

# Enhancing *Balanites aegyptiaca* Seed Germination in Egyptian Deserts Gene Bank by Breaking Dormancy Treatments

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## Abstract

The present study was carried out in Egyptian Deserts Gene Bank (EDGB), North Sinai Research Station, Desert Research Center, Egypt. The present study investigated the responses of *Balanites aegyptiaca* seeds to some chemical and physical factors, such as mechanical scarification, chemical scarification, GA<sub>3</sub> (gibberellic acid), dry heat treatment, potassium nitrate (KNO<sub>3</sub>) in solutions of water, sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), tap and hot water. Results indicated that the highest germination percentage 73.50% was achieved with dry heat treatments at 60 °C for 15 minutes. Treated seeds with dry heat treatments at 60 °C for 15 minutes enhanced standard germination percentage to 70.62 % and germination rate to 1.3. GA<sub>3</sub>. Hot water and mechanical scarification gave the best results in germination percentage, germination rate and standard germination. Some treatments were found to decrease germination percentage, standard germination and germination rate( please check carefully).

**Keywords:** *Balanites aegyptiaca*, gene bank, seed dormancy, viability, germination, gibberellic acid; dry heat, mechanical .

## Introduction

*Balanites aegyptiaca* (L.) Del. known as desert date (Hall and Walker, 1991) or myrobolan and heglig (Bolous, 2000). It is an important tree crop of the savannah zone and semi arid tropical region of Africa. The leaves are used as food, the bark as a substance for fishing and the wood as yoke for draught animals and hand implements. The nut is obtained after the removal of the flesh and pulp of the fruit. It contains a kernel with oil and protein contents ranging from 30% to 60% and from 20% to 30%, respectively (Hall and Walker, 1991).

*Balanites aegyptiaca* has been found to have potential for industrial applications as raw material in the manufacture of soap, candle, chemicals and cosmetics as well as pharmaceutical products. The kernel meal remaining after oil extraction can be used as livestock feed (Abu-Al-Futuh, 1983). The processing of *Balanites aegyptiaca* Fruit involves soaking in water for 3 days and washing off the pulp to obtain the nut. The nut is sun-dried for several hours and the kernel is obtained cracking with stone on top of another stone or a hard flat surface (Aviara *et al.*, 1999)

Seed germination in arid and semi-arid regions has been studied mainly in annual species (Guterman, 1993; Kigel, 1995) but their germination patterns differ widely from those of perennial species. Many perennial species present a combination of endogenous (morphological and/or physiological) and exogenous (physical and/or mechanical) dormancy (Morpeth and Hall, 2000). Seeds with water impermeable coverings are common among perennial species. They have a physical dormancy (Baskin and Baskin, 1998). The process of seed coat breakdown distributes germination of seeds over time to increase chances of successful establishment (Egley, 1993). Immersion in concentrated sulphuric acid increases germination in some species of *Opuntia* (Potter *et al.*, 1984). In this manner, the composition of the seed bank plays a critical role in the

maintenance of the vegetation community in tidal freshwater wetlands (Parker *et al.*, 1989; Leck and Simpson, 1995).

Many species produce seeds that do not germinate shortly after dispersal and require a period of species-specific after-ripening through dry storage (Bewley and Black, 1982; Simpson, 1990; Baskin and Baskin, 1998; Gozlan and Guterman, 1999). Both storage conditions and duration are important factors in regulating the after-ripening process (Paterson *et al.*, 1976; Peishi *et al.*, 1999; Murdoch and Ellis, 2000).

