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Effect of storage conditions and periods of conservation in gene bank on seed viability and germination of *Acacia tortilis* and *Acacia raddiana*

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Abstract

In Egyptian deserts, Acacia tortilis trees play an important role for biodiversity and Bedouins populations, where they used as source of animal fodder, timber, fuel wood, charcoal, gums and other products as well as contributing to soil stabilization of sand dunes. This work aimed to study the effect of storage conditions and storage periods of preservation in gene bank on germinability and viability of two wild economic native Acacia species, which were collected from two different regions of the Egyptian deserts (Wadi Tekuila - Gabel Elba - Red Sea) and (Gabel El-Halal -North Sinai, Sinai). In these areas, Acacia tortilis trees showed high densities and form forest wadis. Acacia plants are exposed to stresses in their natural habitats as insects which feed on their seeds, anthropogenic pressures and the inability of seeds to germinate normally due to some kinds of seed dormancy. The best value of germination percentage (45%) was recorded when seeds of Acacia tortilis subspecies. raddiana from Elba, were conserved in base room at -22 ^{0}C for 24 months.

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Introduction

Acacia is the largest genus in the Leguminosae-Mimosoideae with approximately 1200 species distributed mainly in tropical and subtropical regions (Mabberley, 1997.) Species of Acacia have an ability to flourish under adverse conditions. They can tolerate salinity and seasonal waterlogging and are adapted to environments with little and unreliable rainfall. Moreover, they are adapted to anthropogenic pressures (Ayyad and Ghabbour, 1985; Abdelrahman and Krzywinski, 2008). Species of Acacia have economic values as they could be used as animal fodder and are sources of timber, fuel wood, charcoal, gums and other products as well as contributing to soil stabilization and improvement through nitrogen fixation (Springuel &Mekki, 1993).

Food security refers to the availability of food and one's access to it. A household is considered food-secure when its occupants do not live in hunger or fear of starvation, (FAO, WFP and IFAD, 2012). In Egyptian deserts, both climatic and anthropogenic stresses are reported to play a key role in the decline of Acacia populations (Abdelrahman and Krzywinski, 2008). So, the preservation of Acacia seeds in gene bank is important for the conservation of the speiecs.

Seeds storage conditions and storage periods play a great role in seeds viability, seeds germination and growth parameters. The importance of several factors, which affect seed quality and performance were discussed in previous studies including the physiological and biotechnological features of the seeds and their viability, germination percentage, dormancy pre-treatments for wild species (Lars, 2000).

Acacia tortilis under cold and dry storage conditions retained full seeds viability for 18 months of storage. (Abdelbasit et al., 2012).Genetic erosion of material maintained in gene banks is considered a relevant problem at the International level (FAO/IPGRI, 1994). 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. In general, the combination of 3±7% moisture content and storage temperature below 8^{o}C would permit long-term seed preservation (FAO/IPGRI, 1994). However, even for those seeds stored under controlled conditions, viability may decrease as a result of a deterioration process. Consequently, studies about long-term viability are needed to determine the storability of seed materials in genebanks and to provide conditions, which will maintain the viability of each accession above a minimum value (FAO/IPGRI, 1994).

Fig. 1. Map distance between study sites.

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 ^{o}C with 3-7 % seed moisture content are recommended for long–term secure conservation (Genebank Standards, 1994).
The aim of current study is to determine the conservation measures of Acacia tortilis species in Egyptian Deserts Gene Bank (EDGB), through determine the best conditions and periods of time to conserve seeds of Acacia tortilis species as well as best methods to break seeds dormancy.

Materials and methods

Plant Genetic Resources (PGR): Seed materials of *Acacia tortilis* (Forssk) Hayne sub species *raddiana*

(Savi) Brenan and *Acacia tortilis. (Forssk) Hayne sub species tortilis* were collected from four sites representing two different regions of Egyptian deserts (Wadi Tekuila – Gabel Elba – Red Sea, and Gabel El-Halal –North Sinai area, Table 1 and figure1). Seeds of target species were collected from the studied sites during 2009 season at at seed maturity stage following the guideline of bioversity international and international gene banks and according the procedures of Bennett (1970), Harlan (1975), Marshall (1975), Hawkes (1976, 1980), Arora (1981) and Chang (1985)..

Seeds conservation: According to guidelines of bioversity international and international gene banks and methods set up by Rao *et al.* (2006), the collected seed materials were preserved in the Egyptian Deserts Gene Bank (EDGB) and subjected to drying, cleaning, viability test, packaging and storage.

