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Original Research Article

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ABSTRACT

Islands are well-known to house an incredible biodiversity, higher level of threatened species and extinctions than mainlands. Because of human activities and climate change, these endangered regions required advanced conservation and restoration strategies.

In this study, we investigated seed germination ecology of four native Malvaceae species (*Dombeya acutangula*, *Dombeya populnea*, *Ruizia cordata* and *Thespesia populneoides*) of degraded dry lowlands of Reunion Island. We measured seed imbibition, investigated the dormancy class of seeds, determined light requirements for germination and identified treatments to improve germination of these species. For this purpose, germination tests were performed over 32 days and two photoperiods (12 h/12 h or 0 h/24 h), by using seeds treated with H_2O_2, gibberellic acid and smoke water. Mechanical and chemical scarifications as well as heat treatment were also carried out. Germination percentages were analyzed by fitting generalized linear models (GLMs) with a logit link function.

For the four species, a significant mass increase is detected for scarified seeds after a week imbibition (>60% mass increase). Germination percentages of seeds treated with H_2O_2, GA_3 and smoke water were not significantly different from control seed. On the contrary, scarified seeds exhibited significantly higher germination percentages than control seeds. In addition, germination speed was also significantly higher for scarified seeds. These results are congruent with a physical dormancy phenomenon of seeds of the four Malvaceae shrubs. In addition, no particular light requirement was observed for germination. Artificial methods to break dormancy and stimulate germination described in this study could improve establishment success in arid habitats.

1. Introduction

Human role in global biodiversity loss is nowadays a recognized fact (Chapin et al., 2000). Over the past centuries, human activities such as landscape transformation for urbanization or agriculture, hunting or exotic species introduction have severely increase habitat loss and associated species extinction rates (Barnosky et al., 2011; Brooks et al., 2006). Congruently,

climate change has also been raised as a major threat to biodiversity loss by increasing the effects of habitat destruction (de Chazal and Rounsevell, 2009; Sala et al., 2000). Given the limited international resources (Kier et al., 2009), conservationists now have to set up priorities in conservation activities in order to limit biodiversity loss (Myers et al., 2000) and to develop cost-effective conservation strategies (Mantyka-Pringle et al., 2015).

When compared to mainland areas, oceanic islands are well-known to house an incredible biodiversity and high levels of endemism (Kier et al., 2009). Due to their high endemism rates, islands communities are more sensitive to disturbances and require advanced conservation and restoration strategies (Courchamp et al., 2014; Kaiser-Bunbury et al., 2010). Including Malagasy, the Mascarene archipelago, i.e. Reunion Island, Mauritius and Rodrigues, is recognized as a biodiversity hotspot (Mittermeier et al., 2011). Among these islands, Reunion Island (2512 km^2) conserves by far the largest area of relatively intact habitats in the archipelago (Thébaud et al., 2009). However, invasion caused by introduced species and increasing urbanization pressure in order to fulfill the needs for future human populations are the most influencing factors in habitat loss on the island (Catry and Besnard, 2015; Strasberg et al., 2005). Thus, in order to limit habitats loss and to preserve the remaining ecosystems, the island is admitted as a global priority for conservation (Thébaud et al., 2009). In addition, regulatory tools are also being reinforced for the island plant conservation. The list of protected plants species of Reunion Island was therefore recently extended from 61 to 238 species, in order to facilitate conservation of its exceptional flora (Ministère de la Transition écologique et solidaire, 2017).

The most severe degradation has been observed in lowlands (<1000 m) of the West of the island (coastal habitats, savannas and dry forests) (Strasberg et al., 2005) where their original habitats were reduced to 1% after coffee cultivation, wood exploitation and urbanization (Sarrailh et al., 2008). In these areas, several native (and endemic to the Mascarene archipelago or even to Reunion Island) woody species (especially shrub species) of Malvaceae were common. Nevertheless, their populations severely decreased and now remain in patches or even in isolated individuals. This is the reason for adding four shrub species recently to the list of protected species of Reunion Island (Ministère de la Transition écologique et solidaire, 2017). Thus, *Ruizia cordata* [the only species of *Ruizia*, endemic to Reunion Island (Bosser et al., 1987)], which naturally occurred in dry lowlands of the West of the island, no longer persists in natural habitats (Gigord et al., 1999) and only 5 individuals remain (Augros et al., 2015). *Dombeya* is indigenous to Mascarene archipelago (Gigord et al., 1999), and in Reunion Island, several *Dombeya* species occur in small isolated populations with very few individuals (Humeau et al., 2000). Some species such as *D. populnea* and *D. acutangula* were commonly observed in dry lowlands of the island (Bosser et al., 1987). These species occur in small, relict, fragmented populations in semi-dry tropical forests in the western half of the island and are extremely rare in natural habitats where they have mostly disappeared and are protected by law (Gigord et al., 1999; Humeau et al., 1999). *Thespesia* is a pantropical genus consisting of 16−17 species (Areces-Berazain and Ackerman, 2016). In Reunion Island, *T. populneoides* which is indigenous (Boullet, 2015) was found on coastal habitats of the West of the island (Bosser et al., 1987). Due to the confusion often made with an introduced species *T. populnea*, the natural populations of this species are difficult to estimate (Boullet, 2015). Nevertheless, recently a few individuals have been observed on the West shore of the island (Boullet, 2015).

