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IMPROVING ACACIA TORTILIS SEEDS GERMINATION BY BREAKING DORMANCY TREATMENTS

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ABSTRACT

Acacia tortilis trees are very important in Egypt and Qatar. The tree plays an important role as a source for animals feeding, and for environmental enhancement by combating desertification and firewood. This study was carried out in, Genetic Resources Department, Biotechnology Center, Ministry of Environment, Qatar and Egyptian Deserts Gene Bank, Egypt. The aim of this study was to enhancing the responses of *Acacia tortilis subspecies tortilis* seeds to some chemical and physical treatments, such as Mechanical Scarification, Chemical Scarification, GA₃ (Gibberellic acid), dry heat treatment, Potassium nitrate (KNO₃) in solutions of water, Sulphuric acid (H₂SO₄), Hydrogen peroxide (H₂O₂), tap water and boiling water to increase germination percentage. The obtained results revealed significant differences in the germination percentage of *Acacia tortilis* seeds treated by different dormancy treatments, the highest germination percentage was 76 % achieved with boiling water treatment, treated seeds with 98% concentrated sulphuric acid for periods 10, 20, 25, 30 Min. enhanced germination Percentage to 33%, 67%, 70%, 75% respectively, standard germination percentage and germination rate. The lowest germination percentage with untreated seeds was observed 25%.

KEYWORDS: *Acacia tortilis*, seed germination, breaking dormancy

INTRODUCTION

In the recent years, many woody plant species have become endangered due to increasing aridity and human activities. The continuous overgrazing, overcutting, and uprooting are leading to the disappearance of pastoral plant communities, a reduction of plant cover and soil erosion. In general, trees and shrubs severe deterioration include *Acacia tortilis*, *Retama reatam*, *Moringa peregrina*, *Nitraria retusa* and *Salvadora persica* (Helmy *et al.*, 1996). *Acacia* is a widespread genus of tropical-subtropical trees and shrubs ranging from through Africa to Southeast Asia (Ross, 1981). Species of *Acacia* are of considerable social and economic importance throughout the warm and tropical regions of the world. Their use as fuel wood, for agro-forestry and for industry is well established. Some species of *Acacia* are fast growing and can withstand high stress environments. Tackholm (1974) found that the genus *Acacia* comprised eleven different species growing in Egypt. The great biological diversity in *Acacia* is reflected in its wide distribution and ecological amplitude, and particularly in the tolerance of some species to extremes of drought and salinity. Its value, however, lies not only in its ability to flourish under adverse conditions, but also in the range of useful products that it provides. Among these are high quality of animal fodder, timber, fuel wood, charcoal, gums and other products as well as contributing to soil stabilization and improvement through nitrogenfixation, (Springuel & Mekki, 1994).

Acacia tortilis grazed by livestock and used for fodder and firewood. The gum is of economic importance in

some regions. The wood is used for building camel folds (Rizk & El-Ghazaly, 1995). The GCC countries (Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, United Arab Emirates) cover an area of about 2.56 million km². The climate of most of the region is hot and dry throughout much of the year, ranging from hyper arid to arid (Middleton & Thomas, 1997). Qatar is a peninsula located half away along the western coast of the Arabian Gulf. It covers an area of 11,437 sq. kms including a number of coastal islands. The total coastline including the island is over 700 kms. The land area is largely flat and stony desert with a climate hot and arid. Humidity is high in summer, and the average annual rainfall is around 75 mm. Both desertification and ecosystem degradation problems are common in Arabian Peninsula regions, particularly in the State of Qatar. Seed dormancy is most common in wild flora mainly for survival purposes under unfavorable environmental conditions (Hilhorst 1995; Bewley 1997; Geneve 2003; Baskin and Baskin 2004). Seed dormancy can be described as a temporary inability to germinate under conditions in which all the requirements for germination are fulfilled. 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). Teketay (1996) remarked that treated 20 leguminous species seeds with sulphuric acid, improved germination in all the species. H₂SO₄ was effective to overcome seed coat imposed dormancy in the species studied. the main problem of seeds dormancy for *Acacia albida*, *Acacia*