Seed viability was assessed by germination tests according to FAO, IPGRI (1994). The germination tests were performed according to the International Seed Testing Association ISTA (1996) as well as Association of Official Seed Analysis AOSA (1978). Germination percentage (G %) was determined and seed viability was also evaluated by chemical staining for seeds using Tetrazolium test (Bewley and Black, 1994).Chemical staining for seeds viability tetrazolium test using (Tetrazolium salt (2, 3, 5-triphenyltetrazolium chloride, $C_{19}H_{15}CIN_4$, TTC red). Used TTC with a concentration of 0.1%)

Seed sampling and timing: Four replicates in separated bags for each treatment were used. Seeds were divided into small packages (200 grams for each bag) in *Egyptian Deserts Gene Bank*.

Seed storage periods: Seeds were stored in conservation room at Egyptian Deserts Gene Bank for ((Fresh seeds (zero time), 6 months, 12 months, 18 months and 24 months)).

Seeds storage conditions: Seeds were subjected to storage at room temperature, mean temperature ranged from 20 °C to 25 °C in cloth bags, storage at refrigerator temperature (+4 °C, 40% relative humidity - active room – short term), in vacuum sealed aluminum polyethylene bags, storage at refrigerator temperature (-5 °C, no frost – medium term conservation), in vacuum sealed aluminum polyethylene bags, and storage at (–22 °C, no frost – Base room – long term) in vacuum sealed aluminum polyethylene bags.

Statistical analysis: The experimental design was split plot design with four replicates. Data were statistically analysed according to Snedecor and Cocharn (1980). The Duncan's new multiple range test (Duncan, 1955) at $P \leq 0.05$ was employed to separate the treatment means.

Results and discussion

Effect of storage conditions on germination percentage of Acacia tortilis SPP. tortilis and A.t. SPP. raddiana

Effect of different storage conditions on germination percentage of Acacia seeds without using any treatments to break seed dormancy are presented in Table (2). Seeds A. tortilis spp. raddiana from Sinai showed the highest value of germination percentage (41.60 %) when stored at room temperature. On the other hand, storage of seeds of Acacia tortilis sub species tortilis from Elba area at freezing room -5 ^{0}C gave the lowest value of germination percentage (31.80%). Similar results were indicated by Lewis et al. (1998) and Reiad et al. (1995).

Effect of storage periods on germination percentage of Acacia tortilis SPP. tortilis and A.t. SPP. raddiana

The effect of interaction between Acacia tortilis species and storage periods on germination percentage are indicated in Table (3). Fresh seeds of Acacia tortilis sub species tortilis from Elba showed the highest germination percentage (40.88%), while Acacia tortilis spp. raddiana from Sinai showed the lowest germination rate (33.00%) when seeds were stored for 24 months.

Table 1. The scientific names, family, sites and the location of species.

No.	*Sp.*	*Spp.*	Site	Latitude	Longitude	Altitude
1	*Acacia tortilis*	*tortilis*	Wadi Tekuila, Gabel Elba, Red Sea	N 22°15'3.51"	E 036°22'15.04"	223 M
2			Gabel El-Halal, North Sinai	N 30°48'45.63"	E 034°9'6.72"	185 M
3		*raddiana*	Wadi Tekuila, Gabel Elba, Red Sea	N 22°15'3.51"	E 036°22'15.04"	223 M
4			Gabel El-Halal, North Sinai	N 30°48'45.63"	E 034°9'6.72"	185 M

Table 2. Effect of storage conditions on germination percentage of *Acacia tortilis* spp. *tortilis* and *A. t. spp. raddiana*.

Species Storage conditions	*Acacia t. t.* Elba	*Acacia t. t.* Sinai	*Acacia t. r.* Elba	*Acacia t. r.* Sinai
Room temperature	35.20 DEF	39.00 ABC	39.00 ABC	41.60 A
Active room +4 °C	32.60 FG	36.20 CDE	35.20 DEF	35.30 DEF
Freezing -5 °C	31.80 G	36.80 BCD	37.35 BCD	39.50 AB
Base room -22 °C	33.20 FG	33.80 EFG	35.40 DEF	36.80 BCD

LSD value = 2.535

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

Table 3. Effect of interaction between *Acacia* species and storage periods on germination percentage.

Storage periods species	Fresh (control)	6 months	12 months	18 months	24 months
Acacia t. t. Elba	40.88 A	38.94 AB	38.63 ABC	38.44 A-D	36.63 B-E
Acacia t. t. Sinai	35.94 B-F	34.19 EF	33.88 EF	35.06 DEF	35.06 DEF
Acacia t. r. Elba	34.13 EF	36.38 B-F	37.00 B-E	37.19 B-E	37.13 B-E
Acacia t. r. Sinai	34.44 EF	36.06 B-F	35.38 C-F	35.13 DEF	33.00 F

LSD value = 2.835

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 interaction between Acacia Sub species, storage conditions and storage periods on germination percentage.