Bass *et al.* (1966) conducted germination studies using water, KNO<sub>3</sub> in water, and GA<sub>3</sub> in water as moistening agents for *L. fendleri*, *L. gordonii*, and *L. palmeri*, and as pregermination soaking treatments for *L. gordonii* and *L. palmeri*. Seeds were germinated under light and in total darkness at various temperature regimes. When used as a moistening agent, GA<sub>3</sub> solution had a beneficial effect on germination of seeds of all these species and appeared to replace the light requirement in *L. fendleri* in the dark and improve the germination response over the temperature range evaluated. GA<sub>3</sub> was applied to *L. gordonii* and *L. palmeri* by both soaking and spraying methods. Inhibition of germination in the dark was overcome by treating *L. fendleri* seeds with 400 ppm of GA<sub>3</sub> for 20 h (Sharir and Gelmond, 1971). Seed dormancy in *L. gordonii* was broken by dusting the seed with 90% gibberellic powder (15- 20 mg per gram of seeds) according to (Sharir and Gelmond, 1971). In both studies (Bass *et al.*, 1966; Sharir and Gelmond, 1971), soaking seeds with GA<sub>3</sub> was more effective than spraying or dusting with GA<sub>3</sub> powder.

## Materials and methods

### Seed collection:

Seeds of *Balanites aegyptiaca* (L.) Del. were collected from Paris village, New valley, Egypt which located at latitude 24° 40' 82 N, longitude 30° 36' 86 03 E and altitude 51 M. Fruits were collected in maturity stage while seed were spread on filter paper and dried in dry room of +22 °C and 10 %RH.



**A. *Balanites aegyptiaca* plant**

**B. *Balanites aegyptiaca* fruit**

**Figure (1). A and B illustrate *Balanites aegyptiaca* plant and fruit**

### Drying seeds:

Ripened fruits were collected from the standing trees, cleaning pulp and dried in Egyptian Deserts Gene Bank, after processing seeds to conservation room. Seeds moisture for active conservation should be between 3% and 7% and Seeds moisture for Base conservation should be between 3% and 8% (Rao *et al.*, 2006).

### **Germination test:**

Germination test was carried out according to the guidelines of the Association of Official Seed Analysis (AOSA, 1978). Germination tests were done under germination incubator. Seeds were placed in white plastic container (15cm wide, 23.2cm length, 10cm. depth) filled with mixture media of clay and sand (1:1) which were used in different treatments. Each treatment used 100 seeds divided into four replicates; i.e., twenty-five seeds were sown in each replicate. Two climatic conditions were applied to examine the environmental conditions; 8h dark, 30 °C, 16 h light, 35 °C with relative humidity of 85% using parafilm (laboratory film) to close the container well turned. Distilled water was used to irrigate the media. Seed germination were counted after 7, 10, 15, 20 and 25 days. Growth chambers model (Challenge 500, CH500 VL S/N 7250) was used. The following parameters were measured:

1. T.Z viability %
2. Germination Percentage (G %) was calculated as (total number of germinated seeds)/ (total number of seeds) X 100 according to Bewley and Black (1994).
3. Standard Germination Percentage (SG %) was calculated as (Total number of normal seedlings)/( Total number of seeds used) X 100 according to ISTA (1996).
4. Viability Percentage (V %) was also calculated as (Number of normal germination seeds + number of abnormal germination seeds + hard seeds) / (total number of seeds) X 100.
5. Dormancy Percentage (D %) was obtained as (hard seeds) / (total number of seeds) X 100.

6. Germination rate (GR) was calculated as (Number of germinated seeds)/(Days to first count)+ ...+ (Number of germinated seeds)/(Days of final count) according to Maguire (1962).

### Tetrazolium test

was conducted to assess the percent viability of seeds that were stored under different storage conditions and periods. **That was done using** Tetrazolium salt (2-, 3-, 5-triphenyltetrazolium chloride, C<sub>19</sub>H<sub>15</sub>CIN<sub>4</sub>, TTC red) **and** TTC with concentration of 0.1%. **Staining pattern of the seeds was evaluated** under a low-powered binocular microscope; viable tissues stain **appeared** bright red while pink and very dark red stains indicate dead tissue (AOSA 2005).

