Maximizing seed germination in five species of the genus Acacia (Fabaceae)

Abdenour Kheloufi[1]✉, Faiza Zineb Boukhatem[2], Lahouaria Mounia Mansouri[1], Mohammed Djelilate[3]

[1]Department of Ecology and Environment, University of Batna2, Batna, Algeria
[2]Department of Biotechnology, University of Oran, Oran, Algeria
[3]Department of Biology, University of Relizane, Relizane, Algeria

✉ abdenour.kheloufi@yahoo.fr

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Abstract

Seeds of many tree-species possess a hard seed coat which is impervious to water. These seeds often take a long time to germinate, resulting in heterogeneity and a delay in seedlings development which is an inconvenience for reforestation success. The aim of the present work was to determine the possibilities to improve the germination of five leguminous trees of the genus *Acacia* that have been recorded in the arid and the desert region of Algeria using sulphuric acid. A duration of 30 min of immersion in sulphuric acid improved the seed germination up to 97.5% and 99% for *A. albida* and *A. laeta*, respectively. Increasing the time of immersion (from 30 to 90 min) improved the germination percentages for *A. ehrenbergiana* and *A. seyal* seeds to 92.5% and 93.7%, respectively. Increasing this duration to 120 min had a positive effect on *A. tortilis* seed germination, improving the final germination rate up to 97%. Understanding of seed germination requirements is very important for regeneration and successful tree establishment in forest nurseries as well as for direct plantation in arid and semi-arid lands.

Keywords

Acacia; Germination; Seed coat; Sulphuric acid; Reforestation; Trees

Contents

1 Introduction

Germination is the first stage of seedling growth and is one of the most vulnerable stages for the establishment of any species (Moles and Westoby 2006). The hardness of the seed coat then imposes on the seed a physical dormancy. It is an ecological mechanism that allows the induction of germination only under favorable conditions to ensure the survival of seedlings (Venier et al. 2012). This generally applies to trees and forest shrubs of the leguminous family (Gill and Beardall 2001; Kheloufi et al. 2018a). So, seeds require specific pretreatment before sowing to obtain fast and high germination rate (Burrows et al. 2009). Several researches have been done to develop effective artificial treatments to break dormancy and to ensure that the seeds germinate quickly. According to Kheloufi et al. (2017), the intensity of dormancy for the same species may vary depending on the genotype and the environment in which the seeds are produced.

Treatments such as cold stratification, mechanical scarification, hot water or sulphuric acid treatment are widely used because they can improve the seed germination rate in a relatively short period of time (Azad et al. 2010; Kheloufi et al. 2017). The effectiveness of scarification with sulphuric acid to overcome the impermeability of the seed coat and increase seed germination has been reported for different species. However, the effectiveness of this treatment varies with acid concentration, plant species and duration of treatment (Kheloufi 2017). Indeed, the duration of immersion must be determined to enhance the best time required to increase the chances of breaking seed coat dormancy. In this study, we evaluated seed germination kinetics of five species of the genus Acacia that were recorded in the arid and desert regions of Algeria in a recent inventory established by Kheloufi et al. (2018b) (*A. albida*, *A. ehrenbergiana*, *A. laeta*, *A. seyal* and *A. tortilis*). The acacia genus belongs to the Fabaceae family. The main advantage of these species is the ability to make symbiosis with soil microorganisms (rhizobium and mycorrhizae) conferring them the capacity to survive in grounds very poor in nutritional elements (Bashan et al. 2012; Boukhatem et al. 2016). The evaluation and improvement of germination took place under pretreatments operated by concentrated sulphuric acid at different times of immersion. The positive response of these seeds to these pretreatments is crucial for a better and faster regeneration for integration into a reforestation program to ensure perennial and dense tree species in the arid and semi-arid regions where there is a significant decline in vegetal cover.

2 Material and methods

2.1 Seed sources and collection

Seed provenances of the five acacia species of this study (*A. albida*, *A. ehrenbergiana*, *A. laeta*, *A. saligna*, *A. seyal* and *A. tortilis*) are shown in Table 1. The experiment was conducted at the Laboratory of the Department of Ecology and Environment at the University of Batna 2 (Algeria). The mature pods were harvested from five trees for each species of *Acacia* Mill. Pods already dried naturally were crushed manually to release the seeds. After harvest, the seeds were mixed to minimize inter-genetic variation. Once dried, the seeds were stored in glass containers at a temperature of 4°C for 2 months (simulation of the vernalization period). The seed sample intended for our experiment was obtained by mixing the seeds and removing impurities such as vegetable matter (remains of seed coat, stems, and broken cotyledons), animals (dead insects) or minerals (sand, gravel).

