Breaking seed coat dormancy of six tree species

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Abstract

Breaking physical dormancy in some forest seeds is a challenge for scientists and forest managers to obtain a homogeneous germination for larger seed samples. The role played by the seed coat in seed dormancy of six trees with great interest in agroforestry (*Robinia pseudoacacia*, *Leucaena leucocephala*, *Erythrostemon gilliesii*, *Styphnolobium japonicum*, *Acacia dealbata* and *Brachychiton populneus*) was tested by the effects of the pretreatment and its duration on the performance of seed germination, by considering the final germination percentage (FGP) and the mean germination time (MGT). These parameters are estimated at various times of incubation (5, 10 and 15 days) in Petri dishes and stored in darkness at (25 ± 2°C). The pretreatment consists of an immersion of seeds in concentrated sulphuric acid during 30, 60 and 90 minutes. Sowing without pretreatment (control) revealed no germination induction for *B. populneus* and *A. dealbata*, except for *R. pseudoacacia*, *L. leucocephala* and *S. japonica* where the germination does not exceed 12% for all the experience duration. Generally, pretreatments were very useful to improve seed germination. The time of immersion into sulphuric acid significantly affected ($P < 0.0001$) the FGP and the MGT in all studied species. A duration of 30 minutes of soaking was adequate to give a very high rate of germination for *L. leucocephala*, *E. gilliesii*, *S. japonica* and *A. dealbata* with respective FGP of 100%, 95%, 100% and 82,5%. However, an extended duration of pretreatment of 60 minutes was necessary for a maximal germination for *R. pseudoacacia* and *B. populneus* with FGP of 85% and 100%, respectively. A prolonged duration of 90 minutes of presowing was very fatal for *L. leucocephala*, *A. dealbata* and *B. populneus*. An excellent germinative strength is characterized by a higher FGP and a reduced MGT.

Keywords

Germination; Seed coat dormancy; Reforestation; Sulphuric acid; Agroforestry

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1 Introduction

The final phase of seed development involves the loss of water, cessation of reserve synthesis where after the seed enters a metabolically inactive state. Seed dormancy has been defined as a temporary failure of a viable seed to germinate in conditions that favour germination (Bewley 1997). These conditions are a complex combination of water, light, temperature, gasses, mechanical restrictions, seed coats, and hormone structures. Dormancy in nature serves to protect the seed from conditions which are temporarily suitable for germination, but which quickly revert to conditions too harsh for survival of the tender young seedling (Koornneef et al. 2002). Thus, a seed coat relatively impermeable to moisture prevents germination during isolated showers in the middle of a long dry season, while permitting it during a sustained rainy season (Vázquez-Yanes and Orozco-Segovia 1993).

The seed coat has been shown to be a multifunctional organ which supplies nutrients to the embryo sac throughout development (van Dongen et al. 2003) and is functional during drying of the seed (Howe and Smallwood 1982). The structural and chemical properties of the seed coat impose impermeability (Rolston 1978), regulate water entry once dormancy has been broken (Serrato-Valenti et al. 1993), provide a barrier against fungi (Mohamed-Yasseen et al. 1994), and reduce leakage from the embryo during imbibition (Simon and Harun 1972). So, understanding this type of dormancy and identifying natural ways of breaking it becomes economically important.

From the forester's point of view dormancy has some disadvantages. Delayed and irregular germination in the nursery is a serious constraint on efficient nursery management (Schmidt 2000). Much research has therefore gone into devising effective artificial treatments to remove dormancy, in order to ensure that the seeds germinate quickly. According to Kheloufi et al. (2017), the intensity of dormancy for the same species may vary according to genotype and environment in which seeds are produced. The efficiency of scarification with sulphuric acid to overcome seed coat impermeability and increase seed germination has been reported for different species. However, the efficiency of this treatment varies with the acid concentration, plant species and treatment duration (Kheloufi 2017).

