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Germination dynamics of *Acacia* species under different seed treatments

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Abstract
Establishment of *Acacia* is typically inhibited by hard seed coat. This study was conducted to evaluate effective seed stratification methods for fourteen *Acacia* species, using water (control), boiling water, sulphuric acid and mechanical scarification techniques. Highest germination was observed with sulphuric acid treatment. Average non-germinated seeds were 95% in the control. The highest germination percentage was recorded in *A. victoriae, A. deanii ssp deanii, A. deanii ssp paucijuga, A. farnesiana, A. karroo, A. pruinocarpa, A. saligna* under sulphuric acid treatment, *A. ligulata, A. aneura, A. blakei, A. estrophiolata* when using hot water, *A. kempeana, A. pendula, A. sparsiflora* using mechanical scarification. These results indicate the importance of proper seed stratification in *Acacia* species.

Key words: Germination index, Semi-arid, Seed size, Seed coat dormancy, Stratification technique

Introduction
Species of the genus *Acacia* are widely distributed in arid and semi-arid regions throughout the world (Aref *et al.,* 2003). One trait common among *Acacia* is the production of highly palatable leaves and pods that provide an important resource for human populations such as, food, fuel, medicinal products and wood. It has high nutritional value and used as feed for livestock (Graham and Vance, 2003). Additionally, *Acacia* has important ecological influences by improving soil characteristics and increasing soil fertility (Pearson and Vitousek, 2001), reducing soil erosion, moderating sub-canopy microclimate conditions, contributing to afforestation, and creating natural windbreaks.

Seeds of several *Acacia* species exhibit poor germination or experience delayed germination due to thick, hard, or wax-covered seed coats that inhibit water imbibition and oxygen uptake by the developing embryo (Grubb and Coomes, 1997). Omari (1992) indicated that the mechanical resistance of the seed coat is the dominant form of dormancy in this genus. To break seed dormancy or enhance germination, *Acacia* seeds often require pre-treatment stratification (Teketay, 1998; Warrag and Eltigani, 2005). Numerous techniques have been used to increase seed permeability. During the 19th century in Australia, seeds were submersed in boiling or hot water which increased the permeability of the seed coat (Teketay, 1998). In other locations, sulphuric acid has been used to weaken the outer coating with promising results (Baskin and Baskin, 1998). Mechanical scarification has been used to overcome seed coat imposed dormancy (Teketay, 1998). According to Auld (1986), germination of *Acacia suaveolens* was greater when scarification was used to reduce seed coat-linked innate dormancy. Earlier studies have found that seed size and age can also influence *Acacia* dormancy (Pathak *et al.,* 1980).

The purposes of this study were 1) to evaluate several seed stratification treatments and the time required for maximum germination in fourteen different *Acacia* species, 2) to test the germination dynamics over time comparing different seed treatments, and 3) to relate dormancy and germination to seed size.

Material and Methods
Study area: The study was conducted in the laboratory of the International Centre for Agricultural Research in the Dry Areas (ICARDA) research facility located in Tel Hadya, north-west Syria (36^{o}01'15.61" N, 36^{o}57'20.23").

Germination tests were conducted on fourteen *Acacia* species: *Acacia aneura* F. Muell. ex Benth, *Acacia blakei* Pedley, *Acacia deanei* subsp. *deanei* (R. T. Baker) M. B. Welch *et al., Acacia deanei* subsp. *paucijuga* (F.Muell. ex N.A.Wakef.) Tindale, *Acacia estrophiolata* F. Muell, *Acacia kempeana* F. Muell, *Acacia pendula* A. Cunn. ex

G. Don, *Acacia pruinocarpa* Tindale, *Acacia saligna* (Labill.) H. L. Wendl, *Acacia sparsiflora* Maiden, *Acacia farnesiana* (L.) Willd, *Acacia karroo* Hayne, *Acacia ligulata* A. Cunn. ex Benth, and *Acacia victoriae* Benth. The seed were acquired from a commercial Australian seed company "NINDETHANA" in September 2008. Upon arrival to ICARDA's headquarters the seed were stored in the freezer at –18°C.

Treatments applied to each seed included 1) water (control), 2) mechanical scarification using sand paper (Sand paper 212, P80, Aluminium oxide), 3) soaking in boiling water for ten minutes, and 4) soaking in concentrated sulphuric acid (H_2SO_4) for one hour followed by a rinse with tap water. All seeds were initially placed in pleated filter paper, put in plastic boxes with transparent covers for light exposure, irrigated with distilled water every other day (10-15 ml per tray with 25 seeds), and kept in the incubator at 20±2°C for a light/dark regime of 16:8 hours a day. The seeds remained in the incubator for 21 days. Results for each treatment were reported as germinated or failed to germinate. All germination testing has been conducted according to rules established by the International Seed Testing Association (ISTA) (Anonymous, 2007).

