### Effect of Periods and Conditions of Preservation in Gene Banks on Seed Viability of *Balanites aegyptiaca* (L.) Del.

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#### ABSTRACT



The present study was carried out in the Egyptian Deserts Gene Bank (EDGB), North Sinai Research Station, Desert Research Center, Egypt, during the period of 2005 to 2008. The aim of this study was to investigate the seed viability of *Balanites aegyptiaca* (L.) Del. Under different storage conditions (room temperature, +4°C and -22°C) and various storage periods (fresh seeds (control), 6 months, 12 months and 5 years "seeds were stored up to 5 years ago in Egyptian Deserts Gene Bank" ). Different breaking dormancy treatments were used to encourage, germination of *Balanites aegyptiaca* (L.) Del seeds. The results showed significant effects for conservation conditions, storage periods and dormancy breaking treatments on seed viability parameters. Germination percentage was the highest when seeds were stored under +4°C for six months and when treated by dry heat at 60°C for 15 minutes to break dormancy. The fresh seeds (control, the seeds new collected) gave the highest viability percentage. The seeds, which stored at -22°C gave the highest value of all germination parameters when were stored for 12 months. The increase in germination parameters were greatest for seeds treated with 60°C for 15 min. This significantly denotes the seeds requirement for high temperature to achieve greater viability after storage. **Key words:** *Balanites aegyptiaca*, storage conditions, storage periods, Gene Bank, seed preservation, seed dormancy, seed viability, conservation.

INTRODUCTION

Balanites aegyptiaca (L.) Del. is an important tree crop of the savannah zone and semi arid tropical region of Africa. It is known as desert date (Hall and Walker, 1991), as well as myrobolan and heglig (Bolous, 2000). Leaves are used as food, bark as a substance for fishing and 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 ranges between 30 to 60% and 20 to 30%, respectively (Hall and Walker, 1991). The kernel 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 fodder (Abu Al-Futuh, 1983). The processing of Balanites aegyptiaca (L.) Del. 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 by cracking (Aviara et al., 1999)

The seed phase is the most important stage in the life cycle of higher plants when survival, dormancy and germination are important natural mechanisms to ensure this cycle.

Many species produce seeds that do not germinate shortly after dispersal and require a species-specific after-ripening periods through dry storage (Baskin and Baskin, 1998). Both storage conditions and duration are important factors in regulating the after-ripening process (Murdoch and Ellis, 2000). The values for all germination parameters were attained when seeds were stored at -22°C for 12 months.

Seed germination in arid and semi-arid regions has been studied mainly in annual species (Gutterman, 1993) 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 in this manner, the composition of the seed bank plays a critical role in the maintenance of the vegetation community in tidal freshwater wetlands (Leck and Simpson, 1995).

Genetic erosion of material maintained in genebanks is considered a relevant problem at the International level, for this reason, the monitoring of the main factors causing genetic erosion in *ex situ* collections is strongly recommended to minimize the loss of genetic diversity. These factors include low quality of the original material, over drying of seeds before storage, increase of storage temperature or moisture content of seeds during preservation, lack of regeneration, losses of germplasm in multiplication, physiological changes in seed during storage and no detected loss of germination caused by lack of viability monitoring (FAO, 1997). In general, the combination of  $3\pm7\%$  moisture content and storage temperature below 8°C would permit long-term seed preservation (FAO/IPGRI, 1994).

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The short-term storage was used for small seed samples, the seeds being stored for up to 5 years at  $+4^{\circ}$ C and 30- 45% RH. Large samples of seeds were dried to 4 - 6% water content and stored at -10°C for as long as, routine germination tests indicate no significant fall in viability (Krauss, 1983).

The optimal conditions required within the store depend upon the ultimate use of the stored seeds and the required duration of storage. For storage of base collections, which are rarely removed from store, temperature of less than -18°C with 3-7% seed moisture content are recommended for long-term secure conservation (Genebank Standards, 1994).

This study was carried out to investigate the effect of conservation periods and storage conditions on seed viability of *Balanites aegyptiaca* stored in a gene bank facility.

#### MATERIALS AND METHODS

#### Seed collection

*Balanites aegyptiaca* seeds were collected from Paris village, New Valley, Egypt, at Latitude  $24^{\circ}$  40' 82 N, Longitude  $30^{\circ}$  36' 86 03 E and Altitude 51 M. Fruits were collected in maturity stage and seeds were spread on filter paper and dried in dry room (+22°C & 10% RH).

#### **Drying seeds**

Seeds moisture for active conservation should be between 3% and 7% and Seeds moisture for Base conservation should be between 3% and 8%. *Balanites aegyptiaca* seeds moisture before drying (17%). In this study seeds were dried in moisture controlled dry room (+22°C, 10% RH), to save time and to give accurate results, and were dried immediately in order to minimize seed deterioration. Seeds were placed as a thin layer on the plastic mesh trays to maximize air circulation.