Consequently, these species are targeted for conservation and for cultivation of individuals in greenhouses for their reintroduction. In addition to conservation and propagation, native species could also be used in rehabilitation and/or restoration of damaged ecosystems (Baskin and Baskin, 2014). Indeed, using native species that are eco-physiologically adapted to local conditions (Ammondt and Litton, 2012) is a widely proposed method for rehabilitation of degraded areas (García-Palacios et al., 2010; Kiehl et al., 2010; Tinsley et al., 2006).

Germination ecology studies contribute to a better understanding of biological concepts such as plant reproductive strategies, life history traits and adaptations to habitats (Baskin and Baskin, 2014). Such information can have several applications and can benefit to conservationists and also allow applications in restoration ecology. Indeed, low germination capacity (which may be related to the viability, vigor or dormancy of seeds) is one of the reasons that have been previously suggested to determine success or failure of native species installation on degraded areas (Bochet et al., 2010; Oliveira et al., 2012, 2013). More precisely, limited knowledge about seed dormancy breaking and other germination requirements hinders revegetation programs with many species targeted for that purpose (Alday et al., 2010; Bochet et al., 2007; Marty and Kettenring, 2017; Matesanz et al., 2008; Oliveira et al., 2013). In Reunion Island, knowledge about seed germination ecology (especially about dormancy and photoblasty) of native species is scarce and only a few published research papers are available (e.g. Gigord et al., 1999; Leperlier et al., 2018).

Seed dormancy is a thoroughly studied phenomenon which is known to be set by species to adapt to the environment and to wait for suitable environmental conditions to maximize chances of seedlings survival and establishment (Finch-Savage and Leubner-Metzger, 2006; Née et al., 2017). Seed dormancy can be defined as the failure of an intact viable seed to complete germination (Bewley, 1997). In dormant seeds, germination is prevented at a time of the year when the environment does not remain favorable long enough for seedlings to survive (Baskin and Baskin, 2014).

Numerous Malvaceae species produce dormant seeds [reviewed in (Baskin and Baskin, 2014)]. Physical dormancy (PY) is one of the most occurring dormancy classes within this family and concerns several tropical habitats (e.g. tropical rainforests, dry woodlands, savannas …) and several life forms (e.g.herbaceous species, shrubs and trees). In savanna, Malvaceae is also one of the families in which PY is commonly seen in seeds.

Light is an important germination regulation factor (Probert et al., 1985), being one of the environmental cues that can promote germination (Baskin and Baskin, 2014; Thompson and Ooi, 2010). Although dormancy is a thoroughly studied phenomenon, light requirements are often missing in germination ecology studies. Light requirements are linked to ecological functions such as soil depth detection, avoiding germination too deep in the soil making seedling unable to emerge

([Benvenuti et al., 2001](); [Bond et al., 1999]()). It also serves as gap sensing after disturbance, in order to indicate that conditions are now suitable for seedlings to establish ([Jiménez-Alfaro et al., 2016]()). Therefore, in germination studies, assessing light requirements for seed germination seems critical to a better understanding of germination dynamics.

The purpose of this study was to provide knowledge about dormancy and light requirements of seeds of four shrub species in Malvaceae that are naturally distributed in lowland arid habitats (dry woodlands and coastal habitats) of the West of Reunion Island. Despite the similarities observed among the four species (e.g. habitats, life forms, fruit types), data about germination ecology of these species is scarce (e.g. [Gigord et al., 1999]()). Acquiring such germination data would be suitable for applications such as economical valorization due to esthetic values of native shrubs, coastal habitat soil rehabilitation (e.g. for *T. populneoides*), conservation and/or even ecological restoration (e.g. *D. acutangula*, *D. populnea*, *R. cordata*). In order to fulfill these objectives, we aimed:

(1) To assess whether the seeds of *D. acutangula*, *D. populnea*, *R. cordata* and *T. populneoides* are dormant or not;
(2) If dormant, to determine the dormancy class of these seeds;
(3) To determine light requirements for germination of these species;
(4) To identify treatments to improve germination of these species.

2. Material and methods

2.1. Study species

Dombeya acutangula Cav. is a heterophyllous hermaphrodite shrub which can reach 4 m. It is one of the 12 species from this genus that naturally occur in Reunion Island. *Dombeya* genus contains around 200 species distributed in Africa, Madagascar, and in Mauritius and Rodrigues of Mascarenes archipelago. *D. acutangula* individuals could be mostly found in dry lowland on the West shore of the island but a few individuals have also been found in Cilaos and Mafate at an altitude of 1200 m. Fruits are dry indehiscent capsules with 5 locules and contain around 10 seeds. Fruits are 1 cm large ([Bosser et al., 1987]()) while seeds size is 2.87 ± 0.21 mm (Leperlier et al., unpubl. data). A photograph of a *D. acutangula* seed is presented in [Fig. 1]()a.

Dombeya populnea (Cav.) Baill. is a heterophyllous dioecious shrub which is 10 m high and with a trunk reaching 30 cm. This species is one of the 12 species from this genus that naturally occur in Reunion Island. Individuals were found in several dry lowland areas in the West of the island. As none of the juveniles have been found, the species has been classified as critically endangered (CR) ([UICN France et al., 2013]()). Fruits are dry indehiscent capsules containing 2 or 3 seeds ([Bosser et al., 1987]()). Fruits are 4−6 mm large ([Bosser et al., 1987]()) and seed size of this species is 2.15 ± 0.10 mm (Leperlier et al. unpubl. data). Seeds have a hard testa ([Bosser et al., 1987]()). A photograph of a *D. populnea* seed is presented in [Fig. 1]()b.