There are two types of frequently used pretreatments, mechanical nature (break) and wet nature (immersion in a corrosive solution). The soaking into hot water or acids presents the advantage to treat an important quantity of seeds at the same time. However, the duration of the immersion must be determined to conclude the best time required to raise the coat dormancy. The present study aimed at identifying easily applied pretreatments (Time of soaking into concentrated sulphuric acid) that can be used to treat large quantities of seeds to assure fast, homogeneous and synchronized seed germination of six forest species. Five are from the family Fabaceae (*Robinia pseudoacacia*, *Leucaena leucocephala*, *Erythrostemon gilliesii*, *Styphnolobium japonicum*, *Acacia dealbata*) and one from the family Malvaceae (*Brachychiton populneus*).

2 Material and methods

2.1 Collection and origin of seeds

Seeds of all species were collected in 2017. Seed morphological characteristics and provenances of the six-tree species used in this study (*R. pseudoacacia*, *L. leucocephala*, *E. gilliesii*, *S. japonicum*, *A. dealbata* and *B. populneus*) are presented in Table 1 and Table 2. The experiment was conducted at the Laboratory of Ecology and Environment, University of Batna 2 (Algeria). The mature pods were collected from 5 trees each. Pods already dried up naturally were manually crushed to free seeds. After harvesting, seeds were mixed in view of minimizing inter genetic variations. Once dried, seeds were stored in glass bowls at a temperature of 4 °C for 3 months (simulation of vernalization period). The seed sample for our experiment was obtained by mixing the seeds with a wooden spoon and removing impurities such as plant matter (remains of integument, stems, broken cotyledons), animal (dead insects) or mineral (sands, gravels). Taxonomy update for the studied species by GRIN-Taxonomy (2018) is indicated on Table 3.

Table 1. Seeds morphological characteristics (Mean ± SD).

	Seed color	Weight of 1000 seeds (g)	Seed size (n=20)		
			Length (mm)	Width (mm)	Thickness (mm)
R. pseudoacacia	Black	26	5.11 ± 0.22	3.22 ± 0.14	1.99 ± 0.12
L. leucocephala	Brown	44.8	7.37 ± 0.20	5.40 ± 0.18	1.40 ± 0.09
E. gilliesii	Fadded green	95.4	9.00 ± 0.28	8.62 ± 0.53	2.25 ± 0.15
S. japonica	Black	105.7	7,75 ± 0.36	5,94 ± 0.26	3.70 ± 0.17
A. dealbata	Black	26.3	5.95 ±0.32	3.23 ± 0.13	2.00 ± 0.12
B. populneus	Light yellow	126	7.91 ± 0.40	5.31 ± 0.35	5.20 ± 0.29

Table 2. Seed provenances (5 trees per species).

Species	Vernacular names	Region	Geographical coordinates
R. pseudoacacia	Black locust	University of Batna 1	35°32'27.97"N ; 6° 9'8.09"E
L. leucocephala	White popinac	University of Batna 2	35°38'7.46"N ; 6°16'36.22"E
E. gilliesii	Yellow Bird of Paradise	University of Batna 2	35°38'3.49"N ; 6°16'19.96"E
S. japonica	Japanese pagoda	University of Batna 1	35°32'28.52"N ; 6° 9'8.15"E
A. dealbata	Silver wattle	Municipal Park of Oran	35°41'21.11"N ; 0°38'53.07"W
B. populneus	Bottle tree	Municipal Park of Oran	35°41'19.32"N ; 0°38'47.34"W

Table 3. Taxonomy update for the studied species by GRIN-Taxonomy (2018).

