applied biosciences

MDPI

Article

Seed Morpho-Anatomy and Germination Enhancement of the Australian Native Species *Lomandra longifolia* Labill. and *L. hystrix* (R.Br.) L.R. Fraser & Vickery

Fernanda Caro Beveridge *, Alwyn Williams, Robyn Cave, Sundaravelpandian Kalaipandian and Steve W. Adkins

School of Agriculture and Food Sciences, The University of Queensland, Gatton, QLD 4343, Australia
* Correspondence: fernanda.carobeveridge@uq.net.au

Abstract: *Lomandra* species are an important understory component of many Australian native ecosystems, contributing to the floristic richness and stabilizing soils. However, a limited understanding of their germination biology currently hinders their efficient use in seed-based restoration and ornamental plant production. The present study investigated *Lomandra longifolia* and *L. hystrix* diaspore morpho-anatomy and evaluated different mechanical and/or chemical treatments (nicking, leaching, smoke water and gibberellic acid [GA_3]) and under light or dark conditions to enhance germination. Embryos of both species were small and linear with a low embryo to seed ratio (<0.45). Germination rates of both species were significantly hastened by leaching seeds in running water for 36 h as compared to a non-leached seed. The results suggest that pre-treating both *Lomandra* species by leaching could maximize the effectiveness of seed used by resulting in faster, more uniform and, therefore, reliable germination of these species. Finally, seeds of *L. longifolia* had low final germination (<40%), with a high presence of viable but dormant seeds. The ecological cues that promote germination in nature for both species should be further examined.

Keywords: direct seeding; Australian native seed; seed-based restoration; seed dormancy; seed ecology; seed pre-treatments

Citation: Beveridge, F.C.; Williams, A.; Cave, R.; Kalaipandian, S.; Adkins, S.W. Seed Morpho-Anatomy and Germination Enhancement of the Australian Native Species *Lomandra longifolia* Labill. and *L. hystrix* (R.Br.) L.R. Fraser & Vickery. *Appl. Biosci.* **2023**, *2*, 222–235. https://doi.org/10.3390/applbiosci2020016

Academic Editor: Adriana Basile

Received: 16 March 2023
Revised: 28 April 2023
Accepted: 11 May 2023
Published: 16 May 2023

1. Introduction

Lomandra is a genus within the family Asparagaceae [1] that generally consists of small perennial herbs with a rhizomatous growth habit that often form tussocks [2]. *Lomandra longifolia* Labill. (spiny-head mat-rush) is one of the most widely distributed species in Australia [3] (Figure 1) and is particularly common in the south-east region of Australia [4]. It is highly adaptable and can grow in a wide range of habitats, such as on hillsides of dry forests, alongside creeks and coastal headlands. *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery (creek mat-rush) has a more confined natural distribution than *L. longifolia* and is found in the coastal regions of Queensland and New South Wales. Both species are commonly used as ornamental plants as well as for seed-based restoration projects [5]. These species are important understory components of many Australian ecosystems, providing shelter, breeding sites and food resources for native wildlife [4]. They have multiple benefits for seed-based restoration, which include their contribution to floristic richness and their ability to stabilize soils to prevent erosion due to their fibrous root system [6]. Large quantities of *L. longioflia* seeds are used annually to restore degraded Australian bushlands and disturbed plant communities due to human activity such as mining and construction [6]. Both species can be propagated using freshly produced diaspores (seed encased within the pericarp; hereafter, referred to as seeds) when sown in autumn conditions in Australia.

Even though *Lomandra* spp. are an important ecological, ornamental and restoration component in Australia, their seed biology has been poorly studied, hindering efficient seed use. It is well known that many Australian native species have diverse dormancy

mechanisms [7], which manipulates germination through time and space. The most common form of dormancy in dryland Australian natives is physiological dormancy (PD) [7,8]. Physiologically dormant seeds are permeable to water and have fully developed embryos, but the embryo has low growth potential and cannot overcome the mechanical constraints of its covering layers [9]. For this reason, they usually take >28 days to germinate [10]. *Lomandra* seeds often have very slow germination, taking up to 60 days to germinate [11–13]. Grant et al. [13] reported that although germination of *L. longifolia* was very low after 28 days of imbibition, there was a significant increase (63%) in germination after this period. Plummer et al. [2] suggested that this delay in germination is due to dormancy mechanisms, possibly within the pericarp. Further, they found that *Lomandra sonderi* (F.Muell.) Ewart. and *Lomandra drummondii* (Benth.) Ewart. reached full water imbibition within 24 h and concluded that *Lomandra* spp. do not possess water-impermeable seed coats and, therefore, do not present physical dormancy (PY).

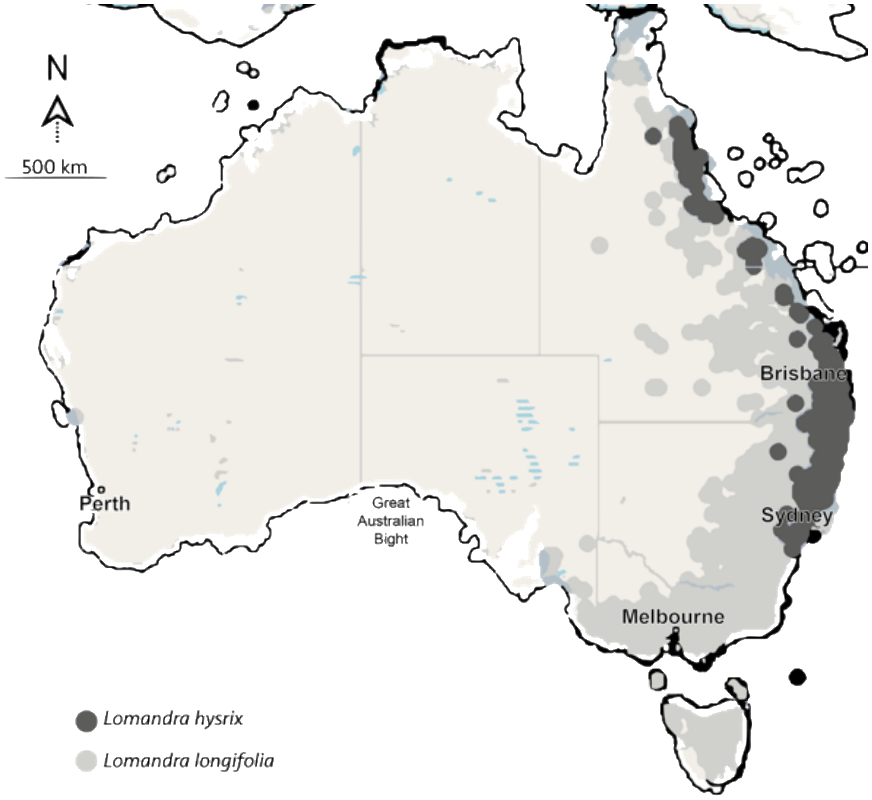

Figure 1. Natural distribution of *Lomandra longifolia* Labill. (light grey) and *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery (dark grey) in Australia (Source: Atlas of Living Australia).

