

GERMINATION RESPONSE OF ACACIA (*ACACIA TORTILIS* (FORSSK.) HAYNE) TO SEED TREATMENTS AND INCUBATION TEMPERATURES

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ABSTRACT

Germination of *Acacia tortilis* seeds in response to some seed treatments was studied under four incubation temperatures. A factorial experiment was laid out in a completely randomized design with six replicates was used under controlled incubator conditions. Seed treatments had significant effects on *Acacia* seed germination. Mechanical scarification for one minute and acid scarification for 15 minutes resulted in the highest seed germination. On the other side, the lowest germination percentage appeared in control and washing treatments without significant difference. Seed germination was significantly affected by incubation temperatures. The highest germination percentage was reported at 25-35 °C, but the lowest at 5-15 °C. The interaction between incubation temperature and seed treatments significantly affected seed germination percentage. Mechanical scarification for one minute and incubation temperature at 25-35 °C produced the maximum germination percentage under the conditions of this study.

Key words: Dormancy; acid scarification, germination, scarification, scalding stratification, washing, *Acacia tortilis*, incubation temperature.

INTRODUCTION

Environmental conditions and seed dormancy may be responsible factors controlling diversity of *Acacia* species. Seed dormancy plays a great role in enhancing chances of survival by delaying germination until conditions in the external environment become optimum for germination and survival (Bewley & Black, 1994; Vleeshouwers *et al.*, 1995).

Dormancy of hard coat seeds varies from species to another and the mechanisms could be adversely affecting germination involve interference with water imbibitions and gas exchange, the presence of chemical inhibitors, barriers against the escape of inhibitors and mechanical restraint of the embryo (Bewley and Black, 1982). Among these restriction, water imbibition seems to be the most appeared cause of dormancy in hard coated seeds.

Intact seeds of *Acacia* exhibit very low germination percentages, which may be due to physical seed dormancy. This dormancy cause water impermeable due to hard seed coats which has been reported to cause dormancy and delay germination for many *Acacia* species (Everitt, 1983; Sy *et al.*, 2001; Girase *et al.*, 2002; Venkatesh *et al.*, 2002; Rincon-Rosales *et al.*, 2003). Numerous seed treatments have been used to overcome seed impermeability of seeds including exposure to sulphuric acid, washing, mechanical scarification, cold stratification and wet heating. Some of these seed treatments induce better seed germination via controlling water uptake and increase seed water imbibitions (Moreno-Casasola *et al.*, 1994; Teketay, 1998; Venkatesh *et al.*, 2002; Rincon-Rosales *et al.*, 2003).

Temperatures have been, also, demonstrated as important factor which interact with seed germination and, subsequently, may contribute in controlling seed impermeability and germination (Moreno-Casasola

et al., 1994). A complicating effect is that temperature had a dual role. It regulates the seasonal changes in dormancy and, also, germination (Vleeshouwers *et al.*, 1995). However, temperature requirement of both processes are quite different. Dormancy of many plant species can be overcome at temperature that may never allow germination (Leadem, 1995).

This investigation was performed to study the effect of some seeds treatment and incubation temperatures on germination percentage of *Acacia tortilis* seeds to understand the role of these factors in controlling physical seed dormancy.

MATERIALS AND METHODS

Pods of *Acacia* (*Acacia tortilis* (Forssk.) Hayne) were collected from well developed populations in the eastern province of Saudi Arabia during May, 2003. Collected pods were air dried for two weeks and shattered manually, immature and unfit seeds such those affected by insects were removed. Healthy proper seeds were taken and kept in bags at 4±1 °C. During the first week of September, 2003, stored seeds were exposed to the following seed treatments.

- Control (T1), cleaned seeds without any treatment.
- Washing (T2), where seeds occupying 50 cm³ were placed in 150 ml. beaker covered with cheese cloth and exposed to continuously tap water at 15-20 °C and a flow rate of 5 ml/second for 24 hours.
- Cold stratification (T3) was achieved by maintaining imbibed seeds on germination blotter at 2 °C for four weeks.
- Mechanical scarification was achieved by placing seeds with an amount of 50 cm³ wood sawdust in a scarifier for half (T4) and one minute (T5).
- Wet heating, where seeds were plunged in 600 ml boiling water for one (T6), two (T7) and three (T8) minutes.

- Acid scarification, where seeds were immersed in concentrated sulphuric acid (95%) for 15 (T9), 30 (T10) and 45 minutes (T11) followed by thorough rinsing with running water for 10 minutes.

