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Physiological dormancy in forbs native to south–west Queensland: Diagnosis and classification

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Abstract

Seed physiological dormancy (PD) is reportedly the primary reason why many Australian native plants are not currently used for revegetation of degraded land. However knowledge of germination and dormancy of forb species from semi-arid environments is lacking. Consequently, we investigated germination of 15 Australian forb species from four families, particularly Asteraceae, native to south–west Queensland (Qld). Seeds were tested for viability using tetrazolium chloride (TZ) and sown at 5 to 35 °C. Nine species, including seven Asteraceae, achieved germination exceeding or not significantly lower ($P>0.05$) than TZ test results. Despite spring dispersal, the majority of species had optimal germination at temperatures reminiscent of winter months. Only six species exhibited low germination across all temperatures investigated when compared to TZ results ($P<0.05$), i.e. low germination could not be attributed to low seed viability. Of these, *Actinobole uliginosum* (Asteraceae) had non-deep PD since seeds responded to gibberellic acid (GA_3) and dry after-ripening. In contrast, *Goodenia fascicularis* appeared to exhibit deep PD since seeds did not respond to GA_3 or dry after-ripening, and scarification led to germination of abnormal seedlings. It appears that, contrary to expectations, seeds of many forbs native to south–west Qld (9 of 15 in this study), possess negligible or no dormancy and may therefore be suitable for use in land rehabilitation. Other species e.g. *G. fascicularis* require further work to investigate dormancy mechanisms and develop reliable germination techniques before seeds can be used effectively.

Keywords: Australia; Germination; Physiological dormancy; Seed; Temperature

1. Introduction

Dormancy is a seed characteristic, the degree of which defines the conditions necessary for seed germination. This block or series of blocks(s) within a dormant seed prevents germination, despite adequate water availability, temperature and gaseous conditions for germination (Benech-Arnold et al., 2000). Therefore dormancy is not due to the absence of environmental factors required to initiate germination and must be considered separately from germination requirements (Vleeshouwers et al., 1995). For both conservation and restoration, it is crucial that dormant seeds are distinguished from non-viable seeds. Viability can be underestimated if, in the absence of germination, seed dormancy is not diagnosed. In addition, reliable methods for alleviating (or at least bypassing) dormancy are essential for effective use and conservation of seeds. Once dormancy is diagnosed, classification of the type and level of dormancy can aid investigation of 'dormancy breaking' treatments and germination requirements (Baskin and Baskin, 2004).

It is often possible to bypass the block(s) to germination and stimulate PD seeds to germinate by applying agents such as gibberellins, e.g. GA_3 (Cohn et al., 1989; Foley, 1992). However, such 'germination inducing factors' do not affect dormancy status (Vleeshouwers et al., 1995) and can result in seedling abnormalities. In contrast, PD can be alleviated altogether by applying treatments that regulate temperature and seed moisture content (Benech-Arnold et al., 2000; Vleeshouwers and Bouwmeester, 2001). Such approaches are also likely to promote the development of normal, healthy seedlings.

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Table 1
Forb species and their collection sites in south–west Queensland

Family	Authority	Species	Collection site GPS co-ordinates		Collection date
			S	E	
Asteraceae	(A. Gray) H. Eichler	*Actinobole uliginosum*	−28°06′ 87.0″	145°76′ 39.2″	22.10.04
Asteraceae	Sond. and F. Muell. ex Sond.	*Brachyscome melanocarpa*	−28°06′ 10.7″	145°81′ 88.6″	22.10.04
Asteraceae	R. Br.	*Calotis cuneifolia*	−27°98′ 00.5″	148°33′ 37.8″	21.10.04
Asteraceae	N.T. Burb.	*Camptacra barbata*	−28°02′ 49.4″	148°43′ 69.5″	21.10.04
Asteraceae	Turcz.	*Gnephosis arachnoidea*	−28°00′ 48.8″	146°39′ 33.4″	09.12.04
Asteraceae	(F. Muell.) Paul G. Wilson.	*Leiocarpa brevicompta*	−28°06′ 10.7″	145°81′ 88.6″	22.10.04
Asteraceae	F. Muell.	*Leptorhynchos baileyi*	−28°00′ 48.8″	146°39′ 33.4″	09.12.04
Asteraceae	(DC.) Benth.	*Minuria integerrima*	−26°98′ 57.6″	146°03′ 26.6″	23.10.04
Asteraceae	(Schltdl.) Sond.	*Pycnosorus chrysanthes*	−28°02′ 70.0″	148°46′ 05.4″	07.12.04
Asteraceae	(DC.) Paul G. Wilson	*Rhodanthe floribunda*	−28°00′ 48.8″	146°39′ 33.4″	09.12.04
Asteraceae	(DC.) Paul G. Wilson	*Rhodanthe moschata*	−28°06′ 87.0″	145°76′ 38.2″	22.10.04
Asteraceae	(F. Muell. ex Benth.) J.M. Black	*Vittadinia pterochaeta*	−27°97′ 90.3″	148°01′ 36.1″	08.12.04
Campanulaceae	P.J. Sm.	*Wahlenbergia timidifructa*	−28°06′ 10.7″	145°81′ 88.6″	22.10.04
Goodeniaceae	F. Muell. and Tate.	*Goodenia fascicularis*	−28°06′ 10.7″	145°81′ 88.6″	24.10.04
Plantaginaceae	Decne.	*Plantago cunninghamii*	−28°06′ 10.7″	145°81′ 88.6″	22.10.04