Identifying the mechanisms that control dormancy and germination, together with finding new ways to hasten seed germination and seedling emergence, could be of great importance to achieve cost-effective usage of *Lomandra* seeds. For example, studies have shown that a combination of smoke water and stratification can increase seed germination to 50% in *Lomandra preisii* (Endl.) Ewart. [7], which could be associated with an overcoming of PD. Moreover, leaching of inhibitors from *L. longifolia* seeds with five cycles of soaking and rinsing combined with warm stratification significantly improved its germination [14]. Similarly, leaching seeds with running tap water or pericarp removal could overcome dormancy in *L. sonderi* and could achieve germination of *ca.* 20% of the seeds. Similar treatments increased germination from 40 to 80% in *L. drummondii* [2]. The authors related the increases in germination with the removal of germination-inhibiting chemicals found in the tissues surrounding the embryo. Gibberellic acid (GA_3) and smoke water have also been used successfully in promoting germination in *L. sonderi* [2]. Gibberellic

acid is known to stimulate endosperm weakening and stimulate embryo germination [15]; smoke water has been shown to promote germination in a wide range of Australian native species [7,16,17]. Furthermore, scarification and seed nicking (a small cut through the tissues surrounding the embryo) of the pericarp and seed coat can be effective in overcoming PD or morpho-physiological dormancy (MPD) for several species. These treatments can relieve mechanical restrictions of the fruit tissues and/or seed coat, allowing embryo growth [18,19]. This can be particularly helpful in seeds that have a low embryo growth potential.

Microscopic investigation of seed internal morpho-anatomy is useful to characterize the seed and help elucidate germination biology aspects. Such analysis can determine embryo size, shape and location within the seed to determine the embryo to seed ratio (E:S) and size of the endosperm and/or cotyledons [10]. Documentation of embryo characteristics can also be used in determining the presence of morphological dormancy (MD) or MPD associated with embryo development [20]. Underdeveloped embryos have differentiated organs and tend to have low E:S ratios [21]. Embryos in seeds with MD need to undergo a growth period prior to radicle protrusion [10] (for example, in [22]). There are few studies that focus on morpho-anatomy characterization of *L. longifolia* and *L. hystrix* seed (such as [5,6]). This information could provide helpful insights into explaining the dormancy and slow germination rates observed for both species.

The current study describes the seed morpho-anatomy of both *Lomandra* species and determines methods for elevating seed germination to enable a more cost-efficient use of these seeds in seed-based restoration projects and ornamental plant production. Thus, the objectives were to (1) identify relationships between germination, seed fill and seed morpho-anatomical structures (embryo and seed size); and (2) develop methods to speed up the rate and increase the final seed germination by investigating chemical and mechanical seed treatments to overcome possible PD at optimum alternating temperatures in light or dark conditions. This information will provide an understanding of the dormancy mechanisms that are preventing germination and will help in developing improved seed germination protocols for *L. longifolia* and *L. hystrix*. Improved germination would also lead to increased use of these highly beneficial species in land regeneration projects and reduce the costs associated with seed wastage.

2. Materials and Methods

2.1. Seed Lots

Lomandra longifolia and *L. hystrix* seeds were obtained from a commercial seed supplier (Native Seeds and Land Repair, Maleny, QLD, Australia). *Lomandra longifolia* seeds were collected from the suburb of Redlands, part of the Brisbane metropolitan area in south-east Queensland during December 2017, while *L. hystrix* seeds were collected from the suburb of Caloundra, Sunshine Coast Region in south-east Queensland during January 2017. Both seed batches had >90% viability as determined by the supplier. After delivery, seeds were stored in a seed storage cabinet at 15 ± 1 °C temperature and 15 ± 3% relative humidity until used. Seed age was 16 months for *L. longifolia* and 27 months for *L. hystrix* when used.

2.2. Seed Fill, Weight and Morpho-Anatomy

Seeds of both species were examined by using an X-ray machine (Faxitron MX-20 Imaging system, Lincolnshire, IL, USA) to determine seed fill percentage. Seed samples (5 replicates of 25 seeds per species) were exposed to 18 Kv of X-ray tube voltage for 20 s and images were captured using Bioptics software (Olympus, Tokyo, Japan) at 2× magnification of resolution. The percentage of filled seeds was determined by counting the number of seeds that had a full-sized endosperm and embryo. Filled seeds had a white color, and damaged or unfilled seeds were indicated by black areas inside the seed. The percentage of filled, partially filled (seeds with parts of their endosperm and/or embryo missing) and unfilled seed was determined. Partly filled and unfilled seeds were considered non-viable. Filled seeds with intact and healthy-looking embryos were considered viable. To determine

the 100-seed mean weight, 5 samples of 100 seed from each species were randomly selected and weighed.

Seed anatomical structure was determined by photographic analysis by using a light microscope (Olympus SZX7, Mornington, TAS, Australia) with a digital camera attached. Seed and embryo size—specifically, length, width and area—were measured using CellSens software. To determine the E:S ratio, seeds were dissected longitudinally, and the embryo length was divided by seed length [23]. Embryo development was classified based on its anatomy (size and shape) according to Martin [21]. The presence of a developed or underdeveloped embryo was evaluated to identify if MD was present [24].

2.3. Germination Stimulation Using Mechanical and Chemical Treatments

Eight mechanical and/or chemical treatments were used *viz.* seed leaching, seed nicking, chemical treatment with smoke water at three different concentrations (Regen 2000 Smokemaster, batch no. 11957R, Tecnica, Bayswater, VIC, Australia), GA_3 (90% gibberellin A3, Sigma-Aldrich, lot BCBD6798V, St. Louis, MO, USA) or a combination of treatments (Table 1). Prior to treatments, all seeds were surface sterilized in 2% (*v*/*v*) sodium hypochlorite (NaOCl) solution for 10 min [25] with two drops of Tween 20 (Labchem, Zelienople, PA, USA) added as a surfactant. Seeds were then washed four times with sterile water and blotted dry. To undertake leaching, seeds of each species were transferred to several mesh ball infusers (diameter of 5 cm) and placed individually into 250 mL glass beakers for 36 h under running, turbulent cold tap water (ambient from main town water supply). To nick seeds, a small cut on the embryo end of the seed was undertaken using a scalpel blade (Figure 2). Chemical treatments (5 mL) were applied to each Petri dish (9 cm diameter) containing two Whatman No. 1 filter papers.

Table 1. The germination stimulant treatments applied to seeds of *Lomandra longifolia* Labill. and *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery consisted of both mechanical and chemical methods involving leaching, nicking, smoke water (SW) and gibberellic acid (GA_3).

