

The Physiological Dormancy and Germination Responses of *Brunonia australis* and *Rhodanthe floribunda* to Gibberellic Acid Treatment

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Abstract

Brunonia australis (Goodeniaceae) and *Rhodanthe floribunda* (Asteraceae) are two potential Australian native flower species desired by floricultural markets. However, the species are difficult to propagate. This study examined internal factors that constraint seed germination, seed viability and physiological dormancy. The study was conducted during April to May 2009 at the Gatton nursery, The University of Queensland Gatton Campus to identify the underlying reasons for poor germination and to provide recommendations for improving propagation effectiveness. Seeds of *B. australis* collected in 2003 germinated readily irrespective of germination media, whereas seeds collected in 2007 and 2008 with high proportion of viable seeds could only germinate with the presence of GA₃ (100 mg.L⁻¹) in germination media though with relatively low rates (8.75% and 10.00% respectively) of seed germination. Seeds of *R. floribunda* collected in 2003 showed a significant improvement in germination in agar media supplemented with 100 mg.L⁻¹ GA₃ (67.5%) compared to control treatment (10.0%). The results suggest that physiological dormancy occurs in both species. The use of GA₃ is recommended for improving germination rates of the two species. Further studies on the effects of different GA₃ concentrations to improve seed germination should be conducted.

Keywords: Dormancy, germination, gibberellic acid, TZ, viability.

Introduction

Brunonia australis (Goodeniaceae) and *Rhodanthe floribunda* (Asteraceae) are recognized as economically important wildflower species of Australia (Barker et al., 2002; Bunker, 1994; Joyce, 2005; Peacock and Smith-White, 1978; Stewart, 1996). Nonetheless, like other native flowers the two species have a poor ability to germinate and this is considered as a barrier for commercial development (Bunker, 1994; Johnston et al., 1999). Seed germination is controlled by both internal and external factors (Benech-Arnold and Sanchez, 2004; Khan, 1982), in which the former comprises physiological dormancy (genetic control of

dormancy) and physical dormancy (a coat-induced dormancy) (Bewley and Black, 1994). In addition, seed viability, i.e. the ability of seed to germinate, is also an internal factor that affects germination of seeds (Mayer and Poljakoff-Mayber, 1989).

Gibberellic acid (GA), particularly GA₃ has recently been proven to play an important role in regulating seed germination. GAs can replace the requirement for environmental factors that are required for germination of dormant seeds, including light and temperature (da Silva et al, 2005). According to Benech-Arnold and Sanchez (2004) an increase in the seed GA level can stimulate metabolism processes in the embryo as well as in the endosperm, leading to germination.

Research carried out at The University of Queensland Centre for Native Floriculture (CNF) has shown that some samples of the two aforementioned species failed to germinate regardless of favorable external factors (temperature, humidity, light and nutrient) (Cave, 2009; Johnston, 2009; Robert, 2003). Consequently, the internal factors including seed dormancy and/or viability are assumed as the limitations for seed germination which need further studies.

For this reason, the research direction was focused on investigating the mechanisms leading to low germination rates and to formulate suitable technical methods to improve propagation efficiency. This is also in line with the recent strategies of the Queensland government and the CNF, a joint initiative of the government and the University of Queensland (UQ). The strategies focus on the discovery and development of potential floricultural species to foster germplasms available to the industry via exploitation, selection, breeding and overcoming technical obstacles such as germination constraints, diseases problems and poor postharvest quality (Johnston, 2003; Joyce, 2005).

The overall objective of this research was to formulate reliable seed germination strategies for *Brunonia australis* and *Rhodanthe floribunda* based on understanding their physiological characters and germination responses. Seed viability (TZ) test and germination experiments with the use of GA₃ at 100 mg.L⁻¹ were carried out to determine whether or not seed

viability and/or seed dormancy are the limiting factors of seed germination in the two species.

Materials and Methods

Selecting and processing seeds

Seeds of *Brunonia australis* tested in this study consist of three groups: (i) seeds collected from the wild (GPS coordinate: 27°42'748"S; 145°47'790"E) on 20 November 2003 (BA1); (ii) BA2 (19 November 2007) and (iii) BA3 (4 April 2008) collected at the UQ Gatton campus. Seeds of *Rhodanthe floribunda* (RF) was collected on 16 February 2003 from Site 3 (GPS coordinate: 27°57'748"S; 148°00'834"E) on the

roadside of the Moonie Highway, Queensland. Seeds of each group were collected from at least 50 individual plants. Only mature seeds close to the point of natural dispersal were collected.

