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Research Note

Physical seed dormancy in *Collaea argentina* (Fabaceae) and *Abutilon pauciflorum* (Malvaceae)

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Summary

Collaea argentina (Fabaceae) and *Abutilon pauciflorum* (Malvaceae) are of high medicinal and ornamental value and are collected for pharmaceutical and ornamental purposes. However, one obstacle for plant production is the occurrence of seed dormancy. Here, we confirmed the occurrence of dormancy in these species, identified possible methods for breaking dormancy and assessed the dormancy condition after seed storage. Wet heat, physical and acid scarification were effective methods for breaking physical dormancy in both species. After four years of dry storage, a high proportion of *C. argentina* seeds were able to germinate (i.e. physical dormancy levels had reduced), whereas *A. pauciflorum* seeds continued to be dormant but were more sensitive to dormancy-breaking treatments. These results should aid plant production and seed conservation of these two species.

Experimental and discussion

Collaea argentina Griseb. (Fabaceae) and *Abutilon pauciflorum* A. St.-Hil. (Malvaceae) are two Argentinean native species that have medicinal and ornamental value (Barboza *et al.,* 2006). Consequently, many of them are threatened by over-collecting, in addition to changes in land use. Knowledge of the factors that control seed germination may contribute to increase plant production in the greenhouse and at the same time assist conservation management decisions for these species.

Physical dormancy (PY) is currently known in at least 15 angiosperm families, including the Fabaceae and Malvaceae. However, the presence of PY in a family does not

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mean that all species have this type of dormancy, and therefore the presence of PY should be tested in each species individually (Baskin and Baskin, 1998). Consequently, we here investigate whether physical dormancy is present in *C. argentina* and *A. pauciflorum* and determine possible methods for breaking it. Several methods have been developed to break physical seed dormancy and stimulate germination, such as mechanical scarification, chemical scarification, dry storage, hot water or dry heat (Baskin and Baskin, 1998). For example, in some Malvaceae species, it has been demonstrated that water uptake is facilitated by the removal of a chalazal plug, which can be achieved using hot water and acid scarification (Daws *et al.,* 2006).

The specific objectives of our study on seeds of *C. argentina* and *A. pauciflorum* were: (1) to verify whether PY is present in these two species; (2) to investigate the effects of wet heat, mechanical and acid scarification on dormancy alleviation and seed germination; and (3) to evaluate the seed dormancy condition after four years of dry storage.

Ripe fruits of *C. argentina* were collected in the Punilla Valley (30°51'S; 64°32'W, province of Córdoba), while *A. pauciflorum* fruits were collected both in this region and in the Calchaquíes Valleys (25°05'S; 65°30'W, province of Salta). Collections were made during March and May 2005, from a minimum of 30 individual plants per species and population. Seeds were separated from fruits, cleaned and stored at 15°C and 15% relative humidity (RH). Immediately prior to starting the experiments in July 2005, seed moisture content was determined gravimetrically by drying seeds at 103°C for 17 hours and expressed on a fresh weight basis (ISTA, 2009).

To test for the presence of PY, 25 seeds of intact and mechanically scarified (achieved by cutting the seed coat with a scalpel blade opposite to the micropyle) were weighed to the nearest 0.1 μg using a digital balance. Seeds were then sown in Petri dishes on the surface of 1% agar in water at 20°C under white light (8 h light/16 h dark). Seeds were removed from the dishes at 1–2 h intervals for the first 12 h and then every 24 h, blotted on filter paper to remove any surface moisture, and reweighed. The experiment was continued until mechanically scarified seeds germinated.

To investigate the effects of wet heat, mechanical and acid scarification on dormancy alleviation and seed germination, the following 14 treatments were performed: 1) wet heat treatments (20°, 30°, 40°, 50°, 60°, 70°, 80°, 90° and 100°C), in which seeds were immersed in water for two minutes; 2) acid scarification by immersing seeds in concentrated sulphuric acid for 10, 20 or 30 minutes; 3) mechanical scarification and 4) intact seeds (used as a control). Four replicates of 25 seeds for each treatment were then sown on the surface of 1% water agar in Petri dishes and germinated at 20°C (8-h light/16-h dark). The proportion of seed germination was recorded daily for 30 days, with germination defined as radicle protrusion by at least 1 mm. Mean time to germination (MTG) in days was also calculated:

$$\Sigma\,(D\,n)\,/\,\Sigma\,n$$

where n was the number of seeds that germinated on day D and D was the number of days from the start of germination test (Pritchard and Miller, 1995).

To evaluate seed dormancy after four years of dry storage, seeds of *C. argentina* and *A. pauciflorum* (Salta population) were stored at 15% RH and 15°C until May 2009, and the imbibition experiment and dormancy-breaking treatments repeated.