### Breaking Dormancy treatments:

Seeds of *Balanites aegyptica* (L.) Del. were subjected to the following pre- treatments before sowing:

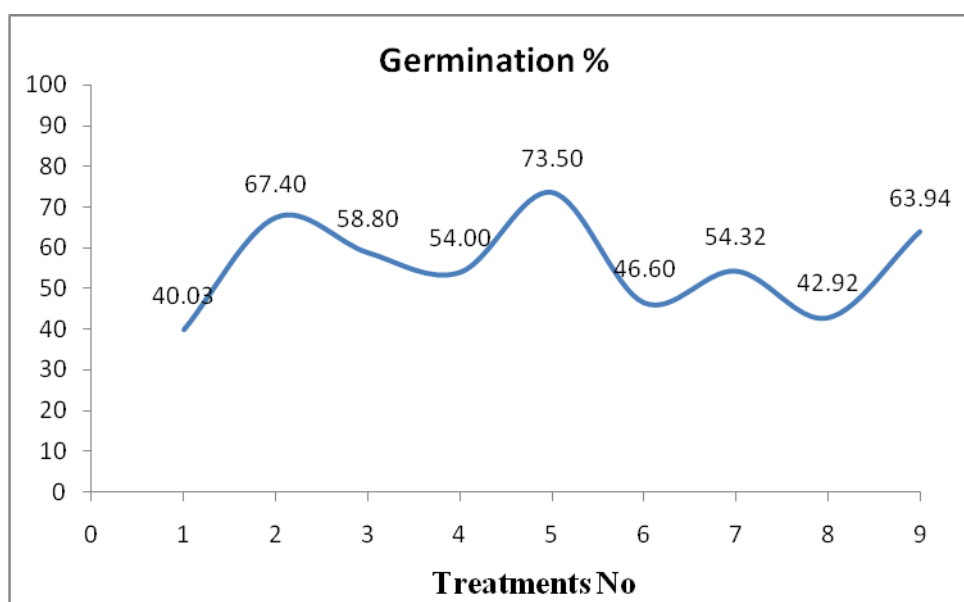
Table (1). Breaking dormancy treatments for *Balanites aegyptica* (L.) Del.

| No | Treatments                         | Time of treatments                 |
|----|------------------------------------|------------------------------------|
| 1  | Control                            | 0                                  |
| 2  | Hot water 70 °C                    | For 24 h                           |
| 3  | GA <sub>3</sub> 10 <sup>-3</sup> M | For 24 h                           |
| 4  | H <sub>2</sub> SO <sub>4</sub> 98% | For 10 min                         |
| 5  | Dry heat 60 °C                     | For 15 min                         |
| 6  | KNO <sub>3</sub> 0.2%              | Used to apposition of sowing water |
| 7  | Normal water                       | For 72 h                           |
| 8  | H <sub>2</sub> O <sub>2</sub> 1%   | For 24 h                           |
| 9  | Mechanical scarification           | 0                                  |

## Results

### Effect of dormancy treatments on germination percentage of *Balanites aegyptiaca*:

Variation in germination responses across different breaking dormancy treatments were elucidated in figure (2). Results showed increasing germination percentage from 40.03% to 67.40 % when seed treated with hot water at 70 °C for 24 hours. Treated seed with GA<sub>3</sub> 10<sup>-3</sup>M for 24 hours increased germination percentage from 40.03% to 58.80%. Treated seeds with 98% H<sub>2</sub> SO<sub>4</sub> for 10 Min and 1% H<sub>2</sub>O<sub>2</sub> for 24 hours increased germination percentage from 40.03% to 54% and 42.92% on succession, while treated seeds with dry heat of 60 °C for 15 Min, gave the highest germination percentage. Using 0.2% KNO<sub>3</sub> to appose sowing water gave little increments in germination percentage (46.60%). Normal tap water for 72 hours resulted in lower germination (54.32%) while mechanical scarification enhances seed germination in *Balanites aegyptiaca* from 40.03% to 63.94%.

