Seed Science Research

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Research Paper

Cite this article: Beveridge FC, Williams A, Cave R, Kalaipandian S, Dayananda B, Adkins SW (2024). Interactions between seed functional traits and environmental factors and their influence on germination performance of Australian native species. *Seed Science Research* **34**, 56–71. https://doi.org/10.1017/S0960258523000235

Received: 15 August 2023
Revised: 9 November 2023
Accepted: 19 December 2023
First published online: 25 March 2024

Keywords:
climate change; dormancy; germination ecology; plant recruitment; salinity; seed functional traits; water stress

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Interactions between seed functional traits and environmental factors and their influence on germination performance of Australian native species

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Abstract

Climate variability is expected to increase due to climate change, with projected increases in temperature and erratic rainfall patterns. These changes will alter the environmental cues sensed by seeds, and therefore will impact plant recruitment. This study investigated the effects of seed functional traits (germinability, germination time, synchrony and seed mass) on germination responses of several sub-tropical native Australian plant species under different environmental factors (water stress, salinity and pH). The effect of a hot water pre-treatment was also tested on Fabaceae seeds with known physical dormancy. Seed traits, environmental factors and seed pre-treatments had significant effects on final germination percentage and germination time. Seed mass and time to 50% germination (t_{50}) were also positively correlated. In contrast, pH did not affect germination and there was no interaction between pH and any of the measured seed functional traits. Some species showed a high thermal tolerance to germination and germination was indifferent to light conditions for all species. Results showed that certain seed functional traits interact with environmental factors to influence germination percentage and time. These findings highlight the importance of considering seed functional traits when determining a species germination response under a changing climate. In addition, the findings provide important knowledge to better guide seed-based land restoration programmes.

Introduction

Plant functional traits are morpho-physio-phenological traits that affect growth, reproduction and survival, and therefore impact plant fitness (Violle et al., 2007). They are a useful tool to explain and forecast how different species that occur in a common environment will perform over time (McGill et al., 2006). However, there is a lack of knowledge on the links between these functional traits and key recruitment stages (Larson et al., 2015), such as the link between seed germination traits and seedling emergence. Seed functional traits include morphological, physiological and biochemical characteristics, which are related to the ecological functions of seed dispersal, persistence, germination and seedling establishment (Saatkamp et al., 2019). Germination timing is coordinated by several morphological and physiological traits and is regulated to occur at a time that maximizes the chances for successful seedling establishment (Long et al., 2015). Seed morphological traits, such as seed shape, mass and size, are also related to dispersal and other functions, such as seed persistence in the soil seed bank (Fenner and Thompson, 2005). Seed germination traits can also include critical environment-regulated physiological processes (Lambers and Oliveira 2019), such as temperature and water potential thresholds to germination, the speed of germination and dormancy-breaking cues.

Seed mass is an important functional trait relating to germination (Zhang et al., 2014) and is used in the prediction of plant community regeneration patterns (Daws et al., 2008). Larger seeds have been correlated with improved seedling establishment and survival when compared to smaller seeds (Moles and Westoby, 2004) and have also been related to greater seedling survival under conditions of soil water stress (Lloret et al., 1999). Therefore, it has been suggested that plant species producing large seeds might be better adapted to survive under unpredictable rainfall patterns (Arène et al., 2017), by being able to germinate under lower base water potentials (ψ_b) (Daws et al., 2008). In some instances, it has also been observed that some smaller seeds tend to have a faster germination rate, which may be important during unfavourable environmental conditions (Arène et al., 2017). In addition to seed mass, functional traits like germination time (such as mean germination time [MGT] and time to 50% germination

[t_{50}]) and synchrony are also important traits. Germination time is often related to seed vigour (Pollock and Roos, 1972) and can influence establishment success and subsequent plant fitness, especially in environments with strong resource competition (Verdú and Traveset, 2005). Germination synchrony (the synchronization of the germination event) is used to understand dormancy patterns and seed adaptation to the environment (Maleki et al., 2023). For example, having low germination synchrony can be an adaptation to unpredictable environments, by spreading the germination risk over time.

The seed germination niche is the range of environmental conditions under which a plant can germinate and establish successfully (Grubb, 1977). The germination niche is influenced by multiple environmental factors acting together, which include temperature, water, chemical signals and light (Baskin and Baskin, 2014). Germination affects the environmental niche experienced by the plant in later life stages and can be used to avoid stressful environments where plant establishment potential is low (Baskin and Baskin, 2014). Germination niche breadth is defined as the amplitude of germination cues that the seeds will respond to (Fernández-Pascual et al., 2017; Sexton et al., 2017) and is calculated by measuring germination performance across different environmental gradients. Specialized species have a narrow niche, whilst generalist species have a broad niche and tend to perform better across different conditions. It is believed that species with broad niches might be more resilient to climate change than those with narrow niches (Walck et al., 2011; Sheth and Angert, 2014). Understanding inter- and intra- species germination niche variation and its relationship to climatic conditions can be useful to identify climatically vulnerable species and aid in species conservation and species selection for restoration programmes (Finch et al., 2019).

Temperature, water availability and light are crucial environmental drivers for seed germination, plant growth and development. Germination timing of non-dormant seeds can be modelled as a function of time, base temperature and water potential (Bradford, 2002). Species tend to germinate over a variety of temperatures, but will usually have an optimum where the fastest rate of germination occurs and the total germination is greatest (Probert, 2000). Cardinal temperatures (the minimum and maximum temperatures that define the limits of growth and development of a plant) have also been used to describe the minimum/base (T_b), optimum (T_o) and maximum/ceiling (T_c) temperature over which seeds can germinate (Bewley and Black 1994; Bewley et al., 2013). Optimal germination temperatures often coincide with favourable times for successful seedling growth and establishment in natural habitats (Alvarado and Bradford, 2002).

Besides temperature, soil moisture is another crucial factor modulating seed germination (Baskin and Baskin, 2014). Germination will usually occur when non-dormant seeds have accumulated sufficient thermal time at an appropriate water potential (Bradford, 2002), with the minimum water potential necessary for germination being species-specific (Allen et al., 2000; Daws et al., 2002). Declining soil moisture negatively impacts physiological events of the germination process as there is less water available for seed imbibition (Hegarty, 1978), and therefore, delays and reduces germination (Cochrane et al., 2015). Additionally, light is another important environmental factor influencing germination, which can be used as a detection mechanism to inform seeds of their temporal and spatial location (such as burial depth, presence of competitors or disturbance) (Fenner and Thompson, 2005; Jiménez-Alfaro et al., 2016). Seed response to light is species-specific, with some species having a light requirement to complete germination (such as several small-seeded species), others a dark requirement, and some are indifferent and can complete germination under either light or darkness conditions (Baskin and Baskin, 2014).

Variability in weather patterns is projected to increase due to climate change, with periods of prolonged warmer temperatures, reduced moisture availability, increased fire frequency and changing soil moisture conditions (CSIRO and Bureau of Meteorology, 2020; IPCC 2021). These trends are already occurring around the globe (IPCC 2021). Tropical and sub-tropical regions of the world, including those of south-east Queensland (QLD) and northern New South Wales (NSW) in Australia, are predicted to have more frequent drier and warmer periods, with longer dry seasons and lower amounts of precipitation (IPCC 2021). Even small increases in mean air temperature due to climate change can increase soil temperatures to considerably higher levels (Ooi et al., 2009, 2012). Therefore, rising temperatures are likely to result in shifts in germination timing (Fernández-Pascual et al., 2015). Although, in general, decreased precipitation is predicted to be a more serious threat to germination success than increased temperatures (Baskin and Baskin, 2022). Having a better understanding of the seed germination niche of a species, and how shifts in climate patterns will impact germination and seedling establishment, will allow for a better-informed prediction on how sub-tropical Australian plant communities will respond to climate change in the longer term.

In addition to climate change, other human-induced soil factors will also impact the success of natural community regeneration, such as soil salinity (Mukhopadhyay et al., 2021) and pH (Rengel, 2011). Soil salinity (the presence of salt and bicarbonates in soil and water) is a common problem in Australia, which has increased due to human activities, such as agriculture and urban land development (Queensland Government, 2022). Soil salinity is also predicted to increase with climate change (Corwin 2021). Salinity can slow the rate and reduce the final germination percentage by causing less water to be available for seed imbibition and by increasing the risk of toxicity due to accelerated ion uptake (Khan and Gulzar, 2003; Sosa et al., 2005; Baskin and Baskin, 2014). The pH thresholds for germination are species-specific, with some species having a broad range of pH thresholds (Nakamura and Hossain, 2009) while others have a specific pH optimum (Stokes et al., 2011). Land acidification due to agricultural production could potentially affect seed germination of species with a narrower pH range.

To predict seedling recruitment due to shifting temperature, moisture and other soil constraints, it is crucial to understand how interactions between seed functional traits (such as seed mass, germinability, germination time and synchrony) and the environmental factors convert into realized germination niches (Arène et al., 2017). To date, there is limited knowledge on the relationship between seed functional traits and germination performance under stressful conditions (Huang et al., 2016; Jiménez-Alfaro et al., 2016). This impedes our ability to predict plant community responses to climate change and develop management protocols for seed-based restoration projects (James et al., 2013). Thus, the current study aims to investigate thresholds for germination across a range of sub-tropical Australian native species commonly used in seed-based restoration programmes, to elucidate the interactions between seed functional traits and environmental factors. The specific objectives of this study were to determine (1) optimum temperature and light conditions for germination across a range of Australian native plant species with contrasting seed mass; (2) germination responses and niche breadth of selected species across a range of naturally occurring environmental factors including water stress, salinity and pH; and (3) how seed functional traits affect species germination tolerance to environmental factors.