Species	Synonym	Family
***Robinia pseudoacacia* L.**	*Robinia pseudoacacia L.*	Fabaceae
***Leucaena leucocephala* (Lam.) de Wit**	*Acacia glauca Willd.*	Fabaceae
***Erythrostemon gilliesii* (Hook.) Klotzsch**	*Caesalpinia gilliesii (Hook.) D. Dietr.*	Fabaceae
***Styphnolobium japonicum* (L.) Schott**	*Sophora japonica L.*	Fabaceae
***Acacia dealbata* Link**	*Acacia decurrens var. dealbata (Link) F. Muell.*	Fabaceae
***Brachychiton populneus* (Schott & Endl.) R. Br.**	*Poecilodermis populnea Schott & Endl.*	Malvaceae

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 and 90 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 10 seeds × 4 treatments × 6 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 the laboratory temperature (25 ± 2°C) 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 (Kheloufi et al. 2017). The counts of germinated seeds were made on the 5th, 10th and 15th day of incubation and were expressed in percentage (The criterion of germination was 2 mm radicle protrusion).

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

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

where FGP is 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).

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

where MGT is 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).

The effects of pretreatments on both 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). All statistical methods were performed using were calculated using SAS Version 9.0 (Statistical Analysis System) (2002) software.

3 Results and discussion

According to the results of ANOVA and Duncan's test, highly significant (P < 0.0001) differences were found among species and among pretreatments, resulting in a highly significant (species x treatment) interaction (Table 5).

Germination and seedlings development are illustrated on figure 1 and figure 2, respectively.

The seed FGP in different species after treatments (control, sulphuric acid) yielded significant differences. According to the results of the ANOVA and Duncan's test, significant germination variations were found between the species and among the duration of treatment.

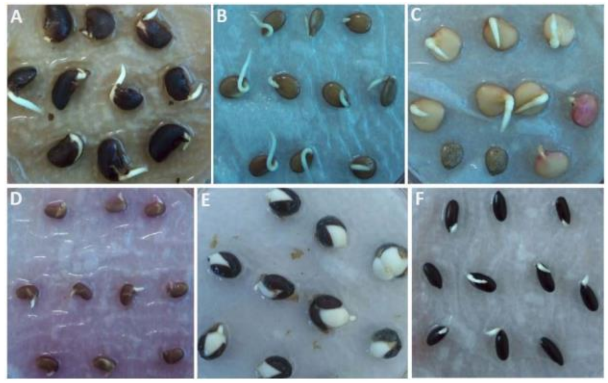

Figure 1. Germination of (A) *S.japonicum*, (B) *L.leucocephala*, (C) *E. gilliesii*, (D) *R. pseudoacacia*, (E) *B. populneus* and (F) *A.dealbata*.

Table 4. Final germination percentage (FGP) and mean germination time (MGT) for forest species exposed to different pre-sowing treatments. For each species, the same alphabet along the column indicates no significance difference (Duncan Multiple Range Test).

Species	Pretreatment	FGP (%)	MGT (days)
R. pseudoacacia	Untreated	7.50 ± 5.00^{C}	4.67 ± 2.88^{A}
	30 min SA	67.5 ± 9.57^{B}	3.21 ± 0.42^{AB}
	60 min SA	85.0 ± 5.77^{A}	2.58 ± 0.41^{AB}
	90 min SA	85.0 ± 5.77^{A}	2.24 ± 0.15^{B}
L. leucocephala	Untreated	10.0 ± 8.16^{B}	3.67 ± 0.57^{B}
	30 min SA	100 ± 0.00^{A}	2.00 ± 0.00^{D}
	60 min SA	85.0 ± 17.3^{A}	2.27 ± 0.12^{CD}
	90 min SA	10.0 ± 9.54^{B}	5.00 ± 0.00^{A}
E. gilliesii	Untreated	82.5 ± 5.00^{A}	2.28 ± 0.41^{AB}
	30 min SA	95.0 ± 5.77^{A}	2.00 ± 0.00^{B}
	60 min SA	92.5 ± 15.0^{A}	2.19 ± 0.14^{AB}
	90 min SA	47.5 ± 12.6^{B}	2.53 ± 0.32^{A}
S. japonica	Untreated	12.5 ± 5.00^{C}	5.50 ± 1.73^{A}
	30 min SA	100 ± 0.00^{A}	3.40 ± 0.08^{B}
	60 min SA	97.5 ± 5.00^{A}	2.21 ± 0.09^{B}
	90 min SA	92.5 ± 15.0^{A}	2.31 ± 0.12^{B}
A. dealbata	Untreated	0^{C}	--
	30 min SA	82.5 ± 9.57^{A}	2.11 ± 0.15^{B}
	60 min SA	55.0 ± 23.8^{B}	2.63 ± 0.72^{B}
	90 min SA	0^{C}	--
B. populneus	Untreated	0^{C}	--
	30 min SA	12.5 ± 5.00^{B}	3.50 ± 2.72^{A}
	60 min SA	100 ± 0.00^{A}	4.13 ± 0.18^{A}
	90 min SA	0^{C}	--