Ruizia cordata Cav. is a heterophyllous dioecious shrub of about 10 m and a trunk reaching a 40 cm diameter. It is the only species of *Ruizia* in the island. Rare individuals can be found in dry lowland of the West of the island. Fruits are dry indehiscent capsules with 10 locules containing 1 or 2 albuminous seeds each ([Bosser et al., 1987]()). Fruits are 7 mm large ([Bosser et al., 1987]()) while seeds size is 2.22 ± 0.20 mm (Leperlier et al. unpubl. data). Seeds have a relatively hard testa ([Bosser et al., 1987]()). A photograph of a *R. cordata* seed is presented in [Fig. 1]()c.

Thespesia populneoides (Roxb.) Kostel. is a shrub which is 7 m high and a trunk reaching a 30−35 cm diameter. In Mascarene archipelago, 2 species exist, *T. populneoides* and *T. populnea*, a pantropical species. *T. populneoides* is often mistaken in Reunion Island with *T. populnea* due to their similar characteristics. It was found on sandy beaches on the West coast of the island. Fruits are indehiscent dry fruits with 5 locules and contain around 10−15 seeds ([Bosser et al., 1987]()). Fruits are 3.5 cm large ([Bosser et al., 1987]()) while seeds size is 10.52 ± 0.55 mm (Leperlier et al. unpubl. data). Seeds are hairy with a hard seed coat ([Bosser et al., 1987]()). A photograph of a *T. populneoides* seed is presented in [Fig. 1]()d.

2.2. Seed collection

The seeds used in this study came from hand-harvested fruits directly from the shrubs. Based on preliminary phenological monitoring, the harvests took place in urban areas of the South of Reunion Island in July 2016 for *D. acutangula*, in October 2016 for *D. populnea* and in June 2017 for *R. cordata* and *T. populneoides*. Right after the harvest, the fruits were stored in plastic bags in the CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) laboratories in the South of the island (21° 19′ 16″ S; 55° 29′ 06″ E) at ambient room temperature ($26,6 \pm 0,8$ °C) with a relative humidity of $64,2 2 \pm 3,4\%$ until being used for the experiments.

2.3. Classification used for the study

Comparison of dormancy among species can be difficult. In order to simplify data communication among studies, several classifications have been proposed. The classification system proposed by [Baskin and Baskin (2004)]() was used in order to identify dormancy in this study.
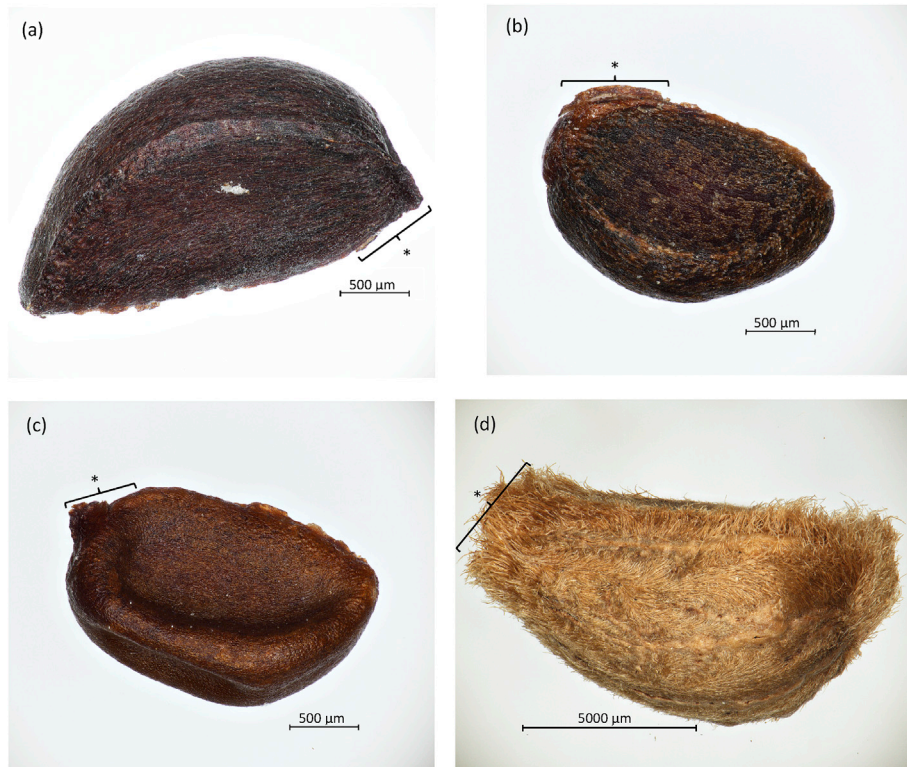
Fig. 1. Seed morphology of (a) *D. acutangula*, (b) *D. populnea*, (c) *R. cordata* and (d) *T. populneoides.* *Position of hilum.

2.4. Imbibition

To determine whether the seed coat is water-permeable or water-impermeable, 5 replicates of 20 seeds from three samples (one lot without scarification (control) and two lots with two different scarification methods: mechanical scarification by sand paper for *D. acutangula*, *D. populnea* and *R. cordata* and by a razor blade for *T. populneoides*; chemical scarification by sulfuric acid (H_2SO_4) during 10 min for *D. acutangula*, *D. populnea* and *R. cordata* and during 60 min for *T. populneoides*) were weighed before imbibition, using the weighing scale (with a 0.001 g precision) of the moisture content analyzer (Denver Moisture Analyzer − Model IR120, Denver Instrument). Each seed sample was placed on moist filter paper (Whatman no. 1) in a Petri dish at ambient room temperature for 7 days. At the end of the imbibition period, seeds were weighed again. A substantial increase in mass would indicate that seeds have water-permeable coats (Bansal et al., 1980).