Following collection, seeds were stored in paper envelopes at 15°C and 15 – 20% relative humidity in a cold room of the CNF laboratory until required.

Experimental flow chart

As discussed above, this study was carried out to test the two possibilities, dormancy and viability, as the internal factors that influence germination of the two species (Figure 1).

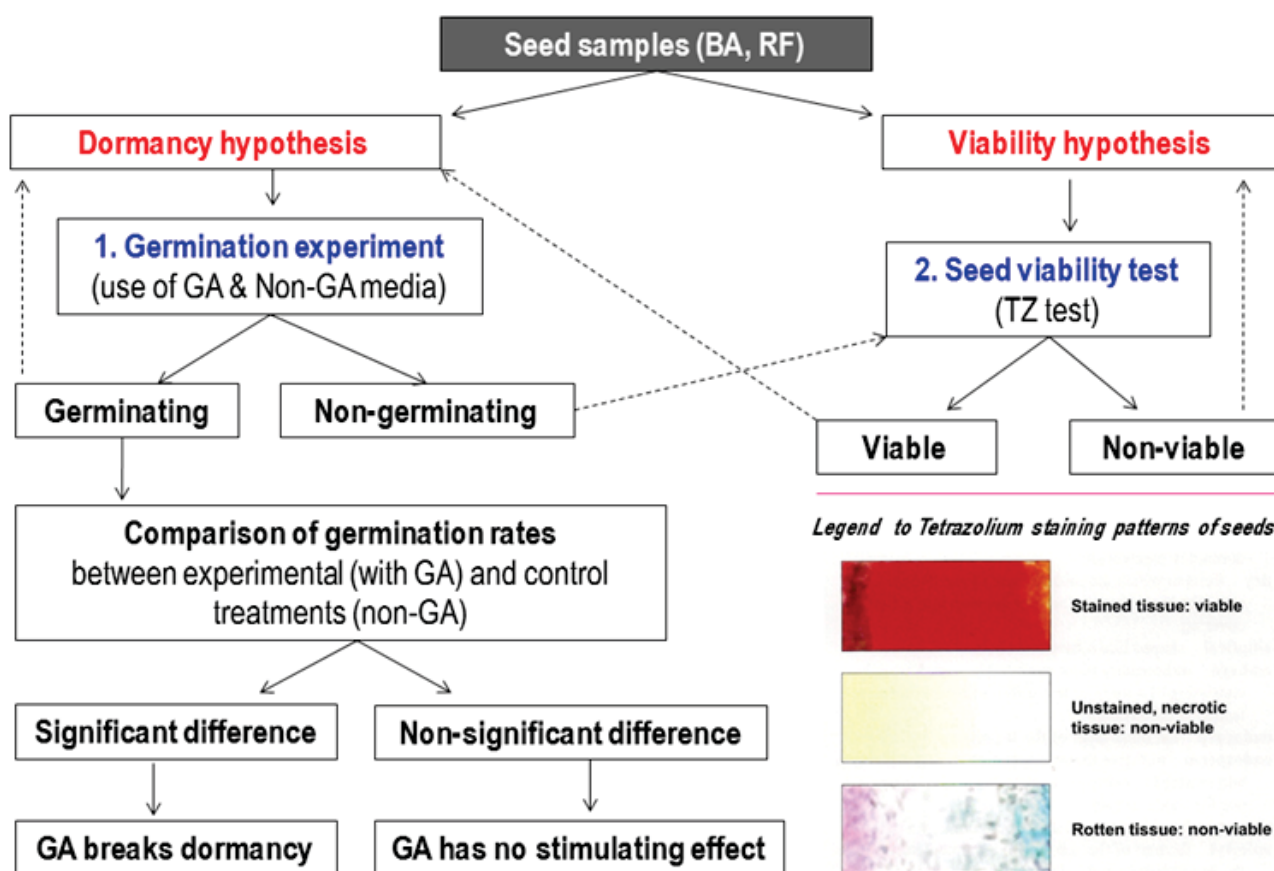


Figure 1. Experimental flow chart to examine poor germination of *Brunonia australis* (BA) and *Rhodanthe floribunda* seeds. The photo of TZ staining patterns of seeds was obtained from International Seed Testing Association (ISTA) Handbook on Flower Seed Testing (Ripka, 2008). Dotted arrows represent conclusions based on the test results.