The experiment was conducted using a completely randomized block design with three replications per block. Each experimental unit (polyethylene bag) contained 25 seeds of one species. The number of seeds germinated was recorded on three separate occasions: 7, 14, and 21 days following the start of each trial period (between 31 January to 21 February 2010). Seed were classified into three categories based on the weight of 100 seeds: large (>3 gm/100 seeds), medium (2-3 gm/100 seeds and small (<2 gm/100 seeds).

Parameters used to express the power of germination were computed with Germination Index (GI), calculated as described by the Association of Official Seed Analysts (AOSA, 1983). The following formula was used to calculate the GI.

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + \frac{\text{No. of germinated seeds}}{\text{Days of the second count}} + \cdots + \frac{\text{No. of germinated seeds}}{\text{Days of the final count}}$$

The predicted number of days required to reach 70% and 90% germination were also calculated for each of the three sample dates using a generalized linear model with binomial errors and a logit link that was fitted to the number of seeds germinated. Percent germination for each species and seed treatment combination were estimated using Genstat (Payne *et al.,* 2009). The analysis of deviance resulting from the model fitting gave an overall assessment of main effects of species and seed-treatments, and their interaction in terms of the *P*-value. For each combination of a species and date, the seed-treatment giving highest estimated germination was identified for studying the dynamics of the germination over time. In order to describe the dynamics of seed germination, we fitted linear and quadratic regressions of seed-germination on time, each passing through origin. Better of the two models was selected on the basis of higher percentage variance accounted for seed germination. Using the selected model, we estimated the time required to achieve a given percent (90%) of the observed potential germination. The differences in the time to germination will characterize the species for their ability to germinate or the germination dynamics.

The germination potential of the species was measured as the highest germination over the seed-treatment on the 21st day (final observation) and its association with size of the seed was evaluated using a correlation between them.

Results and Discussion

Results indicate that plant genotype and pre-sowing treatments can have variable effects on seed germination for the fourteen different species of *Acacia* examined. The effect of the four treatments on the germination of each of the fourteen *Acacia* species following 7, 14 and 21 days is presented in Table 1 and their association with time is exhibited in Fig 1. As can be expected, the lowest germination was observed in the control while the highest germination of 97% was observed for *A. victoriae* at 21 days under H_2SO_4. In the control treatment, the average percentage of non-germinated seeds from the fourteen species was 95% ranging from 80 to 100%. Over the three week period, noticeably high germination was observed for *A. victoriae* in the range 87 – 97% under H_2SO_4 and 81-96% under boiling water, and for *A. farnesiana* in the range 87 – 92% under H_2SO_4. The species with lowest germination were *A. blakei* under H_2SO_4 (1- 11%), *A. farnesiana* and *A. karroo* under boiling water (up to 1%) and *A. karroo* (no germination) and *A. deanii ssp paucijuga* (up to 3%) under scarification.

The earliest germination was observed in the sulphuric acid pre-sowing treatment except for *Acacia blakei* and the most delayed germination was observed in the control treatment (Fig. 1). Seed germination started during the first week after sowing in all fourteen species tested under H_2SO_4 treatment compared to thirteen species under the scarification treatment, ten species under the boiling water treatment, and only four species under the control treatment. Significant differences (*P*<0.001) were observed between species and treatments over each of the three observation dates (data on analysis of deviance not shown). At the end of the first week, the highest germination percentage recorded involved H_2SO_4 treatments for *A. victoriae* (87%) and *A. farnesiana* (87%). *Acacia victoriae* had 81% germination under the boiling water treatment. The same results were observed after 14 and 21 days as *A. victoriae* recorded the highest germination percentage. In contrast, boiling water had no effect on *A. farnesiana* and *A. karroo*. The delay in seed germination of certain species may reflect slow imbibitions resulting from inherent properties of the seed, in particular low permeability of the seed coat.

The germination index for the species under the four treatments over the three week period has been tabulated in Table 2. The highest germination index calculated for the entire period was for *A. victoriae* under H_2SO_4 followed by *A. farnesiana* (GI = 13.11 and 12.76, respectively). *A. victoriae* also had the highest germination index under boiling water while, *A. ligulata* had the highest germination index under the scarification treatment (Table 2). Thus the percent germination (Table 1) was found consistent with the germination index (Table 2).