2.5. Dormancy breaking treatments

2.5.1. Making seeds permeable

Various treatments were assessed to make physically dormant seeds permeable. First, mechanical scarification using sand paper was performed according to Pérez-García (2009). Seeds were blotted between two sand paper sheets and scarified until stripes were visible to the observer. Chemical scarification was conducted using highly concentrated sulfuric acid (H_2SO_4) (Oldham and Ransom, 2009). For this treatment, seeds were soaked in a beaker filled with concentrated sulfuric acid (98%, v/v) during 1, 5 and 10 min for small seeds of *D. acutangula*, *D. populnea* and *R. cordata* and 15, 30 and 60 min for bigger seeds of *T. populneoides*. As another treatment, immersion in hot water was carried out according to Baskin and Baskin (2014). Thus, seeds were soaked in heated water (80 °C) in a beaker until cooling down, and let in the same beaker for 16 h.

2.5.2. Enhancing germination

Germination of seeds with PD can be increased by chemicals (Baskin and Baskin, 2014). So, prior to sowing, seeds were treated with a 10 mg.L^{-1} gibberellic acid solution (GA_3), or with a 0.3% (v/v) hydrogen peroxide solution (H_2O_2) or with smoke infused water.

The smoke infused water solutions were prepared in CIRAD laboratories according to Coons et al. (2014). In their process, the smoker fuel was burned inside a stainless steel bee smoker. The smoke flows from the smoker through a heat-resistant

rubber hose. The end of the rubber hose is placed in a 1000-mL side-arm flask containing 300 mL of water. A water aspirator is attached to the side arm of the flask. The vacuum generated by the aspirator is used to help draw the smoke from the smoker into the water. In order to prepare the high volumes needed (4L per preparation), the process was carried out using an aquarium pump instead of running water. During the process, 150 g of smoker fuel (CHARM'COMBUST, Thomas Apiculture) were burnt. The combustion was carried out until total plant material combustion (150 min). After cooling down, the smoke water solution was stored at 5 °C until being used. Between each preparation, all the material was cleaned up in order to retrieve all the smoke residues and ashes.

2.6. Germination tests

Germination tests were carried out in July 2017. For each of the pre-sowing treatments, 5 replicates of 20 seeds were sown in Petri dishes (60 × 15mm or 94 × 16mm, Greiner Bio-One International GmbH) filled with white pool filter sand, moisturized until saturation. The sand was heated to 140 °C for 2 h in a drying oven prior to sowing in order to ensure limited pathogen growth. To assess the effect of treatments on germination, untreated seeds (5 replicates of 20 seeds with no treatment) were also tested for germination. The Petri dishes were then placed in an incubation chamber by setting the temperature at the constant temperature of 25° ± 0.3 °C to approximate natural annual mean temperature of dry lowlands of the West of Reunion Island.

To determine light requirements for germination, two experiments were carried out, the first one in daily photoperiod (12 h/12 h) with all treatments and the second one after obtaining results from the first. Only the treatment which allowed the highest final germination percentages (GP) in first experimental run was kept for this one. In this experiment, GPs of such treated seeds were compared among 2 light conditions: daily light (12 h/12 h) and constant darkness (0 h/24 h) (Baskin and Baskin, 2014). The darkness was induced by putting the Petri dishes in a black box at the beginning of the tests.

In both experiments, germination was monitored daily (or at 2 days intervals) for Petri dishes in light conditions and once in a week for the ones in darkness to limit light exposure. A seed was considered germinated at the emergence of the radicle. During the observation, moisture was also visually checked and adjusted with filtered water if necessary. Each test was ended after 32 days. Germinated seeds were retrieved from the dishes. Mean GP (%) was calculated at the end of the test by calculating the mean (±SE) of all the replicates of each treatment. Subsequently, to determine germination speed of seeds under different treatments in light, the time needed to reach 50% of the final germination (T_{50}) was also interpolated (Pritchard and Manger, 1990). The final germination was defined as 100% to measure the speed independently from the total amount of germinated seeds of each treatment (Leiblein-Wild et al., 2014).

2.7. Statistical analyses

One-way analysis of variance (ANOVA) was performed to analyze seed mass increase data over treatments. Multiple pairwise means comparisons were then conducted using Tukey's range test in order to separate means.

Germination percentages were analyzed by fitting generalized linear models (GLMs) with a logit link function. Binomial distribution is appropriate for proportion-based data such as germination data (Hay et al., 2014). In order to overcome residuals over-dispersion a quasi-binomial error distribution was thus used to analyze germination percentages. Germination percentages were compared among treatments for seeds placed in daily light. Germination percentages were then compared among light conditions for the second run. For the analyses, only germination data for treatments that exhibited germination percentages different from 0% were tested. In order to separate means in all experiments, multiple comparisons were conducted using parametric models multiple comparisons procedures, with the "glht" function of the "multcomp" package in R.

In order to compare germination speed (T_{50}) among treatments for seeds placed in daily light for the four species, GLMs were performed using Gamma error distribution with a log link function (Chamorro et al., 2013). Means were then separated using the same procedures as for GPs analyses.

