Application and use of sulphuric acid pretreatment to improve seed germination of three acacia species

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Abstract

The experiments were led to study the effect of pretreatments and their duration on germination behavior of three *Acacia* species *A. cyanophylla* Lindl., *A. farnesiana* L. and *A. decurrens* Willd. by analyzing three parameters (GP: germination percentage; MGT: germination mean time (days) and GRI: germination rate index) for various times of incubation (5, 10 and 15 days) in Petri dishes. Pre-sowing treatment included immersion in concentrated sulphuric acid for 60, 90 and 120 minutes. The sowing in distilled water (Control) had no positive effect on the germination induction. Generally, the seed pretreatments were very useful to improve germination. Time of immersion significantly ($P < 0.0001$) affected GP, MGT and GRI in all species. Increasing the duration of sulphuric acid immersion (from 60 to 120 min) improved the germination percentages for *A. cyanophylla* and *A. farnesiana* seeds to (98% and 99%), respectively. However, increasing this duration had a negative effect on *A. decurrens* seed germination, reducing the final germination percentage from 97% at 60 minutes of immersion to 43% at 120 minutes.

Keywords

Dormancy; Sulphuric Acid; Scarification; Tree Reproduction; Reforestation

Contents

1 Introduction

In the dry and desert zones of Algeria, we attend during the last decades a fast-backward movement of the natural plant cover associated with erosion of the biological diversity (Slimani et al. 2010; Vicente-Serrano et al. 2012). This degradation is set by stressful environmental conditions translated by harmful effects on economic and ecological plans (Poff and Zimmerman 2010). Moreover, in the situations where the degradation of the plant cover setting reached the threshold of irreversibility and where the natural regeneration cannot lead to the restoration setting, the request to the systems of rehabilitation becomes an absolute necessity (Melo et al. 2013). However, before beginning any reforestation, it is necessary to select which species and which origins are advisable to choose according to stations to be restored (Duan et al. 2010). A mass of information on the ecological peculiarities of several species of reforestation is described in the mini-monographs presented to the technical consultation FAO on broad-leaved trees with fast growth for the plantation in the Mediterranean and temperate regions. Some of this information can be systematically presented in form of lists of resistant species to certain extreme ecological conditions. On the other hand, the study of germinal requirements of the usable species in rehabilitation allows reasoning with the choice of the best plant materials adapted to the implementation of this system (Cao et al. 2010). Indeed, the seed germination and the first phases of the plant development are critical to succeed quite typical of plant production which will determine directly the density of the populating and consequently the rate of the yield (Kawakami et al. 2009).

Various trees are fast-growing and are classified as better producers of biomass, while the majority of the species have seeds which have difficulties of germinating by presenting certain impermeability to water (Liu et al. 2011). Thus, they cannot germinate in normal conditions. This problem of the imbibition phase is the important problem of the establishment of the leguminous species forests (de Faria et al. 2010). It is known under the name of integumentary dormancy which combined with the embryonic dormancy, makes an ecological mechanism which allows the seed to germinate only in favorable conditions. This phenomenon applies generally to forest trees or to the leguminous shrubs (Vargas et al. 2015). Consequently, seeds require pretreatments to obtain a fast and a uniform germination with a considerable rate (Azad et al. 2013).

The choice of plant material in this work concerns three acacia species which present some encouraging particularities that could contribute to a good afforestation-reforestation program: *A. cyanophylla* Lindl., *A. farnesiana* L. and *A. decurrens* Willd. 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 very poor grounds in nutritional elements (Bashan et al. 2012; Boukhatem et al. 2016). These species are honey-yielding plants and a considerable sources of shade in the dry regions, and also a site of nesting for several species of birds (Yimam et al. 2012; Zhang et al. 2016). Their wood is hard and heavy and could be also used for the reforestation of degraded lands to improve soil and its landscaped environment. Indeed, because they have proven very hardy and tolerant of drought and salinity, they are widely planted for windbreaks and soil erosion control (Myers et al. 2000; Kheloufi et al. 2016; Kheloufi et al. 2017). In this work, we do not intend to develop or to resume all the methods or the ways used to activate the germination activity; we shall restrict to define and to compare only one pretreatment which is the sulphuric acid scarification applicable to the seeds of these species. The understanding of these aspects is crucial for the regeneration and the successful integration in tree nurseries as well as for the direct plantation in arid and semi-arid lands.