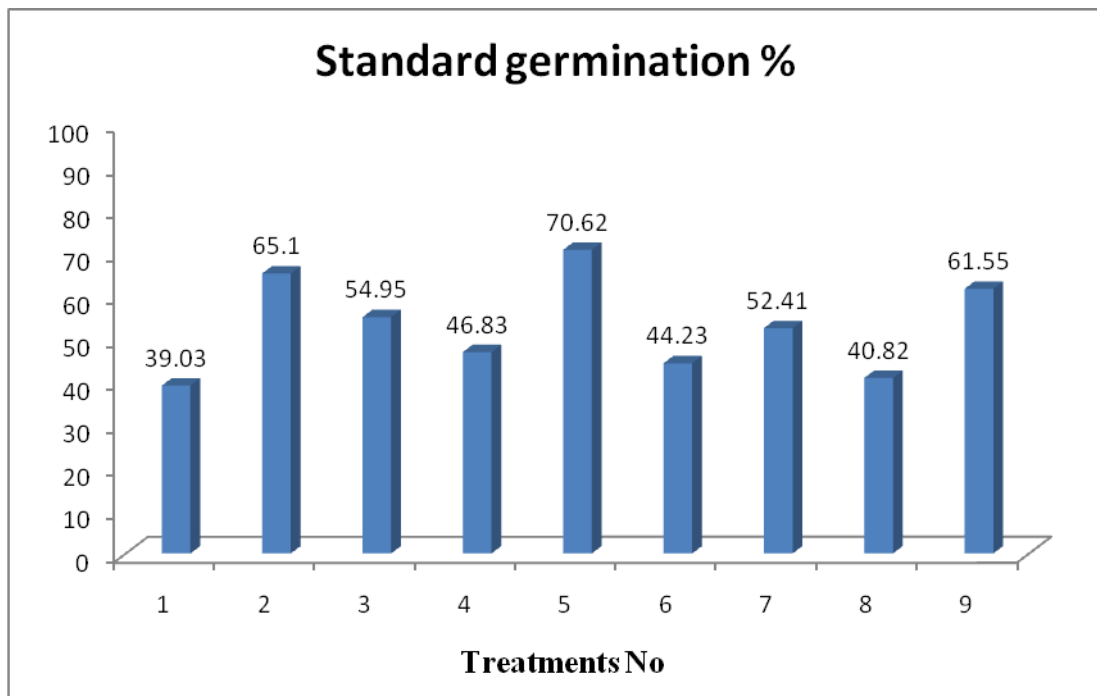


1: Control, 2: hot water 70 °C for 24 hours, 3: GA<sub>3</sub> 10<sup>-3</sup>M for 24 hours, 4: H<sub>2</sub> SO<sub>4</sub> 98% for 10 Min, 5: Dry heat 60 °C for 15 Min, 6: KNO<sub>3</sub> 0.2%, 7: normal water for 72 hours, 8: H<sub>2</sub>O<sub>2</sub> 1% for 24 hours and 9: mechanical scarification.

**Figure (2).** The response of germinating *Balanites aegyptiaca* to dormancy treatments.

## Effect of dormancy treatments on standard germination percentage of *Balanites aegyptiaca*:

Data presented in figure (3) showed that treated seeds of *B. aegyptiaca* with dry heat of 60 °C for 15 Min treatments gave the highest standard germination percentage while treated seed with hot water of 70 °C for 24 hours improved germination from 39.03% in untreated seed (control) to 65.1%. Soaked seed in 1% H<sub>2</sub>O<sub>2</sub> for 24 hours gave the least value of standard germination percentage while soaked seeds in 98% H<sub>2</sub> SO<sub>4</sub> for 10 Min and using 0.2% KNO<sub>3</sub> to appose sowing water تعني استخدام الماء بدلا من ماء الريه الاولى للتجربة Treated seed with Gibberellic acid increased standard germination up to 54.95%. **The best results for improving the germination percentage were attained by treated seeds with dry heat of 60 °C for 15 Min.**



1: Control, 2: hot water 70 °C for 24 hours, 3: GA<sub>3</sub> 10<sup>-3</sup> M for 24 hours, 4: H<sub>2</sub> SO<sub>4</sub> 98% for 10 Min, 5: Dry heat 60 °C for 15 Min, 6: KNO<sub>3</sub> 0.2%, 7: normal water for 72 hours, 8: H<sub>2</sub>O<sub>2</sub> 1% for 24 hours and 9: mechanical scarification.