Table 5. Variance analysis for the traits investigated of *six tree* seeds in response to different pre-sowing at different durations for 15 days-period.

Parameters	Sources of variation	*Df*	*F*	*P*
FGP	TRT	3	183.61	< 0.0001
	Sp	5	109.95	< 0.0001
	TRT × Sp	15	60.12	< 0.0001
MGT	TRT	3	6.62	0.0001
	Sp	5	4.90	0.0007
	TRT × Sp	15	3.87	< 0.0001

The overall mean germination for 30 minutes immersion into sulphuric acid was over 80%, indicating that this was the most efficient treatment in *L. leucocephala*, *E. gilliesii*, *S. japonica* and *A. dealbata* (Table 4). 90 minutes of pretreatment killed most if not all the seeds of *L. leucocephala*, *E. gilliesii*, *A. dealbata* and *B. populneus*, but not those of *R. pseudoacacia* and *S. japonica* (Table 4). This wide variation in responses to the treatments indicates considerable differences among species in the structure of the seed coat as protective barrier. It was observed that many seeds died indicating that long exposure with concentrated sulphuric acid made contact with the embryos (Teketay 1996). This occurred because the seed coats, which normally regulate the uptake of water, had been damaged and the rapid increase in water caused irreversible damage (Kheloufi 2017). This result demonstrated that above mentioned treatment had the destructive effect on embryo.

Mean germination time varied significantly across different species (P = 0.0007) and due to pretreatments (P = 0.0001), (Table 5). Reduced mean germination time in acid treated seeds implies that the period of dormancy in seeds was reduced due to pretreatment of seeds in sulphuric acid. It is reported that acid treatment is an efficient method of enhancing seed germination of species with hard impermeable seed coat (Sy et al. 2001; Pérez-García and González-Benito 2006; Hu et al. 2009). It stimulates fast and uniform germination of seeds.

Robinia pseudoacacia L.

Black locust is a North American tree species introduced in Europe in the early seventeenth century and used for ornamental and forestry purposes (Cierjacks et al. 2013). Several researchers have shown that application of H_2SO_4 is more effective treatments than other treatments for *R. pseudoacacia* seed germination (Zoghi et al. 2011; Mirzaei et al. 2013). Our findings join these results with an average of (85.0 ± 5.77%) FGP in (2.58 ± 0.41 days) MGT for 60 minutes of immersion (Table 4).

Laucaena leucocephala (Lam.) de Wit

L. leucocephala is a multipurpose perennial legume tree species used as livestock feed due to its high nutritive value and palatability (Jones 1979), to improve soil characteristics and in soil erosion control, in agroforestry systems as shade crops or windbreaks and as fuel wood (Soedarjo and Borthakur 1996; Brewbaker 2013). Seed germination of *L. leucocephala* was influenced by the different treatment techniques applied (Table 4). These actions break dormancy by disrupting or softening the seed coat or by fracturing specialized tissues in the seed coat to allow water penetration (Bosman et al. 1995). In the same study by Tadros et al. (2011), blade scarification treatment of *L. leucocephala* resulted in 97% seed germination which was just as effective as the soaking treatments. The use of concentrated sulphuric acid has been successfully demonstrated to increase germination rates in Leucaena species (Teketay 1996; Koobonye et al. 2018). Our results showed that 30 minutes of soaking was enough to have 100% of FGP in just 2 days of MGT (Table 4).