2 Material and methods

2.1 Seed sources and collection

The provenances coordinates and one thousand-seeds weight of the three-species used in this study (*A. cyanophylla, A. farnesiana* and *A. decurrens*) are presented in table (Table 1). The experiment was conducted in the Laboratory of Ecology and Environment, University of Batna 2 (Algeria). The mature pods were collected from 10 trees each. Pods that were already dried up naturally were manually crushed to free seeds. The sieving and the flotation were used to sort out seeds. The test of the flotation aims at eliminating seeds that were empty, broken and damaged by insects. The clean seeds were then spread on filter paper to dry. Once dried, seeds were stored in glass bowls at a temperature of 4°C for 2 months (simulation of vernalization period).

Table 1. Seed characteristics and provenances.

Species	1000-seed weight (g)	Seed Sources
A. cyanophylla	14.75 ± 0.09	Temouchent, Algeria (35°26'32.26"N; 1°13'42.80"W)
A. farnesiana	56.44 ± 0.11	Relizane, Algeria (35°42' 40.58"N; 0°18'28.76"E)
A. decurrens	17.55 ± 0.07	El-Taref, Algeria (36°51'51.60"N; 8°25'55.01"E)

2.2 Experimental design and treatments

Seeds of every species underwent several pretreatment durations which consisted of the immersion in sulphuric acid (98%) at various durations (60, 90 and 120 min) followed by a good soaking in distilled water. The sowing (4 replicates of 20 seeds × 4 treatments × 3 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 a culture chamber in the obscurity at laboratory temperature during 15 days of incubation. The Petri dishes were arranged every two days, according to a randomized design to eliminate any effect of the position in the seed culture room. The counting of germinated seeds was made on the 5^{th}, 10^{th} and 15^{th} day of incubation and was expressed in percentages (the criterion of germination was 2 mm radicle protrusion). Several parameters were considered:

The kinetics of germination: To estimate better the physiological meaning of the germination, the number of germinated seeds was counted on the 5^{th}, 10^{th} and 15^{th} day.

The germination rate index (GRI): It reflects the percentage of germination on each day of the germination period (Maguire 1962). GRI is calculated by the formula [1]:

$$\mathrm{GRI}\ (\%) = \sum \frac{\text{No: of Germinated Seeds}}{\text{No: of Days}} \qquad [1]$$

The mean germination time (MGT): It represents the mean time, a seed lot requires to initiate and end germination (Orchard 1977). MGT is calculated by the formula [2]:

$$\text{MGT (days)} = \frac{\sum \text{n.D}}{\sum \text{n}} \quad [2]$$

Where:
n - number of seeds newly germinated at time D;
D - days from the beginning of the germination test;
Σn - final germination.

2.3 Statistical analysis

Experiments were set up in a completely randomized design with 4 replications of 20 seeds. The mean, one-way, two-way ANOVA and repeated measures analysis of variance (Generalized Linear Model: GLM) were calculated using SAS Version 9.0 (Statistical Analysis System) (2002) software. The mean separations were carried out using Duncan's multiple range tests and significance was determined at $p < 0.05$.

3 Results

The effect of the four treatments on the germination of each species (*A. cyanophylla, A. farnesiana* and *A. decurrens*) following 5, 10 and 15 days and their association with time are shown in table (Table 2).

Table 2. Germination kinetics (%), germination rate index (GRI) and mean germination time (MGT) for three acacia species exposed to different pre-sowing treatments. The same alphabet along the column indicates no significant difference (Duncan Multiple Range Test).