Materials and methods

Study species, seed provenance and preparation

Eight species from two angiosperm families were selected based on (1) their role in seed-based restoration projects in sub-tropical Australia; (2) lack of information on their germination niche; (3) their widespread geographic range; and (4) differences in their diaspore (hereafter referred to as seed) mass and morphology (Fig. 1) and plant life forms (Table 1). All species occur naturally in sub-tropical Australia, with some also occurring in other climates. Species were sourced from south-east QLD and northern NSW (Table 2). Both regions have a sub-tropical climate, characterized by hot humid summers and dry and mild winters, with higher rainfall during the summer months.

Seeds of all species were examined by two-dimensional X-ray imaging (Faxitron MX-20 Imaging system, Lincolnshire, Illinois, USA) to determine the seed fill percentage for each seed lot. Randomly selected seed samples (4 replicates of 25 seeds per species) were exposed to 18 Kv for 20 s. The percentage of fully filled seeds was determined by counting the number of seeds that exhibited full-sized endosperm or cotyledonary tissues and a visually healthy embryo. Partly filled and empty seeds were classified as unfilled. To determine the 100-seed mean weight of each seed lot, 5 samples of 100 seeds randomly selected from each seed lot were weighed. To alleviate physical dormancy (PY) in the Fabaceae species, seeds were pre-treated by soaking in hot water (95 ± 2°C) for 2 min. The hot water exposure time required to remove PY had been predetermined by a preliminary experiment and a review of published literature on similar seeds (Baskin et al., 1998; Hu et al., 2009; Erickson et al., 2016).

Experiment 1: effect of light and temperature on germination

Prior to the germination treatments being applied, all seeds were surface sterilized in 2% (v/v) sodium hypochlorite (NaOCl)

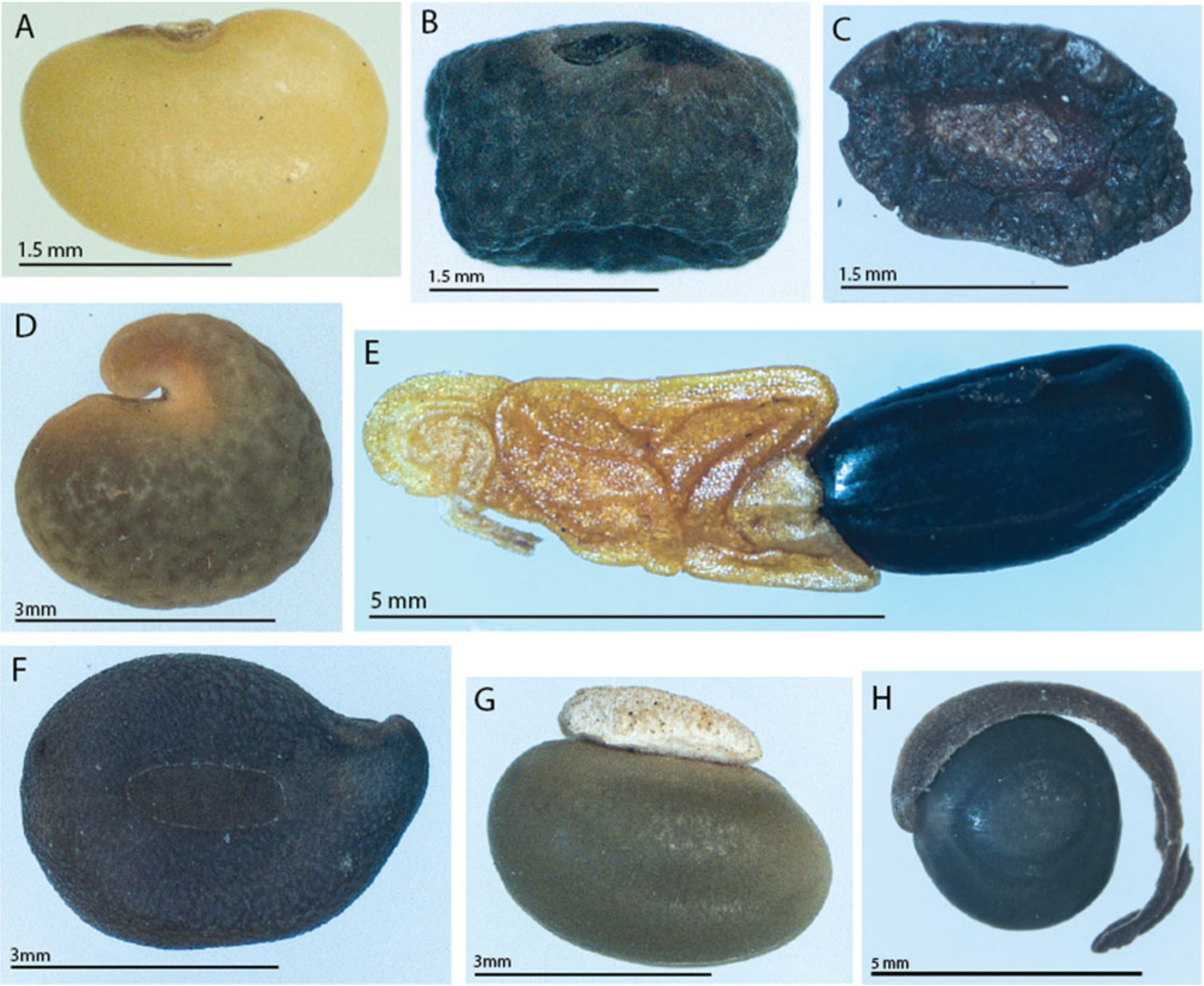

Figure 1. Seed morphology of the study species, placed in order from lightest to heaviest seed (A–H). (A) *Desmodium brachypodum*; (B) *Indigofera australis*; (C) *Corymbia citriodora*; (D) *Swainsona galegifolia*; (E) *Acacia leiocalyx*; (F) *Senna barclayana*; (G) *Hardenbergia violacea*; (H) *Acacia complanata*.

Table 1. Characteristics of the species studied, including, life form, habitat, diaspore type, dormancy class and seed pre-treatment needed to overcome dormancy. PY, physical dormancy; ND, non-dormant; HW, hot water.

Species	Common name	Family	Life form	Habitat	Diaspore	Dormancy	Pre-treatment
Acacia complanata A.cunn. Ex Benth.	Long-pod wattle	Fabaceae	Shrub	Dry sclerophyll forest, woodland and heath	Legume, sub-globular	PY	HW
Acacia leiocalyx (Domin) Pedley	Black wattle	Fabaceae	Shrub/Tree	Widespread in eucalypt woodlands	Legume, large and longitudinal	PY	HW
Corymbia citriodora (Hook.) K.D.Hill & L.A.S.Johnson	Lemon-scented gum	Myrtaceae	Tree	Undulating country in open forests and woodlands, dry ridges and plateaux of coastal sub-tropical Queensland	Ovoid small seed	ND	Not required
Desmodium brachypodum A. Gray	Large tick trefoil	Fabaceae	Perennial herb	Woodlands, dry forests and grassy areas	Legume, small and rounded	PY	HW
Hardenbergia violacea (Schneev.) Stearn	False sarsaparilla	Fabaceae	Climbing perennial	Widespread in a variety of habitats from coast to mountains, usually in open forests and woodlands	Legume, large and semi-spheroid	PY	HW
Indigofera australis Willd.	Austral indigo	Fabaceae	Slender perennial shrub	Open forests and woodlands in southern Australia	Legume, small and cuboid	PY	HW
Senna barclayana (Sweet) Randell	Pepperleaf senna	Fabaceae	Perennial herb/sub shrub	Forest, woodland, grassy areas and roadsides	Legume, medium and semi-deltoid	PY	HW
Swainsona galegifolia (Andrews) R.Br.	Darling pea	Fabaceae	Perennial shrub	Variety of habitats, mostly ranges and tablelands	Legume, medium and spherical but flattened	PY	HW

solution for 10 min with two drops of Tween 20 surfactant (Labchem, Zelienople, Pennsylvania, USA). Seeds were then washed three times with sterile water and blotted dry. Seeds were placed in plastic Petri dishes (9 cm diameter) containing two Whatman No. 1 filter papers moistened with 5 mL of deionized water or treatment solution (when required). The Petri dishes were closed and placed into a transparent plastic box (40 × 20 × 10 cm; l/w/d) lined with three layers of saturated paper towel, then closed with an airtight lid to reduce water evaporation from the Petri dishes. For each experiment, 3 replicates of 25 seeds each were used.

The untreated (control) and pre-treated (to break dormancy) batches of seeds from each of the eight species (Table 2) were placed into germination incubators (TRIL-750 Illuminated Refrigerator Incubator, Thermoline, Wetherill Park, Australia) set at four alternating (day/night) thermoperiods (15/5 ± 1°C [Winter], 25/15 ± 1°C [Spring/Autumn], 30/20 ± 1°C [Summer] and 35/25 ± 1°C [a simulated climate change Summer]), each with a matching 12/12 h (light/dark) photoperiod. In a second study, a thermogradient bar (T-bar; Lindner and May Pty. Ltd., Windsor, Brisbane, QLD, Australia) was used to incubate pre-treated (non-dormant) seeds at constant temperatures ranging from 11.6 ± 0.5 to 36.7 ± 0.5°C (11.6, 14.6, 17.0, 19.5, 23.4, 24.9, 27.4, 29.7, 33.2 and 36.7°C). The T-bar consisted of 10 insulated, equal-sized individual chambers and the ambient temperature and humidity inside each of the chambers was monitored hourly using Tinytag TGP 4017 data loggers (Hastings Ltd., Port Macquarie, NSW, Australia). Seeds were exposed to cool white, fluorescent light (*ca.* photosynthetic photon flux density [PPFD] of 100 µmol m^{-2} s^{-1}) with a 12/12 h, day/night photoperiod or to darkness (Petri dishes wrapped with two layers of aluminium foil).