After this step, moisture content should be measured and this was done by digital humidity sensors which measure the amount of water vapour in the air at equilibrium with a sample of seeds enclosed in a sealed chamber, the reading is generally expressed as equilibrium relative humidity (eRH), and can be related to conventional percentage moisture content by using standard curves. This instrument called portable hygrometer (Fig. 1), which is non-destructive method to measure moisture content of seeds before storage (Rao *et al.*, 2006).

#### Seed storage conditions

In order to get information on preservation of seed viability for a long period, treatments of seed storage conditions were as follow:

a. Seed storage at average room temperature, of (20 - 25°C) in cloth bags.

- b. Seed storage at cold temperature of +4°C and 40% Relative humidity (Active Room Short term storage), in vacuum-sealed aluminum polyethylene bags.
- c. Seed storage at -22°C and no frost (Base Room Long term storage) in vacuum-sealed aluminum polyethylene bags.



**Figure (1):** Portable hygrometer (Digital Humidity Sensors). The Rotronic Palm 3 display unit with AWVC-dio workstation utilises a dry state hygrolytic sensor and in-built fan for rapid equilibration. Range: +5 to +50 °C (+/-0.2°C), 0 to 100% RH (+/- 1.5% RH)

#### **Storage periods**

Seeds were stored under each storage room for 6, 12, and 60 months. The effect of storage period on seed viability was then compared with that of fresh seed.

#### Germination test

Germination test was carried out under germination incubator according to the guidelines of the Association of Official Seed Analysis (AOSA, 1978) the following parameters were measured:

#### T.Z viability%

a. Tetrazolium test for seed viability

The Tetrazolium test can be used as a backup procedure to identify viable but dormant seeds that have failed to germinate at the end of a germination test. The procedure for this test is indicated below.

The Tetrazolium test is not an absolute test of seed viability. To gain reliability, the test must be compared with the results of germination tests for each species.

b. Preconditioning

1. Remove the seed-covering structures (glumes, etc.).

2. Precondition the seeds by soaking in water or by placing them in a moist medium at 30 °C for 24-48 hours. No preconditioning is necessary when ungerminated seeds are evaluated at the end of a germination test.

c. Tetrazolium salt used in testing

Tetrazolium salt (2, 3, 5-triphenyltetrazolium chloride,  $C_{19}H_{15}CIN_4$ , TTC red). Used TTC with a concentration of 0.1%.

d. Staining

- 1. Use 100 seeds for each species, each storage condition and each storage period. Divide seeds to four replications, 25 for each replicate.
- 2. Bisect the seeds longitudinally through the embryo with a razor blade.
- 3. Discard half of each seed and place the other half in the staining solution at the recommended concentration in a glass vial.
- 4. Place the vials in an incubator in a dark area at the recommended temperature and duration for each species for 24-48 hours.
- 5. After staining, wash the seeds several times in a distilled water to remove excess stain.
- 6. Immerse the seeds in lactophenol solution (1 liter of lactophenol prepared from 200 ml phenol, 200 ml lactic acid, 400 ml glycerin, and 200 ml water) for one to two hours before evaluating the seeds.
- 7. Evaluate the seeds for a staining pattern under a lowpowered binocular microscope; viable tissues stain bright red. Pink and very dark red stains indicate dead tissue, (Moore, 1973).
- 8. Classify the seeds into three categories depending on staining pattern:
- I. Completely stained seeds that are viable;
- II. Completely unstained seeds that are nonviable; and
- III. Partially stained seeds that will produce either normal or abnormal seedlings, depending on the intensity and pattern of staining.

#### **Germination Percentage (G %)**

$$(G \%) = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

#### Standard Germination Percentage (SG %)

$$(SG \%) = \frac{\text{Total number of normal seedlings}}{\text{Total number of seeds used}} \times 100$$

#### Viability Percentage (V %)

(V %) = (Number of normal germinated seeds + number of abnormal germinated seeds + hard seeds) / total number of seeds  $\times$  100

#### **Dormancy Percentage (D %)**

(D %) = Hard seeds / total number of seeds  $\times 100$ 

GR =	N of germinated seeds	+	N of germinated seeds
OK	days to first count	'	days to final count

To assess seed germination, growth model Challenge 500, CH500 VL S/N 7250 were used. Seeds of different treatments were placed in white plastic containers (15 cm width, 23.2 cm length, and 10 cm depth) filled with mixture media of clay and sand (1:1). Four replicates comprised of 100 seed represented each storage periods and storage condition. Hence, each replicate contained

25 seeds placed in a plastic container. Containers were closed tightly using parafilm and placed under 30 or 35°C in the growth chambers. The rest of the environmental conditions were the same in both chambers (8 hours dark, 16 hours light, and 85% RH). The distilled water was used to irrigate the growth media in the plastic containers.

