest percentages of seed germination of *H. thebaica* Mart were obtained when seeds were treated with freezing storage in deep-freezer at -18°C for 12 hours then soaked in hot water at 60°C for 1 hour. These findings are in agreement with those reviewed on effect of freeze-thaw scarification that breaks the seed coat by exposing seeds to temperature alternations between low and high (*Stout, 1990; Rutar et al 2001*). None of the previously studied research on seed priming of Doum palm tried the effect of freeze. *Idohou et al (2015)* on Doum *H. thebaica* Mart seeds reported that, the time of germination and water soaking had significant effects on seed germination. Also, *Al-fredan and Ali (2008)* reported that on Doum palm seeds gave the highest seed germination percentage (93%) when seeds were mechanically scarificated then soaked in water for 24 h. Non treated seeds gave the lowest germination percentage as well as hot water and treated seeds with gibberellic acid followed by H₂SO₄. The sulpharic acid treated seeds resulted in a low germination percentage (32%). *Tahir et al (2007)* on *H. thebaica* Mart, *Çırak et al (2007)* on *Hypericum aviculavifolium*. Also, *Kasera et al (2002)* found that *Leptadaenia reticulate* seeds subjected to soak in water for 24 h gave the highest seed germination percentage 100%. *Sreerama et al (2000)* on *Cassia fistula, C. spectabilis* and *Delonix regia* seeds, *Moussa et al (1998)* on Doum palm *Hyphaene thebaica* Mart seeds.

These above results may be explained by the fact that seed scarification might have removed the mechanical resistance of seed coat and this enabled the embryo to germinate without any mechanical obstacle.
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Mechanism behind the reduction of seed hardness by a freeze-thaw scarification is to make tiny scars on hard seed coat and make seed coat brittle to enhance germination (*Busse, 1930; Stout, 1990; Hall et al. 1993*). A force that produces scars on seed surface through this technique is depending on the size, shape and water content of seeds and treatment intensity and durations (*Kimura and Islam, 2012*). Methods for cooling in freeze-thaw scarification include freezer, (*Shibata and Hatakaya, 1995*); carbon dioxide (CO₂) snow, dry ice, liquid air (*Busse, 1930*); ultra-low freezer (*Stout, 1990*); acetone (*Rutar et al. 2001*); liquid N₂ (*Patane and Gresta, 2006*). These findings are in agreement with those reviewed by Witte (*1977*) on *Zamia integri-folia*. The result of freezing on young growing seedling showed similar trend to cold water treatment. Also, similar results in agree on importance of lower temperature on seed germination *Tilki and Dirik (2007)* on *pinus brutia*. Millaku (*et al. 2012*) on Gentiana lutea L. and Koloteo (*et al. 2001*) on some Conifer species.

**Cracker and Barton (1953)** concluded that low vigor seeds may need more water to germinate than high vigor seeds, and Hegarty (*1978*) contended that loss of vigor precedes loss of viability. Whereas, *Martinková and Honěk, (2007)* on *Taraxacum officinale* as the manner of seed storage has no effect on percentage of germination, frozen seed may be used safely for experiments studying this quality of seed materials. Thus, of lower germination % resulted from non-treated or cold water seeds, did not negative their effect for better growing young seedling.

*Priming* methods such as heat, freeze, mechanical and acid scarification are useful tools to soften hard seeds, improve germination and enhance seedling establishment. However, effectiveness of the methods varies depending on the duration of imposed treatments and species or cultivars to be used (*Taia, 2004*). Over treatment or longer time scarification may impose negative impacts on or injury to the seeds.

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Tocopherol and Sterols in Dates of the Date Palm (Phoenix dactylifera L.)

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The relationship between the level of tocopherol and sterols, with the different dates in the date palm (Phoenix dactylifera L.) was investigated. The results showed that there is a significant difference in the level of tocopherol and sterols, with different dates in the date palm (Phoenix dactylifera L.). The highest level of tocopherol and sterols was recorded in the date palm (Phoenix dactylifera L.) with the date of the middle of the season, while the lowest level was recorded in the date palm (Phoenix dactylifera L.) with the date of the beginning of the season.

Key words: Tocopherol, Sterols, Date palm, Season, Dates.