senegal, *Acacia tortilis* and *Acacia nilotica* was caused by an impermeable seed coat. Presowing treatments in hot water in the four species and duration of water soaking treatments did not appreciably increase level of germination Chris, (1994). He concluded the seeds dormancy of *Acacia albida*, *Acacia senegal*, *Acacia tortilis* and *Acacia nilotica* was caused by an impermeable seed coat. Seed coat dormancy was observed in three of the four species. The sulphuric acid treatments increased 1. germination to 80 %. Chris, (1994) concluded that the 2. main problem of seeds dormancy for *Acacia albida*, *Acacia senegal*, *Acacia tortilis* and *Acacia nilotica* was caused by an impermeable seed coat. Pre sowing 3. treatments in hot water in the four species and duration of water soaking treatments did not appreciably increase level of germination. 4.

MATERIALS AND METHODS

Seed collection

Seeds of *Acacia tortilis* were collected Beside Rawdat 5. Rashed Entrance road, Qatar, which located at latitude N 25° 09 54", longitude E 051° 09 204" and altitude 32 M. 6. Pods were collected in maturity stage while seed were spread on dry newspaper and dried in dry room of +22 °C and 10 %RH.

Drying seeds

Ripened pods were collected from the standing trees, cleaning cover and dried in plant genetic resources unit, 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 Petri dishes 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, 27 °C, 16 h light, 30 °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 was counted after 7, 10, 15, 20 and 25 days. The following parameters were measured:

T.Z viability %

Germination Percentage (G %) was calculated as (total number of germinated seeds) / (total number of seeds) X 100 according to Bewley and Black (1994).

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.

Dormancy Percentage (D %) was obtained as (hard seeds) / (total number of seeds) X 100.

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 collected and germinated. That was done using Tetrazolium salt (2-, 3-, 5-triphenyltetrazolium chloride, C₁₉H₁₅CIN₄, 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 1978).

Breaking Dormancy treatments

Samples of seeds were selected randomly and treated with each of the treatments. Seeds of *Acacia tortilis* treated with were subjected to the following pre- treatments before sowing:

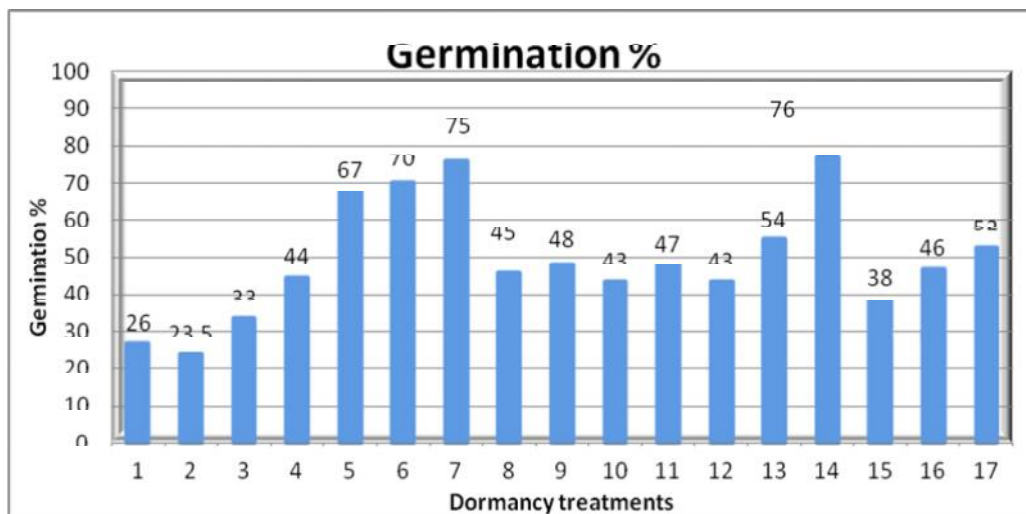
TABLE 1: Breaking dormancy treatments for *Acacia tortilis*