Treatment	Type
Leaching	Mechanical
Nicking	Mechanical
SW1: Smoke water (50 mL L^{-1})	Chemical
SW2: Smoke water (100 mL L^{-1})	Chemical
SW3: Smoke water (200 mL L^{-1})	Chemical
GA3: gibberellic acid (289 µM)	Chemical
Nicking + SW2	Combination
Nicking + GA3	Combination

Previcure® (2% *v*/*v*; Bayer Crop Science) was added to the Petri dishes to inhibit fungi growth [19,26]. Then, following the addition of seed, each Petri dish was sealed with Parafilm to prevent evaporation of solutions. This was undertaken in a laminar air flow hood to reduce possible microbial contamination. All treatments were applied under light (with a 12/12 h day/night photoperiod) or dark conditions to simulate the seed being placed on the soil surface (light) or seed burial (dark). For seeds imbibed under light, the 12/12-h photoperiod used had a light intensity of 100 µmol m^{-2} s^{1} (produced by cool white, fluorescent tubes). Dark conditions were achieved by wrapping the Petri dishes with two layers of aluminum foil. Petri dishes were placed in an incubator (TRIL-750 Illuminated Refrigerator Incubator, Thermoline, Wetherill Park, NSW, Australia) using a matching 12/12-h thermoperiod of 20/10 ± 1 °C. The thermoperiod was selected from earlier studies carried out on both *Lomandra* species (unpublished data) and from the published literature (maximum germination for *L. longifolia* was at 20 °C and for *L. hystrix* it was at 15 °C [1]).

Figure 2. Location where seed nicking was applied to seed (pericarp + seed) of *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery to help relieve mechanical pressure on the embryo by the surrounding tissues. A small nick was formed by applying gentle pressure with a scalpel blade (marked as a black line in the image), resulting in a cut through the pericarp.

2.4. Data Collection and Analysis

For both species, each treatment was replicated 4 times, and each replication had 25 seeds per Petri dish. A completely randomized design was used. All seed germination tests were run for 60 days. Petri dishes were examined for germination twice weekly. Germinated seeds (radicle protrusion $\geq$ 2 mm) were recorded and removed. Seeds germinated under dark conditions were observed under a green safety light (Lion 24 LED Magnetic work lamp, covered with a green plastic sheet) in a dark room. Cumulative germination over time for the different seed treatments for each species was determined using a non-linear regression model fitted with the drm function in package drc [27] using R software, version 3.5.3 [28]. A three-parameter log-logistic model was used [29]. The germination rate index (GRI) was determined according to Maguire [30] (Equation (1)). Germination data (final germination percentage, percentage of dormant seeds and GRI) for each species were analyzed using a two-way factorial analysis of variance (ANOVA). When significant differences were identified, a Tukey's honest significance difference (HSD) test was used as a post-hoc analysis to identify significant differences between treatment means.

Equation (1): Germination rate index (GRI; Maguire [30])

$$\text{GRI}\left(\%\text{d}^{-1}\right) = \frac{\sum(G_i - G_{i-1})}{i} \quad (1)$$

i: Day of germination count
G_i: Percentage of seeds germinated in day i
G_{i-1}: Percentage of seeds germinated the previous count day

3. Results

3.1. Seed Fill and Seed Morpho-Anatomy

Lomandra longifolia had a 100% and *L. hystrix* had a 99% seed fill (Figure 3a,b, respectively). The seed cross-sectional area was 7.5 mm^2 for *L. longifolia* and 11.8 mm^2 for *L. hystrix* (Table 2). Embryos of both species were fully differentiated and seemed to be fully developed. They were small and linear and located in the basal part of the seed (Figure 4). Both species had a large proportion (>70%) of the seed consisting of endosperm tissue surrounding the embryo (Figures 3c and 4). The E:S ratio was 0.4 for both *L. hystrix* and *L. longifolia* (Table 2). The 100-seed weight was 900 mg for *L. hystrix* and 860 mg for *L. longifolia*.

Table 2. Seed characteristics as determined from 10 randomly selected seeds of *Lomandra longifolia* Labill and *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery. An endosperm was present in both species, and the embryo type was linear. The E:S ratio, seed and embryo length, area, perimeter and width were measured. Mean ± SE.

Tissue	*Lomandra longifolia*	*Lomandra hystrix*
E:S ratio	0.4 ± 0.03	0.4 ± 0.04
Seed length (mm)	3.9 ± 0.1	4.9 ± 0.8
Seed width (mm)	2.3 ± 0.2	2.5 ± 0.4
Seed area (mm^2)	7.5 ± 0.6	11.8 ± 5.2
Embryo length (mm)	1.4 ± 0.1	2.2 ± 0.5
Embryo area (mm^2)	0.3 ± 0.0	0.6 ± 0.1
100 seed weight (mg)	860 ± 20	900 ± 20

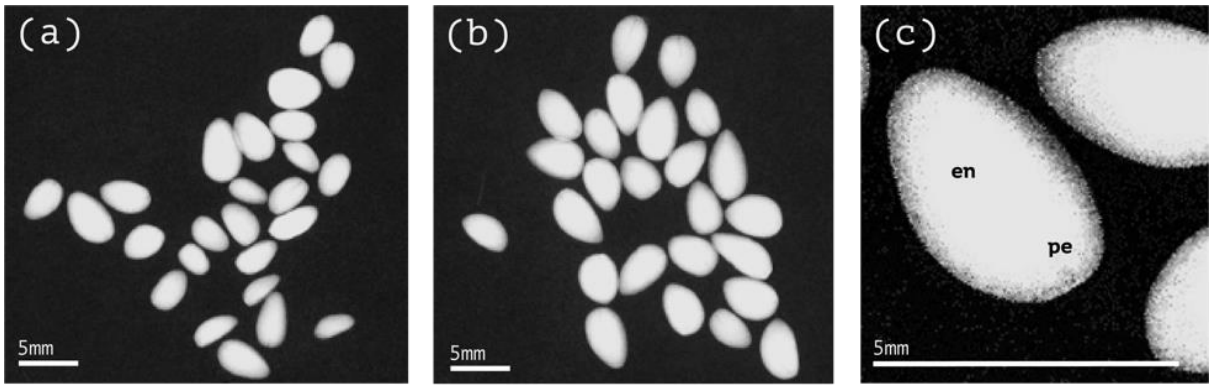

Figure 3. X-ray images (Faxitron MX-20) showing seed fill of (**a**) *Lomandra longifolia* Labill. (100 ± 0% seed fill); (**b**) *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery (99 ± 1% seed fill); (**c**) seed endosperm (en; white tissue) can be distinguished from the seed pericarp (pe; grey border area) surrounding the seed in *L. hystrix*. Seed fill was calculated by averaging the results of 5 replicates of 25 seeds from each species. All seeds shown are considered filled.