**Table (1):** Seeds of *Balanites aegyptiaca* were subjected to the following pre-treatments before sowing.

No	Treatments	Time of treatments
1	Control	
2	Hot water 70 °C, Veena et al. (2001)	24 h
3	GA <sub>3</sub> 10 <sup>-3</sup> M, Schelin <i>et al.</i> (2003)	24 h
4	H <sub>2</sub> SO <sub>4</sub> 98%, Teketay (1996)	10 min
5	Dry heat 60 °C, Schelin et al. (2003)	15 min
6	KNo <sub>3</sub> 0.2%, Aroonrungsikul <i>et al.</i> (2002)	used to irrigate
		the growth media
7	Normal water, Çırak et al. (2007)	72 h
8	H <sub>2</sub> O <sub>2</sub> 1%, Grange <i>et al.</i> (2003)	24 h
9	Mechanical scarification, Schelin et al. (20	03)

#### Statistical analysis

The experimental design was Split Split Plot Design with four replicates. Data were statistically analyzed according to Snedecor and Cocharn (1980). The Duncan's new multiple range test (Duncan, 1955) at  $P \le 0.05$  was employed to separate the treatment means. Transformation data to ARC SIN

#### **RESULTS AND DISCUSSION**

#### Effect of seed storage conditions

Data in Table (1) showed the effect of seeds storage conditions (room temperature, +4°C & 40% RH and -22°C) on viability parameters of *Balanites aegyptiaca* (L.) Del. Results revealed that there were significant differences between the storage conditions in all parameters, unless dormancy (D%). The highest value of germination percentage (62.96%), standard germination percentage (58.11%), viability percentage (98.98%) and germination rate (1.12) were achieved when seeds were stored under -22°C. While room conditions gave the least value in all parameters unless dormancy percentage which appeared with base room (34.01%). It could be concluded that decreasing storing temperature -22°C caused an increase in germination, standard germination, viability percentages and germination rate in *Balanites aegyptiaca*. These results are in agreement with those obtained by Genebank Standards, (1994) and Lewis et al. (1998) as they concluded that lower temperatures associated with improved viability in base collection over active collection.

#### Effect of seed storage periods:

Data in Table (2) indicated that there were significant differences between all storage periods in all

parameters. Seeds stored up to 6 and 12 months gave the highest germination percentage (66.95 and 68.19 respectively), standard germination percentage (62.16 and 65.27 respectively) and germination rate (1.17), while the least values of these parameters appeared with fresh seeds. As for dormancy percentage (55.31%) This parameter gave the least value with seeds, which were stored for 5 years. These results are in agreement with those obtained by Baskin and Baskin (1998) and Murdoch and Ellis (2000).

From data in Table (2), it was noticed that the viability percentage was very high immediately after harvesting. The percentage of viability gradually decreased further with time lapse. The freshly harvested seeds exhibited 99.99% of viability. This high percentage of viability was lowered to 98.68% after one-year, and to 71.70% after five years in this concern.

# Effect of the interaction between seed storage conditions and seed storage periods

Data in Table (4) indicted the effect of the interaction between storage conditions and storage periods on germination, standard germination, viability, dormancy percentages and germination rate of *Balanites aegyptiaca* seeds.

Storing seeds under room temperature for one year gave the highest value of germination percentage followed by storing up to 6 months as well as seeds conserved either under  $-22^{\circ}$ C or  $+4^{\circ}$ C for one year or 6 months as gave the highest value. On the other hand, when seeds were stored for five years or as freshed the germination percentage was decreased. Similar results were confirmed by Lewis *et al.* (1998). A similar trend was observed for standard germination percentage.

Storage seeds under base room for 12 months gave the highest percentage of standard germination, as well as when seeds were stored under active and room temperatures. The least value of standard germination in fresh collected seeds.

Fresh seeds under room temperature gave the highest value of viability percentage and germination rate, as well as seed stored under +4°C which gave the same trend for conservation of seeds under different conditions. Gupta *et al.* (2005) noticed similar trends.

Although the fresh seeds gave the highest values for viability percentage when stored under different conditions, they gave the highest values of dormancy percentage, it may be concluded that Balanites aegyptiaca (L.) Del. seeds contain the hard seed coat which led to increase the dormancy percentage and storing seed for different periods led to minimizing the dormancy percentage especially under room temperature for five years. These results are in agreement with those obtained by Yogeesha et al. (2005). According to germination rate, there was an increase in the germination rate with decreasing storage

**Table (2):** Effect of seed storage conditions on germination percentage, standard germination percentage, viability percentage, dormancy percentage and germination rate, of *Balanites aegyptiaca* seeds.

Storage conditions	G%	SG%	V%	D%	GR
Room temp. (20-25 °C)	57.72 <sup>ь</sup>	54.72 <sup>b</sup>	96.58 <sup>b</sup>	34.58 <sup>a</sup>	1.07 <sup>b</sup>
Active room (+4 °C& 40% RH)	59.32 <sup>b</sup>	56.04 <sup>b</sup>	98.16 <sup>a</sup>	34.42 <sup>a</sup>	1.08 <sup>b</sup>
Base room (-22 °C)	62.96 <sup>a</sup>	58.11 <sup>a</sup>	98.98 <sup>a</sup>	34.01 <sup>a</sup>	1.12 <sup>a</sup>

\* Means followed by the same letter within the same column are not significantly different (P = 0.05, Duncan's new multiple range test). G%: germination percentage, SG%: standard germination percentage, V%: viability percentage, D%: dormancy percentage, and GR: germination rate.