Table 2. Seed lot information, including provenance, collection date and seed fill (as determined by X-ray).

Species	Provenance	Collection date	Seed fill (%)
Acacia complanata	Gympie	Nov-21	88 ± 5
Acacia leiocalyx	Bundaberg	Nov-20	95 ± 2
Corymbia citriodora	Newcastle/Cutt	Jun-20	93 ± 1
Desmodium brachypodum	Narrabri	Mar-20	99 ± 1
Hardenbergia violacea	Esk	Dec-21	100 ± 0
Indigofera australis	Esk	Dec-21	100 ± 0
Senna barclayana	Narrabri	Feb-21	88 ± 3
Swainsona galegifolia	Gunnedah	Nov-14	92 ± 4

Experiment 2: germination under environmental factors

Effect of water potential on germination

Seeds were germinated at target water potentials of 0.0, −0.1, −0.2, −0.4, −0.6, −0.8, −1.0 or −1.5 MPa (Lewandrowski et al., 2018; Frischie et al., 2019). The water potentials were achieved using polyethylene glycol BioUltra 8,000 (PEG; Sigma-Aldrich, 3050 Spruce Street, St. Louis, MO, USA) and with distilled water as the control treatment. The PEG solutions were prepared according to Michel (1983) and made up to the appropriate strength with sterile deionized water. The PEG concentrations were calculated using the following equation (Michel 1983):

$$[\mathrm{PEG}] = [4 - (5.16\psi T - 560\psi + 16)^{0.5}]/(2.58T - 280)$$

where:

Temperature (T) is expressed in degrees Celsius ($^\circ$C);
Osmotic potential (Ψ) is expressed in bar (1 bar = 0.1 MPa);
PEG amount, [PEG], is expressed as g of PEG/g of H_2O

This equation is the result of a model that considers ψ varying quadratically with concentration and linearly with temperature of solution. The selected water potential values are target nominal values, given that water potential can vary slightly during testing. Seeds were then placed to germinate at 25/15°C following the germination protocol described in Experiment 1. The same germination protocol was used for seeds exposed to the salinity and pH treatments described below, with each Petri dish receiving 5 mL of its respective solution.

Effect of salinity on germination

To determine how salinity can affect seed germination, sodium chloride (NaCl; AnalaR NORMAPUR®, lot 15K300021) solutions of 0 (control), 100, 200 or 400 mM were used (Cochrane 2018). The salinity range was selected based on salinity levels found in Australian soils (Rengasamy 2006, 2010).

Effect of pH on germination

To determine the effect of pH on seed germination, pH levels were selected based on naturally occurring pH ranges in Australian soils (4.0–10.0) (Rengasamy 2006; de Caritat et al., 2011). To obtain solutions of pH 4.0 and 6.0, a 2 mM solution of MES [2-(*N*-morpholino) ethanesulphonic acid] was adjusted with 0.1 M hydrogen chloride (HCl) or sodium hydroxide (NaOH). For pH 8.0, a 2 mM solution of HEPES [*N*-(2-hydroxymethyl) piperazine–*N*–(2-ethanesulphonic acid)] was adjusted with 0.1 M NaOH. Finally, for pH 10, buffer solutions were prepared with 2 mM tricine [*N*-Tris (hydroxymethyl) methylglycine] and adjusted with 0.1 M NaOH. Five mL of the corresponding solution was added to each Petri dish and 5 mL of unbuffered deionized water (pH 6.4) was used as a control. This experiment was not undertaken for *A. complanata* given the limited number of seeds available for this species.

Germination niche breadth

Germination niche breadth (B_n) was estimated using final germination proportions for the following environmental factors: alternating and constant temperatures, water stress, salinity and pH, using a normalized version of the reciprocal of the Simpson index:

$$B_n = \frac{1}{(R\sum_i p_i^2)}$$

with p_i denoting the proportion of seeds germinated in each state at ith experimental condition; R denoting the total number of states (experimental conditions for each treatment); B_n values range from 1/R (narrow niche) to 1.0 (broad niche) (Feinsinger et al., 1981). For both temperature parameters (alternating and constant), light and dark results were pooled together to estimate B_n, and this was only done for non-dormant seeds.

Experimental design and statistical analysis

A completely randomized design was used for all experiments with all seed germination tests conducted for 28 days (Baskin and Baskin, 2014). Germination, defined as radicle protrusion of 1–2 mm (Pedrini and Dixon 2020), was recorded three times a week with all germinated seeds immediately removed, to avoid interaction with ungerminated seeds. Seeds showing bacterial or fungal contamination were also removed to avoid further contamination. Seeds imbibed under darkened conditions were observed for germination in a dark room under a green light (PPFD *ca.* 0.14 μmol m^{-2} s^{-1}; Lion 24 light emitting diode [LED] magnetic work lamp covered with a plastic sheet transmitting green light 530–580 nm). At the end of each experiment, a cut test was conducted to visually assess the seed viability of all non-germinated and healthy-looking seeds (*i.e.*, those free of bacterial or fungal contamination). Seeds were considered viable when the presence of a firm, white endosperm and embryo were observed.

All germination results were adjusted for seed fill (as an estimate of seed viability, using the viability adjusted germination [VAG] equation) (Tieu et al., 2001):

$$\mathrm{VAG} = \frac{\text{Final germination}}{\text{Viability}} \times 100$$

The t_{50} (the median germination time) was calculated for germination under optimum alternating temperatures (of 25/15°C), for water stress, salinity and pH, using a three-parameter log-logistic model by fitting cumulative germination data over time, using the drc package (Ritz and Streibig 2012) in the R statistical software environment (R Core Team 2022):

$$\text{Germination} = \frac{G_{\max}}{1 + \exp[b(\log(\text{time}) - \log(t_{50}))]} \times 100$$

with $G_{\max}$ denoting the maximum germination; t_{50} denoting the time required for 50% of the seeds that germinated during the trial to germinate; b denoting the slope of the equation at t_{50}.

The MGT (the reciprocal of the rate of germination) was calculated for water stress, salinity and pH as:

$$\mathrm{MGT} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \times 100$$

with n_i denoting the number of seeds germinated in the ith time; k denoting the last day of germination evaluation; t_i denoting the time from the beginning of the experiment to the ith observation.

Seed synchrony was calculated for water stress, salinity and pH using the synchronization index, which varies from 0 to 1 (with a synchrony of 1 meaning that seed germination occurs at the same time):

$$\mathrm{SYN} = \frac{\sum C_{n_{1,2}}}{N}$$

where $C_{n_{1,2}} = \frac{n_i\ (n_i - 1)}{2}$ and $N = \frac{\sum n_i \left(\sum n_i - 1\right)}{2}$

and n_i is the number of seeds germinated in the ith time.

Seed mass was analysed for significant differences between species by analysis of variance (ANOVA), followed by a Tukey *post-hoc* test for mean comparison between species' seed mass. Final seed germination for each experiment was analysed using binomial logistic regressions (generalized linear models [GLM] fitted with a logistic link function and a binomial error structure) to determine factorial effects, using the GLM function in R. For each environmental factor, a fully factorial model including all factors and interactions were computed. Final germination was analysed as the response variable, with environmental factors (water stress, salinity, or pH) and species, environmental factor and seed mass, environmental factor and MGT as explanatory variables. Then a Tukey *post-hoc* test was undertaken for mean comparison between treatments. The MGT and seed synchrony for germination under the different environmental factors were analysed using ANOVA and Tukey *post-hoc* for mean comparison between treatments.

To fit hydro- and thermal-time germination models, the seedr R package was used (Fernández-Pascual et al., 2020). The hydro-time model of Bradford (Bradford, 2002; Bewley et al., 2013) was used to determine the ψ_b and the hydro-time constant (θ_H). The thermal-time model of Garcia-Huidobro (Garcia-Huidobro et al., 1982; Bewley et al., 2013) was used to calculate the cardinal temperatures T_b, T_c, T_o and the thermal-time values (θ_T). The constant temperatures from Experiment 1 (with light and dark results pooled together) were used to calculate the thermal-time models. All values for the hydro- and thermal-time model parameters were calculated based on the 50th percentile. It was not possible to calculate cardinal temperatures for *A. leiocalyx*, *H. violacea* and *S. galegifolia* (due to their low germination percentages), and θ_T for *S. barclayana* (due to its low germination percentage) and *D. brachypodum* (although germination percentages were high for this species, most germination events occurred on the same count date which compromised model fitting). It was not possible to calculate ψ_b for *A. complanata, H. violacea, S. barclayana* and *S. galegifolia* (due to their low germination). A correlation matrix was carried out (using the R corrplot package) to determine the correlation between the different seed functional traits measured (seed mass, t_{50}, final germination, ψ_b, T_b, T_c and T_o).

Results

Seed mass

Seed mass ranged from 0.27 to 2.54 g 100 seed^{-1} ($F_{7,24} = 1779$, $p < 0.001$; Table 3). Most species showed significantly different seed masses, except for *D. brachypodum* and *I. australis*, *I. australis* and *C. citriodora*, and between *A. leiocalyx* and *S. barclayana*.

Table 3. Mean 100-seed mass and time to 50% germination (t_{50}; ± SEM) for eight sub-tropical Australian native species. Species are arranged in order of increasing seed mass. Mean values followed by the same superscript letter are not significantly different ($p < 0.05$).