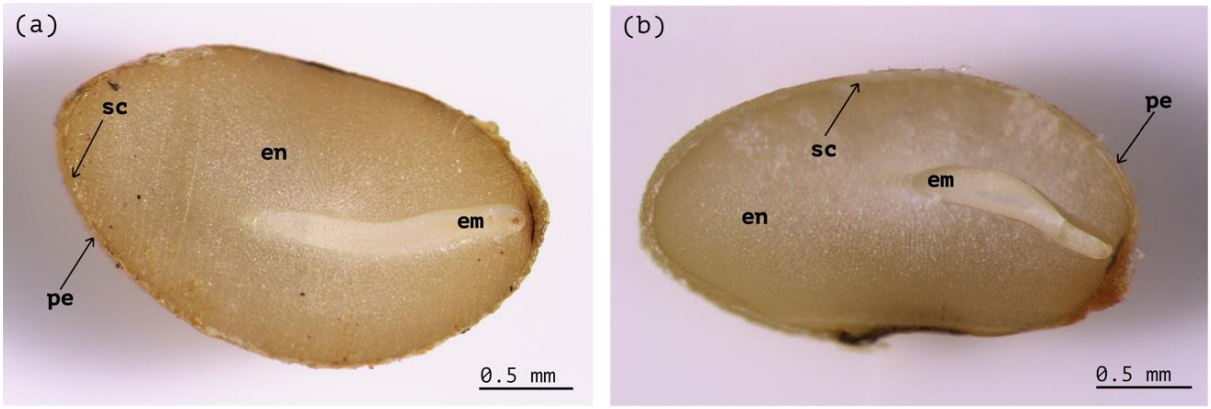

Figure 4. Light microscope images of longitudinal sections of seed (pericarp + seed) of (**a**) *Lomandra longifolia* Labill; and (**b**) *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery. In image (**b**), the embryo of *L. hystryx* is not shown in full as it sinks into the endosperm. Seeds had a small, linear basal embryo (em). The endosperm (en) filled a large proportion of the seed. Seed coat (sc) and pericarp (pe) are indicated with arrows. Magnification × 160.

3.2. Germination Enhancement Using Mechanical and Chemical Treatments

Leaching significantly increased ($p \leq 0.001$) the GRI for both species in comparison to untreated seeds and other pre-treatments regardless of light conditions (Table 3). Leached

seeds were also the first to start germination (Figure 5). For *L. longifolia*, seeds leached in darkness had a higher GRI (1.0 ± 0.1% day^{-1}) as compared to leached seeds under light (0.5 ± 0.1% day^{-1}). Leached seeds for *L. hystrix* incubated under light conditions had GRI of 3.7 ± 0.1% day^{-1} as compared to control of 1.7 ± 0.2% day^{-1} (Table 3). However, the GRI for leached seeds incubated under light was significantly higher ($p < 0.0001$) than in darkness (2.4 ± 0.2% day^{-1}).

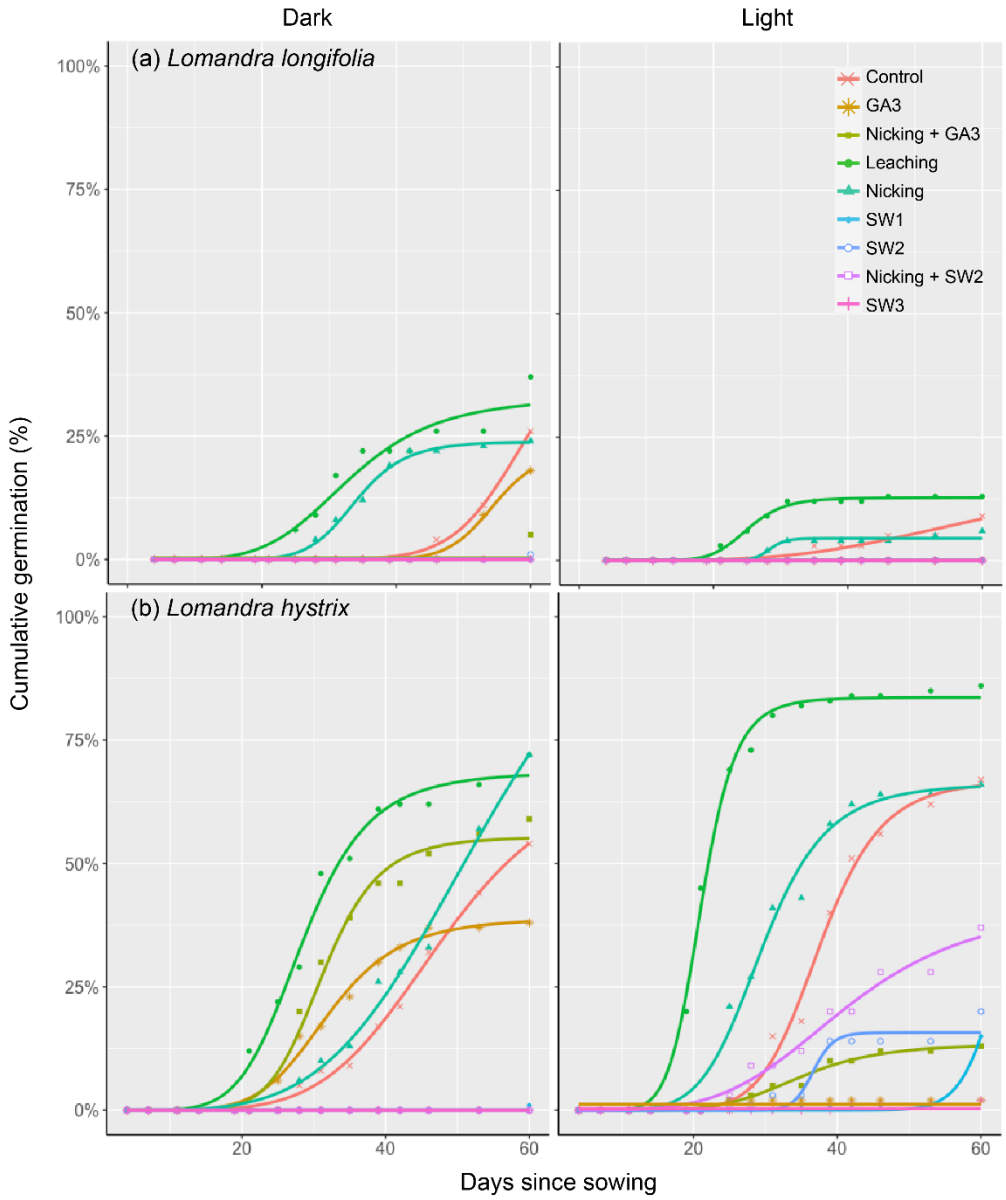

Figure 5. Mean germination percentage during 60 days of incubation (20/10 °C, 12/12-h thermoperiod, light [12/12-h photoperiod] or dark) incorporating chemical and/or mechanical treatments applied to (**a**) *Lomandra longifolia* Labill; and (**b**) *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery. Four replications per treatment, with twenty-five seeds per replicate per species were used.

Leaching or nicking (mechanical treatments) did not improve the final germination percentage for either species when compared to the untreated seeds (Figure 5). *Lomandra longifolia* had a maximum germination of 37.0 ± 1.9% (Figure 5a; leached seeds incubated in darkness). Furthermore, untreated *L. longifolia* seeds under darkness had a significantly higher final germination percentage (26.0%) as compared to seeds exposed to light (9%; $p \leq 0.01$). In *L. longifolia*, the germination percentages for leached seeds were significantly

higher as compared to nicked seeds (7% higher under light and 13% higher under darkness; $p \leq 0.01$). *Lomandra hystrix* had a maximum of 86.0 ± 2.6% germination (Figure 5b; leached seeds incubated under illuminated conditions), and unlike *L. longifolia*, no significant differences ($p > 0.05$) were observed for untreated seeds of *L. hystrix* incubated under light or dark conditions. In *L. hystrix*, untreated seeds, leached seeds and nicked seeds had significantly higher final germination percentages (>53%) than for the remainder of the treatments (<40%; $p \leq 0.05$).