2.2 Experimental design and treatments

Seeds of every species underwent several pretreatment durations consisting of an immersion into sulphuric acid (98%) at various durations (30, 60, 90 and 120 minutes) followed by a good soaking in distilled water. For control, seeds were not treated. It was conducted to be able to compare the effect of no pretreatment on germination. The sowing (4 replicates of 25 seeds × 5 treatments × 5 species) was realized in Petri dishes of 10 cm diameter, papered with two layers of Whatman filter paper and soaked with 20 ml of distilled water and then placed in the obscurity at the laboratory temperature (25 ± 2°C) during 18 days of incubation. The Petri dishes were arranged and moistened every two days, according to a randomized design to eliminate any effect of the position in the seed culture room (Kheloufi et al. 2017). The counts of germinated seeds were done daily from day 5 to day 18 of incubation and were expressed as a percentage. The criterion of germination was 2 mm radicle protrusion.

In the germination tests, final germination percentage (FGP), mean germination time (MGT) and germination rate index (GRI) for each species and pretreatment were calculated using the following procedures and formulas:

$$\text{FGP (\%)} = \frac{\sum ni}{N} \times 100$$

where FGP is the final germination percentage, ni is the number of germinated seeds at final day of test, and N is the total number of incubated seeds per test (Côme 1970).

$$\text{MGT (jours)} = \frac{\sum (ti.ni)}{\sum ni}$$

where MGT is the mean germination time, *ti* is the number of days from beginning of the test, *ni* is the number of germinated seeds recorded at time *t(i)*, and Σ*ni* is the total number of germinated seeds (Orchard 1977).

$$\text{GRI (\%)} = \sum \frac{\text{Number of germinated seeds}}{\text{Number of days}}$$

where GRI is the germination rate index. It is calculated according to Maguire (1962).

The effects of pretreatments on the three variables were tested by analysis of variance (ANOVA). Differences between treatments after ANOVAs were carried out through mean comparison contrasts. Multiple comparisons of means were performed with Duncan's test (α = 0.05). The Pearson correlation coefficient was also calculated for the three variables studied ($p \leq 0.05$). All statistical methods were performed using were calculated using SAS Version 9.0 (Statistical Analysis System) (2002) software.

Table 1. Provenances of acacia seeds in Algeria.

Species	Regions	Geographic coordinates	Elevation (m)
A. albida	Tindouf	28°45'00''N ; 8°40'00''W	399
A. ehrenbergiana	Tamanrasset	22°35'54.64"N ; 5°23'36.73"E	1200
A. laeta	Tamanrasset	22°35'54.64"N ; 5°23'36.73"E	1170
A. seyal	Biskra	34°44'29.14"N ; 5°53'30.14"E	51
A. tortilis	Tamanrasset	22°46'57.09"N ; 5°34'35.00"E	1409

3 Result and discussion

The effect of pretreatment with sulphuric acid on the kinetics of germination of the different species of Acacia during 18 days is illustrated in Figure 1. Seeds exhibit variable behaviors with different durations of immersion in concentrated sulphuric acid.

Indeed, the treatment affects very significantly ($P < 0.0001$) the evolution of germination over time (Table 2). The results obtained show the influence of the treatment which plays a very important role in the induction of the germinative activity. According to the same table, the factors: treatment (TRT), species (SP) and time (T) as well as their correlation (T×TRT×SP) very significantly ($P < 0.0001$) affect the kinetics of germination.

Table 2. Variance analysis for the traits investigated of five acacia species in response to different durations of sulphuric acid pretreatment and after 18 days of sowing.

Parameters	Sources of variables	Degree of freedom	Sum of squares	Mean square	F of Fisher	*P*
FGP	TRT	4	100138,68	25034,67	901,01	< 0,0001
	SP	4	13465,80	1496,20	53,85	< 0,0001
	TRT × SP	16	129393,12	3594,25	129,36	< 0,0001
MGT	TRT	4	89,84	22,46	181,47	< 0,0001
	SP	4	19,62	2,18	17,62	< 0,0001
	TRT × SP	11	136,57	4,40	35,59	< 0,0001
GRI	TRT	4	99454,20	24863,55	889,33	< 0,0001
	SP	4	14010,64	1556,73	55,68	< 0,0001
	TRT × SP	16	128526,07	3570,16	127,70	< 0,0001
Kinetics of Germination	**Generalized Linear Model (GLM) Repeated Measures Analysis of Variance**					
	Between Subjects Effects					
	TRT	4	1299535,00	324883,75	889,33	< 0,0001
	SP	4	183072,44	20341,38	55,68	< 0,0001
	TRT × SP	16	1679407,42	46650,20	127,70	< 0,0001
	Within Subjects Effects					
	T	14	516910,81	36922,20	7357,09	< 0,0001
	T × TRT	56	95726,11	1709,39	340,61	< 0,0001
	T × SP	56	14757,97	117,12	23,34	< 0,0001
	T × TRT × SP	224	131059,25	260,03	51,82	< 0,0001

FGP (final germination percentage), MGT (mean germination time), GRI (germination rate index), TRT (treatment), SP (species), T (time).