**Table (3):** Effect of seed storage periods on germination percentage, standard germination percentage, viability percentage, dormancy percentage and germination rate, of *Balanites aegyptiaca* seeds.

Storage periods	G%	SG%	V%	D%	GR
Fresh (control)	44.69 c	42.10 c	99.99 a	55.31 a	1.17 a
6 months	66.95 a	62.16 a	98.82 a	30.15 b	1.16 ab
12 months	68.19 a	65.27 a	98.68 a	27.50 b	1.08 b
5 years	59.62 b	55.39 b	91.70 b	27.45 b	0.96 c

\* Means followed by the same letter within the same column are not significantly different (P = 0.05, Duncan's new multiple range test).

**Table (4):** Effect of the interaction between seed storage condition and seed storage periods on germination percentage, standard germination percentage, viability percentage, dormancy percentage and germination rate, of *Balanites aegyptiaca* seeds.

Storage conditions						
Storage periods	Room temp.		Base room			
	(22-25 °C)	(+4 °C & 40% RH)	(-22 °C)			
	Germinati	on percentage				
Fresh (control)	44.30 <sup>d</sup>	44.30 <sup>d</sup>	45.46 <sup>d</sup>			
6 months	59.05 °	68.99 <sup>ab</sup>	69.04 <sup>ab</sup>			
12 months	69.09 <sup>ab</sup>	64.80 <sup>a-c</sup>	70.60 <sup>a</sup>			
5 years	58.05 °	58.87 °	62.25 bc			
S	Standard germ	ination percentage				
Fresh (control)	42.10 <sup>d</sup>	42.00 <sup>d</sup>	42.00 <sup>d</sup>			
6 months	55.12 °	66.32 <sup>ab</sup>	64.81 <sup>ab</sup>			
12 months	66.67 <sup>ab</sup>	61.17 <sup>a-c</sup>	67.90 <sup>a</sup>			
5 years	53.43 °	54.30 °	58.42 <sup>bc</sup>			
-	Viability	percentage				
Fresh (control)	99.68 <sup>a</sup>	99.68 <sup>a</sup>	99.68 <sup>a</sup>			
6 months	97.25 <sup>ab</sup>	99.35 <sup>ab</sup>	99.35 <sup>ab</sup>			
12 months	98.49 <sup>ab</sup>	98.23 <sup>ab</sup>	99.43 <sup>a</sup>			
5 years	85.08 <sup>d</sup>	91.83 °	96.45 <sup>b</sup>			
	Dormanc	y percentage				
Fresh (control)	53.40 <sup>a</sup>	53.40 ª	53.40 <sup>a</sup>			
6 months	35.10 <sup>b</sup>	27.82 <sup>b</sup>	27.66 <sup>b</sup>			
12 months	26.75 <sup>b</sup>	29.43 <sup>b</sup>	26.27 <sup>b</sup>			
5 years	24.40 <sup>b</sup>	28.02 <sup>b</sup>	29.98 <sup>b</sup>			
-	Germi	nation rate				
Fresh (control)	1.16 ab	1.16 <sup>ab</sup>	1.17 <sup>ab</sup>			
6 months	1.13 ab	1.22 <sup>a</sup>	1.13 <sup>ab</sup>			
12 months	1.12 ab	0.97 °	1.15 <sup>ab</sup>			
5 years	0.88 °	0.96 °	1.03 bc			

\* Means followed by the same letter within the same column are not significantly different (P.=0.05, Duncan,s new multiple range test).

temperature. This was true under the storing periods of 6 months. Higher rate was obtained at 6 months periods at  $+4^{\circ}$ C. Also, seed storage of *Balanites aegyptiaca* under low temperature could encourage the reservation of protein which plays an important role for enhancing the germination speed. Similar results were confirmed by Reiad *et al.* (1995).

#### Effect of dormancy breaking treatments

Variations in germination responses across different breaking dormancy treatments were elucidated in Table (5). Results showed an increase in germination percentage from 40.03 to 73.50%, standard germination percentage from 39.03% to 70.62% and germination rate from 0.99 to 1.30 when seeds were treated with dry heat at 60°C for 15 min. Howevers the highest dormancy percentage (58.97%) and viability percentage were obtained with control treatment. Schelin et al. (2003) confirmed similar results. Also, when seeds was soaked in normal water for 72 hours and treated with GA<sub>3</sub>10<sup>-3</sup>M for 24 hours gave the highest viability percentage, but the seed untreated (control treatment) achieved highest dormancy value. All pretreatments increased seeds viability parameters compared with untreated seeds (control).