Viability and germination tests

Physical damages of the seeds were checked using a stereomicroscope. Only intact seeds were then selected for germination and viability tests.

To avoid contamination problems during the germination tests reported by previous studies on native floricultural species (Johnston et al., 2004; Mullins et al.,

2002), seeds were disinfected in chlorine (2000 mg.L⁻¹) plus one drop of Tween®20 for 10 minutes then rinsed with sterile distilled water (DW) three times before being placed into sterile germination media. In this test, two treatments were used for each seed sample with four replicates of 20 seeds each sown into 9cm diameter plastic Petri dishes containing (i) 1% agar water (control) and (ii) 1% agar water and GA₃ (100 mg.L⁻¹). Petri dishes were sealed with plastic bands to avoid agar

desiccation. Seeds of all treatments were placed in dark and air-conditioned room with temperature of 25°C. Germination, defined as radical emergence by at least 1 mm, was scored every week.

Seeds that did not germinate by the end of the germination experiment was subjected to germination testing using TZ method. The seeds were imbibed for 24 hours in distilled water (DW) at ambient temperatures. Imbibed seeds were placed into 1.0% 2,3,5-triphenyl tetrazolium chloride (TZ) solution and held at 40°C in dark for 24 hours. After rinsing with DW, seeds were cut longitudinally to expose the embryo. Deep red to deep pink embryos were recorded as viable, whereas white to pale pink embryos were recorded as non-viable (Copeland and McDonald, 1995; Hampton and Tekrony, 1995).

Experimental design and analysis

The study was set up in a completely randomized factorial design with four replicates each for BA1, BA2, BA3 and RF seed group. Each replicate consists of 20 seeds, totaling 80 seeds for each seed group. The data

of germination rates was analyzed by using balanced ANOVA of the IRRISTAT statistical package (IRRI Release 5, Manila, the Philippines, 2005) with least significant differences (LSD) calculated at 5% level of significance.

GraphPad Prism (Release 6, GraphPad Software Inc., La Jolla, CA 92037 USA) was used to illustrate germination trend over time and compare germination rates amongst selected treatments.

Results and Discussion

Brunonia australis

There were noticeable differences in germination rates of the three groups of *Brunonia australis* (BA) seeds (Table 1). BA1 seeds (seeds collected in 2003 from the wild) germinated readily on the first week, i.e. 70% germination on 1% agar medium plus GA₃ (100mg.L⁻¹) and 78.8% on 1% agar without GA₃. The germination increased gradually until the fifth week, i.e. 87.5% and 80.0% for BA1 with and without GA₃, respectively (Table 1).

Table 1. Percentage of *Brunonia australis* seeds germinated over five weeks.

Treatments	Percentage of seeds germinated over time (weeks) (%)					P-value
	Week 1	Week 2	Week 3	Week 4	Week 5	
BA1 with GA ₃	70.00	78.75	85.00	85.00	87.50	n.s
BA1 without GA ₃	78.75	78.75	78.75	78.75	80.00	
BA2 with GA ₃	0.00	0.00	2.50	6.25	8.75	*
BA2 without GA ₃	0.00	0.00	0.00	0.00	0.00	
BA3 with GA ₃	0.00	2.50	5.00	6.25	10.00	*
BA3 without GA ₃	0.00	0.00	0.00	0.00	0.00	

Note: BA1, BA2 and BA3 are seeds of *Brunonia australis* collected in 2003, 2007 and 2008, respectively; GA₃ concentration: 100 mg.L⁻¹; Date sown: 9 April 2009; n.s. not significant; * P < 0.05.

However, there was no significant difference in the germination rates between BA1 with and without GA₃, suggesting that GA₃ at 100 mg.L⁻¹ did not have stimulating effect on germination of the BA1 seeds. BA1 seeds which were collected in 2003 might have experienced a sufficient dormancy period which has enabled the seeds to germinate under the favorable temperature and humidity condition regardless of the GA₃ treatment.

In contrast, BA2 seeds (collected in 2007) and BA3 seeds (collected in 2008) sown on agar with 100 mg.L⁻¹ GA₃ did not germinate until week 2 and week 3, respectively. After that the germination rate rose slightly to 10 % and 8.8%, respectively, by the fifth week (Table 1). BA2 and BA3 seeds without GA₃ did not germinate after five weeks (Table 1). GA₃ treatment significantly

increased both BA2 and BA3 germination (P < 0.05).