All the statistical analyses and figures presented in the result section were performed using R version 3.3.1 (R Core Team, 2016) with ggplot2 (Wickham, 2009), sciplot (Morales, 2012), multcomp (Hothorn et al., 2008) and multcompView (Piepho, 2004).

3. Results

3.1. Imbibition

Both scarified and non-scarified (control) seeds showed a significant mass increase for the four species after a week of imbibition (t.test, data not shown). Significant differences between mean increases in mass were detected after 7 days of imbibition over treatments for *D. acutangula*, *D. populnea*, *R. cordata* and *T. populneoides* (P = 5.00 × 10^{-13}; P = 2.58 × 10^{-8}; P = 4.79 × 10^{-7}; P = 6.70 × 10^{-7} respectively). For each species, scarified seeds exhibit higher increases in mass than control seeds (a three-fold to more than a ten-fold increase). For instance, seeds of *D. acutangula* treated with sulfuric acid exhibit an 86.00 ± 2.76% mean mass increase while control seeds exhibit an increase of 9.00 ± 1.48%. For *D. populnea*, sand paper scarified seeds exhibit a 59.00 ± 2.67% mean mass increase while control seeds exhibit an increase of 6.00 ± 0.91%. For *R. cordata* seeds treated with sulfuric acid exhibit a 61.00 ± 3.70% mean mass increase while control seeds exhibit an increase of

22.00 $\pm$ 1.62%. For *T. populneoides* sand paper scarified seeds exhibit an 88.00 $\pm$ 5.18% mean mass increase while control seeds exhibit an increase of 10.00 $\pm$ 1.24%. Except for *D. acutangula*, there was no significant difference between scarification methods (Fig. 2).

3.2. Germination tests

For the four species, a significant effect of treatments on final germination percentage was detected (P $= 2.47 \times 10^{-11}$; P $< 2.20 \times 10^{-16}$; P $= 5.22 \times 10^{-7}$; P $< 2.20 \times 10^{-16}$ for *D. acutangula*, *D. populnea*, *R. cordata* and *T. populneoides,* respectively). Germination percentages of seeds treated with H_2O_2, GA_3 and smoke water were not significantly different from control seeds, except for *T. populneoides,* where H_2O_2 treated seeds exhibit significantly lower germination percentages than control seeds. On the contrary, scarified seeds (wet heat, sulfuric acid and mechanical scarification) mostly exhibit significantly higher germination percentages than control seeds (Fig. 3).

For instance, seeds of *D. acutangula* treated with sulfuric acid during 10 min exhibit a final germination percentage of 84.00 $\pm$ 3.67% while control seeds exhibit that of 35.00 $\pm$ 4.47% (Fig. 3a). Seeds of *D. populnea* treated with sulfuric acid during 10 min exhibit a final germination percentage of 87.00 $\pm$ 4.06% while control seeds exhibit a final germination percentage of 6.00 $\pm$ 1.87% (Fig. 3b). Seeds of *R. cordata* treated with sulfuric acid during 10 min exhibit a final germination percentage of 42.00 $\pm$ 4.36% while control seeds exhibit a final germination percentage of 13.00 $\pm$ 3.00% (Fig. 3c). Seeds of *T. populneoides*
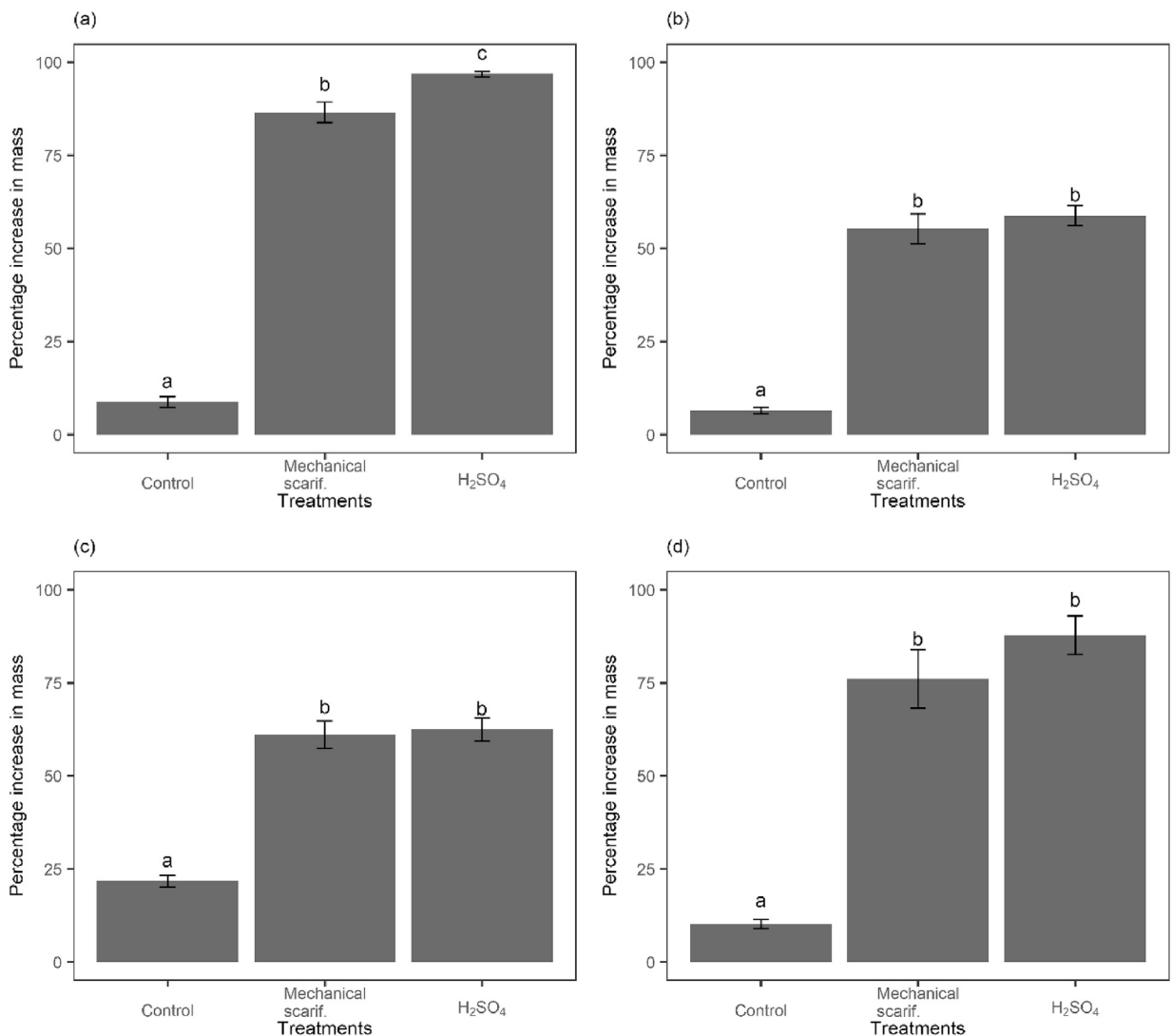