The results illustrated in Figure 1 indicate that in the control, the imbibition with distilled water has no positive action on the initiation of germination in *A. tortilis*, and this throughout the incubation period. However, the seeds of *A. albida*, *A. ehrenbergiana*, *A. laeta* and *A. seyal* have germinated without pretreatment but the germination rate remains below 50% (Figure 1, Table 3).

The Figure 1, representing the dynamics of the germination rates as a function of the increasing duration of the immersion in concentrated sulphuric acid (0 to 120 minutes) shows three phases, a first phase of latency, due to the imbibition, a second exponential phase where there is an acceleration of germination followed by a stationary phase. In the seed lot treated at 30 min of acid immersion, the latency and the exponential phase are spread until the 9th day for the majority of the species except for the seeds of *A. tortilis* that continue until the 13th day. On the other hand, in the batches treated at 120 min, the stationary phase starts on average at the 6th day for all species. The beginning of the stationary phase is proportional to the duration of the pretreatment (T×TRT; $P < 0.0001$) (Table 2).

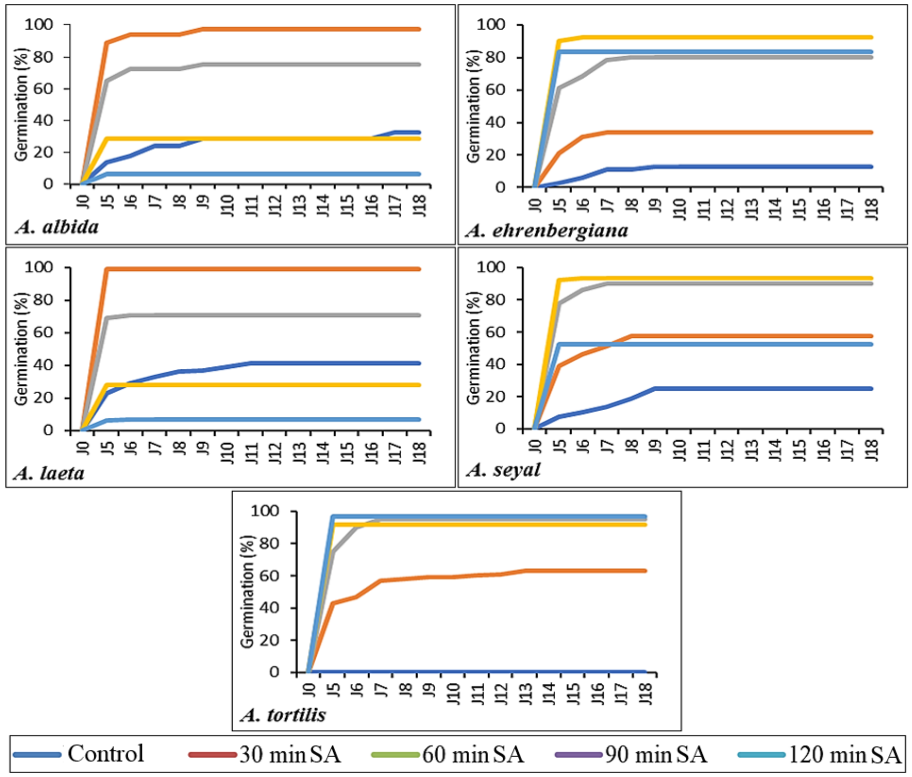

Figure 1. Evolution of the seed germination rate of different Acacia species according to the treatment with concentrated sulphuric acid at different immersion times for a period of 18 days of incubation.

Based on ANOVA and Duncan test results, highly significant differences ($P <$ 0,0001) were found between species and between pretreatments, resulting in a highly significant interaction (TRT×SP) (Table 2). For the different species, the FGP gave significant differences after treatment (control, sulphuric acid).