Non-germinated BA2 and BA3 seeds were subjected to a viability test using the TZ staining technique. The results indicated high proportions of viable seeds, i.e. 82 % in BA2 and 81% in BA3. Therefore, it was concluded that the seeds of *B. australis* collected in 2007 (BA2) and 2008 (BA3) have a dormancy character, and that GA₃ at 100 mg.L⁻¹ had a stimulating effect on germination of these seeds. These results demonstrated that the more recent harvested seeds (BA2 and BA3) experienced a certain dormancy period before being able to germinate under favorable environments. To break dormancy, addition of stimulants such as GAs is necessary.

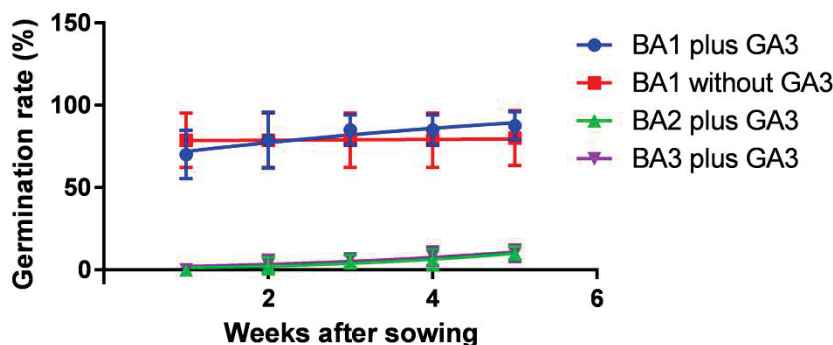


Figure 2. Germination rates over time of *B. australis* (BA) seeds. Vertical bars represent standard errors of means (SE).

As described above, the seeds of *B. australis* collected in 2003 (BA1) germinated readily without a need for addition of GA₃, whereas one- and two-year-old seeds (BA2, BA3) required GA₃ for germination even though only a small percentage of seeds germinated (Figure 2 the same as Table 1). Further studies to test the effects of different GA₃ concentrations on germination of the newly harvested seeds are recommended.

Rhodanthe floribunda

Rhodanthe seeds treated with and without GA₃ started germinating at the first week with the percentages of 60 % and 5 %, respectively. The germination rates increased gradually until the fifth week, reaching 67.5% for GA₃-treated seeds and 10 % for control seeds. Therefore GA₃-treated seeds had a higher germination percentage compared to the control seeds (P <0.05) (Figure 3).

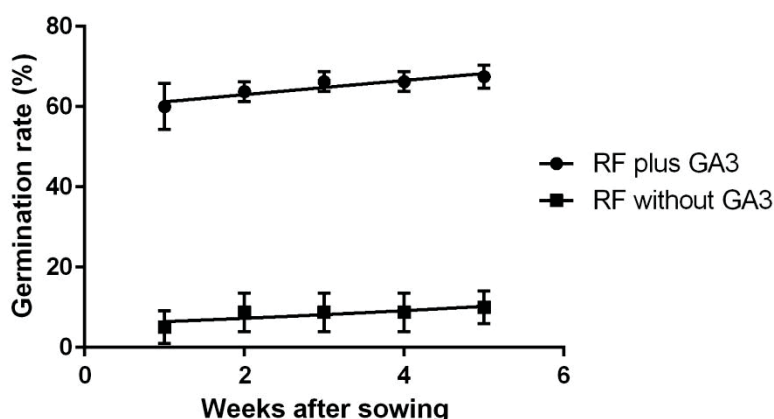


Figure 3. Germination rates of *Rhodanthe floribunda* (RF) seeds over time. Seeds were collected on 16 February 2003. The seeds were sown on 9 April 2009. Vertical bars represent standard errors of means (SE).

It can be concluded that *R. floribunda* has dormancy character, and the use of GA₃ at 100 mg.L⁻¹ can overcome dormancy and stimulate seed germination.

Conclusion

The study has established that both *Brunonia australis* and *Rhodanthe floribunda* seeds have dormancy character. The addition of GA₃ at 100 mg.L⁻¹ to the germination media can stimulate germination of *R. floribunda* collected in 2003 and one- and two-year-old *B. australis* seeds. For this reason, adding GA₃ to

germination media is recommended to increase propagation efficiency of the two species. Different concentrations of GA₃ should be tested to determine the optimum GA₃ concentration to improve seed germination.

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