Species	100-seed mass (g)	t_{50} (days)
Desmodium brachypodum	0.27 ± 0.00^{a}	3.8 ± 04^{a}
Indigofera australis	0.31 ± 0.01^{ab}	4.8 ± 0.9^{a}
Corymbia citriodora	0.42 ± 0.01^{b}	8.0 ± 0.4^{b}
Swainsona galegifolia	0.64 ± 0.03^{c}	11.0 ± 2.1b^{bc}
Acacia leiocalyx	0.89 ± 0.01^{d}	13.7 ± 2.0^{c}
Senna barclayana	0.95 ± 0.04^{d}	2.4 ± 0.8^{a}
Hardenbergia violacea	2.07 ± 0.04^{e}	13.8 ± 12.9^{c}
Acacia complanata	2.54 ± 0.03^{f}	15.4 ± 0.8^{c}

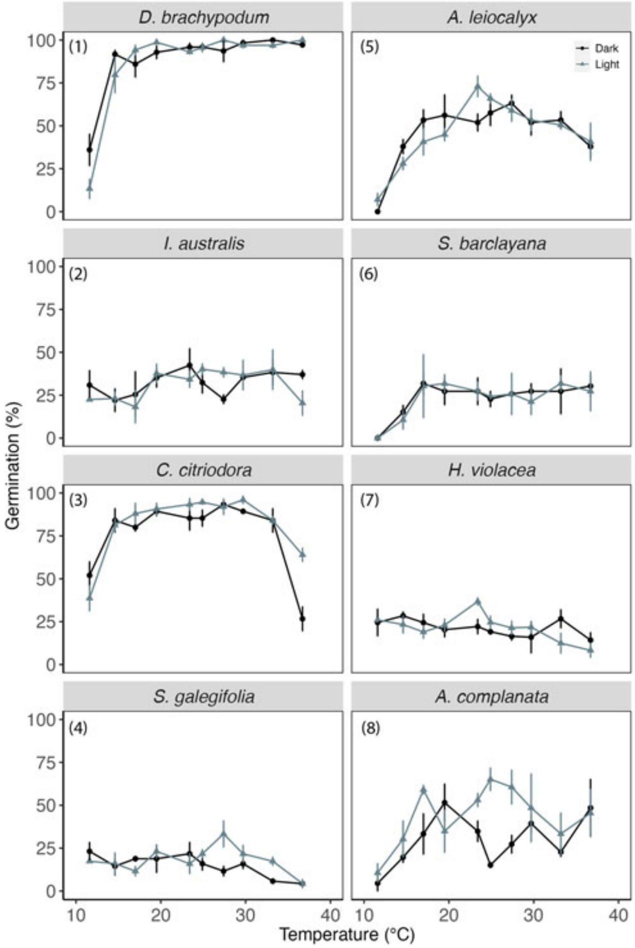

Figure 2. Effects of temperature and an alternating light/dark (–►–) or constant dark (–●–) incubation treatment on mean final germination percentage ± SEM of seeds of eight sub-tropical Australian native species. Pre-treated (non-dormant) seeds (soaking in hot water undertaken for all Fabaceae species) were incubated at temperatures between 12.0 ± 0.5 and 37.0 ± 0.5°C in deionized water and exposed to a 12/12 h light/dark photoperiod or constant dark by wrapping the Petri dishes in two layers of aluminium foil for 28 days. Species are arranged (and numbered) in order of increasing seed mass.

Experiment 1: effect of temperature and light on germination

The final germination percentage varied between species (Figs. 2, 3). *Desmodium brachypodum* and *C. citriodora* had the highest final germination under most alternating and constant temperatures, reaching >95% germination in at least one alternating and one constant temperature. On the other hand, *S. barclayana, S. galegifolia* and *I. australis* had <50% germination for all alternating and constant temperatures. *Swainsona galegifolia* showed the lowest germination percentage, with seeds treated with hot water having a maximum of 36 ± 9% at 25/15°C. Most species germinated to some extent under all alternating temperatures (Fig. 3), with *D. brachypodum* and *S. barclayana* having significantly lower germination at 15/5°C ($F_{3,43} = 28.73$; $p < 0.001$ and $F_{3,\,43} = 18.90$; $p < 0.01$, respectively). All species had some germination at the highest alternating temperature of 35/25°C. At constant temperatures (Fig. 2), germination increased as temperature increased until *ca.* 25°C, with no further increase. A higher germination percentage was observed for most species sown at alternating temperatures (Fig. 3) as compared to their respective constant temperatures (Fig. 2).

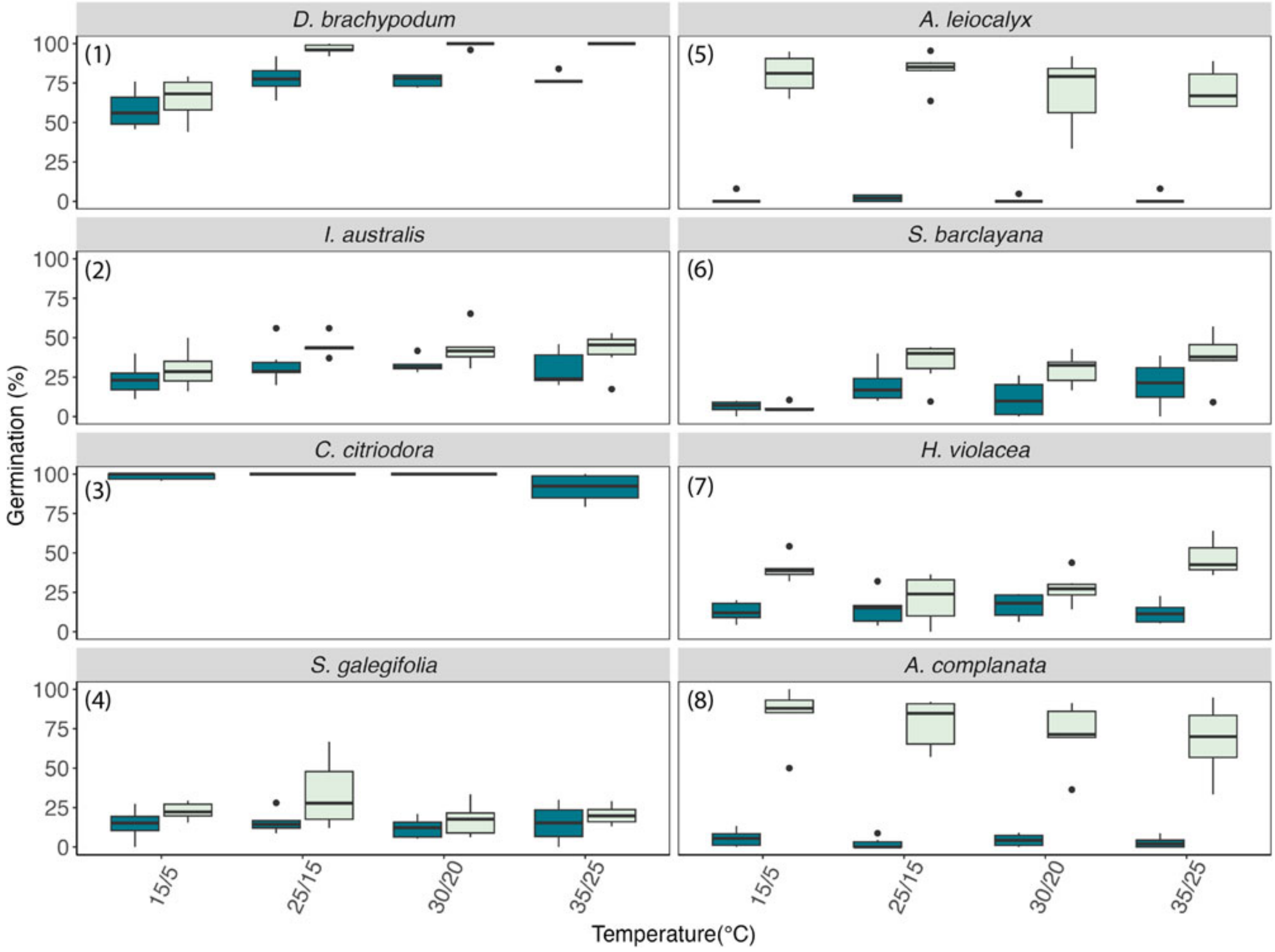

Figure 3. Effects of temperature and a pre-treatment (soaking seeds in hot water for all Fabaceae species; hot water was not applied to *C. citriodora* seeds as they are non-dormant) on mean germination percentage of seeds of eight sub-tropical Australian native species. Control (dormant; dark blue boxplots) and pre-treated (non-dormant; light blue boxplots) seeds were incubated at four alternating temperatures of 15/5, 25/15, 30/20 and 35/25°C in sterile deionized water and exposed to 12/12 h light/dark photoperiod or constant dark by wrapping Petri dishes in two layers of aluminium foil for 28 days. As no significant differences occurred between light or dark treatments, results show light and dark data pooled together. Species are arranged in order of increasing seed mass.

The thermal germination niche breadth varied between species, with *D. brachypodum* and *C. citriodora* having broad breadths for both alternating and constant temperatures (>0.6; Table 4). All other species had narrow thermal breadths (≤0.3). In general, for most species, the thermal niche breadth was broader for alternating as compared to constant temperatures (Table 4). No significant differences were observed between light and dark on final germination percentage for all species, at either constant ($p > 0.1$) or alternating ($p > 0.05$) temperatures (Fig. 2).

For species with PY, hot water treatment increased germination for most species at various temperature regimes (Fig. 3). For example, control seeds of *A. complanata* and *A. leiocalyx* had <9% germination, but when treated with hot water, germination significantly increased ($F_{1,46} = 375.30$; $p < 0.001$ and $F_{1,46} = 491.41$; $p < 0.01$, respectively) to > 57% for all alternating temperature regimes. For *D. brachypodum* and *S. barclayana*, there was no significant difference ($p = 1$) between the control and hot water at the lowest alternating temperature (15/5°C).