Fig. 2. Percentage increase in mass for seeds of (a) *D. acutangula*, (b) *D. populnea*, (c) *R. cordata* and (d) *T. populneoides* over different treatments after a week (168 h) of imbibition on moist Whatman paper at room temperature. Means $\pm$ SE are represented. Means with the same letters are not significantly different at P < 0.05. mechanical scarification.
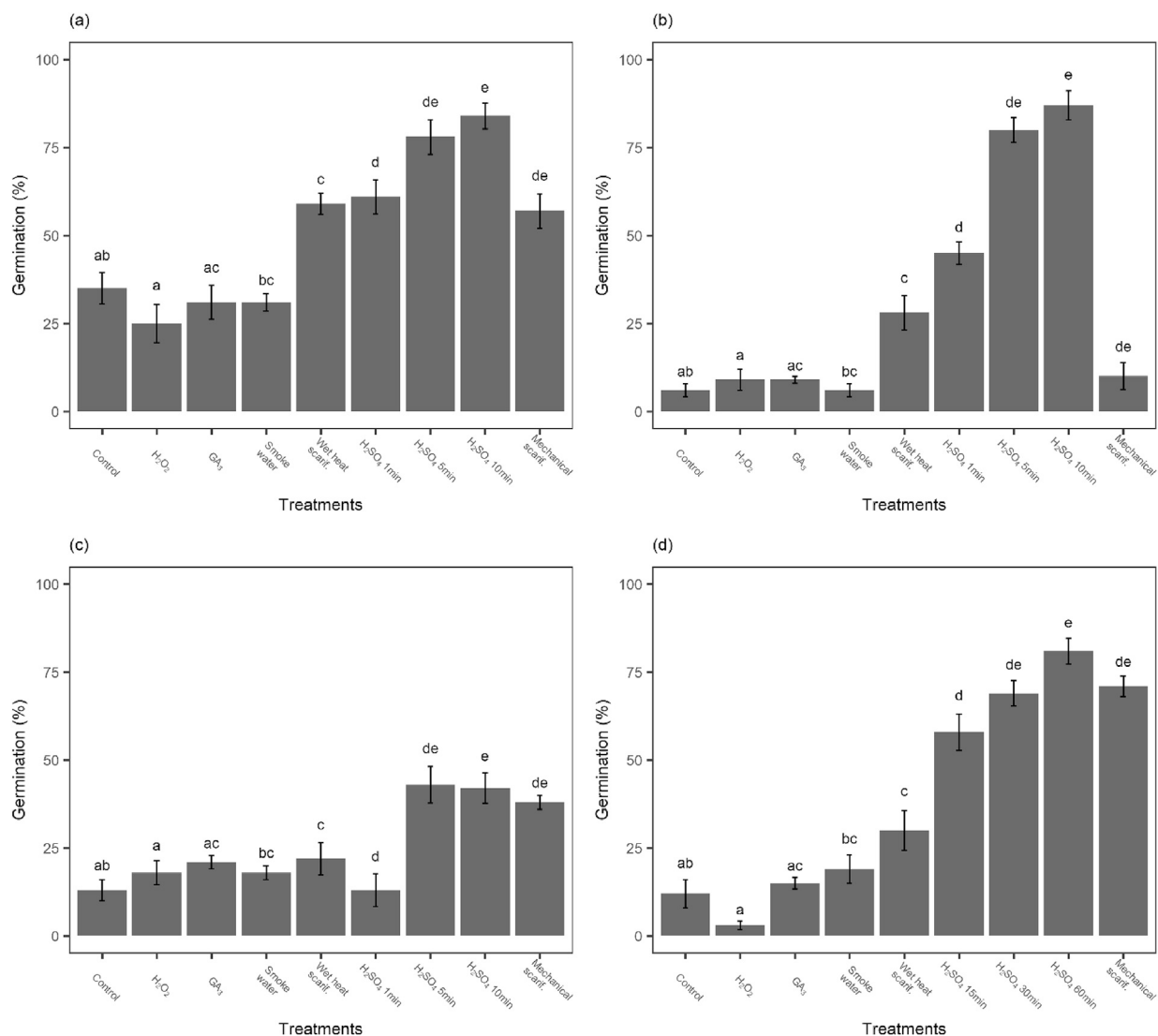
Fig. 3. Cumulative germination (%) within 32 days of seeds of (a) *D. acutangula,* (b) *D. populnea,* (c) *R. cordata* and (d) *T. populneoides* incubated under daily light conditions (12 h/12 h) at 25 °C. Means ± SE are represented. Means with the same letters are not significantly different at P < 0.05. Mechanical scarif: mechanical scarification.