 $GA_3$  enhances seed germination in species exhibiting physiological and morph-physiological dormancy (Baskin and Baskin, 1998). Germination of *B. aegyptiaca* was improved when  $GA_3$  was applied at a concentration of 500 ppm (Zarad *et al.*, 1998) found a negative effect of high concentrations of  $GA_3$  on the germination of *Albizzia grandibracteata*.

Teketay and Tigabu (1996) reported negative effect of boiling water treatment on the germination of *Tamarindus indica*.

The use of  $H_2SO_4$  as a scarification method to overcome physical dormancy is well known and several studies showed improvement of germination with  $H_2SO_4$  treatments (Teketay, 1996, 1998)

Dry heat treatments at 60°C for 15 minutes improved the germination of seeds. Several studies have shown that dry heat treatments (60-100°C) improved germination of hard-seeded species. Dry heat treatment plays a role in the germination of *B. aegyptiaca*. Zarad *et al.* (1998) found that sulfuric acid inhibited germination percentage compared to control of *Balanites aegyptiaca* seeds.

#### Effect of the interaction between storage conditions and storage periods on seeds viability with tetrazolium test

Data tabulated in Table (6) revealed that there were different significant differences between the interaction between storage conditions and storage periods on viability percentage, as stored seeds up to 6 and 12 months under room temperature decreased viability percentage from 100 to 48%, while storing seeds for

**Table (5):** Effect of seed dormancy breaking treatments on germination percentage, standard germination percentage, viability percentage, dormancy percentage and germination rate of *Balanites aegyptiaca* seeds.

Dormancy treatments	G%	SG%	V%	D%	GR
Control	40.03 <sup>g</sup>	39.03 g	99.00 <sup>a</sup>	58.97 <sup>a</sup>	0.99 <sup>d</sup>
Hot water 70 °C 24h	67.40 <sup>bc</sup>	65.10 <sup>bc</sup>	96.63 <sup>b</sup>	25.82 <sup>de</sup>	1.18 <sup>b</sup>
GA310 -3M 24h	58.80 <sup>d</sup>	54.95 <sup>d</sup>	99.63 <sup>a</sup>	37.32 °	1.17 <sup>b</sup>
H <sub>2</sub> SO <sub>4</sub> 98% 10 min	54.00 <sup>e</sup>	46.83 <sup>e</sup>	96.34 <sup>b</sup>	39.52 °	1.02 cd
Dry heat 60 °C 15 min	73.50 <sup>a</sup>	70.62 <sup>a</sup>	98.35 ab	$19.92^{\text{ f}}$	1.30 <sup>a</sup>
KNO <sub>3</sub> 0.2%	$46.60^{\ f}$	$44.23 \ ^{ef}$	97.59 ab	46.32 ab	0.87 °
Normal water 72 h	54.32 °	52.41 <sup>d</sup>	99.91 <sup>a</sup>	42.13 bc	0.99 <sup>d</sup>
H <sub>2</sub> O <sub>2</sub> 1% 24 h	$42.92^{\text{ f}}$	$40.82^{\ f}$	98.62 ab	51.46 <sup>b</sup>	0.80 e
Mechanical scarification	63.94 <sup>cd</sup>	61.55 °	96.90 <sup>b</sup>	27.90 <sup>d</sup>	1.12 bc

\* Means followed by the same letter within the same column are not significantly different (P = 0.05, Duncan's new multiple range test).

 Table (6): Effect of the interaction between seed storage conditions and storage periods on viability percentageof Balanites aegyptiaca seeds with tetrazolium test.

<b>Room conditions</b>	Fresh seed	6 months	12 months	5 years
Room temperature	100 <sup>a</sup>	89 <sup>bc</sup>	80 <sup>d</sup>	48 <sup>e</sup>
Active room +4 oC	100 <sup>a</sup>	96 <sup>ab</sup>	91 <sup>b</sup>	84 <sup>cb</sup>
Base room -22 oC	100 <sup>a</sup>	96 <sup>ab</sup>	92 <sup>b</sup>	90 bc

\* Means followed by the same letter within the same column are not significantly different (P =0.05, Duncan, s new multiple range test).

five years under  $-22^{\circ}$ C enhanced the viability percentage but storing seed under room temperature for five years decreased the viability percentage. It could be concluded that when seeds are to be stored under room temperature it must decrease the period to less than one year. However, the storing conditions available must be conserved under  $-22^{\circ}$ C or  $+4^{\circ}$ C for any time, as well as using fresh seeds for germination directly. These results are in agreement with those obtained by Magdalena *et al.* (1999) and Genebank Standards, (1994).

#### Effect of the interaction between (storage conditions) room temperature, seed storage periods and breaking dormancy treatments on germination percentage

Data presented in Figure (2) show the effect of interaction between room temperature, storage periods and breaking dormancy treatments on germination percentage of Balanites aegyptiaca (L.) Del. In Figure (2) the germination response to pre-sowing treatments gave the highest value (87.76%) when seeds were treated with dry heat (60°C) for15 min, and also, when seeds were stored for 12 months under room temperature conditions. On the other hand, when seeds were stored for 12 months without any treatments under room temperature conditions the germination percentage was increased from 72.85 to 80.52%. While, the germination percentage gave the least value when seeds were stored under room temperature for 5 years.