**Figure (3).** The response of *Balanites aegyptiaca* standard germination% to dormancy treatments.

### Effect of dormancy treatments on viability percentage of *Balanites aegyptiaca*:

Table (3) indicated that the viability of seed after exposure to different breaking dormancy treatments tended to increase / decrease (please check??). Untreated seeds gave 99% viable seeds. Tetrazolium test showed that seeds were 99.91% viable when treated with normal water for 72 hours and 99.63 % viable when treated with  $GA_3 10^{-3}M$  for 24h. Treated seeds with 98%  $H_2SO_4$  for 10 min decreased the viability of seed to 96.34%. Results showed significant differences between viable seeds preconditioned with various treatments where most seeds that did not germinate were in a state of dormancy; these ungerminated seeds are 96% viable (seeds ungerminate but seed stile viable, seeds content any dormancy type). The best results for enhancing the viability percentage were attained by treated seeds with normal water for 72 h.

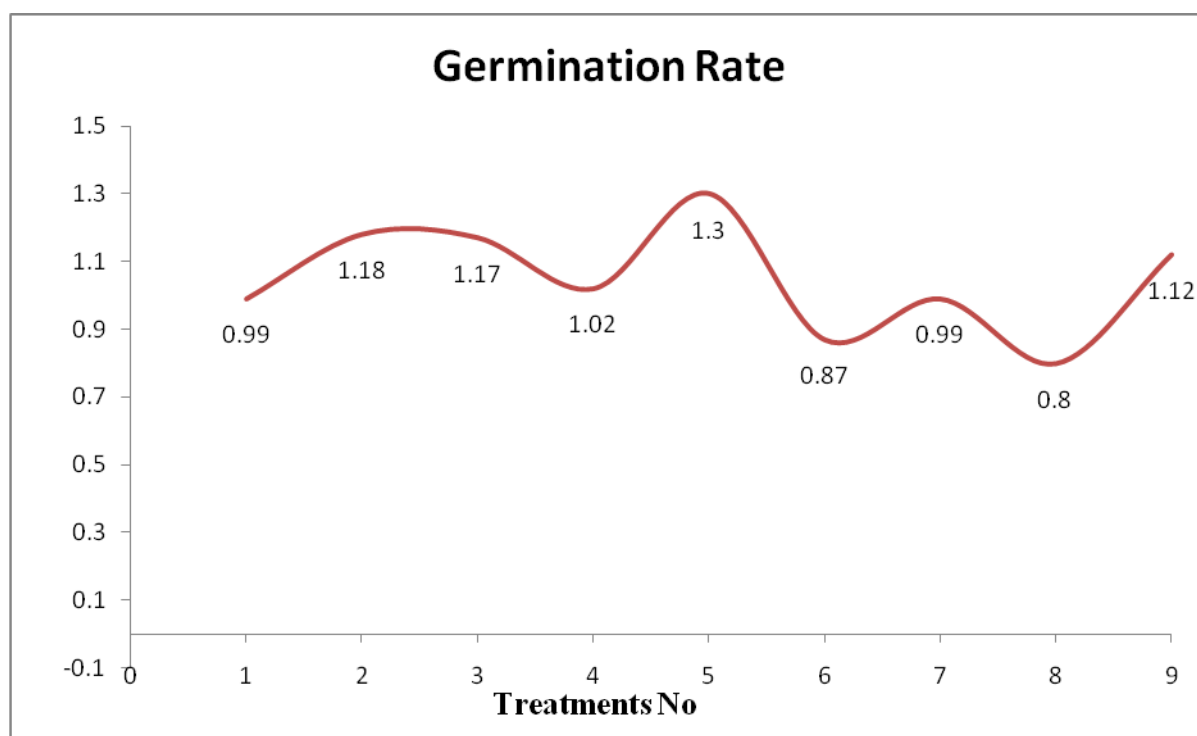
Table (3). The **viability** of seeds after pretreatments (Tetrazolium staining test was applied to seeds that failed to germination).