treated with sulfuric acid during 60 min exhibit a final germination percentage of 81.00 ± 3.67% while control seeds exhibit a final germination percentage of 12.00 ± 4.06% (Fig. 3d). However, for *D. populnea,* germination percentages of mechanically scarified seeds are not significantly different from control seeds (Fig. 3b). For *R. cordata,* germination percentages of scarified seeds during 1 min in sulfuric acid and of wet heat treated seeds are not significantly different from control seeds (Fig. 3c). For *T. populneoides,* germination percentages of wet heat treated seeds are not significantly different from control seeds (Fig. 3d).

For the four species, a significant effect of treatments on germination speed (T_{50}) was revealed for *D. acutangula, D. populnea, R. cordata, T. populneoides* ($P = 2.89 \times 10^{-10}$; $P = 1.06 \times 10^{-7}$; $P = 1.61 \times 10^{-2}$; $P < 2.20 \times 10^{-16}$, respectively). In each case, T_{50} is significantly lower for seeds with a scarification method (Table 1).

For *D. acutangula,* seeds treated with sulfuric acid during 5 min exhibit a T_{50} of 4.64 ± 0.48 days, while control seeds (and other treated seeds such as H_2O_2, GA_3 and smoke water treated seeds) exhibit the highest T_{50}. The same observations can be made for *D. populnea, R. cordata* and *T. populneoides* seeds. For *D. populnea,* T_{50} is the lowest for seeds treated with sulfuric acid during 10 min (11.37 ± 0.52 days), for *R. cordata,* T_{50} is the lowest for mechanically scarified seeds (13.05 ± 0.44 days) and for *T. populneoides* T_{50} is the lowest for mechanically scarified seeds (3.92 ± 0.54 days).

No significant effect of light conditions on germination percentages was detected for *D. acutangula*, *D. populnea* and *R. cordata* ($P = 0.067$, $P = 0.123$ and $P = 0.359$, respectively). However, for *T. populneoides,* light conditions had significant effect on germination percentages ($P = 5.92 \times 10^{-3}$). For this species, germination percentages of seeds placed in daily light germinated to 46.00 ± 4.30%, while those placed in darkness only germinated to 25.00 ± 3.54% (Table 2).

Table 1

Germination speed (T_{50}) of seeds under different treatments for seeds placed in daily light for the four species. Means ± SE are represented Means with the same letters are not significantly different at P < 0.05.

Treatments	*D. acutangula*	*D. populnea*	*R. cordata*	*T. populneoides*
Control	13.77 ± 0.78^c	24.96 ± 3.28^c	17.70 ± 1.53^{ab}	18.24 ± 2.36^d
H_2O_2	14.90 ± 1.04^c	16.50 ± 2.48^{bc}	20.00 ± 2.68^{ab}	21.70 ± 2.91^d
GA_3	14.97 ± 2.22^c	21.10 ± 2.81^c	17.70 ± 1.60^{ab}	22.95 ± 4.28^d
Smoke water	18.90 ± 3.12^c	25.32 ± 2.36^c	21.85 ± 1.93^b	16.70 ± 3.77^{cd}
Wet heat scarification	16.01 ± 2.86^c	23.40 ± 2.25^{bc}	23.43 ± 2.94^{ab}	18.00 ± 3.45^d
H_2SO_4 1min (15min)[a]	10.80 ± 0.44^{bc}	16.43 ± 1.43^{bc}	13.90 ± 2.81^{ab}	8.90 ± 1.35^{bc}
H_2SO_4 5min (30min)	4.64 ± 0.48^a	14.45 ± 0.57^{ab}	15.90 ± 1.54^{ab}	5.22 ± 0.18^{ab}
H_2SO_4 10min (60min)	7.06 ± 1.61^{ab}	11.37 ± 0.52^a	15.18 ± 1.09^{ab}	4.04 ± 0.53^a
Mechanical scarification	10.74 ± 2.40^{bc}	23.75 ± 3.88^c	13.05 ± 0.44^a	3.92 ± 0.54^a

[a] Indications between parentheses are duration treatments for seeds of *T populneoides* treated with sulfuric acid.

Table 2

Final germination percentages (GP) of scarified seeds under different light conditions for the four species. Means ± SE are represented. Bold P-values are significant.