Fresh seeds under room temperature gave normal germination percentage without any treatments. While, storage seeds under room temperature for 6 months

gave an increase in germination percentage especially with dry heat ( $60^{\circ}$ C) for 15 min. Also, storage of seed in room temperature conditions for 12 months and trating with dry heat ( $60^{\circ}$ C) for 15 min gave better germination percentage in room temperature. On the other hand, storage of seeds up to 5 years gave the least germination percentage.

Generally stored seeds under room temperature gave normal germination percentage, and saved the germinability for seeds up to 1 year only. We found they the seeds storage at room temperature the germinability of seeds was gradually reduced and the best germination lasted until 12 months, and the lowest seed germination percentage was obtained when stored for a period of five years.

# Effect of the interaction between (storage conditions) active room at +4°C, seed storage periods and breaking dormancy treatments on germination percentage

In Figure (3) the effect of the interaction between active room  $+4^{\circ}$ C, storage periods and breaking dormancy treatments on germination percentage of *Balanites aegyptiaca* (L.) Del. The results showed that when storing seed up to 6 months under  $+4^{\circ}$ C and treated with dry heat of 60°C for15 min, gave the highest values. Storing seeds up to 12 months under the previous same conditions and treatments gave the same trend. On the other hand, when seeds were stored under ( $+4^{\circ}$ C) up to 6months gave the highest germination percentage value with all dormancy breaking treatments.

# Effect of the interaction between (storage conditions) base room at -22°C, seed storage periods and breaking dormancy treatments on germination percentage

In the Figure (4) the germination percentage showed the highest value when seed were stored under -22 °C for 6 months and treated with dry heat 60 °C for 15 min. while storing seeds up to 12 months under -22°C achieved the highest values in all treatments, without dry heat at 60°C for 15 min.

#### Conclusion

The results from this study provided that: Storing seeds under room temperature (control) were saved seeds viability up to one year. Storing seed under +4°C (active) or -22°C (base) were saved the viability of seeds to 5 years. Physical dormancy were a major hurdle for completed and rapid germination seeds of *Balanites aegyptiaca*, Pre-treated seeds of *B. aegyptiaca* with dry heat 60°C for 15 min enhancing the seed germination. The best storage conditions were freezing conservation for all seeds types under studies.

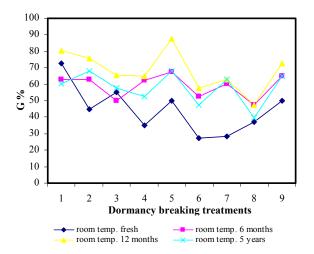
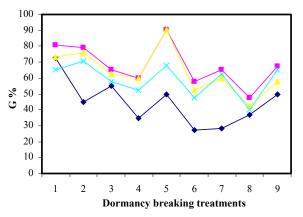
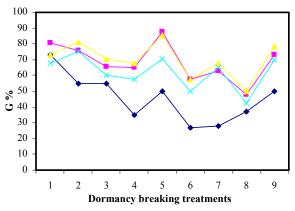


Figure (2): Effect of the interaction between room temperature, seed storage periods and breaking dormancy treatments on germination percentage of *B. aegyptiaca* seeds. 1 = Control; 2 = Hot water 70°C for 24h; 3 = GA<sub>3</sub>10 -<sup>3</sup>M for 24h; 4 = H<sub>2</sub>SO<sub>4</sub> 98% for 10 min; 5 = Dry heat 60 °C for15 min; 6 = KNO<sub>3</sub> 0.2%; 7 = Normal water for 72 h; 8 = H<sub>2</sub>O<sub>2</sub> 1% for 24 h; and 9 = Mechanical scarification.



**Figure (3):** Effect of the interaction between active room +4°C, seed storage periods and breaking dormancy treatments on germination percentage of *B. aegyptiaca* seeds.



**Figure (4):** Effect of the interaction between base room at -22°C, seed storage periods and breaking dormancy treatments on germination percentage of *B. aegyptiaca* seeds.

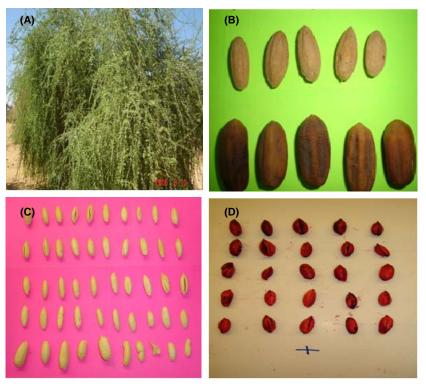


Figure (5) (A) *Balanites aegyptiaca* tree, (B) Fruit and seeds, (C) Embryo, and (D) Fresh seed of after TZ test.