Species with lighter seeds tended to have faster germination (Pearson correlation coefficient of 0.67; Table 3) compared with heavier seeds at 25/15°C (optimum germinating conditions, irrespective of light). For example, *D. brachypodum* and *I. australis* had t_{50} of 3.8 ± 0.4 and 4.8 ± 0.9 days, respectively, significantly shorter ($p < 0.04$) than heavier seeds of *C. citriodora* (8.0 ± 0.4), and those three species had significantly faster ($p < 0.07$) germination than heavier-seeded species *A. leiocalyx* and *A. complanata*, which had $t_{50} > 13$ days, respectively (Table 3). However, there was no relationship between seed mass and final germination percentage for most species at optimum conditions, except for the small-seeded *D. brachypodum* and *C. citriodora* that showed higher germination compared to the other larger-seeded species.

Cardinal temperatures (T_b, T_c and T_o) varied between species (Table 5). The estimated T_c was 41–53°C (*C. citriodora* and *A. complanata*, respectively), and the T_b varied from 8°C (*C. citriodora*) to 12°C (*D. brachypodum* and *I. australis*). Optimum germination temperatures were between 24 and 29°C (*I. australis* and *D. brachypodum*, respectively). The θ_T values differed for sub-optimal and supra-optimal values, with *C. citriodora* having the lowest values (faster germination) for both sub-and supra-optimal θ_T (48 and 63°C hour [°C h], respectively). *Indigofera australis* had the highest value for sub-optimal θ_T (392°C h) and *A. complanata* had the highest value for supra-optimal θ_T (593°C h). The ψ_b varied between −0.1 MPa (*A. leiocalyx*) and −1.5 MPa (*D. brachypodum*; Table 5). The

Table 4. Germination niche breadth (Bn) for eight sub-tropical Australian native species, for the environmental factors: constant temperatures, alternating temperatures, water stress, salinity and pH. pH was not tested for *Acacia complanata* (given low seed numbers). Species are arranged in order of increasing seed mass. *R* is the number of total states per environmental factor.

Species	Environmental factor	1/*R*	Bn
Desmodium brachypodum	Constant temperature	0.25	0.70
	Alternating temperature	0.10	0.80
	Water stress	0.13	0.76
	Salinity	0.25	0.58
	pH	0.20	0.96
Indigofera australis	Constant temperature	0.25	0.09
	Alternating temperature	0.10	0.13
	Water stress	0.13	0.08
	Salinity	0.25	0.03
	pH	0.20	0.16
Corymbia citriodora	Constant temperature	0.25	0.62
	Alternating temperature	0.10	0.78
	Water stress	0.13	0.38
	Salinity	0.25	0.19
	pH	0.20	0.62
Swainsona galegifolia	Constant temperature	0.25	0.03
	Alternating temperature	0.10	0.03
	Water stress	0.13	0.03
	Salinity	0.25	0.03
	pH	0.20	0.09
Acacia leiocalyx	Constant temperature	0.25	0.22
	Alternating temperature	0.10	0.30
	Water stress	0.13	0.08
	Salinity	0.25	0.12
	pH	0.20	0.31
Senna barclayana	Constant temperature	0.25	0.13
	Alternating temperature	0.10	0.06
	Water stress	0.13	0.03
	Salinity	0.25	0.04
	pH	0.20	0.04
Hardenbergia violacea	Constant temperature	0.25	0.04
	Alternating temperature	0.10	0.10
	Water stress	0.13	0.02
	Salinity	0.25	0.02
	pH	0.20	0.14
Acacia complanata	Constant temperature	0.25	0.13
	Alternating temperature	0.10	0.23
	Water stress	0.13	0.10
	Salinity	0.25	0.13

range of hydrotime (θ_H) varied between 1.5 MPa h (*A. leiocalyx*) and 5.5 MPa h (*C. citriodora*).

A correlation matrix (Fig. 4) was undertaken for the species (*D. brachypodum, I. australis, C. citriodora, S. barclayana* and *A. complanata*) where it was possible to compare all seed functional traits. For these species, seed mass was strongly positively correlated with ψ_b ($r = 0.87$ [Spearman's correlation coefficient]), also positively correlated to t_{50} ($r = 0.4$). t_{50} was positively correlated with final germination and T_c ($r = 0.5$), and strongly negatively correlated with T_b ($r = -0.87$). Final germination was strongly negatively correlated with ψ_b and T_b, but positively correlated ($r = 0.6$) to T_o. ψ_b and T_o were negatively correlated ($r = -0.67$) (Fig. 4).

Experiment 2: germination under different environmental factors

There were significant interactions between all environmental factors and species ($p < 0.001$), showing that responses to environmental factors were species-specific (Figs. 5–7). For all species, final germination percentage decreased with decreasing water potential ($F_{49,\ 128} = 4.80$; $p < 0.01$) (Fig. 5A). *Desmodium brachypodum* and *C. citriodora* were the only species to have high germination down to −0.8 MPa (with 93 ± 2 and 64 ± 4%, respectively), and *D. brachypodum* was the only species to germinate down to −1.5 MPa with 36 ± 4% (the lowest negative water potential assessed in this study) (Fig. 5A). On the other hand, from −0.4 MPa onwards germination decreased when compared to the control for several species. For *A. complanata*, germination significantly decreased ($p < 0.01$) when compared to the control for all values lower than −0.1 MPa (Fig. 5A). Germination niche breadth for water stress was broad for *D. brachypodum* (0.76), but narrow for all other species (<0.38).

Most species with faster germination (lower t_{50} at optimum germination conditions) had higher germination under water stress (Fig. 5), such as *D. brachypodum, I. australis* and *C. citriodora* and (with t_{50} of 3.8 ± 0.4, 4.8 ± 0.9 and 8.0 ± 0.4 days, respectively). On the other hand, *A. complanata*, which had slower germination (t_{50} of 15.4 ± 0.8 days), gave a very low germination percentage under water stress. Moreover, MGT and/or t_{50} significantly increased ($p \leq 0.001$; Fig. 5C). As the water stress increased for *D. brachypodum* (from −1.00 MPa), *A. leiocalyx* (at −0.60 MPa), *H. violacea* (at −0.50 MPa), *I. australis* (from −0.04 MPa), *S. barclayana* and *S. galegifolia* (at 0.08 MPa), when compared to the control (Fig. 5B). Germination synchrony did not significantly vary for all species but *D. brachypodum*, where synchrony decreased from −1 MPa. There was also an interaction between seed mass and water potential, as heavier-seeded species (such as *A. complanata*, *H. violacea, S. barclayana* and *A. leiocalyx*) tended to have lower final germination and higher base water potential.

For all species, germination significantly decreased as salinity increased ($F_{21,64} = 3.82$; $p < 0.01$; Fig. 6A). *Desmodium brachypodum* was the only species that germinated at the highest salinity concentration of 400 mM, although germination (of 9 ± 5%) was significantly lower ($p < 0.001$) than for the other concentrations (>70%; Fig. 6A). This species, the lightest-seeded species with the fastest germination, was also the species to have the highest final germination percentage across all salinity levels. On the other hand, there was no significant interaction ($p > 0.05$) between t_{50} or seed mass and germination response under salinity levels for the remaining species. Regardless of the species' seed

Table 5. Seed germination thresholds for eight Australian native species. Cardinal temperatures are defined as base temperature (T_b), optimal temperature (T_o) and ceiling temperature (T_c). The thermal-time model relates to the sub-optimal (θ_T) and supra-optimal (θ_{Tsupra}) range of temperatures. Hydro-time modelling is related to the base water potential (ψ_b) thresholds and hydro time (θ_H) for germination. All parameters from the thermal- and hydro-time models were based on the 50th percentile. Parameters could not be measured for some species due to low germination.

Species	T_b (°C)	θ_T (°C h)	T_o (°C)	T_c (°C)	θ_{Tsupra} (°C h)	ψ_b (MPa)	θ_H(MPa h)
Acacia complanata	11.4	300.8	25.7	52.5	593.0	–	–
Acacia leiocalyx	–	–	–	–	–	−0.1	1.5
Corymbia citriodora	7.9	47.72	28.2	41.3	62.5	−0.9	5.5
Desmodium brachypodum	11.6	–	28.6	45.1	–	−1.5	5.2
Hardenbergia violacea	–	–	–	–	–	0	2.0
Indigofera australis	11.6	329.0	23.8	43.2	519.88	−0.2	4.3
Senna barclayana	14.6	–	27.5	36.7	–	–	–
Swainsona galegifolia	–	–	–	–	–	−0.04	2.8