species	Storage periods / Storage conditions	Fresh (control)	6 months	12 months	18 months	24 months
Acacia t. t. Elba	Room temperature	40.00 A-G	37.00 C-J	36.00 C-K	33.00 G-L	30.00 JKL
	Active room +4 ºC	40.00 A-G	39.00 A-H	38.00 A-I	40.00 A-G	38.00 A-I
	Freezing -5 ºC	42.00 ABC	37.75 B-I	39.00 A-H	36.25 C-K	40.00 A-G
	Base room -22 ºC	41.50 A-E	42.00 ABC	41.50 A-E	44.50 AB	38.50 A-H
Acacia t. t. Sinai	Room temperature	35.25 C-K	33.75 F-L	31.75 H-L	34.25 E-L	28.00 L
	Active room +4 ºC	37.00 C-J	36.00 C-K	34.25 E-L	36.00 C-K	37.75 B-I
	Freezing -5 ºC	37.00 C-J	34.00 F-L	34.50 D-L	34.00 F-L	36.50 C-J
	Base room -22 ºC	34.50 D-L	33.00 G-L	35.00 C-L	36.00 C-K	38.00 A-I
Acacia t. r. Elba	Room temperature	35.00 C-L	34.00 F-L	31.00 I-L	30.00 JKL	29.00 KL
	Active room +4 ºC	36.50 C-J	35.00 C-L	40.00 A-G	37.00 C-J	35.50 C-K
	Freezing -5 ºC	33.00 G-L	37.00 C-J	36.00 C-K	41.75 A-D	39.00 A-H
	Base room -22 ºC	32.00 H-L	39.50 A-G	41.00 A-F	40.00 A-G	45.00 A
Acacia t. r. Sinai	Room temperature	34.00 F-L	38.00 A-I	33.00 G-L	33.00 G-L	28.00 L
	Active room +4 ºC	34.00 F-L	35.00 C-L	32.00 H-L	36.00 C-K	32.00 H-L
	Freezing -5 ºC	34.00 F-L	34.00 F-L	38.00 A-I	35.00 C-L	36.00 C-K
	Base room -22 ºC	35.75 C-K	37.25 C-J	38.50 A-H	36.50 C-J	36.00 C-K

LSD value = 5.670

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

Table 5. Effect of interaction between storage conditions and storage periods on viability percentage of *Acacia tortilis* spp. *tortilis* and *A. t.* spp. *raddiana*.

species	Storage periods / Storage conditions	Fresh (control)	6 months	12 months	18 months	24 months
Acacia t. t. Elba	Room temperature	97.00 AB	99.00 AB	99.00 AB	100.0 A	97.00 AB
	Active room +4 ºC	97.00 AB	96.00 AB	97.00 AB	95.00 AB	96.00 AB
	Freezing -5 ºC	97.00 AB	100.0 A	99.00 AB	98.00 AB	99.00 AB
	Base room -22 ºC	97.00 AB	98.00 AB	99.00 AB	98.00 AB	99.00 AB
Acacia t. t. Sinai	Room temperature	96.00 AB	95.00 AB	96.00 AB	100.0 A	96.00 AB
	Active room +4 ºC	96.00 AB	96.00 AB	94.00 B	95.00 AB	95.00 AB
	Freezing -5 ºC	96.00 AB	97.00 AB	96.00 AB	96.00 AB	96.00 AB
	Base room -22 ºC	96.00 AB	97.00 AB	97.00 AB	97.00 AB	97.00 AB
Acacia t. r. Elba	Room temperature	97.00 AB	97.00 AB	99.00 AB	100.0 A	97.00 AB
	Active room +4 ºC	97.00 AB	96.00 AB	97.00 AB	96.00 AB	97.00 AB
	Freezing -5 ºC	97.00 AB	97.00 AB	97.00 AB	97.00 AB	97.00 AB
	Base room -22 ºC	97.00 AB	97.00 AB	97.00 AB	96.00 AB	97.00 AB
Acacia t. r. Sinai	Room temperature	99.00 AB	96.00 AB	95.00 AB	94.00 B	95.00 AB
	Active room +4 ºC	99.00 AB	96.00 AB	94.00 B	95.00 AB	95.00 AB
	Freezing -5 ºC	99.00 AB	97.00 AB	96.00 AB	96.00 AB	96.00 AB
	Base room -22 ºC	99.00 AB	97.00 AB	97.00 AB	97.00 AB	97.00 AB

LSD value = 4.219

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

Effect of interaction between storage conditions, and storage periods on germination percentage of Acacia tortilis SPP. tortilis and A.t. SPP. raddiana

Data presented in Table (4) show the effect of interaction between storage conditions, and storage periods on germination percentage *Acacia tortilis SPP. tortilis and A.t. SPP. raddiana*. The germination percentage gave the highest value (45%) when seeds of *Acacia tortilis sub species raddiana from Elba,* were conserved in base room (-22 °C) for 24 months. On the other hand when stored seeds of *Acacia tortilis sub species raddiana from Sinai,* under room temperature for 24 months gave the least germination percentage (28%).