#### REFERENCES

- A.O.S.A., ASSOCIATION OF OFFICIAL SEED ANALYSTS. 1978. Rules for testing seeds. D.f. Grade (Ed.) Proc. Association Official Seed Anal. 3. (3), Washington, D.C.
- ABU-AL-FUTUH, I.M. 1983. *Balanites aegyptiaca*: an unutilized raw material potential ready for agroindustrial exploitation. United Nations Industrial Development Organization Report TF/INT/77/021, Vienna.
- AROONRUNGSIKUL, C., S. SUKPRAKARN, E. NAWATA, AND T. SAKURATANI. 2002. Effects of physical treatments on breaking of seed dormancy in Thai cucumber, Thai Journal of Agricultural Science 35 (3): 237-243.
- AVIARA, N.A., M.A. HAQUE, AND M.I. GWANDZANG. 1999. Physical properties of guna seeds. Journal of Agricultural Engineering Research 73: 105–111.
- BASKIN, J.M., AND C.C. BASKIN. 1998. Seeds. ecology, biogeography and evolution of dormancy and germination. Academic Press Inc., San Diego, CA.
- BEWLEY, J.D., AND M. BLACK. 1994. Seeds: physiology of development and germination. 2<sup>nd</sup> Ed. Plenum press, New York.
- BOLOUS, L. 2000. Flora of Egypt 2: 140-218.
- ÇIRAK, C., K. KEVSEROGLU, AND A.K. AYAN. 2007. Breaking of seed dormancy in a Turkish endemic Hypericum species: *Hypericum aviculariifolium* subsp. depilatum var. depilatum by light and some

pre-soaking treatments. Journal of Arid Environments **68 (I.1):** 159-164.

- DUNCAN, D.B. 1955. Multiple ranges and multiple (F) test. Biometries 11: 1-42.
- EGLEY, G.H. 1993. Water-impermeable seed coverings as barriers to germination. In: Taylorson, R.B. (Ed.), Recent Advances in the Development and Germination of Seeds. Plenum Press, New York, NY.
- FAO. 1997. Report of the technical meeting on the methodology of the world information and early warning system on plant genetic resources. FAO, Rome.
- FAO/IPGRI. 1994. Genebank Standards. Food and agriculture organization of the United Nations, and international plant genetic resources Institute, Rome.
- GENEBANK STANDARDS. 1994. Food and agriculture organization of the United Nations, Rome. International plant genetic resources, Rome.
- GRANGE, S., D.I. LESKOVAR, L.M. PIKE, AND B.G. COBB. 2003. Seed coat structure and oxygenenhanced environments affect germination of triploid watermelon. Journal of the American society of horticultural sciences 128 (2): 253-259.
- GUPTA, M.L., D.L. GEORGE, AND I.G.M.A. PARWATA. 2005. Effect of harvest time and drying on supersweet corn seed quality. Seed Science and technology **33**: 167-176.
- GUTTERMAN, Y. 1993. Seed Germination in Desert Plants. Berlin, Springer.

- HALL, J.B., AND D.H. WALKER. 1991. *Balanites aegyptiaca*: a monograph. School of Agricultural and Forest Sciences, University of Wales, Bangor, UK J. Agric. Res. **27:** 373–379.
- I.S.T.A. INTERNATIONAL SEED TESTING ASSOCIATION. 1996. International rules for seed testing. Seed Science and Technology (supplement) **24:** 1-335.
- KRAUSS, A. 1983. Organization of the Seed Processing and Storage Section at Plant Genetic Resources Centre/ Ethiopia. PGRC-E.ILCA-Germplasm-Newsletter 4: 9-12.
- LECK, M.A., AND R.L. SIMPSON. 1995. Ten year seed bank and vegetation dynamics of a tidal fresh water marsh. Annual Journal of Botany **82:** 1547-1557.
- LEWIS, D.N., A.H. MARSHALL, AND D.H. HIDES. 1998. Influence of storage conditions on seed germination and vigour of temperate forage species. Seed Science and Technology **26**: 643-655.
- MAGDALENA, RUIZ; ISAURA MARTOAN, CELIA, DE LA CUADRA. 1999. Cereal seed viability after 10 years of storage in active and base germplasm collections. Field crops research **64**: 229-236.
- MAGUIRE, J.D. 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Science **2:** 176-177.
- MOORE, R.P. 1973. Tetrazolium staining for assessing seed quality. In Heydecker, W. (Ed.), seed ecology, the Pennsylvania State University Press, University Park, Pennsylvania.
- MORPETH, D.R., AND A.M. HALL. 2000. Microbial enhancement of seed germination in Rosa corymbifera 'Laxa'. Seed Science Research **10**: 489– 494.
- MURDOCH, A.J., AND R.H. ELLIS. 2000. Dormancy, viability and longevity. In: Fenner,M. (Ed.), seeds. the ecology of regeneration in plant communities, 2<sup>nd</sup>. ed. CAB International, Wallingford.
- RAO, N. KAMESWARA. JEAN, HANSON. DULLOO, M. EHSAN. KAKOLI, GHOSH. DAVID, NOWELL. AND MICHAEL, LARINDE. 2006. Ipgri Handbooks for Genebanks No. 8, IPGRI, Rome, Italy.