| TR NO. | Dormancy treatments       | Viability % |
|--------|---------------------------|-------------|
| TR 1   | Control                   | 99.00       |
| TR 2   | hot water 70 °C for 24h   | 96.63       |
| TR 3   | $GA_3 10^{-3}M$ for 24h   | 99.63       |
| TR 4   | $H_2SO_4$ 98% for 10 min  | 96.34       |
| TR 5   | Dry heat 60 °C for 15 min | 98.35       |
| TR 6   | $KNO_3$ 0.2%              | 97.59       |
| TR 7   | Normal water for 72 h     | 99.91       |
| TR 8   | $H_2O_2$ 1% for 24 h      | 98.62       |
| TR 9   | Mechanical scarification  | 96.90       |

**Effect of dormancy treatments on germination rate of *Balanites aegyptiaca*:**

Germination rate of *B. aegyptiaca* seeds incurrence by treated with different dormancy breaking treatments, as well as, used some treatments (KNO<sub>3</sub> 0.2%, H<sub>2</sub>O<sub>2</sub> 1% for 24 hours) decrements germination rate

Treated seeds with dry heat of 60 °C for 15 min gave the highest germination rate (1.3) while soaked seeds in hot water of 70 °C for 24h increased germination rate from 0.99 to 1.18. Soaked seeds in GA<sub>3</sub>10<sup>-3</sup>M for 24h increased germination rate from 0.99 to 1.17 while treated seeds with mechanical scarification increased germination rate from 0.99 to 1.12. Soaked seeds in 98% H<sub>2</sub>SO<sub>4</sub> for 10 min increased germination rate from 0.99 to 1.02 while treated seeds of *B. aegyptiaca* with 0.2% KNO<sub>3</sub> and 1% H<sub>2</sub>O<sub>2</sub> for 24 h decreased germination rate from 0.99 to 0.87 and 0.8 with respectively No differences were found when treated seeds with normal water for 72 h compared with the control. The best results for enhancing the germination rate were attained by treated seeds with Dry heat of 60 °C for 15 min.



1: Control, 2: hot water 70 °C for 24 hours, 3: GA<sub>3</sub> 10<sup>-3</sup> M for 24 hours, 4: H<sub>2</sub> SO<sub>4</sub> 98% for 10 Min, 5: Dry heat 60 °C for 15 Min, 6: KNO<sub>3</sub> 0.2%, 7: normal water for 72 hours, 8: H<sub>2</sub>O<sub>2</sub> 1% for 24 hours and 9: mechanical scarification.

**Figure (4).** Effect of dormancy treatments on germination rate of *Balanites aegyptiaca*.

## Discussion

There are significant differences in the germination of *Balanites aegyptiaca* seed of different dormancy treatments compared with the the control (Figure 2) which indicated physical and physiological dormancy of *B. aegyptiaca* seeds. Germination response of improved selection of *Balanites aegyptiaca* seed to soaking in hot water in the present study was similar to that reported by Duval and Nesmith (2000). Several studies have shown improvement in germination with hot water treatments (Teketay, 1996; Kannan *et al.*, 1996; Schelin *et al.*, 2003).

In the present study, soaking seeds in water was found to be as effective in promoting germination as soaking the seeds in GA<sub>3</sub>. These results were confirmed by Tansi (1999) who studied the break of seed dormancy in *Capparis*

*spinosa* using mechanical, chemical and physical treatments under laboratory, greenhouse and field conditions. Tetrazolium test indicated that seeds were 97% viable and exhibited 20.7% germination. The highest germination of 53% was obtained in seeds soaked in 400ppm GA<sub>3</sub> for 120 min after treatment with sulfuric acid for 20 min.