Species	Treatments	Germination kinetics (%)			GRI (%)	MGT (days)
		5th day	10th day	15th day		
A. cyanophylla	Untreated (Control)	0^D	0^D	0^D	0^D	--
	60 min	32^C	43^C	49^C	29.10^C	7.30^A
	90 min	78^B	82^B	82^B	60.97^B	5.25^B
	120 min	95^A	98^A	98^A	73.61^A	5.15^B
A. farnesiana	Untreated (Control)	0^D	0^D	0^D	0^D	--
	60 min	34^C	44^C	47^C	29.86^C	6.71^A
	90 min	55^B	67^B	75^B	47.29^B	6.92^A
	120 min	90^A	99^A	99^A	71.87^A	5.45^B
A. decurrens	Untreated (Control)	0^D	0^D	0^D	0^D	--
	60 min	90^A	97^A	97^A	71.18^A	5.35^B
	90 min	67^B	78^B	84^B	55.83^B	6.40^A
	120 min	43^C	43^C	43^C	32.84^C	5.00^B

The seeds of the three studied species present varied behavior towards treatments in the sulphuric acid at the time of their germination. The treatment effects are highly significant ($P < 0.0001$) on the rate and the mean time of germination (Table 2 and 3). The obtained results highlight the effect of treatment which has a very important role in seed germination.

The results on Table 2 show that control (sowing in distilled water) had no positive effect on the induction of germinal activity. The increase of the soaking time in the sulphuric acid from 60 to 120 min is favorable for the seeds of *A. cyanophylla* and those of *A. farnesiana* improving the increase of the GRI from 29.1% to 73.61% and from 29.86% to 71.87%, respectively. However, the extension of the soaking time showed unfavorable evolution on *A. decurrens* seeds, reducing the GRI from 71.18% to 32.84% (Table 2). Acid treatment exhibited lowest MGT and highest GRI for *A. cyanophylla* and *A. farnesiana* seeds (Table 2). Generally, the highest percentage of germination was obtained by all the species seeds with values of a final germination rate close to 99% but at a different soaking duration. The one-way ANOVA shows that treatment and time affect significantly ($P < 0.0001$) both GRI and kinetics of germination. However, there were no significant differences ($P > 0.05$) between species on the variation of the MGT ($P < 0.0001$) (Table 3). The two-way ANOVA shows that there is also a significant effect ($P < 0.0001$) of the interaction (Treatment × Species) on the variation of the germination rate index and the mean germination time. The repeated measures analysis of variance reveals a significant effect ($P < 0.0001$) of factors "treatment (duration and type), species, time and their correlation" on the kinetics of germination in the case “Between Subjects Effects” and “Within-Subjects Effects” (Table 3). When the germination was observed over time, highest percentage of germination starting from the 10^{th} day.

Table 3. Variance analysis for the traits investigated of *A. cyanophylla, A. farnesiana and A. decurrens* seeds in response to concentrated sulphuric acid pre-sowing at different durations for a period 15 days.

Parameters	Sources of Variation	*Df*	*F*	*P*
Germination Kinetics	Species ‘Sp’	2	2.17	0.1285
	Time after sowing ‘Time’	2	98.39	<0.0001
	Treatment ‘TRT’	3	537.09	<0.0001
	Sp × Time	4	3.88	0.0065
	Sp × TRT	6	87.78	<0.0001
	Time × TRT	6	18.48	<0.0001
	Sp × Time × TRT	12	4.38	<0.0001
MGT	TRT	3	15.23	<0.0001
	Sp	2	5.24	0.0119
	TRT × Sp	6	7.66	0.0003
GRI	TRT	3	487.71	<0.0001
	Sp	2	3.20	0.0528
	TRT × Sp	6	84.41	<0.0001

4 Discussion

As shown in the results section, non-scarified seeds of the three-species subjected to 5, 10 or 15 days of incubation did not germinate, indicating that the seeds have the typical hard, impermeable seed coat of the leguminous species. This is in agreement with the results of Chauhan and Johnson (2009), which reported that non-scarified seeds of the leguminous trees did not reveal any imbibition or germination as a result of the hard seed coat.

The efficiency of the sulphuric acid to raise the integumentary inhibition had been demonstrated by several authors (Cox et al. 1945; Egley 1972; Diouf et al. 2015; Dayamba et al. 2014; Kheloufi 2017). The study of the acid scarification effects for the *Acacia* genus shows a highly significant influence on the rate and the average times of germination (Nasr et al. 2013; Kheloufi and Mansouri 2017). Moreover, the chemical scarification by the concentrated sulphuric acid gives high germination rates compared with the scarification by boiling water or by sandpaper (Burrows et al. 2009; Ghassali et al. 2012).