Erythrostemon gilliesii (Hook.) Klotzsch

It is semi-evergreen shrub or small tree with slender, erect branches; it can grow 8 meters or more tall, but is usually smaller in cultivation. The plant is evergreen in warm climates, becoming deciduous in cool seasons. *E. gilliesii* is an ornamental shrub with showy yellow flowers (Marinho et al. 2014). It was used in folk medicine due to its contents of different classes of secondary metabolites. Its flowers showed a good antioxidant activity (Barbieri et al. 2016). In our study, seeds of *E. gilliesii* have germinated without treatment with (82.5 ± 5.00%) FGP. However, the soaking into sulphuric acid for 30 minutes improved FGP up to (95.0 ± 5.77%) and reduce MGT from (2.28 ± 0.41 days) to (2.00 days), (Table 4).

Styphnolobium japonicum (L.) Schott

Although its Latin name implies that the pagoda tree is a Japanese plant, it is in fact native to China. The species was first described under the name *Sophora japonica*, based on cultivated material from Japan, and thus the choice of species name. It is a deciduous tree, 15-25 m tall, usually branching low down when growing in the open but capable of forming a tall, clean trunk (Wang et al. 2006). The pagoda tree is grown as an ornamental and for its durable timber, which is used for furniture and construction (Bubner 2008). In previous study, the optimum treatment time into sulphuric acid for enhancing germination in *S. japonicum* was 40 minutes (Xu and Gu 1985). Kou and Wang (2008) have worked on the same species and showed that concentrated sulphuric acid played an important role in urging germination, among which, corroding seed coat with concentrated sulphuric acid, was the most effective method, the germination rate could reach 98%. In our investigation, similar results were observed and seed germination of *S. japonicum* reached 100 % by soaking into sulphuric acid for just 30 minutes (Table 4).

Acacia dealbata Link

Most Acacia species have hard seed coats which are impervious to water (Aref et al. 2011). Many experiences have been done on various Acacia species (Kheloufi 2017; Kheloufi et Mansouri 2017; Kheloufi et al. 2017) and showed that seeds of some Acacia species (*A. karroo*, *A. nilotica*, *A. farnesiana*, *A. decurrens* and *A. saligna*) have impermeable seed coats and need a specific duration of soaking into sulphuric acid (30 to 120 minutes) to improve seed germination. These species were selected for reforestation programs in arid and semi-arid regions of Algeria after their inventory and geographical distribution (Kheloufi et al. 2018).

A. dealbata is an evergreen tree growing to 25 m. It is a fast-growing tree, cold tolerant species has been used to control soil erosion, hillside stabilization, gully erosion control and in windbreaks (González-Muñoz et al. 2012). May (1999) showed that 30-35% of seeds germinated with no pretreatment. In our results, no germination was observed with untreated seeds and 30 minutes of immersion into sulphuric acid enhanced germination up to (82.5 ± 9.57%) of final germination (Table 4).

Brachychiton populneus **(Schott & Endl.) R.Br.**

B. populneus is a tree up to 20 m in height, which usually has a relatively short bole and a densely-foliaged crown. It is a relatively slow growing tree that sometimes becomes semi-deciduous during early summer (Buist et al. 2000). The tree is quite popular in farming areas as it can be used as fodder during drought periods (Scarff and Westoby 2006). The seeds are surrounded in the capsule by irritant hairs (Cuadra et al. 2012). Germination success can be increased with immersion in hot water, followed by soaking for 12 hours in the cooled water (Beck and Balme 2003). According to Table 4, 60 minutes of immersion into concentrated sulphuric acid improved seed germination from 0 % (untreated seeds) to 100% in only 4 days.