Table 3. Germination rate index (GRI) for *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery and *Lomandra longifolia* Labill. incubated under complete darkness (24 h) or light/dark (12/12-h photoperiod) conditions following chemical and/or mechanical treatments. All seeds were incubated for 60 days at 20/10 °C with a 12/12-h matching thermoperiod. Treatments were as follows: leaching with running tap water for 36 h, nicking (small cut through pericarp and seed testa), smoke water (SW)—SW1:50, SW2:100, SW3:200 mL L^{-1}; gibberellic acid—GA_3: 289 µM; SW2 + nicking, GA_3 + nicking and control (untreated seeds). Means ± SEM were calculated using 4 replications, 25 seeds per replication. Treatments that had a significantly higher GRI than the control are in bold, and treatments that resulted in zero germination are denoted by a dash.

Treatment	*Lomandra longifolia*		*Lomandra hystrix*	
	Light	**Dark**	**Light**	**Dark**
Leaching	**0.5 ± 0.1**	**1.0 ± 0.1**	**3.7 ± 0.1**	**2.4 ± 0.2**
Nicking	0.2 ± 0.1	0.7 ± 0.0	2.2 ± 0.3	1.6 ± 0.4
SW1	-	-	0.3 ± 0.0	-
SW2	-	-	0.5 ± 0.1	-
SW3	-	-	-	-
GA_3	-	0.3 ± 0.1	-	1.2 ± 0.2
SW2 + nicking	-	-	0.9 ± 0.3	-
GA_3 + nicking	-	0.1 ± 0.0	0.4 ± 0.1	1.7 ± 0.1
Control	0.2 ± 0.1	0.5 ± 0.1	1.7 ± 0.2	1.2 ± 0.1

Treatment with smoke water, at all three concentrations (SW1:50, SW2:100 and SW3:200 mL L^{-1}) and in combination with nicking, gave a significantly lower final germination percentage for both species in light and dark conditions when compared to untreated seeds (≤37% for *L. hystrix* and ≤1% for *L. longifolia*; $p \leq 0.05$). *Lomandra hystrix* and *L. longifolia* seeds treated with a combination of GA_3 and nicking had significantly higher final germination in darkness as compared to light conditions ($p \leq 0.05$; Figure 6). Under light, GA_3 and SW3 gave a significantly lower final germination of <3% than other treatments for *L. hystrix* and *L. longifolia* ($p \leq 0.005$). In *L. longifolia*, significantly higher ($p \leq 0.05$) final germination percentage occurred in dark conditions as compared with light conditions, for leaching, nicking, GA_3 and the control (24, 18, 18, 17% higher, respectively).

After 60 days, ≥50% of *L. longifolia* seeds remained ungerminated (Figure 6a). These seeds were considered dormant as they were filled (determined by X-ray) and firm. No significant differences were observed in dormant seeds of *L. longifolia* between treatments and control ($p \geq 0.05$). For *L. hystrix* (Figure 6b), the number of dormant seeds was significantly higher for seeds treated with smoke water (≥80% dormant seeds in all concentrations), SW2 + nicking (≥63%) and GA_3 + nicking (>40%) (Figure 6). Moreover, for GA_3 and nicking + GA_3 treatments in this species, dormancy was significantly higher in light conditions when compared to darkness (98 vs. 56% and 87 vs. 41% respectively: $p \leq 0.005$) (Figure 6). Seed death occurred mostly in *L. longifolia*, with the highest number observed in SW2 + nicking (51% dead seeds in light conditions) and GA_3 + nicking (>40% for light and dark conditions) (Figure 6). In contrast, a few seeds (≤8%) of *L. hystrix* were dead at the end of the experiment.

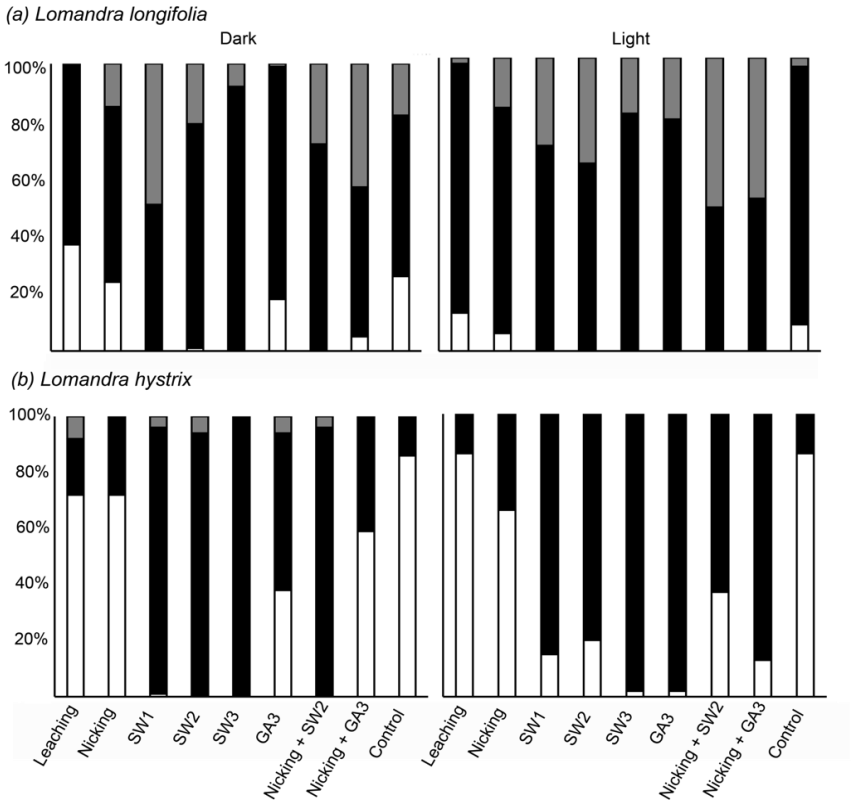

Figure 6. Percentage of germinated (white), dormant (black) and dead (grey) seeds for (**a**) *Lomandra longifolia* Labill. and (**b**) *Lomandra hystrix* (R.Br.) L.R. Fraser and Vickery and *Lomandra longifolia* Labill. germinated under dark or light (12/12-h photoperiod) conditions following chemical and/or mechanical treatments. All seeds were incubated for 60 days at 20/10 °C with a 12/12-h matching thermoperiod. Treatments were as follows: leaching with running tap water for 36 h, nicking (small cut in through the pericarp and seed coat), smoke water—SW1:50; SW2:100; SW3:200 mL L^{-1}; gibberellic acid—GA_3:289 µM, Control: untreated seed. Means of 4 replications with 25 seeds each per species, per treatment are shown.