The most recent attempt to classify the observed diversity of dormancy types and levels (Baskin and Baskin, 2004) builds upon Nikolaeva's comprehensive system (Nikolaeva, 1969). Physiological dormancy (PD), the most common form of dormancy (Baskin and Baskin, 2004), is thought to be caused by a physiological state of the embryo and possibly a decreased gas permeability of seed covering structures (Baskin and Baskin, 2001). Three levels of PD are recognized: non-deep, intermediate and deep (see Baskin and Baskin, 2004). Physical dormancy (PY) is due to a water impermeable seed or fruit coat, morphological dormancy (MD) requires that an underdeveloped embryo grows before germination can commence and combinations of PD and MD also exist (MPD) (Baskin and Baskin, 2001).

In recent years, partly due to increasing demands for biologically diverse land rehabilitation, seed dormancy of Australian native seeds has received considerable attention. It is frequently reported that the majority of Australian plant species possess seed dormancy mechanisms of some sort (Bell, 1999; Koch and Dixon, 2000; Tieu et al., 2002; Merritt et al., 2007), and that species in diverse families, including Euphorbiaceae, Asteraceae and Goodeniaceae (i.e. herbaceous, non-grassy species), have unknown dormancy alleviation and germination requirements. As a result, dormancy is cited as the greatest obstacle to the effective use of native seed in land revegetation (Merritt et al., 2007). Furthermore, the majority of previous studies have focused on native grasses, hard-coated PY seeds, and species native to temperate and Mediterranean-type regions within Australia. Consequently, this study aimed to identify seed dormancy in a range of previously unstudied Australian forb species spanning four families native to the semi-arid tropical region of south–west Qld.

2. Materials and methods

2.1. Seed collecting and processing

Seeds of 15 species across four families of native forbs were collected in south–west Qld between October and December 2004 (see Table 1). Where possible, at least 10,000 seeds/species were collected from >50 individual plants. Only mature seeds close to the point of natural dispersal were collected. Following collection, seeds were treated as if they were to be used in land revegetation by being stored in paper envelopes at 15 °C and 15 to 20% relative humidity (RH) for between 10 and 16 weeks before being used in germination experiments. Non-seed material was removed by hand, aspirator or by rubbing seed capsules/flowers through a sieve using a rubber bung.

2.2. Seed viability and germination testing

Viability of 3 replicates of 20 seeds/species was assessed using the tetrazolium chloride (TZ) staining technique (ISTA, 2003). Seeds were initially hydrated on plain agar for 24 h at room temperature before being scarified (away from the embryo axis) and placed in TZ solution at 30 °C and darkness for 24 h. Seeds were then cut in half and examined. Only uniformly stained red/dark pink embryos were considered 'viable'.

Germination tests used 3 replicates of 15 seeds each sown into 9 cm diameter plastic Petri dishes containing 1% agar-water. Petri dishes were sealed inside plastic bags to avoid agar desiccation. Seeds of all species were placed in germination incubators at 15, 20, 25 and 30 °C, with constant light provided by fluorescent tubes (*ca*. 50 μmol m^{-2} s^{-1}). Five of the species (*A. uliginosum*, *Brachyscome melanocarpa*, *Camptacra barbata*, *Plantago cunninghamii* and *Goodenia fascicularis*) were also sown at 5, 10 and 35 °C. Germination, defined as radicle emergence by at least 1 mm, was scored every 7 d, germination tests ran for 60 d and any non-germinated seeds were assessed using a cut test. Empty or necrotic seeds were excluded when calculating percentage germination.

2.3. Treatment conditions

Based on high viability (results of TZ tests) and low germination percentages, two species (*A. uliginosum* and *G. fascicularis*) underwent further investigation. Gibberellic acid (GA_3) was applied to seeds via incorporation into the agar germination medium; seeds received either constant application of 250, 125 or 62.5 mg/L throughout the germination test, or a pulsed application of 250 mg/L for 24 h before seeds were moved to plain agar for germination. Mechanical scarification