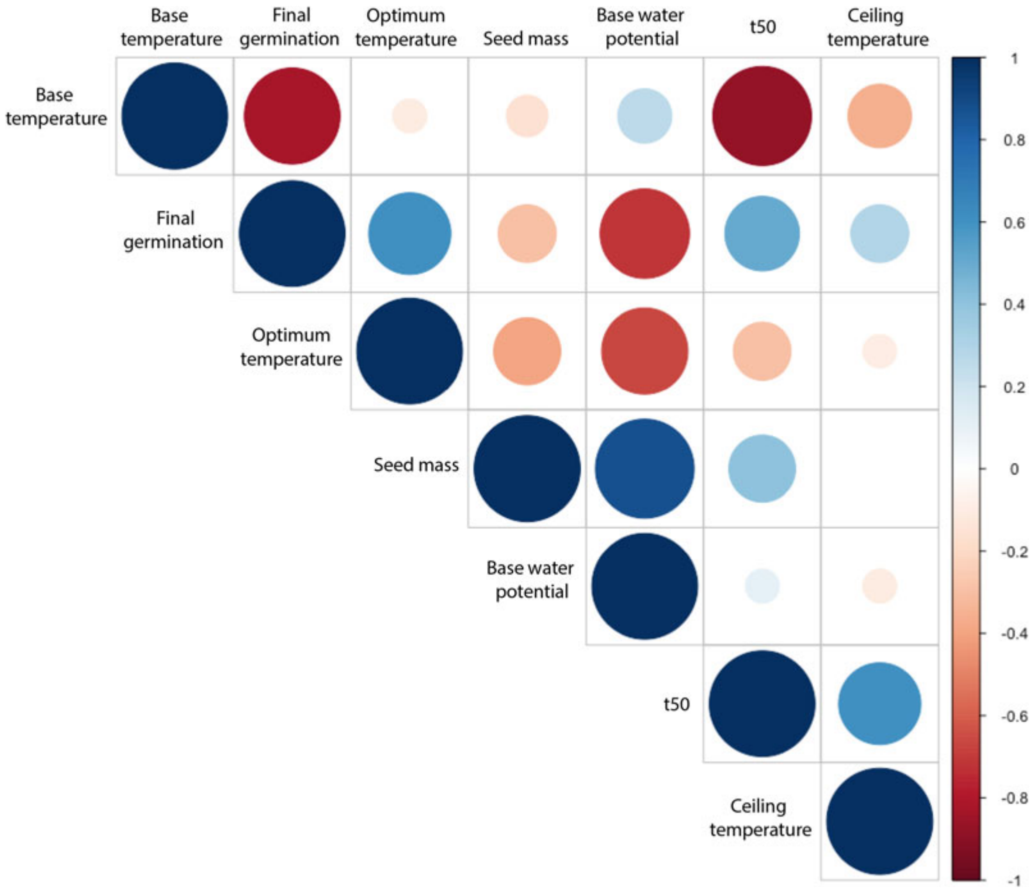

Figure 4. Spearman correlation matrix showing the relationship between seed functional traits of five native species (*Desmodium brachypodum, Indigofera australis, Corymbia citriodora, Senna barclayana* and *Acacia complanata)*: base temperature (°C), final germination (%), optimum germination temperature (°C), seed mass (g), base water potential (MPa), time to 50% germination (t_{50}, days) and ceiling temperature (°C). Circle size and intensity of colour show the strength of the associations, where larger circles show stronger associations. Circle colour displays whether an association is positive (blue) or negative (red). The key to correlation coefficients is shown in the right-hand bar.

mass or t_{50}, final germination decreased in a similar fashion for all species (Fig. 6A). The MGT and/or t_{50} significantly increased with increasing NaCl ($p \leq 0.03$) for *D. brachypodum, I. australis, S. barclayana, S. galegifolia* and *H. violacea* (from 100 mM) (Fig. 6B). Germination synchrony did not significantly vary ($p > 0.05$) for any of the species (Fig. 6C). Germination niche breadth for salinity was broad only for *D. brachypodum* (=0.76), but narrow for all other species (<0.40; Table 4)

There was a significant interaction between pH and species ($F_{24,70} = 2.62$; $p < 0.01$), but pH levels did not significantly ($p > 0.05$) affect germination percentage, MGT, t_{50} or germination synchrony for most species (Fig. 7). *Corymbia citriodora* showed

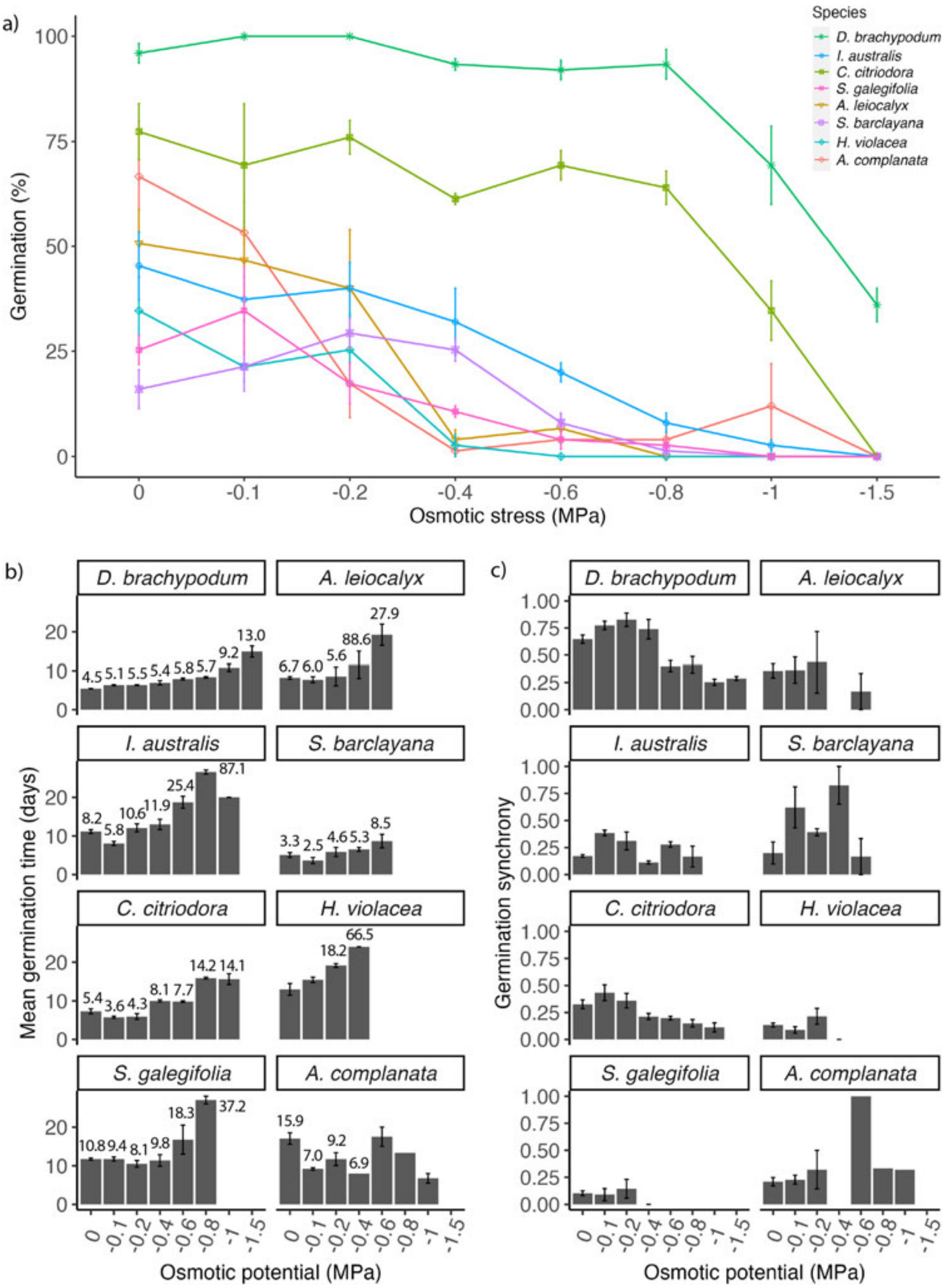

Figure 5. Final germination (A), mean germination time, with the estimated time to 50% germination (t_{50}) values (days) added (when it was possible to estimate them), respectively, to each MGT bar (B) and germination synchrony (C) of eight species incubated in deionized water (control) or in different water potential solutions (achieved using polyethylene glycol [PEG] 8,000) and under a 12/12 h photoperiod and a matching thermoperiod (25/15°C). Species are arranged in order of increasing seed mass.

significantly higher germination ($p < 0.02$) at pH 4.0, 6.0 and 8.0 (≥80%) when compared to the control (56 ± 4%), and germination of *A. leiocalyx* was significantly higher ($p < 0.02$) at pH 4.0, 8.0 and 10.0 (>60%) compared to the control (35 ± 6%) (Fig. 7A). There was no significant interaction ($p > 0.05$) between t_{50} and pH, or between seed mass and pH. Germination niche breadth for pH was broad for *D. brachypodum* and *C. citriodora* (>0.6), but narrow for all other species (≤0.31; Table 4). However, it is important to note that these narrow results might be related to the overall low germination percentages rather than species having a narrow niche for pH levels, given that most species did not have significant differences in germination percentages for different pH levels.

Discussion

By focussing on seed functional traits, rather than on species, it is possible to identify seed characteristics and germination dynamics that can be generalized across groups of species, such as species co-existing in the same habitat or with phylogenetically related species (such as the Fabaceae family). The findings from this study provide insight into the current and future impacts of