Various experiments concerning the seed pretreatments reveal that time is an important factor for the induction of a considerable germinability. Indeed, the evaluation of the germination capacity depends not only on the reached percentage of germination but also its speed as well as its evolution in time. The correlation of these two factors is often used to determine the success of a pretreatment on physical and embryonic dormancy (Nonogaki et al. 2010). The speed of germination depends on the seed harvest moment, resulting from a very crucial biological property for an adaptation in the conditions of the environment (Gardarin et al. 2011). Furthermore, the seed dormancy, whether it is of embryonic or physical nature, is determined by several factors such as dehydration, the content in oxygen, extreme temperatures and the acidity of the culture medium (Ribeiro et al. 2011). These conditions are necessary for a good production of shoots by seeds (Kaydan and Yagmur 2008). The rest is determined by the internal qualities of the seeds themselves among which the metabolism, the content of certain growth regulators as well as the presence of certain inhibitory substances of germination in the integument, preventing and delaying the imbibition which is the first stage of the germination induction (Phartyal et al. 2009).

According to the multitudes of works on the stimulation of the germination in hundreds of forest species, there are various types of dormancy: exogenous (determined by the properties of the integument surrounding the seed); endogenous (determined by the physiological state of the embryo) and the third type is the combined dormancy (determined by a combination of both previously mentioned factors) (Wang et al. 2012). In the case of a mechanical dormancy, the inhibition of the germination is due to the action of certain physical obstacles, creating a negative osmotic pressure and an imbalance in the gaseous exchanges in the seed (Fernandez et al. 2008). The threshold of this dormancy differs from species to species (Bradford et al. 2008). Additionally, this kind of inhibition can be eliminated by using various pretreatments such as immersion in boiling water; soaking in some concentrated sulphuric acid as well as the abrasion of the integument by using some sand (Azad et al. 2012). The effect of the temperature can be added to these treatments by using many modes of freezing as well as thermal shock (Hanley 2009). Tadros et al. (2011) add that it is necessary to increase the duration of the soaking time when the seeds are older. This is valuable for all the seeds with thick and hard integuments (Smýkal et al. 2014). The efficiency of the sulphuric acid to raise the integumentary inhibition in others forest, agro-forestry and pastoral species had been demonstrated by several researchers (Saied et al. 2008; Tanaka-Oda et al. 2009; Zare et al. 2011). However, a prolonged treatment of seeds in the acid can damage the embryo and reduce germinal performances (Kestring et al. 2009). The optimal duration of soaking is proportional to the coat rigidity. Indeed, in this study, the seeds of these three acacia species seem very sensitive to the increase of the duration of soaking in acid.

The soaking time in sulphuric acid depends on the thickness and on the solidity of the coat (de Paula et al. 2012). Our results are in agreement with this conclusion, revealing that the use of sulphuric acid allowed increasing germination percentage even if the soaking duration had sometimes a negative effect especially for a duration of 120 minutes. Kgope et al. (2010) showed that the effect of forest fires would also play an important role on the germination and the establishment of *A. karroo* which is the more-tolerant species.

Finally, seed dormancy has species specificity and varies with stage of seed maturity and degree of seed drying (Li et al. 2013). Thus, pre-treatment must be adjusted on the basis of the status of seed dormancy (Azad et al. 2010).

5 Conclusion

Algerian forest departments actually adopt orientations which consist in using more and more native species. However, exotic species could also contribute to a better afforestation-reforestation program. They just have to study their adaptation and their survival in the environment where the program will be introduced, without forgetting to limit the invasive character of each species. Based on the results presented, it is clear that the seeds of *A. cyanophylla, A. farnesiana* and *A. decurrens* need pre-sowing treatment to enhance germination. The results also show that the type of seed dormancy in these species is physical dormancy. Concentrated sulphuric acid can be used to pre-treat the seeds in order to break the impermeable seed coat and allow germination activity. This pretreatment could be recommended to nursery gardeners because these solutions are not expensive and simple to realize. Among the pre-treatments, the best treatment was 120 minutes of immersion in sulphuric acid for *A. cyanophylla, A. farnesiana* seeds and only 60 minutes for *A. decurrens* seeds. Our results will assist forestry nurseries in enhancing restoration in arid and semi-arid lands while taking advantage of the economic interests of these species.

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