4. Discussion

This study aimed to characterize seed morpho-anatomy and to determine seed treatments that could enhance the germination of two Australian native *Lomandra* species. The seed traits that influence germination of *L. longifolia* and *L. hystrix* had not been addressed in detail previously and there is a lack of published protocols on how to improve germination for both important seed-based restoration and ornamental species. The current study showed that seeds of both species had PD at the time of sowing, probably due to tissues surrounding the small embryo imposing a mechanical constraint to its protrusion and/or the presence of chemical inhibitors (as shown to occur in other *Lomandra* species). Further results indicate that germination rates of both *Lomandra* species can be significantly improved by leaching seeds under running tap water. Having a deep understanding of seed biology is crucial for seed-based restoration success [31]: it enables seed treatments to be optimized for improving seed performance [32] and can result in faster, more uniform and, therefore, reliable germination. Additionally, understanding seed germination timing will be crucial for seed use under future climate change [33].

4.1. Seed Morpho-Anatomy

The seed fill percentage in both seed lots studied was 100%, suggesting that other factors besides seed viability or seed fill are limiting germination. Both species presented small (E:S = 0.4), linear and basal embryos (Figure 4) surrounded by a thick layer of endosperm.

Even though the embryos were small, microscopic evaluation showed that embryos in this current study were fully differentiated and seemed to be fully developed, consisting of a well-defined primary axis and cotyledon. In contrast, Ruiz-Talonia et al. [14] identified MPD in *L. longifolia* due to the presence of an underdeveloped embryo. In their study, a combination of leaching and warm stratification was needed to overcome dormancy and improve germination. This present study evaluated the use of stored seeds of *L. Longifolia* and *L. hystrix* (for 27 and 16 months, respectively). Seed age can influence seed dormancy status as seeds can undergo after-ripening, dormancy cycling [10] or overcome MD or MPD during storage. Therefore, there is a possibility that embryo growth occurred during storage, overcoming MD. Although, it is important to note that Baskin et al. [22] proposed that a seed with a small embryo in relation to the endosperm does not strictly mean that the embryo is underdeveloped. Some species, such as in the genus *Nymphaea* (Nymphaeaceae [18]) and *Drosera anglica* (Huds.) LePage and W.Bldw. (Droseraceae [22]), have small embryos with low E:S ratios (e.g., 0.24 ± 0.01 for *D. anglica*). However, these embryos did not exhibit growth inside the seed before germination could occur. Therefore, MD was not present. Consequently, to identify the presence of MD or MPD in both of our study species, further studies should be undertaken with freshly collected seeds to determine whether embryo growth occurs within the seed prior to them becoming germinable.

4.2. Seed Germination Ecology and Enhancement

In *L. longifolia*, seeds incubated in the dark had a significantly higher final germination percentage than those imbibed in light conditions (37 vs. 13%; Figure 5). This suggests that *L. longifolia* can germinate to a higher extent if buried in the soil. On the other hand, there was no significant difference between light and dark treatment in *L. hystrix*, although marginal improvements in germination were observed under light. Seeds of many species are sensitive to light intensity and quality, which is a mechanism to avoid plant competition [34]. Therefore, light detection by seeds can be an important germination cue [35]. In *L. longifolia*, germination inhibition by light could be related to the avoidance of germination near or at the soil surface, ensuring seeds are positioned at sufficient depth where moisture is more reliably obtained. This is a common seed adaptation to environments where moisture is limited [36], such as those where *L. longifolia* naturally grows. Moreover, temperature is moderated at greater soil depths [35], which may be an adapdation of *L. longifolia* to enable germination to occur in a wide range of climates (Figure 1). However, burial at depth does not appear to be a requirement of *L. hystrix* seeds, possibly because this species has adapted to growing along watercourses and in rainforests, where water is usually more abundant and temperature fluctuations are less extreme.

Leaching significantly improved the GRI of both species (Figure 5). The positive effects of leaching in this study are consistent with previous observations made for *L. longifolia* [14] and *L. sonderi*. Plummer et al. [2] suggested that water-soluble germination inhibitors located in the pericarp and embryo could inhibit germination in *L. sonderi*; germination inhibition was successfully overcome by removing the pericarp or by leaching seeds for 24 h in running tap water. This may be an adaptation to regions where occasional, but heavy, rainfall can leach out seed germination inhibitors and break down the seed coat tissues [26,37]. This mechanism ensures that conditions are suitable for germination and seedling establishment. On the other hand, Baskin et al. [18] suggested that leaching can also act as a stratification treatment. Periods of warm or cold stratification have been shown to alleviate PD in seeds [38]. For example, stratification at 26/13 °C or 33/18 °C for 4- or 8-weeks alleviated dormancy in *L. preisii* when seeds were germinated at 18/7 °C [7]. Moreover, warm stratification achieved >80% germination in *Acanthocarpus preisii* Lehm. (Asparagaceae) as compared with <20% when seeds were not stratified [39]. Further studies should be directed at identifying if the use of stratification treatments or wet/drying cycles [40] could be involved in overcoming dormancy of *Lomandra* seeds in the soil seedbank.

The improvement in germination of seeds with non-deep PD after scarification has been related to overcoming a mechanical barrier to embryo growth imposed by the tissues

surrounding the embryo [10,41]. Likewise, seed nicking has also been shown to relieve embryo growth restrictions [19]. Although in this study, nicking did not significantly promote germination, a positive trend was observed (Figure 5). In seeds presenting PD, the fully developed embryo has a low growth potential; therefore, it cannot overcome the mechanical constraints imposed by its surrounding tissues [10]. Once treatments such as nicking are performed, the embryo can gain sufficient expansive force to protrude through the surrounding tissues. In nature, embryos with low growth potential need cues from the environment to initiate internal chemical signaling which promote certain covering tissues to breakdown and increase the growth potential [10]. However, this resistance can also be weakened over time in the seeds' natural environment by the production of tissue-softening enzymes released by the embryo or by weakening through physical biotic factors such as temperature, fire, animal ingestion, seed burial and saprophytic fungi [37,42]. Embryo germination resistance also varies according to imbibition conditions such as temperature and light/dark interactions [10], and further studies could be undertaken to determine the influence these parameters can have on endosperm resistance.

Constant exposure to smoke water throughout the incubation period significantly inhibited germination of both species, with ≤1% final germination for *L. longifolia* and ≤20% final germination for *L. hystrix* (Figure 6). This contrasts with the findings of Merritt et al. [43], where smoke water was reported to promote germination in other *Lomandra* species. For example, imbibing seeds in smoke water (1:10 [*v*/*v*]) for 24 h enhanced seed responses to warm stratification providing the highest germination for *L. preisii* seeds (*ca.* 50% [7]). In contrast, Vening et al. [44] reported that smoke water used in an agar-based germination medium at 1:10 (*v*:*v*) had no significant effect on germination in Australian native forbs from fire prone environments. However, seed sensitivity to smoke water can be a complex process [7], as active constituents of smoke water (such as karrikins) can vary between different stock solutions and species react differently [45]. Furthermore, Adkins et al. [45] found that caryopsis of wild oats (*Avena fatua* L.) had greater germination when exposed to smoke water for 7 days prior to incubation with distilled water as compared to caryopsis that received smoke water before and during incubation.