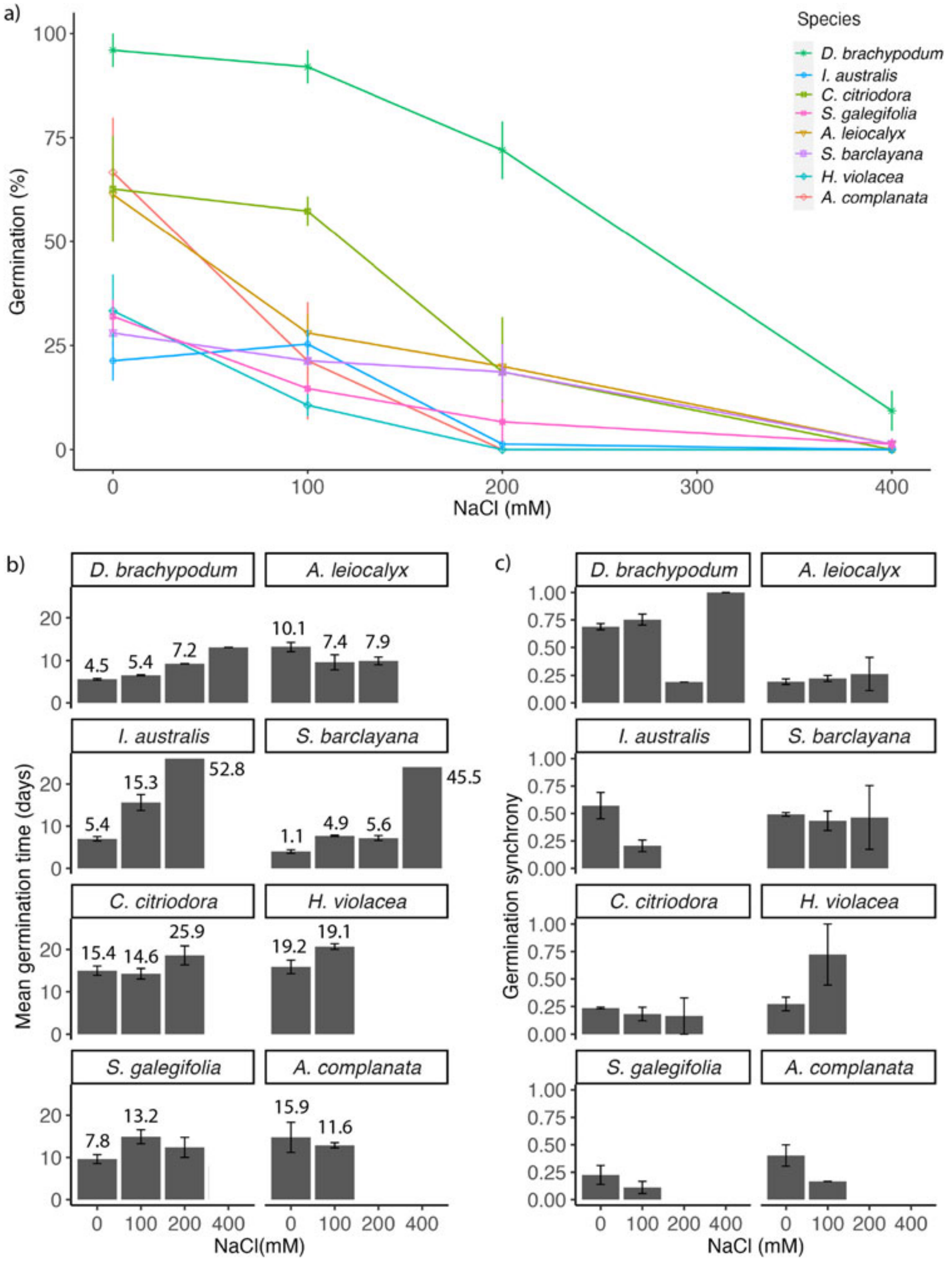

Figure 6. Final germination (A), mean germination time, with the estimated time to 50% germination (t_{50}) values (days) added (when it was possible to estimate them), respectively, to each MGT bar (B) and germination synchrony (C) of eight species incubated in deionized water (control) or in different salinity solutions (achieved using sodium chloride [NaCl]) and under a 12/12 h photoperiod and matching thermoperiod (25/15°C). Species are arranged in order of increasing seed mass.

climate change on seed recruitment of sub-tropical Australian species and allow determination of the range of regeneration conditions required. Consequently, the results have important implications for seed-based restoration programmes.

This study firstly examined the temperature thresholds and light conditions for germination of eight Australian native species with different seed masses. Secondly, it examined the germination response (final germination percentage, germination time and synchrony) under various conditions of water stress, salinity and pH, with the aim of identifying interactions between seed functional traits and their responses to environmental factors, together with determining germination niche breadths for each environmental factor. Results show that, in general, lighter seeds had faster germination and therefore a better germination response to water stress and salinity (Figs. 5, 6, respectively) when compared to heavier seeds that had slower germination. Additionally, germination was indifferent to light conditions, the thermal niche breadth was broad for some species (Table 4), but most species were able to germinate even at high-temperature conditions (Figs. 2, 3). Most species had narrow germination niche breadths for water stress and salinity (Fig. 5, 6, Table 4).

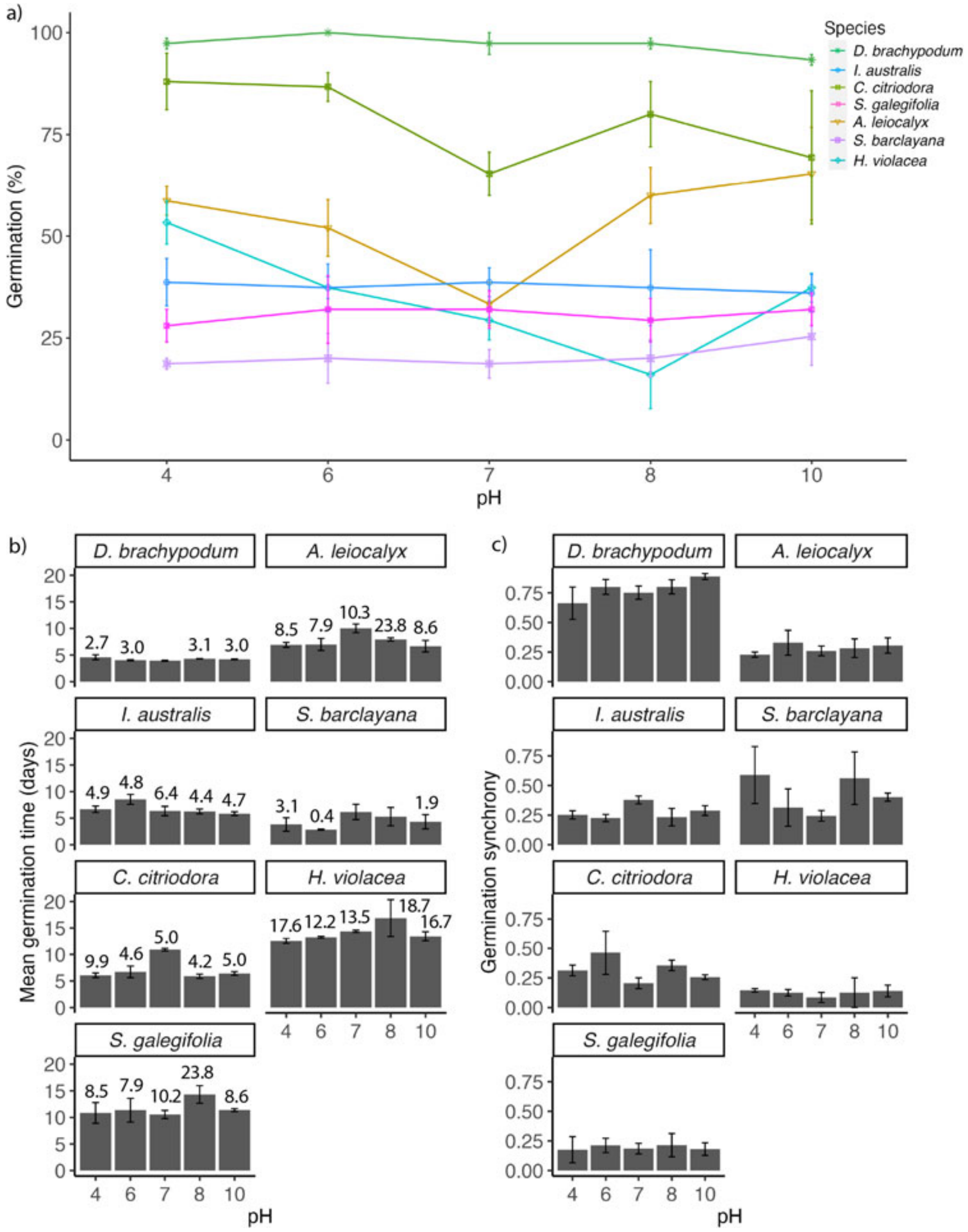

Figure 7. Final germination (A), mean germination time, with the estimated time to 50% germination (t_{50}) values (days) added (when it was possible to estimate them), respectively, to each MGT bar (B) and germination synchrony (C) of seven species incubated in deionized water (control) or in different pH solutions (achieved using different buffer solutions) and under a 12/12 h photoperiod and matching thermoperiod (25/15°C). Species are arranged in order of increasing seed mass.

Effect of light and temperature on germination

Under optimum germination conditions (25/15°C; regardless of light) there was a positive correlation ($r = 0.67$) between seed mass and t_{50}, with lighter seeds germinating faster than heavier seeds (Table 3, Fig. 4). Previous studies have shown that seed mass can have a positive, negative, or no effect on final germination (Shipley and Parent 1991; Daws et al., 2008; Kahmen and Poschlod 2008; Barak et al., 2018). Although earlier emergence and faster growth have been identified as the main advantages of large-seeded species, studies investigating several species have shown that smaller-seeded species frequently have higher germination rates (e.g. Norden et al., 2009; Merino-Martín et al., 2017). The positive correlation between seed mass and t_{50} could be related to species with lighter seeds having evolved over time for faster germination and emergence to escape unfavourable environmental conditions. Fast germination might also be a competitive strategy for small (lighter) seeded species to establish in favourable micro-sites before larger-seeded (heavier), more competitive, species (Norden et al., 2009).

Some species under study had a wide thermal germination niche (Figs. 2, 3; Table 4), with most species having a higher germination percentage achieved under alternating temperatures as compared to the constant temperatures. This has been previously observed in the literature (Pérez and Chumana 2020), as alternating conditions represent better the temperature fluctuations seeds sense in nature. The T_b (predicted minimum temperature that needs to be exceeded for germination to occur) ranged from 8 to 12°C, T_o (temperature at which the highest germination speed is achieved) was 24 to 29°C and T_c (maximum temperature under which germination can occur) was 43 to 53°C (Table 5). Results showed that germination responses were not significantly affected by warmer temperatures, as germination percentage was not reduced considerably (except for *C. citriodora*) for most species as temperatures increased, and most species had a very high T_c. In a germination trait analysis carried out by Dürr et al. (2015) on 243 species of different ecosystems around the world, the study found that T_c values for wild species were limited to 35–40°C, a range that is lower than some of the species in this current study. Having high T_c might provide species with a competitive advantage when competing with other species to colonize fragmented habitats, which can reach high temperatures (Sampayo-Maldonado et al., 2019).