In 2005, low germination (< 30%) was recorded for wet heat treatments in the range 20° to 60°C, therefore only water temperatures of 70°, 80°, 90° and 100°C were used.

Prior to commencement of experiments in 2005, seeds of both species and populations showed low initial moisture content of less than 7% (table 1). Following imbibition, it was found that all mechanically scarified seeds of *C. argentina* and A. *pauciflorum* (both populations) took up water and had an increase in mass of at least 118%, whereas intact seeds only increased by a maximum of 24% in mass (table 1). Moreover, seed germination of both species was greater than 75% in mechanically scarified seeds and was completed during the first 3 d, whereas germination in intact seeds was less than 8% and was delayed until 7 d. These results strongly indicate that seeds of both species have a water impermeable seed coat and are therefore physically dormant. Similar results have been reported for other Fabaceae and Malvaceae species (Baskin and Baskin, 1998; Ortega Baes *et al.,* 2002; Daws *et al.,* 2006).

Table 1. Moisture content (% fresh weight basis), water uptake (% initial mass basis) and germination (%) of treated and intact seeds by species prior to experiments in 2005 and 2009. Data are the mean ± 1 standard error.

Species	Population	Year	MC (% fresh weight basis)	Water uptake (% initial mass basis)		Germination (%)	
				Mechanically scarified	Intact	Mechanically scarified	Intact
Collaea argentina	Córdoba	2005	6.0±0.2	118.5±2.5	17.1± 9.7	96	4
	Córdoba	2009	-	163.1±7.0	23.4±14.8	80	0
Abutilon pauciflorum	Córdoba	2005	6.4±0.1	175.4±5.9	24.5± 4.2	80	8
	Salta	2005	6.8±0.19	156.7±6.4	18.4± 6.3	75	4
	Salta	2009	-	193.7±6.7	42.0±13.1	72	8

Germination and MTG of *C. argentina* and both populations of *A. pauciflorum* were significantly different among dormancy-breaking treatments (figure 1). The highest germination proportion was found after 30 min with acid scarification, followed by the other two acid treatments, wet heat (90°-100°C) and mechanical scarification for *C. argentina* and in mechanically scarified and wet heat treated seeds (70°-100°C) for both *A. pauciflorum* populations. For Córdoba's population of *A. pauciflorum*, no germination was observed in wet heat treatments below 50°C. For both species and populations, germination started around the second day for all dormancy-breaking treatments and MTG was longer in intact seeds than in the other treatments (figure 1).

Under natural conditions, both *C. argentina* and A. *pauciflorum* grow in environments disturbed by grazing and fire, where the soil surface may experience high temperatures for brief periods during the day (Daws *et al.,* 2002, 2006) or even higher temperatures (≥ 100°C) when soils are submitted to fire. The dormancy-breaking and stimulation of germination by high temperatures suggest that these species are adapted to germinate in these disturbed environments, similar to other species of these families (Baskin and Baskin, 1998; Daws *et al.,* 2006).

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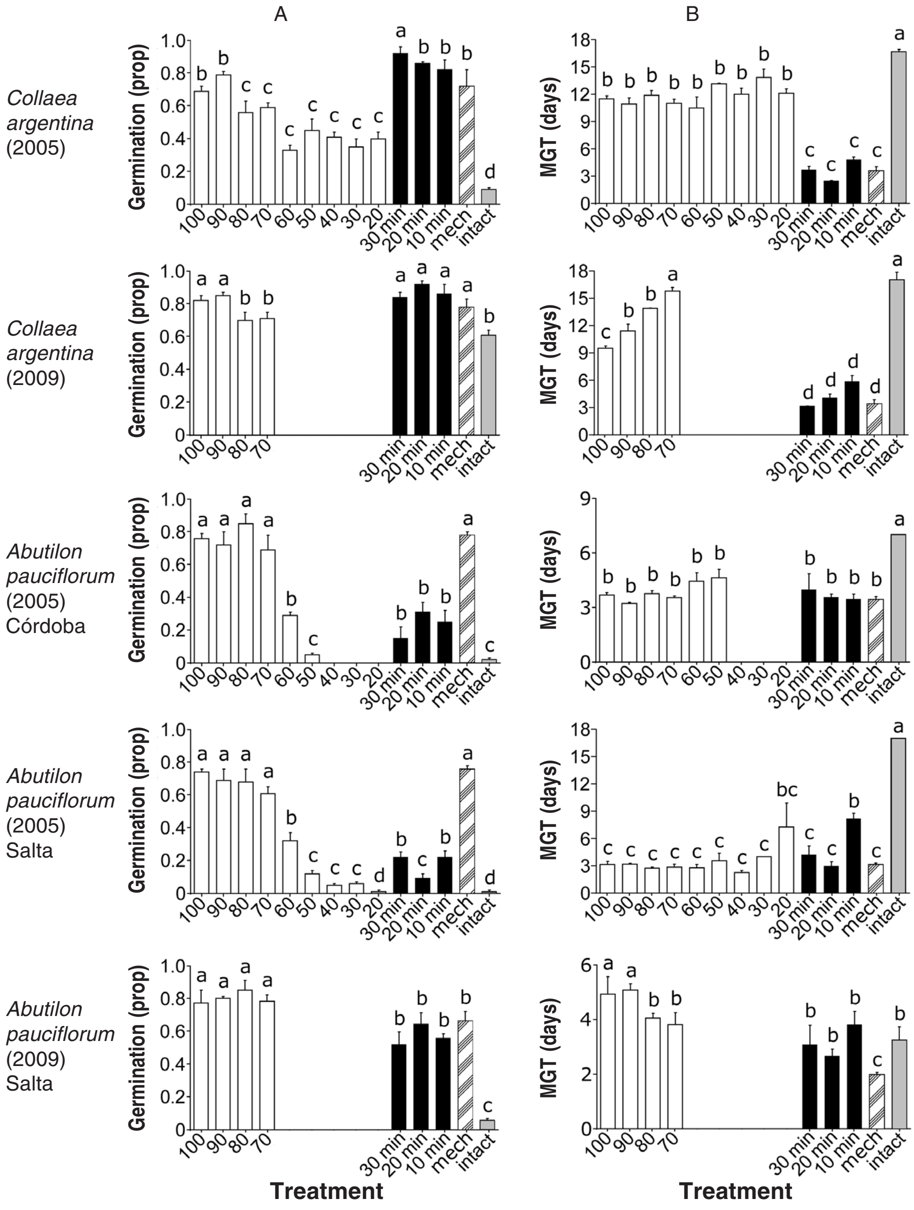