The overall mean germination for a very short immersion of 30 minutes in sulphuric acid was greater than 95%, indicating that it is the most effective treatment in *A. albida* and *A. laeta*, with respective FGP values of 97.5% and 99% (Table 3). A higher pretreatment time for these two species appears to be fatal for the embryo and a clear and progressive reduction of FGP and GRI was observed from 60 minutes of treatment. It has been observed that many seeds lose their viability, indicating that a long exposure to concentrated sulphuric acid has been in contact with the embryos (Teketay 1996). This is due to the seed coat, which usually regulate the absorption of water, have been damaged (Kheloufi 2017).

A pretreatment time of 90 minutes was the perfect treatment for the seeds of *A. ehrenbergiana* and *A. seyal*. These seeds required more time of immersion in order to achieve a maximum and considerable germination percentage of 92.5% and 93.7%, respectively. *A. tortilis* is the only species that required a longer immersion time (120 min) in the acid without it being harmful for its embryo by indicating a very considerable value of 97% (Table 3). This large variation in treatment responses indicates considerable differences between species in the seed coat structure as a protective barrier.

Table 3. Final germination percentage (FGP), mean germination time (MGT) and germination rate index (GRI) for five acacia species exposed to different pre-sowing treatments (time of immersion in sulphuric acid). For each species, the same alphabet along the column indicates no significance difference (Duncan Multiple Range Test) (n = 4 × 25 seeds).

Species	Treatment	FGP (%)	MGT (days)	GRI (%)
A. albida	Untreated	32,5 ± 2,88^{c}	7,47 ± 0,89^{a}	26,7 ± 2,33^{c}
	30 min	97,5 ± 2,88^{a}	5,20 ± 0,10^{b}	96,1 ± 2,54^{a}
	60 min	75,0 ± 5,77^{b}	5,23 ± 0,16^{b}	73,7 ± 6,03^{b}
	90 min	28,7 ± 4,78^{c}	5,00 ± 0,00^{b}	28,7 ± 4,78^{c}
	120 min	6,25 ± 2,50^{d}	5,00 ± 0,00^{b}	6,25 ± 2,50^{d}
A. ehrenbergiana	Untreated	12,5 ± 2,88^{d}	6,50 ± 0,40^{a}	11,2 ± 2,61^{d}
	30 min	33,7 ± 4,78^{c}	5,45 ± 0,19^{b}	32,7 ± 4,92^{c}
	60 min	80,0 ± 4,08^{b}	5,39 ± 0,03^{b}	77,8 ± 4,08^{b}
	90 min	92,5 ± 5,00^{a}	5,02 ± 0,05^{c}	92,3 ± 5,13^{b}
	120 min	83,7 ± 7,50^{b}	5,00 ± 0,00^{c}	83,7 ± 7,50^{d}
A. laeta	Untreated	41,0 ± 8,24^{c}	6,17 ± 0,48^{a}	37,5 ± 7,40^{c}
	30 min	99,0 ± 2,00^{a}	5,00 ± 0,00^{b}	99,0 ± 2,00^{a}
	60 min	71,0 ± 3,82^{b}	5,02 ± 0,05^{b}	70,8 ± 3,98^{b}
	90 min	28,0 ± 10,3^{d}	5,00 ± 0,00^{b}	28,0 ± 10,3^{d}
	120 min	7,00 ± 3,82^{e}	5,25 ± 0,50^{b}	6,92 ± 3,90^{e}
A. seyal	Untreated	25,0 ± 4,08^{c}	6,93 ± 0,54^{a}	21,4 ± 2,64^{c}
	30 min	57,5 ± 12,5^{b}	5,60 ± 0,33^{b}	54,9 ± 11,7^{b}
	60 min	90,0 ± 4,08^{a}	5,18 ± 0,05^{cb}	88,8 ± 4,09^{a}
	90 min	93,7 ± 4,78^{a}	5,01 ± 0,02^{c}	93,6 ± 4,88^{a}
	120 min	52,5 ± 9,57^{b}	5,00 ± 0,00^{c}	52,5 ± 9,57^{b}
A. tortilis	Untreated	0,00 ± 0,00^{c}	--	0,00 ± 0,00^{c}
	30 min	63,0 ± 5,03^{b}	5,95 ± 0,63^{a}	58,7 ± 5,54^{b}
	60 min	92,0 ± 3,82^{a}	5,26 ± 0,06^{b}	92,0 ± 3,41^{a}
	90 min	95,0 ± 3,26^{a}	5,00 ± 0,00^{b}	93,2 ± 3,26^{a}
	120 min	97,0 ± 3,82^{a}	5,00 ± 0,00^{b}	97,0 ± 3,82^{a}

The MGT varies significantly between the different species ($P < 0.0001$) and pretreatments ($P < 0.0001$) (Table 3). The reduction of MGT in acid treated seeds implies that the dormancy period in these seeds has been reduced due to pretreatment. It is reported that sulphuric acid treatment is an effective method to improve seed germination of species with a hard and impermeable seed coat (Nasr et al. 2013). It stimulates a fast and uniform germination of the seeds.