After treatments application, fifty seeds were sown in 1.0 L plastic air tight disposable container, containing dry washed sand and were covered with 3.0 mm sand. Containers were irrigated with distilled water to field capacity and incubated in a programmed refrigerated incubator on 12 hrs light: 12 hrs dark (2000 L x sylvonic cool white florescent lamps) with four incubation temperatures; namely 5 - 15, 10 - 20, 15 - 25 and 25 - 35 °C (Light - dark). Treatment combinations were replicated six times and arranged as a factorial experiment in a completely randomized design. Germination percentages were recorded every three days for thirty days after sowing. No further germination was observed six days later (36 days). Thereafter, the experiment was terminated. A seed considered germinated when the seedling had emergence from the soil. Germinated seeds were discarded after counting.

Collected data were subjected to the analysis of variance (ANOVA), according to Gomez and Gomez (1984). Treatment means were compared using the Bays Least Significant Difference test (BLSD) according to Waller and Duncan (1969). Computations and statistical analysis were done, using SAS (SAS, 2001).

RESULTS

Data in Fig. 1 and 2 showed that seed germination of *Acacia tortilis* (Forssk.) Hayne was significantly affected by seed treatments and incubation temperatures. Mechanical scarification for one minute (T5) showed the highest seed germination (75.7 %), followed by acid scarification for 15 minutes, T9 (74.9 %) without significant difference. Both of the aforementioned treatments surpassed the

control (T1) with 322.9 and 318.4 %, respectively, in seed germination. The mechanical scarification for 0.5 minute (T4) and acid scarification for 30 minutes (T10) ranked third (68.0 %) and fourth (63.4 %), respectively, and surpassed the control with 279.9 and 254.2 %. Wet heating of seeds for one, two and three minutes (T6, T7 and T8) showed seed germination of 42.0, 57.0 and 59.2 %, surpassing the control (T1) with 134.6, 218.4 and 230.7 %, respectively. The minimum seed germination was appeared with the control (T1) and washing (T2) treatments.

Seed germination was markedly affected by the evaluated incubation temperatures. Germination percentage was significantly higher at 25-35 °C, while the low temperature (5-15 °C) suppressed germination percentage (Fig. 2). Germination appeared after three days at 25-35 °C, while, at 5-15 °C, germination started after six days. The highest germination percentage was reached at day six for all seed treatments at 25-35 °C and day nine, at 15-25 °C, while, at 5-15 °C, a clear difference was detected among seed treatments showing maximum germination at day 21 with acid scarification (Fig. 3).

The interaction between incubation temperatures and seed treatments significantly affected seed germination percentage (Table 1). Mechanical scarification for one minute (T5) at 25 - 35 °C and 15-25 °C showed significantly the highest germination percentage (97.3 and 92.0 %, respectively). Mechanical scarification for one minute (T5) and sulphuric acid for 15 minutes (T9), under incubation temperatures of 10-20 and 15-25 °C, ranked the third in germination percentage (90.0 %). *Acacia tortilis* seeds failed to germinate in the control under the lowest incubation temperature (5-15 °C), while germination percentage under the highest incubation temperature (25-35 °C) reached 34.6 %. The remaining seed treatments gave variable germination percentages ranging between the previous values under the different incubation temperatures (Table 1).

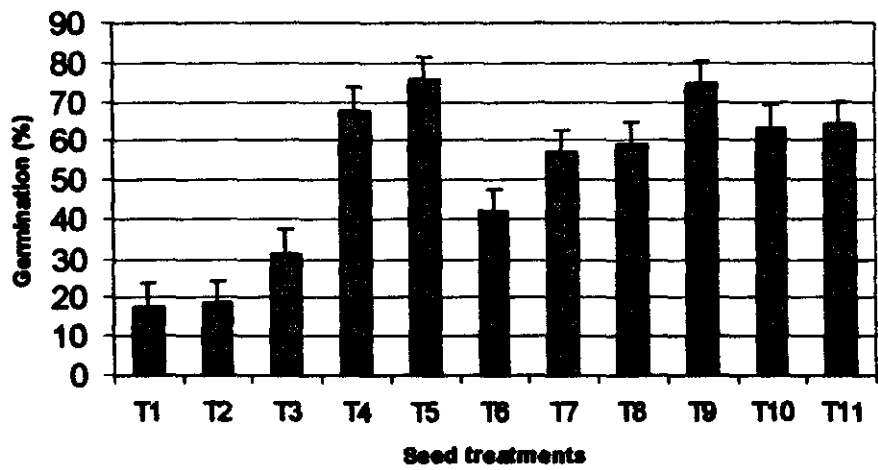


Fig.(1): Seed germination of *Acacia tortilis* as affected by seed treatments. Bars= LSD (5%)