No	Treatments	Time of treatments
1	Control	0
2	H ₂ SO ₄	5 min
3	H ₂ SO ₄	10 min
4	H ₂ SO ₄	15 min
5	H ₂ SO ₄	20 min
6	H ₂ SO ₄	25 min
7	H ₂ SO ₄	30 min
8	H ₂ O ₂ 1%	12 h
9	H ₂ O ₂ 1%	24 h
10	KNO ₃ 0.1%	Used to apposition of sowing water
11	KNO ₃ 0.2%	Used to apposition of sowing water
12	GA ₃ 200ppm	Used to apposition of sowing water
13	GA ₃ 300ppm	Used to apposition of sowing water
14	Boiling Water	Up to cold + 24 h
15	tab water	24 h
16	dry heat 60 °C	15 min
17	Mecanical Scarification	0

RESULTS**Effect of dormancy treatmentson germination percentage of *Acacia tortilis***

Variation in germination responses across different breaking dormancy treatments were elucidated in figure (1). Results showed increasing germination percentage from 26 % to 52 % when seed treated with mechanical scarification. Treated seed with 98% H_2SO_4 for 30 Min. increased germination percentage from 26% to 75 %. Treated seeds with 98% H_2SO_4 for 20 Min and 25 min

increased germination percentage from 26 % to 67 and 70 % Respectively, while treated seeds with Boiling Water up to cold and keep in water for 24 hours, gave the highest germination percentage 76 %. Using 0.2% KNO_3 to oppose sowing water gave little increments in germination percentage (47 %). Normal tap water for 24 and 98% H_2SO_4 for 5 min resulted in lower germination (23.5%) while treated seeds with H_2O_2 1% for 24 hours enhance seed germination in *Acacia tortilis* from 26 % to 48 %.



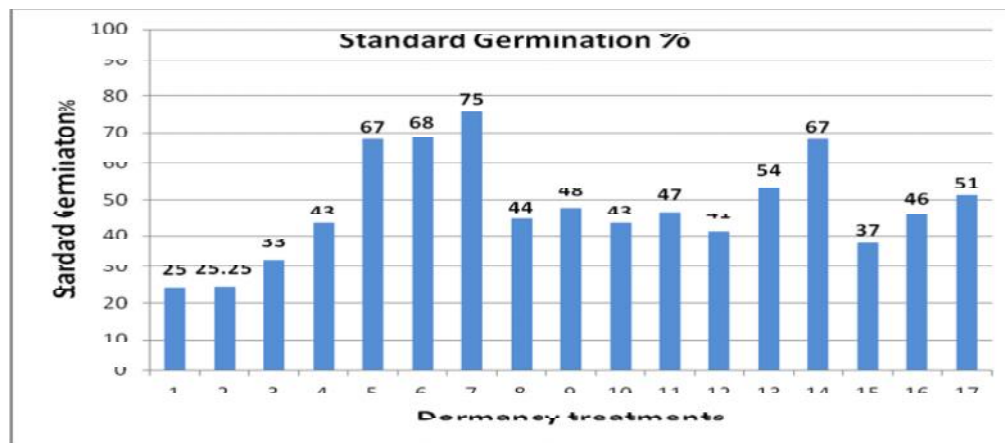
1: Control, 2: H_2SO_4 for 5 Min, 3: H_2SO_4 for 10 Min, 4: H_2SO_4 for 15 Min, 5: H_2SO_4 for 20 Min, 6: H_2SO_4 for 25 Min, 7: H_2SO_4 for 30 Min, 8: H_2O_2 1% for 12 h, 9: H_2O_2 1% for 24 h, 10: KNO_3 0.1%, 11: KNO_3 0.2%, 12: GA_3 200ppm, 13: GA_3 300ppm, 14: Boiling Water, 15: tap water, 16: dry heat 60 °C for 15 Min, 17: Mechanical Scarification

FIGURE 1: The response of germinating *Acacia tortilis* to dormancy treatments.

Effect of dormancy treatmentson standard germination percentage of *Acacia tortilis*

Data presented in figure (2) showed that treated seeds of *Acacia tortilis* with H_2SO_4 for 30 Min treatments gave the highest standard germination percentage while treated seed with boiling improved germination from 25 % in untreated seed (control) to 67 %. Treated seeds with H_2SO_4 for 20 Min, and H_2SO_4 for 25 Min enhanced S.G%

to 67 % and 70%. Soaked seed in 1% H_2O_2 for 24 hours gave 48 % standard germination percentage while soaked seeds in 0.2% KNO_3 to increasing S.G% to 47 %, treated seed with Gibberellic acid 300 ppm increased standard germination up to 54%. The best results for improving the germination percentage were attained by treated seeds with H_2SO_4 for 30 Min.