According to our results, the seed germination depends on the growth potential of the embryo. This potential depends especially on the structure of the seed surrounding the embryo (endosperm, pericarp, and glume) (Germanà et al. 2014). Other factors such as hormones and environmental factors also affect the development of the embryo (Shu et al. 2016). Seed dormancy is determined by several factors such as dehydration, oxygen content, extreme temperatures and the pH of the growth media. Several studies have shown that wet scarification (acid or hot water) and dry scarification (mechanical abrasion) applied to seeds with very hard seed coat have allowed imbibition and improved respiration in the seed (Chen et al. 2007). These conditions are necessary for a good shoot production. The next step is determined by the internal qualities of the seeds, including the metabolism, the content of certain growth regulators and the presence of certain germination-inhibiting substances like Abscisic acid (Teketey 1996).

It is possible that poor germination observed in untreated seeds may have been partly attributed to reduced pretreatment severity, interpreted by a short time of immersion in the acid. De plus, l'imperméabilité des graines à l'eau et au gaz a été attribuée à des obstacles physiques et biochimiques du tégument (Bewley 1997; Allen et al. 2007). According to the results obtained, the scarification treatments with sulphuric acid (98%) were effective, which caused the breaking of dormancy and the induction of seed germination of all species studied.

Table 4. Pearson correlation between final germination percentage (FGP), mean germination time (GMT) and germination rate index (GRI) for different acacia species exposed to different pretreatment durations in sulphuric acid (TRT) (Df = 200).

	FGP	MGT	GRI
FGP	1,00000	-0,39330	0,99845
P		< 0,0001	< 0,0001
MGT	-0,39330	1,00000	-0,42276
P	< 0.0001		< 0,0001
GRI	0,99845	-0,42276	1,00000
P	< 0,0001	< 0,0001	

According to Table 4, the correlation between the different variables studied (FGP, MGT and GRI) was very highly significant. Indeed, when the FGP increased, the GRI also increased (positive correlation) but the MGT tends to decrease (negative correlation). In order to characterize the best pretreatment for a given species, several researchers suggest that, an FGP and/or GRI variable must be calculated with a time-related variable such as MGT or T_{50} (time when the germination rate reaches 50%) (Kheloufi 2017; Kheloufi et al. 2018a).

Many seed treatment experiments have shown that time is an important factor for the induction of good germinative activity. In fact, the evaluation of the germination capacity does not only depend on the percentage of germination achieved but also on its speed and its evolution over time (Norden et al. 2009). These two factors combined together are often used to determine the success of a pretreatment on breaking dormancies (integumentary or embryonic) (Blakesley et al. 2002). This confirms our results which indicate that the best treatment is characterized by a high FGP and GRI and a very low MGT compared to other treatments.

As indicated in the results section, the non-scarified seeds of *A. tortilis*, even during a long incubation period up to 18 days; did not germinate, indicating the inhibitory effect of the seed coat making them impermeable to water, a phenomenon typical of forest legume species. The seed coat dormancy often concerns species adapted to alternating dry and wet seasons, and particularly several genera and species in Fabaceae, such as *Acacia*, *Prosopis*, *Ceratonia*, *Robinia*, *Albizia*, and *Cassia*. The study of the effects of pretreatments in the genus Acacia showed a very significant influence on the rate and mean germination times (Van der Burg et al. 2014). In addition, chemical scarification by concentrated sulphuric acid gives very high germination rates compared with scarification by boiling water or sandpaper (Germanà et al. 2014; Kheloufi 2017).

4 Conclusion

In Algeria, forest departments are adopting guidelines that involve the use of more and more native species. However, exotic species could also contribute to the success of a good reforestation program to ensure forage for several animal species. Forestry departments and research institutes need only to study their adaptation and survival in the environment where the program will be implemented, as well as to limit the invasiveness of each species. Based on the results presented, it is clear that seeds of different species of Acacia need pretreatment to improve their germination. The germination rate thus increased from 5-10% in nature to more than 90% after treatment with sulphuric acid for a short period of time. The results also agree that the type of dormancy in seeds of this genus is of a physical type. These pretreatments could be recommended to foresters and nursery growers because this solution is cheap and simple to apply, which would promote land restoration in arid and semi-arid areas.

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