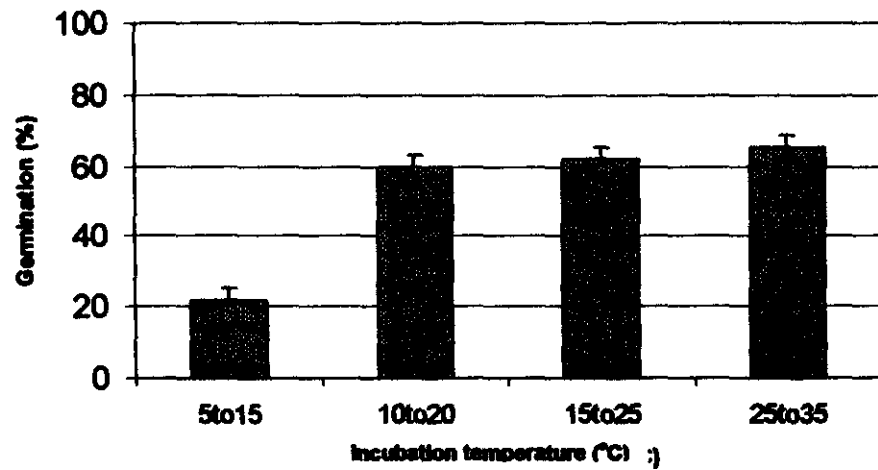


Fig.(2): Seed germination of *Acacia tortilis* as affected by incubation temperatures. Bars= LSD (5%)

Table (1): Germination (%) of *Acacia tortilis* Forsk in response to seed treatments and incubation temperatures.

Seed treatments (A)	Incubation temperature in °C (B)			
	5-15	10-20	15-25	25-35
T1. Control	0.0	9.1	28.2	34.6
T2. Washing (W)	7.3	19.3	22.7	29.3
T3. Stratification (S)	22.7	22.7	36.7	44.0
T4. Mechanical scarification 0.5 min.	18.7	80.0	86.7	86.7
T5. Mechanical scarification 1.0 min.	23.3	90.0	92.0	97.3
T6. Boiling in water 1.0 min.	18.1	56.7	51.3	42.0
T7. Boiling in water 2.0 min	17.3	70.0	64.0	76.7
T8. Boiling in water 3.0 min	26.7	76.0	57.3	76.7
T9. Acid scarification 15 min.	42.7	82.7	90.0	84.0
T10. Acid scarification 30 min.	30.0	72.0	77.3	74.0
T11. Acid scarification 45 min.	33.3	77.3	73.3	73.3
BLSD for A*B (5%)	11.6			