1: Control, 2: H_2SO_4 for 5 Min, 3: H_2SO_4 for 10 Min, 4: H_2SO_4 for 15 Min, 5: H_2SO_4 for 20 Min, 6: H_2SO_4 for 25 Min, 7: H_2SO_4 for 30 Min, 8: H_2O_2 1% for 12 h, 9: H_2O_2 1% for 24 h, 10: KNO_3 0.1%, 11: KNO_3 0.2%, 12: GA_3 200ppm, 13: GA_3 300ppm, 14: Boiling Water, 15: tap water, 16: dry heat 60 °C for 15 Min, 17: Mechanical Scarification

FIGURE 2: The Effect of dormancy treatments on standard germination percentage of *Acacia tortilis*

Effect of dormancy treatments on viability percentage of *Acacia tortilis*

Table (2) indicated that the viability of seed after exposure to different breaking dormancy treatments. Results showed non-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 100 % viable (seeds ungerminate but seed stipe viable, the seeds dormancy of *Acacia tortilis* was caused by an impermeable seed coat).

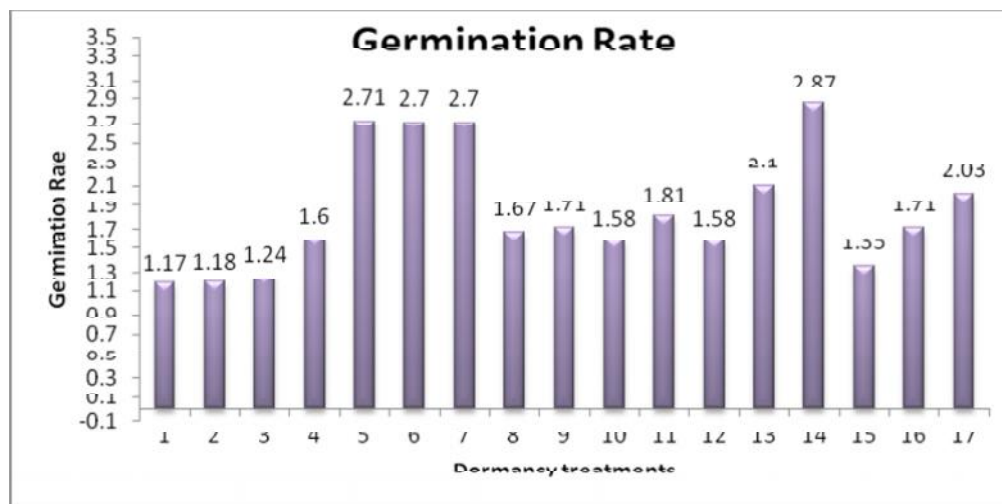
TABLE 2: The viability of seeds after pretreatments (Tetrazolium staining test was applied to seeds that failed to germination)

TR NO.	Dormancy treatments	Viability %
TR 1	Control	99.25
TR 2	H ₂ SO ₄ for 5 Min	99.5
TR 3	H ₂ SO ₄ for 10 Min	98.5
TR 4	H ₂ SO ₄ for 15 Min	98.75
TR 5	H ₂ SO ₄ for 20 Min	100
TR 6	H ₂ SO ₄ for 25 Min	98.25
TR 7	H ₂ SO ₄ for 30 Min	97.75
TR 8	H ₂ O ₂ 1% for 12 h	99.5
TR 9	H ₂ O ₂ 1% for 24 h	99.25
TR 10	KNO ₃ 0.1%	99
TR 11	KNO ₃ 0.2%	98.5
TR 12	GA ₃ 200ppm	98.5
TR 13	GA ₃ 300ppm	99.5
TR 14	Boiling Water	98.5
TR 15	tab water	99
TR 16	dry heat 60°C for 15 Min	99
TR 17	Mechanical Scarification	97.75