Significant enhancing in the germination responses to GA<sub>3</sub> treatments was attained in the present study. Similar results were found in *B. aegyptiaca* seed (Baskin and Baskin, 1988).

It appeared that dry heat treatments of 60 °C for 15 min enhancing germination percentage and germination rate. Many studies have shown that dry heat treatments (60 °C -100 °C) tended to improve germination of hard seeds of some leguminous species (Teketay, 1996). Schelin *et al.* (2003) reported that *Balanites aegyptiaca* seeds possess physical, physiological or combined dormancy when seeds were subjected to different dry heat treatments at 60 °C, 80 °C and 100 °C for 15, 30 and 60 min. Moreover, it seems that low intensity heat shock elicited Increased germination.

Mechanical scarification tends to improve germination percentage, standard germination and germination rate. Similar results were obtained for hard seed coat (Stilinovic and Grabic, 1988).

Hydrogen peroxide appeared to increase germination percentage with no significant difference compared with the control. Duval and Nesmith (2000) treated seeds of triploid watermelons with hydrogen peroxide of 0%, 1%, 2%, 4% or 8% aqueous in agar seeds and germinated on agar at constant 28 °C in the dark. All H<sub>2</sub>O<sub>2</sub> treatments increased final percentage germination relative to the control by as much as 70%. Furthermore, H<sub>2</sub>O<sub>2</sub> treatments at >2% severely injured germinating seeds.

Treatments with sulphuric acid tend to improve germination percentage, standard germination and germination rate. **Patane and Gresta (2006)** reported that chemical scarification with sulphuric acid was effective in reducing the hardness of the seed but at the highest studied concentration of 70% and the longest time studied exposure of 60 min.

## **Conclusion**

*Balanites aegyptiaca* seed appeared to be affected by pre-treatment breaking dormancy. Treated seeds with dry heat at 60 °C for 15 minutes resulted in the highest germination percentage of 73.50%, enhanced standard germination percentage to 70.62 % and germination rate to 1.3. The highest viability percentage was attained when seeds treated with normal (tap) water for 72 hours. The present study concluded the possibility of *Balanites aegyptiaca* seeds to be responded to breaking dormancy treatments.

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# تحسين أنبات بذور بلح العبيد المحفوظ ببنك الصحاري المصرية للجينات النباتية باستخدام معاملات كسر السكون

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## الملخص:

تم إجراء الدراسة الحالية في بنك الصحاري المصرية للجينات النباتية – محطة بحوث شمال سيناء - مركز بحوث الصحراء – مصر و ذلك لدراسة استجابة معاملة بذور بلح العبيد (بلح السكر) ببعض معاملات كسر السكون الكيميائية والميكانيكية مثل الخدش الميكانيكي والكيماوي، حمض الجبريلليك ، الحرارة الجافة ، نترات البوتاسيوم ، فوق أكسيد الهيدروجين ، حمض الكبريتيك ، الماء العادي والماء الساخن.

ومن أهم النتائج التي تم التوصل إليها أنه باستخدام المعاملة بالحرارة الجافة عند 60 م لمدة 15 دقيقة تحققت أعلى نسبة للانبات (73.5%) كما أدت لتحسين الأنبات القياسي إلي 70.62% ومعدل الإنبات إلى 1.3. ومن ناحية أخرى، أدت المعاملات بـحمض الجبريلليك والماء الساخن والخدش الميكانيكي للحصول علي أفضل النتائج لكل من نسبة الإنبات والإنبات القياسي ومعدل الإنبات. كما أدت بعض المعاملات إلى تدهور نسبة الإنبات والإنبات القياسي والحيوية ومعدل الإنبات مثل المعاملة بـحمض الكبريتيك.