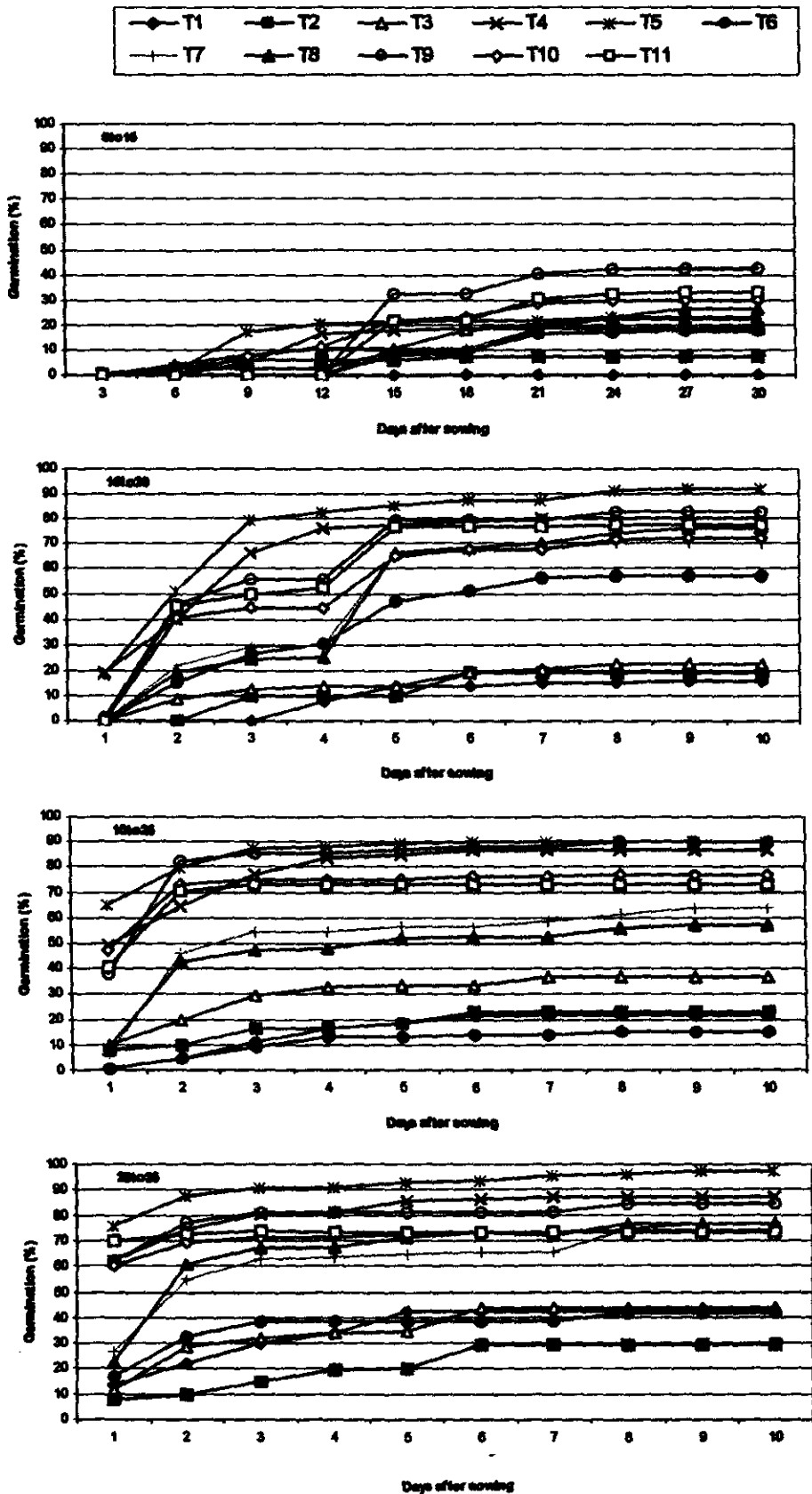


Fig.(3): Seed germination of *Acacia tortilis* Forsk under incubation temperatures of 5 to 15 °C (A), 10 to 20 °C (B), 15 to 25 °C (C) and 25 to 35 °C (D) in response to different seed treatments.

DISCUSSION

The hard seed coat of many legumes species including *A. tortilis* showed physiological seed dormancy which delay germination (Teketay 1998; Sy *et al.*, 2001). Several seed treatments have been used to break physical seed dormancy in *Acacia* species. Washing failed to improve seed germination of *Acacia tortilis*. Similar results were found on seed of other legume species (Sy *et al.*, 2001). It seems that washing for 24 hours was not quite enough to soften the seed coat of *A. tortilis* and thereafter increased their permeability. However, Nasim *et al.* (1996) found that washing seeds of *A. nilotica* in running water for 24 hours increased germination from 40 to 70 %.

Stratification had a very low effect on seed germination of *A. tortilis*. Seeds with physiological dormancy may require cold stratification to overcome dormancy (Baskin and Baskin, 1998). However, exposing seeds with physical seed dormancy to cold stratification did not show effective seed dormancy relief (Horok and Wax, 1991).

Mechanical scarification resulted in higher seed germination percentages, particularly under the higher incubation temperature. Mechanical scarification treatments permits seed coats of legumes to become permeable to water and oxygen and promotes germination (Baskin and Baskin, 1998; Herranz *et al.*, 1998; Girase *et al.*, 2002; Venkatesh *et al.*, 2002).

Wet heating up to three minutes promoted germination which might be due to increasing imbibitions. The efficiency of using wet heating seems to be depend on the duration of boiling treatment. Short period of one minute showed significant increase in seed germinations but to a lesser extent than two or three minutes. However, it is expected that longer periods of wet heating might cause seed death. Similar germination response has been reported in some legumes (Herranz *et al.*, 1998; Rincon-Rosales *et al.*, 2003). Exposure seeds to hot summer of Saudi Arabia although it is always dry season it may be a natural mean of breaking seed dormancy of *A. tortilis*. Mucunguzi and Oryem-Origa (1996) found that dry heat significantly promoted germination of *A. gerrardii* and *A. sieberina*. However, prolonged dry heat treatment of hard seed coat may reduce seed germination (Muccunguzi and Oryem-Origa, 1996; Susko *et al.*, 2001).