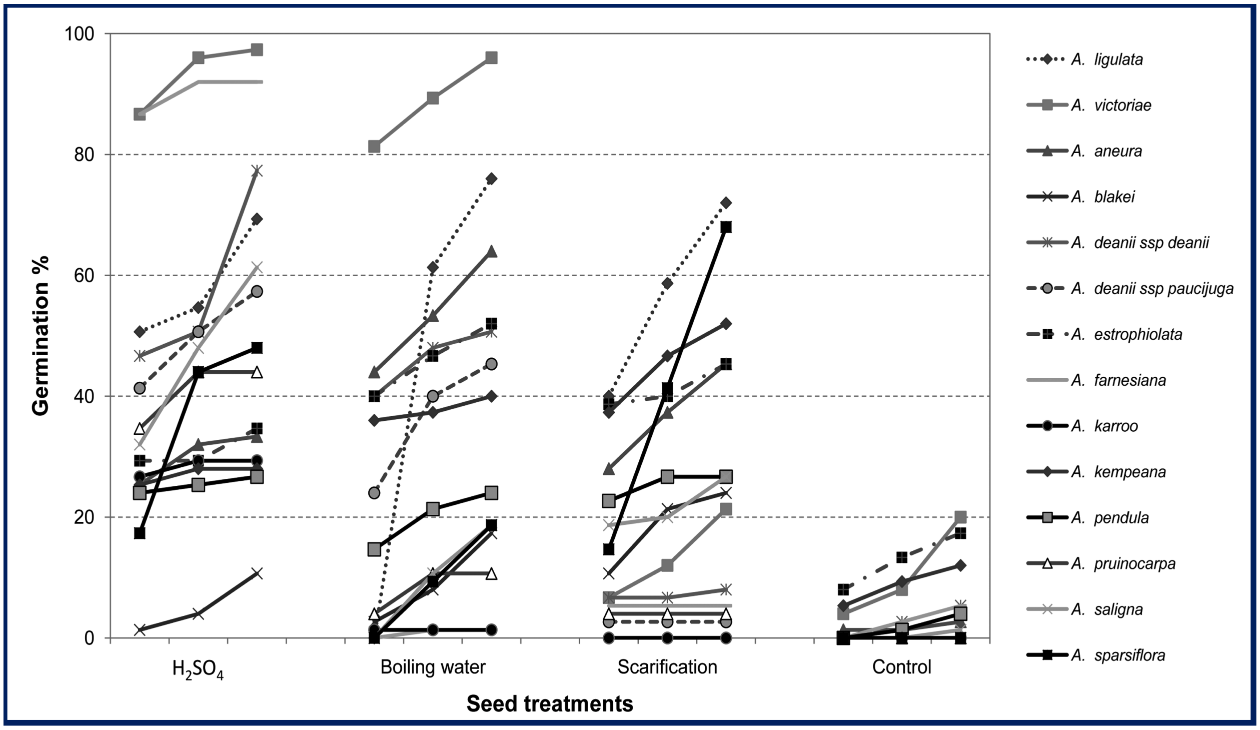

Fig. 1. Accumulated germination percentage for 14 *Acacia* species under different seed treatments and three observations

When using H_2SO_4 treatment, the seed-coat and embryo might have been damaged for four of the species tested (*A. aneura*, *A. blakei*, *A. estrophiolata* and *A. kempeana).* Similar results were obtained by Ghorbanli *et al.,* (2001) who applied different concentrations of sodium chloride to *Acacia* seed and other tree and shrub species for enhanced seed germination. When no treatments were used, the highest germination occurred in *A.victoriae* (20%) followed by *A. estrophiolata* (17%). Thus, the hard coat of these two species does not appear to inhibit germination rates. In contrast with the recommendation of Tadros *et al.,* (2011) boiling water had a negative effect on *A. farnesiana* which might be due to the differences in the water temperature and the period of soaking.

Low germination percentages in *A. blakei* and *A. karroo*

Table 1. Germination % in 14 *Acacia* species in four seeds treatments after 7, 14 and 21 days

Species	H_2SO_4 Days			Boiling water Days			Scarification Days			Control Days		
	7	**14**	**21**	**7**	**14**	**21**	**7**	**14**	**21**	**7**	**14**	**21**
A.ligulata	51	55	69	00	61	76	40	59	72	00	00	00
A.victoriae	87	96	97	81	89	96	07	12	21	04	08	20
A. aneura	25	32	33	44	53	64	28	37	45	01	01	03
A.blakei	01	04	11	2.7	08	17	11	21	24	00	00	01
A.deanii ssp deanii	47	51	77	40	48	51	07	07	08	00	03	05
A.deanii ssp paucijuga	41	51	57	24	40	45	03	03	03	00	00	00
A. estrophiolata	29	29	35	40	47	52	39	40	45	08	13	17
A.farnesiana	87	92	92	00	01	01	05	05	05	00	03	05
A.karroo	27	29	29	01	01	01	00	00	00	00	00	00
A.kempeana	25	28	28	36	37	40	37	47	52	05	09	12
A.pendula	24	25	27	15	21	24	23	27	27	00	01	04
A.pruinocarpa	35	44	44	04	11	11	04	04	04	00	00	00
A.saligna	32	48	61	00	11	19	19	20	27	00	00	01
A.sparsiflora	17	44	48	00	09	19	15	41	68	00	00	00
Mean	38	45	51	21	31	37	17	23	29	01	03	05