Figure 1. (A) Germination proportion and (B) mean time to germination of seeds under wet heat (white bars); acid scarification (black bars); mechanical (mech) scarification (hatched bars) and intact seeds (grey bars) treatments. Seeds were of *C. argentina* in 2005, *C. argentina* in 2009, *A. pauciflorum* from Córboda in 2005, *A. pauciflorum* from Salta in 2005 and *A. pauciflorum* from Salta in 2009. In each graph values with the same letters are not significantly different at $p > 0.05$ (assessed in graphs A and B by One-way ANOVA followed by post hoc DGC's test and in graphs C-E by GLM procedures followed by post hoc DGC's test). Data are the means of 4 replicates (+ 1 standard error).

In some species, seeds with PY stored dry at room temperature or at 5°C for several months or years become permeable to water (Baskin and Baskin 1998; Jayasuriya *et al.,* 2008). For example, some legumes (Papilionoideae) become permeable during dry storage because cells in the strophiole break (Cavanagh, 1987). In this study, experiments were repeated after four years of storage to evaluate seed dormancy after dry storage. Mechanically scarified seeds of both species took up water and increased in mass greater than 160% and with germination greater than 70%. In contrast, intact seeds only took up between 20-40% water and less than 8% of seeds germinated (table 1). After four years of dry storage at 15°C, 60% of intact seeds of *C. argentina* germinated compared to just 10% in 2005, although the MTG remained the same at 17d. Seeds treated at 70°C and 100°C, and with acid for 20 min, also had higher germination than in 2005 (*t*-test, $p < 0.05$; figure 1). In contrast, intact seeds of *A. pauciflorum* only increased in germination from 1-6%, whereas MTG was approximately six-times faster after dry storage. For this species, high germination in acid treatments was observed in 2009 compared with 2005 (*t*-test, $p < 0.05$; figure 1), which could mean that the seeds were more sensitive to physical dormancy breaking treatments after several years of dry storage, as has also been also reported for other species (Jayasuriya *et al.,* 2009).

It has been argued that species with physical dormancy, capable of forming persistent soil seed banks, are associated with unpredictable environments (Fenner, 1985). For both species: (1) the dispersal time is in the autumn (April-June); (2) the next wet season is the summer; (3) fire events occur during the end of winter and beginning of spring; and (4) seeds remain dormant but with a proportion able to germinate after 4 years of storage. Consequently, it is to be expected that seeds of these species remain dormant in the soil seed bank for long periods of time until conditions are suitable for germination, although this remains to be tested.

In conclusion, *C. argentina* and *A. pauciflorum* show physical seed dormancy and wet heat (≥ 70°C), mechanical and acid scarification are effective methods to dormancy-breaking. After four years of dry storage, a high proportion of *C. argentina* seeds can germinate, whereas for *A. pauciflorum* seeds remain dormant but are more sensitive to dormancy-breaking treatments; which means that they could form persistent soil seed banks. The determination of seed dormancy type, the development of effective dormancy-breaking treatments and an understanding of the dry seed storage response, should aid plant production and seed conservation programmes on these two species with ornamental and medicinal value.

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