It is believed that hot water, which is a form of thermal scarification (Budy *et al.*, 1986), overcome physical dormancy in seeds, causing cracks in the seed coat. These cracks allow water to enter to initiate germination (Baskin and Baskin, 1998). Alternatively, hot water may cause thermal shock to the embryo and leaching inhibitors (Budy *et al.*, 1986).

Acid scarification significantly enhanced seed germination of *A. tortilis*, particularly with increase incubation temperature and the optimum duration was

15 min. Similar germination responses to acid scarification have been reported in other *Acacia* species (Girase *et al.*, 2002; Venkatesh *et al.*, 2002; Rincon-Rosales *et al.*, 2003). It seems that time duration required to overcome dormancy, using sulphuric acid, depends on the hardness of the seed coat (Budy *et al.*, 1986). According to Baskin and Baskin (1998), acid scarification increased seed permeability to water, which promoted germination. However, there are many other effects of acid treatments like heating, build up due to reaction of the acid with rinse water. Budy *et al.* (1986) reported that acid treatment not only increased the intake of water, but also caused hydrolysis of seed tissues, which might promote the induction of seed germination.

The optimum germination temperature for *Acacia tortilis* appeared with raising incubation temperature to 25 – 35 °C. Temperatures of 10 – 20 and 15 – 25 showed moderately higher germination percentage (Fig. 2). The corresponded values were relatively similar to those found for other *Acacia* species (Danthu *et al.*, 1992; Abdulfatih, 1995; Sy *et al.*, 2001; Girase *et al.*, 2002). Temperatures of 10 – 20 to 25 – 35 are prevailing with natural condition at the time of *Acacia* emergence in Saudi Arabia during winter or spring, respectively. High temperature, therefore, seems to improve germination of *Acacia tortilis* under natural conditions, indicating that incubation temperatures partially regulated seed germination in this species.

High temperatures may regulate seed germination in *A. tortilis* through increase water velocity, which may interact with seed permeability showing significant enhancement in seed germination. Alternatively, high temperature has been reported to effect membrane permeability (Salisbury and Ross, 1994). This trend may contribute positively in seed permeability and, consequently, affects both germination percentage and time required to reach maximum germination.

In conclusion, coat seed of *A. tortilis* is probably the primary mechanism restricting germination. Mechanical scarification, wet heating treatment and sulphuric acid, which either scarified or softened the seed coat, were effective in breaking physical seed dormancy of *A. tortilis*. The effectiveness of these seed treatments depend on the duration of treatment exposure. Mechanical scarification for one minute was the most effective method of breaking dormancy and improving germination. Incubation temperature for maximum germination percentage and germination rate was 25-35 °C. Mechanical scarification under laboratory conditions probably reflects condition in arid environment. Seeds can potentially be exposed to mechanical scarification under arid environment due to teeth of grazing animals, severe drought and mechanical damage due to sand movement by wind.

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الملخص العربي

استجابة الإنبات في السم (*Acacia tortilis* (Forssk.) Hayne)
لمعاملات البذور ودرجات حرارة تعريضها

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تم دراسة إنبات بذور السم (*Acacia tortilis*) كاستجابة لتأثير بعض معاملات البذور ودرجات حرارة تعريضها تحت ظروف حاضنات متحكم بها. استخدم للتأثير الدراسة تجربة عاملية في تصميم تام العشوائية وكررت كل معاملة ست مرات. أُنشئت نتائج الدراسة أن معاملات البذور أثرت معنوياً على نسبة إنبات بذور السم وأن الخشخيش الميكانيكي لمدة دقيقة واحدة والخشخيش بحمض الكبريتيك المركز لمدة ١٥ دقيقة سجلت أعلى نسبة إنبات، وعلى عكس ذلك ظهرت أقل نسب إنبات مع معاملة المقارنة ومعاملة الغسيل بالماء بدون فرق معنوي بينهما. أثرت درجة حرارة تعريض البذور معنوياً على نسبة الإنبات وكانت أعلى نسبة عندما حُضنت البذور على درجة حرارة ٢٥-٣٥ درجة مئوية، في حين ظهرت أقل نسبة إنبات بتعريض البذور على درجة حرارة ٥-١٥ درجة مئوية. كما أُنشئ التفاعل بين معاملات البذور ودرجة حرارة التعريض معنوياً على نسبة الإنبات، حيث أُنشئت معاملة الخشخيش الميكانيكي لمدة دقيقة واحدة وتعريض البذور على درجة حرارة ٢٥-٣٥ درجة مئوية إلى ظهور أعلى نسبة إنبات لبذور السم تحت ظروف هذه الدراسة.