	Photoperiod	GP (%)	P-value
D. acutangula	Constant darkness (0 h/24 h)	81 ± 4.30	0.067
	Daily light (12 h/12 h)	67 ± 4.90	
D. populnea	Constant darkness (0 h/24 h)	83 ± 4.07	0.123
	Daily light (12 h/12 h)	72 ± 4.90	
R. cordata	Constant darkness (0 h/24 h)	57 ± 4.06	0.359
	Daily light (12 h/12 h)	48 ± 8.31	
T. populneoides	Constant darkness (0 h/24 h)	25 ± 3.54	**5.92×10^{-3}**
	Daily light (12 h/12 h)	46 ± 4.30	

4. Discussion

Imbibition of non-scarified seeds of the four species remained low (<23%) after one week on the moistened paper. On the other hand, the values strongly increase after scarification (>60% mass increase). These differences suggest that the seeds possess a hard coat making them water-impermeable (Baskin and Baskin, 2014).

Germination percentages (GP) was lower and germination speed (T_{50}) was higher in untreated seeds place in light for the four species. Low germination levels and speed of control seeds are consistent with seeds having a dormancy (Baskin and Baskin, 2014). Furthermore, for all the species, scarification allowed significant improvement in GP (with the exception of mechanical scarification for D. populnea, for which GP were low. These results were attributed to the thicker seed coat on seeds of this species (pers. obs.)) and reduced T_{50}. According to the revised criteria for dormancy determination from Baskin and Baskin (2014), these observations altogether with the seed water-impermeability we observed allow us to conclude that seeds of the four studied Malvaceae shrub species possess a physical dormancy. Same observations were made on other species in this family, in relation with the permeability of seed coat (e.g. Galíndez et al., 2010; Williams et al., 2005).

However, even untreated seeds imbibed water in slower rates, and were also able to germinate to some extent. Environmental factors such as mineral nutrition, water availability or day length can influence seed maturation (Hudson et al., 2015; Jaganathan and Liu, 2014) and cause changes in dormancy state (Baskin and Baskin, 2014). Variation in dormancy level allow seed germination to distribute widely within time across seasons and maximize chances of species establishment (Jaganathan et al., 2017).

Early germination and high germination percentages are usually linked to higher chances of installation of seedlings in an area (Donohue et al., 2010; Luis et al., 2008). However, early germination does not always guarantee survival (Quintana et al., 2004). In dry lowlands, where temperatures can be high and water availability can be low, species have to be able to develop strategies to maximize chances of seedling emergence and survival. Physical dormancy is one of the most often observed strategies among shrubs in arid habitats. Forty-two percent of dry lowlands shrubs possess seeds with PY (Baskin and Baskin, 2014). Species whose seeds have PY usually have the ability to form long-lived seed reserves and spread germination over seasons and maybe over years (Baskin and Baskin, 2014).

PY release involves formation of small opening(s) called water gap(s) (Baskin et al., 2000; Jayasuriya et al., 2009). In seeds with PY, water-gaps serve as environmental signal detector in determining the suitable timing for breaking dormancy and thus allow water uptake and germination (Jaganathan et al., 2017). In Malvaceae species in which seeds have PY, dormancy alleviation often involves the chalazal area (reviewed in Baskin and Baskin, 2014) and water can enter the seed after the chalazal plug is disrupted (Nandi, 1998; Seal and Gupta, 2000). The disruption of occluding structures is often due to two main factors: high temperatures and their fluctuation (Baskin and Baskin, 2014). The high temperatures allow the weakening of the occluding structure, while the high fluctuating temperatures allow expansion and contraction and at last a separation of the cells composing the structure (Taylor, 2005). Further studies are required to observe the occluding structure in seeds of the four species, and their disruption resulting in germination.

Seed response to light is often related to their ability to persist in the soil (Saatkamp et al., 2011) and act as a detection mechanism to soil depth or to a gap after disturbance (Jiménez-Alfaro et al., 2016). In our study, no light effect on germination was observed among the species except for seeds of *T. populneoides* for which GP was higher for seeds placed in light. However, we hypothesize that the lower germination percentages observed in darkness for this species could be due to the lower water supply during the experiment. To avoid biases in the experiment in darkness, moisture was adjusted only once a week. Nevertheless, when germination was checked in darkness, sand seemed drier for this species. Thus, a low water supply could have influenced the germination capacity of *T. populneoides* seeds in these light conditions.

There are numerous studies reporting the absence of light requirements for germination of seeds with PY after dormancy is broken (naturally or artificially) (e.g. Zida et al., 2005). Therefore, PY seems to be the most important factor controlling germination for the four species. Indeed, the success of plants whose seeds have PY tend to be more dependent to physical environment and rely on temperature more than light (Baskin et al., 2004). Dormancy breaking requiring high temperatures (Baskin and Baskin, 2014), they have to be exposed to light for their germination. A light requirement after PY is broken would thus be superfluous here (Baskin et al., 2004).

5. Conclusions

Our study contributes to a better understanding of germination ecology of four native shrub species (Malvaceae) of dry lowlands of the island. More precisely, based on our results, we can conclude that seeds of the four species have PY. This result is congruent with observation made on other shrub species from dry lowlands where species tend to set this germination strategy in order to escape harsh environmental conditions and to maximize establishment chances. In addition, mostly, no light requirement was observed for germination of these species in our study, which is similar to observation with other species whose seeds have PY.

Moreover, this study also allowed providing information on artificial methods to break dormancy and stimulate germination. Scarification methods described in this study could be used to overcome PY and promote seed germination of these shrub Malvaceae species of tropical dry lowlands. Because of the complementary goals of conservation and restoration, the methods could thus benefit professionals, which are interested in propagation of such species for their conservation or for restoration programs.

Declaration of competing interest

None.

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