Table 2. Germination Index for different *Acacia* species under different seed treatments

Species	H_2SO_4	Boiling water	Scarification	Control
A. ligulata	8.22	5.08	7.68	0.00
A. victoriae	13.11	12.51	1.78	1.43
A. aneura	4.16	7.46	5.05	0.25
A. blakei	0.70	1.21	2.41	0.06
A. deanii ssp deanii	8.22	6.41	1.02	0.32
A. deanii ssp paucijuga	6.89	4.83	0.38	0.00
A. estrophiolata	4.44	6.44	5.87	1.71
A. farnesiana	12.76	0.10	0.76	0.32
A. karroo	4.00	0.19	0.00	0.00
A. kempeana	3.81	5.37	6.25	1.18
A. pendula	3.59	2.70	3.52	0.22
A. pruinocarpa	5.62	1.05	0.57	0.00
A. saligna	6.35	1.14	3.08	0.06
A. sparsiflora	4.57	1.11	5.27	0.00
Mean	6.17	3.97	3.12	0.40

indicate that seed treatments did not have a significant effect on germination where maximum percent germination was 24 and 29, respectively. Further experimentation on these species is needed to identify alternative treatments that could increase germination response.

The correlation between percent germination and seed weight was statistically significant and highest for H_2SO_4 treatment at 7 days (correlation = 0.63, $P<0.05$). There was significant negative correlation for scarification at 21 days (correlation = -0.58, $P<0.05$) (data not shown). Correlations, negative in magnitude, were not significant for boiling water. Thus it may be argued that the high germination under H_2SO_4 is due to species with large seed weight while under scarification and boiling water due to species with small seed weight (Table 3).

The slopes of the linear regression of percent germination with time (b) and the estimated number of days for getting 90% of the potential or the maximum germination observed is given in Table 3. The slope which measures the increase in germination over one week was highest (40.8%) for *A. victoriae* and lowest (9%) for *A. blakei.* The large seeded species and *A. pendula* (in the medium group) reached within 15 days (earliest) 90% of their potential germination, while the small seeded *A. sparsiflora* took the longest (20 days). The time taken to reach 90% of potential germination was in the range 15-18 days for the four medium seeded species and in the range 16-20 days for the small seeded ones. Considering the species, there is a significant negative trend in the time of germination with seed size (correlation = -0.621, $P<0.05$).

Conclusion

This study found that each of fourteen *Acacia* species required a stratification treatment to break hardened seed coats and to increase the rate of germination. *Acacia victoriae* had the highest germination percentage regardless of the seed treatment applied. Low germination percentages in *A. blakei* and *A. karroo* indicate that these four seed treatments did not have a significant effect on germination where maximum percent germination was 24 and 29, respectively. These species may require different treatment schedules. Further experimentation on these species is needed to identify alternative treatments that could increase germination response. Germination percentage in relation to the seed weight revealed that the largest seed size responded better to the H_2SO_4 treatments while the medium and small seed weight responded better to the boiling water and scarification treatments. The results can help researchers select optimal seed treatment types for different *Acacia* species, enhancing restoration efforts and farming practices in arid and semi-arid environments.

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Table 3. Species seed weight, slope of the regression line through origin and estimated number of days for getting 90% of the maximum germination observed under the best treatment

Species	Best treatment	Seed category	Weight 100 seeds (g)	Slope (b) of the linear regression	90% of observed maximum germination	Estimated days for 90% of observed maximum germination
A. farnesiana	H_2SO_4	Large	9.04	39.1	82	15
A. karroo	H_2SO_4	Large	5.01	12.4	26	15
A. pruinocarpa	H_2SO_4	Large	4.36	18.2	39	15
A. victoriae	H_2SO_4	Large	3.90	40.8	87	15
A. pendula	H_2SO_4	Medium	3.04	11.1	24	15
A. estrophiolata	Hot water	Medium	2.42	20.7	46	16
A. deanii ssp paucijuga	H_2SO_4	Medium	2.40	22.5	51	16
A. deanii ssp deanii	H_2SO_4	Medium	2.03	27.1	69	18
A. saligna	H_2SO_4	Small	1.71	22.3	55	17
A. kempeana	Scarification	Small	1.41	20.5	46	16
A. aneura	Hot water	Small	1.28	24.5	57	16
A. ligulata	Hot water	Small	1.15	25.1	68	19
A. sparsiflora	Scarification	Small	0.83	21.5	61	20
A. blakei	Scarification	Small	0.55	9.0	21	17

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