Considering the above, constant exposure of *Lomandra* seeds to smoke water in this current study could have caused germination inhibition. This may be due to the dual effect of smoke water on the seed germination process reported by Light et al. [46] for 'Grand Rapids' lettuce (*Lactuca sativa* L.). This study proposed that smoke water had an inhibitory component that enters the seed and a promotor component that remains in the seed, inactive until sufficient rainfall has leached out the inhibitor. Additionally, it is important to note that smoke has been proposed to act as a germination enhancer, rather than a dormancy breaker [7,47]. Therefore, smoke water effects might only act to enhance germination after dormancy has been overcome. Further studies on both *Lomandra* species could be undertaken by applying smoke water as a pre-treatment prior to incubation, but after seeds have been treated for dormancy.

Although leaching improved the GRI for *L. longifolia*, the final germination percentage achieved in all treatments was low (≤37% germination). This low germination percentages of *L. longifolia*, even when exposed to GA_3, could suggest the presence of a deeper level of PD. In many species with intermediate or non-deep PD, germination is stimulated by GA_3, while those with deep PD fail to germinate in GA_3 treatments that would normally promote germination [48]. To alleviate PD in *L. sonderi*, the pericarp needed to be removed from the seed (presumably to remove germination inhibition imposed by these tissues); then, GA_3 (145 µM) was applied (to relieve embryo dormancy [2]). Further studies on *L. longifolia* seeds should be undertaken such as excision of the pericarp and seed coat [42] and excision of the embryo [10]; and then, applying GA_3 to identify if the pericarp or endosperm are preventing GA_3 from reaching the embryo. Although GA_3 did not improve germination in both *Lomandra* species, there was a significant interaction between GA_3 and darkness, where seeds treated with GA_3 had higher germination in the dark as compared to light. This could be related to GA_3 interacting with the phytochrome system within seeds [37].

5. Conclusions

This study is one of the first to investigate techniques to enhance germination of the Australian native species *L. longifolia* and *L. hystrix*. The innate slow and initially low germination of both *Lomandra* seeds requires a significantly large number of viable seeds to be sown to achieve the plant density required for ornamental plant production or seed-based restoration projects. Results from this study show that both species had small, linear embryos and a high proportion of endosperm tissue. Slow germination of both species is most likely associated with the presence of at least one mechanism of PD present in the seed. Leaching seeds prior to incubation was the only treatment to significantly hasten seed germination in both *Lomandra* species. To determine the mechanisms by which leaching functioned on hastening germination and to correctly classify dormancy in both species, further studies are now needed on freshly collected seeds. Future areas of research include measuring embryo growth during incubation to test for MPD, undertaking warm stratification prior to germination incubation, and seed treatments with GA_3. Understanding the factors that influence seed germination and pre-treating seeds accordingly, or ensuring these requirements are met in the natural environment (in the case of seed-based restoration), is crucial for the success and cost-efficient use of these seeds. It is also important to consider the scaling-up of treatments for large restoration projects or ornamental plant production and how that could affect seed tissues and the overall cost-effectiveness of the treatment. Moreover, the possibility of providing similar effects naturally in the field by sowing seeds when long periods of rain are forecasted should also be examined.

Author Contributions: Conceptualization, F.C.B. and S.W.A.; original draft preparation, F.C.B., R.C., A.W. and S.W.A.; review and editing, F.C.B., S.K., R.C., A.W. and S.W.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Seed Information Database (SID), Royal Botanical Gardens Kew. Available online: http://data.kew.org/sid/ (accessed on 14 September 2018).
2. Plummer, J.; Crawford, A.; Taylor, S. Germination of *Lomandra sonderi* (Dasypogonaceae) promoted by pericarp removal and chemical-stimulation of the embryo. *Aust. J. Bot.* **1995**, *43*, 223–230. [CrossRef]
3. Lee, A.; Macfarlane, T. Lomandra. *FI Aust.* **1986**, *46*, 100–141.
4. Bonney, N.; Miles, A. *What Seed Is That?* N. Bonney: Beverley, Australia, 1994.
5. Ahmad, N.M.; Martin, P.M.; Vella, J.M. Embryology of the dioecious Australian endemic *Lomandra longifolia* (Lomandraceae). *Aust. J. Bot.* **2009**, *56*, 651–665. [CrossRef]
6. Ahmad, N.M.; Martin, P.M.; Vella, J.M. Clonal propagation of *Lomandra longifolia* by somatic embryogenesis. *Sci. Hortic.* **2014**, *180*, 102–110. [CrossRef]
7. Merritt, D.; Turner, S.; Clarke, S.; Dixon, K. Seed dormancy and germination stimulation syndromes for Australian temperate species. *Aust. J. Bot.* **2007**, *55*, 336–344. [CrossRef]
8. Erickson, T.; Barrett, R.; Merritt, D.; Dixon, K. *Pilbara Seed Atlas and Field Guide: Plant Restoration in Australia's Arid Northwest*; CSIRO Publishing: Clayton, Australia, 2016.
9. Baskin, C.; Baskin, J. Types of seeds and kinds of seed dormancy. In *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*; Elsevier: Amsterdam, The Netherlands, 2014; pp. 37–77.
10. Baskin, C.C.; Baskin, J.M. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*, 2nd ed.; Academic Press: London, UK, 2014.
11. Bonney, N. Understanding botanical pathlines for improved germination of native plant seed. In Proceedings of the Fourth Australian Workshop on Native Seed Biology for Revegetation, Mildura, Australia, 3–4 September 2001; pp. 105–111.
12. Gibson-Roy, P.; Delpratt, J.; Moore, G. Restoring the Victorian Western (Basalt) Plains grassland. 1. Laboratory trials of viability and germination, and the implications for direct seeding. *Ecol. Manag. Restor.* **2007**, *8*, 114–122. [CrossRef]