- REIAD, M.SH., R.TH. ABDRABOU, AND M. YASSIN. 1995. Effect of grain maturity and storage period on growth at different stages of wheat plants. Journal of agriculture Science. Mansoura University **20** (6): 2713-2722.
- SCHELIN, M., M. TIGABU, I. ERIKSSON, L. SAWADOGO, AND P.C. ODEN. 2003. Effects of scarification, gibberellic acid and dry heat treatments on the germination of *Balanites aegyptiaca* seeds from the Sudanian savanna in Burkina Faso. Seed Science and Technology **31 (3)**: 605-617.
- SNEDECOR, G.W., AND W.G. COCHRAN. 1980. Statistical methods. 6th Ed. Iowa State Univ. Press Ames., Iowa, USA.
- TEKETAY, D. 1996. Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. Forest Ecology and Management **80:** 209-223.
- TEKETAY, D., AND M. TIGABU. 1996. The effect of presowing seed treatments, temperature and light on the germination of *Tamarindus indica* L., a multipurpose tree. Journal of Tropical Forsety **12:** 73-79.
- VEENA-GUPTA, ANJALI-KAK, VIVEK-MITTER, AND SINGH, B.B. 2001. Techniques to remove hard seededness in the wild medicinal plant Abutilon indicum, Journal of Medicinal and Aromatic Plant Science 23 (3): 369-371.
- YOGEESHA, H.S., T.N. SHIVANANDA, AND K. BHANUPRAKSH. 2005. Effect of seed maturity, seed moisture and various pre-treatments on seed germination of annatto (*Bixa orellana* L.). Seed Science and technology **33**: 97-104.
- ZARAD, S.S., Y.A. HOSSNY, AND H.M. EL-BAGOURY. 1998. Effect of various treatments on seed germination and water application on vegetative growth of desert date (*Balanites aegyptiaca* Linn) seedlings grown in different soils. Egyptian Journal of Physiological Science **21** (2): 147-159.

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## تأثير فترات وظروف الحفظ في بنوك الجينات على حيوية بذور بلح العبيد

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### الملخص العربسي

تم إجراء الدراسة الحالية في بنك الصحاري المصرية للجينات النباتية بمحطة بحوث شمال سيناء مركز بحوث الصحراء مصر أثناء الفترة من 2005 حتى 2008 ، وتهدف إلي دراسة استجابة بلح العبيد (الهجليج، بلح السكر) لكل من ظروف الحفظ (درجة حرارة الغرفة العادية22 - 25° م، + 4°م و40% رطوبة نسبية، - 22°م ولا توجد رطوبة) وفترات حفظ البذور (التخزين لمدة 6 أشهر، التخزين لمدة 12 شهر، التخزين لمدة 5 سنوات مقارنة بالبذور حديثة الجمع)، مع استعمال العديد من معاملات كسر السكون وقد أوضحت النتائج ما يلى (1) أن هناك فروق معنوية في الاستجابة لظروف وفترات الحفظ ومعاملات كسر السكون علي بذور بلح العبيد في جميع القراءات التي تم دراستها، (2) أعلي نسبة أنبات تحصل عليها عندما تخزن البذور عند المدون علي بذور بلح العبيد في جميع القراءات التي تم دراستها، (2) أعلي نسبة أنبات تحصل عليها عندما تخزن البذور عند +4°م لمدة 6 شهور واستخدام معاملة الحرارة 60 °م لمدة 15 دقيقة، (3) البذور الحديثة التي لم تخزن أعطي أعلي نسبة مئوية الحيوية، (4) عند التخزين في -22°م لمدة 12 شهرة نتج عنها القيمة الاعلى في جميع القراءات النور بلح العبيد تحتاج المعاملة بالحرارة العالية معاملة الحرارة 60 °م لمدة 15 دقيقة، (3) البذور الحديثة التي لم تخزن أعلي أعلي نسبة مئوية الحيوية، (4) عند التخزين في -22°م لماته 12 شهرة نتج عنها القيمة الاعلى في جميع القراءات، (5) اتضح أن بذور بلح العبيد تحتاج المعاملة بالحرارة العالية بعد الخروج من التخزين، (6) يمكن تخزين بذور بلح العبيد لمدة سنة واحدة فقط عند الحفظ في الغرفة المعاملة بالحرارة العالية بعد الخروج من التخزين، (6) يمكن تخزين بذور بلح العبيد لمدة سنة واحدة فقط عند الحفظ في الغرفة العادية، وعند توافر غرف الحفظ يجب أن تحفظ البذور في +4°م أو -22°م لمدة تصل لخمسة اعوام