Temperature tolerance, as identified in this study, may indicate resilience to future warming temperatures (Cochrane et al., 2011). Species capacity to germinate under a wide temperature range is an adaptive strategy, providing an advantage under climate change scenarios (Sampayo-Maldonado et al., 2019). It is important to note that although modelling germination performance under environmental stress in the laboratory is useful to predict germination outcomes in the field (Tomlinson et al., 2022), laboratory results from this study should be validated in the field. For example, in this study, a $T_c > 45$°C was observed for *D. brachypodum* and *A. complanata*, which might not be applicable to reality.

Germination responses and seed functional traits

Although all the studied species naturally occur in similar habitats, their seed traits and germination responses varied considerably, suggesting that various seed traits might be responsible for modulating seed stress response (Kos and Poschlod, 2008; Saatkamp et al., 2019). Lighter-seeded and faster germinating species (Table 3) tended to have higher germination percentages under water stress and salinity and there was a strong positive correlation between seed mass and ψ_b. This suggests that seeds with faster germination might have an initial advantage to survive water stress and high salinity levels. Trait-specific responses have also been found in other studies examining Australian native species for water stress (Merino-Martín et al., 2017) and salinity (Cochrane 2018). Merino-Martín et al. (2017) also found that lighter-seeded species germinated better at lower water potentials compared to heavier-seeded species, when studying species from semi-arid environments in Western Australia, where heavier-seeded species could not germinate under lower water potentials. Similarly, Jurado and Westoby (1992) found that species with a seed mass of 0.1–10.0 mg were mostly fast germinators, while species with a seed mass >10 mg were mostly slow germinators. Smaller (lighter) seeds have a larger surface area to mass ratio, which confers seeds a faster capacity to absorb water (Kikuzawa and Koyama 1999). This might explain why *D. brachypodum* and *I. australis*, the species with the lightest seeds (<0.32 g per 100 seeds), had faster germination ($t_{50} < 5$ days).

Additionally, *D. brachypodum* and *C. citriodora* also had the lowest ψ_b (of -1.5 and -0.9 MPa respectively) and had high germination (> 63%) up to −0.8 MPa, with *D. brachypodum* being able to germinate even under −1.5 MPa (36%), which corresponds to very dry soil conditions. On the other hand, heavier-seeded species might not have been able to imbibe enough water to trigger germination when moisture was limited due to water stress or salinity conditions, due to their smaller surface area to mass ratio. Other morphological traits such as seed coat structure and thickness might also be an important factor in modulating germination, by regulating water uptake, by physically constraining embryo protrusion or by limiting gas exchange (Daws et al., 2005; Norden et al., 2009). Although these traits were outside the scope of this study, the authors recommend that they should be considered in future studies. It should be noted that the effectiveness of the hot water treatment to break PY might have varied between treated seeds, as conditions needed to break PY between species can vary considerably and there can also be intraspecific PY variation (Hudson et al., 2015). This may have caused some seeds to remain with a level of dormancy at the time of the experiment.

Germination and seedling emergence rate can be crucial in determining successful plant establishment in stressful conditions (Verdú and Traveset, 2005), such as salinity (Fernández et al., 2016) and drought (Cochrane, 2018). For most species studied, water stress conditions increased the time to germination relative to the control, and germination time significantly increased as water stress and salinity increased for several species. Faster germinating seeds can show increased seedling growth and development of root systems that can better uptake ground water, thus reducing topsoil moisture competition (Cochrane, 2018). Faster seedling growth can also be important for other interspecific competition such as light (Miller et al., 1994), space (Funes et al., 2009) and nutrients. Accordingly, Bochet et al. (2007) found that species that established successfully had higher mean germination rates than unsuccessful species at various water potentials tested. They identified that at water potentials between −0.05 and −0.35 MPa, germination time appeared to represent a critical threshold which could explain the differences in species success on road slope establishment. Furthermore, germination rates decreased with decreasing water potential for four *Piper* species (Piperaceae) from a tropical rainforest (Daws et al., 2002). Species that germinate early can have priority to later germinating species by interfering with their establishment, growth or persistence (Barak et al., 2018), and can therefore be important traits influencing species establishment in restored ecosystems (Pywell et al., 2003).

The ability to germinate under low ψ_b can be an indicator of a species' potential to survive successfully under arid conditions (Briedé and McKell, 1992; Bochet et al., 2007). Having a lower ψ_b allows seeds to germinate faster and earlier under drier soil conditions (Arène et al., 2017), as observed with the lighter-seeded species, *D. brachypodum* and *C. citriodora* (Fig. 5) in this study. Often, water stress can slow down seed water imbibition, therefore preventing germination (Cochrane et al., 2014), as occurred for most of the species under this study. Several studies have shown that arid plant species can germinate up to -1.0 and -1.5 MPa (Schütz et al., 2002; Neil et al., 2003) and have faster germination (Jurado and Westoby, 1992). Jurado and Westoby (1992) found that > 53% of the 105 species studied from central Australian arid zones had fast germination. Moreover, some seeds can remain quiescent until the water potential increases to levels sufficient for water imbibition (Bell et al., 1993). This has also been observed for salinity, where some species have been found to recover successfully from saline pre-treatments after being transferred to a non-saline environment (Cochrane 2018). Soil salinity can reduce germination rate and percentage (Khan and Gulzar, 2003; Baskin and Baskin, 2014) by either osmotic effects or by facilitating ion uptake that could result in toxicity (Ayers 1952). Therefore, examining the recovery (*i.e.,* the ability of seeds to germinate normally after being exposed to stress) of seeds should be incorporated into studies to assess if seeds can survive and germinate successfully after being exposed to stress.

On the other hand, having germination delayed by water stress might promote seed germination under sub-optimal environmental conditions, hampering successful seedling establishment (Cochrane, 2018). It is worth considering that environmental thresholds for germination might differ from those necessary for seedling emergence and establishment, with the transition from seed to seedling being a major bottleneck in the seed recruitment process (James et al., 2011). Although some studies have shown that germination under moisture stress might correlate to seedling drought tolerance traits (Kos and Poschlod, 2008), the next step from this study would be to conduct field studies to investigate how seed functional traits can influence the capacity of germinated seeds to convert into established seedlings.

The effects of pH on seed germination have been widely used for assessing the adaptive ability of species to cope with increased soil acidity or alkalinity (Norsworthy and Oliveira, 2005; Stokes et al., 2011). In this study, there was no interaction between pH and seed functional traits (including seed mass, germination time or synchrony), and pH levels did not influence seed germination patterns. Alkalinity (high pH) is a common characteristic of Australian soils (Ma et al., 2015), therefore these seeds might be adapted to germinating in such conditions. The ability to germinate under a wide range of pH values might indicate the potential ability of our study species to establish in alkaline, neutral, or acidic soils. This knowledge can be useful when restoring sites with extreme pH levels, such as in mine-site rehabilitation. It is important to note that the buffer solution properties and concentration can affect germination (Ma et al., 2015); therefore, field trials using soils with different pH levels should be undertaken to validate the effects of soil pH on seed germination and emergence.

Results from this study showed that most selected subtropical Australian native species have a high thermal tolerance, and some species (such as *D. brachypodum* and *C. citriodora*) had a wide thermal germination niche breadth. Even though projections for Australia show that temperatures in Spring and Summer (when germination of seeds of these species occurs in nature for these species) are predicted to rise more than Autumn and Winter temperatures due to climate change (Hughes 2011), most species from this study might have the capacity to germinate under future global warming. On the other hand, a narrow germination niche breadth in response to water stress and salinity might be the limiting factors that may restrict germination in the future. Predictions for the selected species' geographical locations (eastern Australia) show that rainfall patterns will decrease and become more erratic, with longer drought periods (CSIRO and Bureau of Meteorology 2020) and soil salinity is projected to increase considerably (Hassani et al., 2021). Therefore, careful considerations will have to be taken when using these seeds in seed-based restoration, to ensure water potentials in the field are higher than the critical thresholds for germination and are also sufficient for later seedling establishment. In addition, it is important to take into account that although seeds might be able to germinate in future warmer conditions, these warmer conditions might not be optimal for other plant life stages, as warmer conditions have been found to be detrimental in overall fitness conditions for some Australian native species (Notarnicola et al., 2023).

Conclusion

Understanding how different seed functional traits can benefit the reproductive performance of native species under environmental factors is important to understand plant recruitment and to predict the impacts of climate change on native plant communities and restored ecosystems. Several outcomes of this study have direct application in a restoration context. Pre-treating Fabaceae seeds prior to sowing has a significant positive effect on germination timing which can increase the environmental window for germination and help encourage rapid establishment. Additionally, our results suggest smaller-seeded species might be better suited for seed recruitment in adverse restoration sites. When studying germination responses under environmental factors, assessing a variety of seed functional traits in addition to seed mass is an important approach as this can improve species selection and manipulation in seed-based restoration programmes. Overall, our findings demonstrate that in our study system, light-seeded species can achieve successful germination even under high levels of environmental stress and highlight the sensitivity of heavier-seeded species to water stress and salinity. Light-seeded species, such as *D. brachypodum* and *C. citriodora*, may therefore be potential candidates for seed-based restoration programmes of highly degraded areas or environments that are predicted to be considerably impacted by climate change.

Acknowledgements. We would like to express our gratitude to Mr Clay Stokoe, Native Seeds and Land Repair, for kindly donating seeds for this study. This study was financed by the Graduate School Scholarships (UQGSS) and the School of Agriculture and Food Sustainability (AGFS) of The University of Queensland (UQ) and no external source of funding was used.

Competing of interest. All authors declare no conflict of interest.

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