13. Grant, C.; Campbell, C.; Charnock, N. Selection of species suitable for derelict mine site rehabilitation in New South Wales, Australia. *Water Air Soil Pollut.* **2002**, *139*, 215–235. [CrossRef]
14. Ruiz-Talonia, L.; Whalley, R.; Gross, C.; Carr, D.; Reid, N. Overcoming limitations to propagation from seed of 40 Australian species important for restoration. *New For.* **2022**, 1–20. [CrossRef]
15. Alvarado, V.; Nonogaki, H.; Bradford, K. Expression of endo-β-mannanase and SNF-related protein kinase genes in true potato seeds in relation to dormancy, gibberellin and abscisic acid. In *Dormancy in Plants: From Whole Plant Behaviour to Cellular Control*; Viemont, J., Crabbe, J., Eds.; CABI Publishing: Wallingford, UK, 2000; pp. 347–364.
16. Roche, S. Smoke-a new process for germinating Australian plants. *Aust. Hortic.* **1994**, *92*, 46–47.
17. Dixon, K.W.; Roche, S.; Pate, J.S. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* **1995**, *101*, 185–192. [CrossRef]
18. Baskin, C.C.; Thompson, K.; Baskin, J.M. Mistakes in germination ecology and how to avoid them. *Seed Sci. Res.* **2006**, *16*, 165–168. [CrossRef]
19. Turner, S.; Merritt, D. Seed germination and dormancy. In *Plant Germplasm Conservation in Australia: Strategies and Guidelines for Developing, Managing and Utilising Ex Situ Collections*; Offord, C.A., Meagher, P.F., Eds.; Australian Network for Plant Conservation Inc.: Canberra, Australia, 2009.
20. Baskin, C.C.; Baskin, J.M. A revision of Martin's seed classification system, with particular reference to his dwarf-seed type. *Seed Sci. Res.* **2007**, *17*, 11–20. [CrossRef]
21. Martin, A. The comparative internal morphology of seeds. *Am. Midl. Nat.* **1946**, *36*, 513–660. [CrossRef]
22. Baskin, C.C.; Baskin, J.M. Underdeveloped embryos in dwarf seeds and implications for assignment to dormancy class. *Seed Sci. Res.* **2005**, *15*, 357. [CrossRef]
23. Commander, L.; Merritt, D.; Rokich, D.; Dixon, K. Seed biology of Australian arid zone species: Germination of 18 species used for rehabilitation. *J. Arid Environ.* **2009**, *73*, 617–625. [CrossRef]
24. Ooi, M.K. Dormancy classification and potential dormancy-breaking cues for shrub species from fire-prone south-eastern Australia. In *Seeds: Biology, Development and Ecology*; Adkins, S., Ashmore, S., Navie, S.C., Eds.; CAB International: Wallingford, UK, 2007; pp. 205–216.
25. Merrit, D. Seed storage and testing. In *Australian Seeds: A Guide to Their Collection, Identification and Biology*; Sweedman, L., Merritt, D., Eds.; CSIRO Publishing: Collingwood, Australia, 2006; pp. 53–60.
26. Cochrane, A.; Kelly, A.; Brown, K.; Cunneen, S. Relationships between seed germination requirements and ecophysiological characteristics aid the recovery of threatened native plant species in Western Australia. *Ecol. Manag. Restor.* **2002**, *3*, 47–60. [CrossRef]
27. Ritz, C.; Baty, F.; Streibig, J.C.; Gerhard, D. Dose-response analysis using R. *PLoS ONE* **2015**, *10*, e0146021. [CrossRef]
28. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022.
29. Pedrini, S.; Lewandrowski, W.; Stevens, J.C.; Dixon, K.W. Optimising seed processing techniques to improve germination and sowability of native grasses for ecological restoration. *Plant Biol.* **2018**, *21*, 415–424. [CrossRef]
30. Maguire, J.D. Speed of Germination—Aid in selection and evaluation for seedling emergence and vigor 1. *Crop Sci.* **1962**, *2*, 176–177. [CrossRef]
31. Shaw, N.; Barak, R.S.; Campbell, R.E.; Kirmer, A.; Pedrini, S.; Dixon, K.; Frischie, S. Seed use in the field: Delivering seeds for restoration success. *Restor. Ecol.* **2020**, *28*, S276–S285. [CrossRef]
32. Erickson, T.E.; Muñoz-Rojas, M.; Kildisheva, O.A.; Stokes, B.A.; White, S.A.; Heyes, J.L.; Dalziell, E.L.; Lewandrowski, W.; James, J.J.; Madsen, M.D. Benefits of adopting seed-based technologies for rehabilitation in the mining sector: A Pilbara perspective. *Aust. J. Bot.* **2017**, *65*, 646–660. [CrossRef]
33. Walck, J.L.; Hidayati, S.N.; Dixon, K.W.; Thompson, K.; Poschlod, P. Climate change and plant regeneration from seed. *Glob. Chang. Biol.* **2011**, *17*, 2145–2161. [CrossRef]
34. Yan, A.; Chen, Z. The control of seed dormancy and germination by temperature, light and nitrate. *Bot. Rev.* **2020**, *86*, 39–75. [CrossRef]
35. Fenner, M.; Thompson, K. *The Ecology of Seeds*; Cambridge University Press: Cambridge, UK, 2005.
36. Turner, S.R.; Lewandrowski, W.; Elliott, C.P.; Merino-Martín, L.; Miller, B.P.; Stevens, J.C.; Erickson, T.E.; Merritt, D.J. Seed ecology informs restoration approaches for threatened species in water-limited environments: A case study on the short-range Banded Ironstone endemic *Ricinocarpos brevis* (Euphorbiaceae). *Aust. J. Bot.* **2017**, *65*, 661–677. [CrossRef]
37. Adkins, S.W.; Bellairs, S.M.; Preston, C.; Thompson, L.; Farley, G. Identifying dormancy mechanisms of Australian native plant species. In Proceedings of the Fourth Australian Workshop in Native Seed Biology for Revegetation, Mildura, Australia, 3–4 September 2002; pp. 61–70.
38. Kildisheva, O.A.; Dixon, K.W.; Silveira, F.A.; Chapman, T.; Di Sacco, A.; Mondoni, A.; Turner, S.R.; Cross, A.T. Dormancy and germination: Making every seed count in restoration. *Restor. Ecol.* **2020**, *28*, S256–S265. [CrossRef]
39. Turner, S.; Merritt, D.; Ridley, E.; Commander, L.; Baskin, J.; Baskin, C.; Dixon, K. Ecophysiology of seed dormancy in the Australian endemic species *Acanthocarpus preissii* (Dasypogonaceae). *Ann. Bot.* **2006**, *98*, 1137–1144. [CrossRef] [PubMed]
40. Hoyle, G.; Daws, M.; Steadman, K.; Adkins, S. Mimicking a semi-arid tropical environment achieves dormancy alleviation for seeds of Australian native Goodeniaceae and Asteraceae. *Ann. Bot.* **2008**, *101*, 701–708. [CrossRef]

41. Erickson, T.E.; Shackelford, N.; Dixon, K.W.; Turner, S.R.; Merritt, D.J. Overcoming physiological dormancy in seeds of *Triodia* (Poaceae) to improve restoration in the arid zone. *Restor. Ecol.* **2016**, *24*, S64–S76. [CrossRef]
42. Cochrane, A.; Probert, R. Temperature and dormancy-breaking treatments: Germination of endemic and geographically restricted herbaceous perennials. *Aust. J. Bot.* **2006**, *54*, 349–356. [CrossRef]
43. Merritt, D.; Rokich, D. Seed biology and ecology. In *Australian Seeds: A Guide to Their Collection, Identification and Biology*; Sweedman, L., Merritt, D., Eds.; CSIRO Publishing: Collingwood, Australia, 2006; pp. 19–24.
44. Vening, G.S.; Guja, L.K.; Spooner, P.G.; Price, J.N. Seed dormancy and germination of three grassy woodland forbs required for diverse restoration. *Aust. J. Bot.* **2017**, *65*, 625–637. [CrossRef]
45. Adkins, S.; Peters, N. Smoke derived from burnt vegetation stimulates germination of arable weeds. *Seed Sci. Res.* **2001**, *11*, 213–222.
46. Light, M.; Gardner, M.; Jäger, A.; Van Staden, J. Dual regulation of seed germination by smoke solutions. *Plant Growth Regul.* **2002**, *37*, 135–141. [CrossRef]
47. Thompson, K.; Ooi, M.K. To germinate or not to germinate: More than just a question of dormancy. *Seed Sci. Res.* **2010**, *20*, 209–211. [CrossRef]
48. Baskin, J.M.; Baskin, C.C. A classification system for seed dormancy. *Seed Sci. Res.* **2004**, *14*, 1–16. [CrossRef]