Effect of dormancy treatments on germination rate of *Acacia tortilis*

Figure (3) Germination rate of *A. tortilis* seeds incurrence by treated with different dormancy breaking treatments, treated seeds with Boiling Water up to cold and keep in water for 24 hours gave the highest germination rate (2.87) while treated seeds H₂SO₄ for 20 Min, H₂SO₄ for 25 Min, and H₂SO₄ for 30 Min increased germination rate from 1.17 up to 2.71, 2.7, and 2.71 Respectively but No differences were found between this treatment's. Soaked

seeds in GA₃300ppm M for 24h increased germination rate from 1.17 to 2.1 while treated seeds with mechanical scarification increased germination rate from 1.17 to 2.03. Soaked seeds in 0.2% KNO₃ and 1% H₂O₂ for 24 h increased germination rate from 1.17 to 1.58 and 1.81 with respectively. No differences were found when treated seeds with H₂O₂ 1% for 24 h, dry heat 60°C for 15 Min. The best results for enhancing the germination rate were attained by treated seeds with Boiling Water up to cold and keep in water for 24



1: Control, 2: H₂SO₄ for 5 Min, 3: H₂SO₄ for 10 Min, 4: H₂SO₄ for 15 Min, 5: H₂SO₄ for 20 Min, 6: H₂SO₄ for 25 Min, 7: H₂SO₄ for 30 Min, 8: H₂O₂ 1% for 12 h, 9: H₂O₂ 1% for 24 h, 10: KNO₃ 0.1%, 11: KNO₃ 0.2%, 12: GA₃ 200ppm, 13: GA₃ 300ppm, 14: Boiling Water, 15: tab water, 16: dry heat 60 °C for 15 Min, 17: Mechanical Scarification

FIGURE 3: The Effect of dormancy treatments on standard germination percentage of *Acacia tortilis*

DISCUSSION

There are significant differences in the germination of *Acacia tortilis* seeds of different dormancy treatments compared with the the control (Figure 2) which indicated physical dormancy of *Acacia tortilis* seeds. Germination response of improved selection of *Acacia tortilis* 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). It appeared that mechanical scarification treatments enhancing germination percentage and germination rate. Many studies have shown that mechanical scarification tended to improve germination of hard seeds coat of some Acacia species Stilinovic and Grbic (1988) Reported that *Acacia decurrens*, *A. melanoxylon*, *Rhus typhina* and *Koelreuteria paniculata* seeds possess physical dormancy, treated seeds with mechanical scarification improvement germination percentage. Diangana (1985) showed that soaking the seeds of *Acacia mangium* and *Albizia factoria* in H_2SO_4 raised the germination percentage and increased germination rate. In the present study, soaking seeds in water was found to be as effective in promoting germination as soaking the seeds in GA_3 . These results were confirmed by Toncer (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_3 for 120 min after treatment with sulfuric acid for 20 min. Significant enhancing in the germination responses to GA_3 treatments was attained in the present study. Similar results were found in *B. aegyptiaca* seed (Baskin and Baskin, 1988). Mechanical scarification tends to improve germination percentage, standard germination and germination rate. Similar results were obtained for hard seed coat (Stilinovic and Grbic, 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_2O_2 treatments increased final percentage germination relative to the control by as much as 70%. Furthermore, H_2O_2 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. Soaking seeds in water for 24 hours increasing germination percentage, (Matias and others 1973) reported that Soaking in water for 2 to 48 hours improve seed germination of many tropical tree species such as *Acacia mearnsii*, *A. melanoxylon*, *A. nilotica*, *Adenanthere mirosperma*, *Albizia amara*, *A.*

procera, *Grevillea robusta*, *Trewia nidiiflora*, and *Pinus caribaea*.

CONCLUSION

Acacia tortilis seed appeared to be affected by pre-treatment breaking dormancy. Treated seeds with boiling water up to cold and keep in water for 24 hours resulted in the highest germination percentage of 76 %, enhanced germination rate to 2.87. Seeds of *Acacia tortilis* content high viability percentage. The present study concluded the possibility of *Acacia tortilis* seeds to be responded to breaking dormancy treatments.

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