According to our results, germination of a seed depends on the potential of embryo growth or potentials of growth preventor. These potentials depend particularly on seed structure that surrounded the embryo (endosperm, pricarp, glumes) (Schopfer and Plachy 1985; Germanà et al. 2014). Other factors like hormones and environmental factors also affect embryo growth (Shu et al. 2016). This kind of dormancy happens when factors like water and gas are not permitted to enter the seed, so imbibition is not occurred and consequently, resulting in decreasing seed germination (Bewley 1997). It is possible that the observed poor germination in untreated seeds could have been in part attributed to the less severity of the treatment i.e. short duration of immersion in the acid. In addition, impermeability of seeds to water and gas was attributed to physical and biochemical obstacles of the coat (Allen et al. 2007). According to the obtained results, scarification treatments with sulphuric acid (98%) were effective, which caused dormancy breaking and seed germination induction of all studied species.

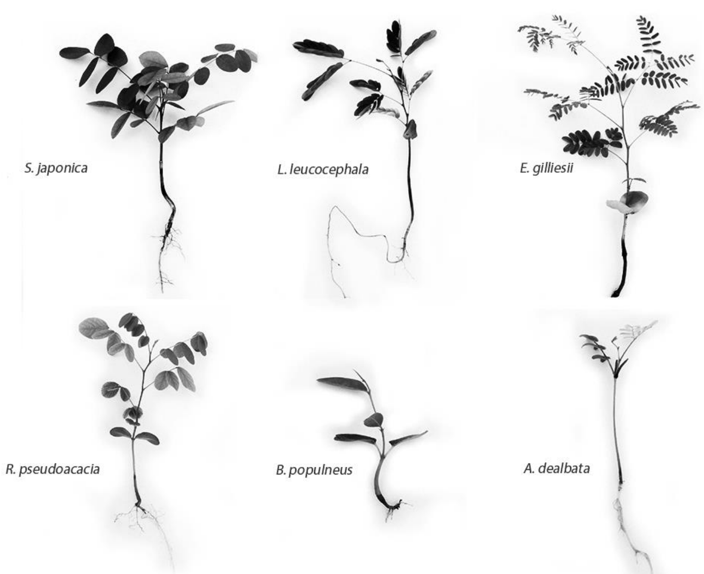

Figure 2. 60 days old seedlings of *Robinia pseudoacacia*, *Leucaena leucocephala*, *Erythrostemon gilliesii*, *Styphnolobium japonicum*, *Brachychiton populneus* and *Acacia dealbata* (grown in pots).

Our results demonstrate that each species has its own characteristic set of germination requirements with a specific threshold of response according to its peculiar degree of heterochrony. Erickson et al. (2016) also stated that acid treatment of seeds removes waxy layer of the seed coat by chemical decomposition of seed coat component which is similar to breakdown process that occurs during microbial attack.

The period of storage according to the seed longevity of the species, seed pretreatment methods and seed size can affect the germination. The larger seeds resulted in higher germination percentage since larger seeds contain more food reserves to support germination (Savage 2015). The characteristic of the mother tree can also affect the behavior of seeds. Moreover, provenances (ecotypes) and harvesting period shall be considered (Petrie et al. 2016).

4 Conclusions

Seed dormancy is known to occur in many tropical tree species. Seeds of most arid and semi-arid tree species areas cannot germinate promptly when subjected to conditions favorable for germination due to impermeable seed coat to water. The study revealed the existence of considerable variation in germination among species with respect to germination percentage and mean germination time subjected to different duration of soaking into sulphuric acid. Insufficient soaking may not be effective enough as it just makes the seed coat glossy. Furthermore, concentration of the acid and time of exposure are very critical and need to be quantified for each species since seeds exposed for a long time get damaged easily. The best duration of immersion may reduce mean germination time and increase final germination percentage.

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