Fig. 2. Germination of *Acacia tortilis* sub species *tortilis*.

Fig. 3. Germination of *Acacia tortilis* sub species *raddiana*.

Generally, the storage of seeds under cold storage room gave high germination percentage, and saved the germinability for seeds up to 2 years. On the other hand, seeds storage at room temperature reduced the germinability of seeds. Similar results were recorded by Abdelbasit *et al.* (2012).

Fig. 4. TZ staining test of *Acacia tortilis* subspecies *tortilis* seeds.

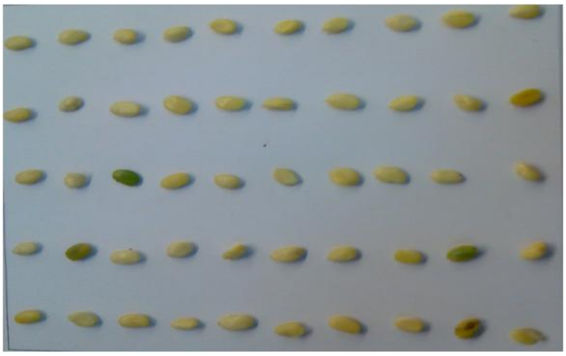

Fig. 5. Seeds of *Acacia tortilis* sub species *raddiana* without coat for TZ test.

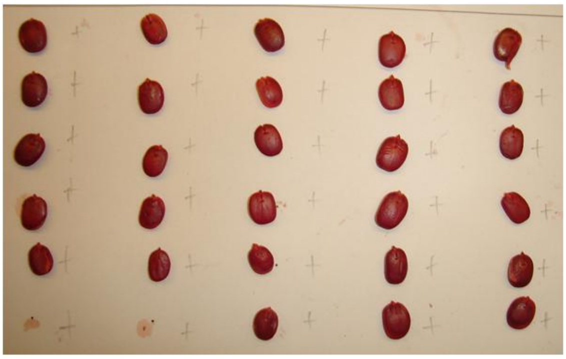

Fig. 6. Seeds of *Acacia tortilis* subspecies *raddiana* + results after staining TZ test.

Effect of interaction between storage conditions, and storage periods on viability percentage of *Acacia tortilis SPP. tortilis and A.t. SPP. raddiana using TZ test*

Data presented in Table (5) confirmed that *Acacia tortilis* seeds have very good viability percentage, and conservation of seeds under cold storage periods for different period gave significant differences.

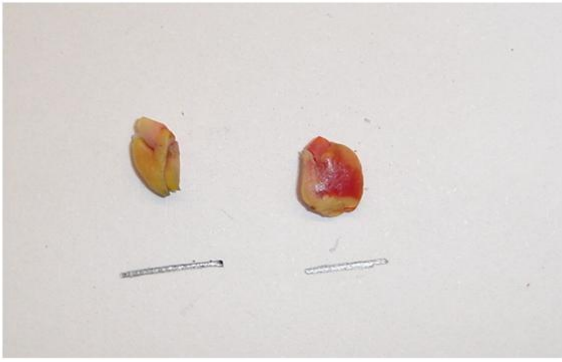

Fig. 7. Seeds of *A. t. t.* - results after staining TZ test.

Results confirmed that *Acacia tortilis* seed would be stored safely at cold storage and it maintained acceptable viability percentage even after storage under suboptimum conditions. The results provide evidence that dormancy plays a major role in regulating germination in *Acacia tortilis* and prevent seeds from dropped their viability during storage for reasonable period of time. The two sub species in the cold and dry storage did not show seeds viability decline after 18 month. However, this is not a long period of storage time to give clue results, of this type of seeds which is characterized by hard seed coat dormancy which regulate the seed germination and prevents the seed from germination during unfavorable conditions. Consequently, studies about long-term viability are needed to determine the storability of seed material in seed gene bank. The results obtained from tests revealed that cold and dry storage conditions are suitable for *Acacia tortilis* seeds preservation in the three provenances. But the cold store excelled the dry storage since the seed did not break dormancy with increasing time the same results recorded with (Abdelbasit *et al.,* 2012).

Conclusion

The best value of germination percentage (45%) was recorded when seeds of Acacia tortilis sub species raddiana from Elba were conserved in base room at -22 oC for 24 months. The best conditions to conserve seeds of Acacia tortilis and raddiana were the cold storage condition of -5 and -22 oC. Seeds of Acacia tortilis spp. tortilis and spp. raddiana could be preserved up to 24 months without significant change in the germination percentage. Physical dormancy was a major hurdle for completed and rapid germination of Acacia tortilis spp. tortilis and spp. raddiana seeds.

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