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Research Note

Seed desiccation tolerance and germination of a potentially threatened Chinese species, *Fosbergia shweliensis*

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Summary

Fosbergia shweliensis is a potentially critically endangered Chinese tree species. However, there is little known about the seed biology of this species. Here we performed germination and desiccation trials of its seeds. Fresh seeds of *F. shweliensis* were not dormant and germinated optimally at 25°C (100% germination). There was 92% germination when seed moisture content (MC) was reduced to 16% (fresh weight basis). Further drying at 15°C and 15% relative humidity, reduced germination to 73% when seed MC was 12% and drying to 7% MC resulted in complete loss in viability. These results indicate that *F. shweliensis* seeds are partly desiccation-tolerant (i.e. intermediate in storage behaviour). The present research supplied seed germination information and guidance about seed storage for the genetic conservation and restoration of this species.

Experimental and discussion

Fosbergia shweliensis (Anth.) Tirveng. and Sastre is a subtropical tree species belonging to the Rubiaceae family. It is endemic to the Gaoligong Mountains region of Yunnan province, south-east China. The plant had been presumed to be extinct in the wild because other than the type specimens, no specimens had been collected for 85 years, until the autumn of 2003 (Li *et al.*, 2006). Up to now, fewer than 200 adult individuals of this species have been found in the wild, in an area of less than 100 km and seedlings were seldom observed (Li *et al.*, 2006; Dao *et al.*, 2011). Consequently, it would be categorised as a critically endangered species, according to the IUCN (2010) red list criteria. In addition, *F. shweliensis* is a potentially important economic species for horticulture due to its large, yellow-white fragrant flowers, giant fruits and plant height (Li *et al.*, 2006). However, there is little seed biology information available for germplasm conservation and use of *F. shweliensis*.

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Ex situ seed bank conservation by dry and low temperature storage is possible for conserving a large proportion of seed plants. However, the storage conditions are only suitable for orthodox seeds, not for intermediate or recalcitrant seeds. Orthodox seeds can tolerate desiccation to low moisture contents (< 7%) with little negative effect on viability (Roberts, 1973). In contrast, recalcitrant seeds are killed by drying to moisture contents as high as 20–30% (Pritchard, 2004). Intermediate seeds tolerate desiccation to moisture contents of approximately 10–12%, but further desiccation reduces viability and/or there is a more rapid loss in viability during storage at cooler as opposed to warmer temperatures (Hong *et al*., 1998). To store the seeds of *F. shweliensis* successfully, it is necessary to determine seed storage behaviour to enable suitable environments for storage to be defined. The present research aims to determine the best germination temperature and the seed desiccation tolerance of *F. shweliensis*. The results will be useful for genetic preservation and population restoration of this endangered species.

Mature seeds of *F. shweliensis* used in this study were randomly collected from about 20 trees in Gaoligong Mountains. After harvest, the fresh fruits were packed in a cloth bag and transported to the laboratory within two days. Upon reaching the seed bank, the fruits were processed and washed under running tap water to extract the seeds.

Seed equilibrium relative humidity (eRH) was determined by pouring a sample of seeds into an approximately 3.2 cm^3 sample holder which was placed into the measuring chamber of an AW-D10 water activity station used in conjunction with a HygroLab 3 display unit (HygroLab3-set40, Rotronic, Switzerland) at 15°C. The eRH of fresh seeds was 94.8%. The moisture content of the seeds, estimated by the low constant oven method (103 ± 1°C for 17 hours; ISTA, 2007) using five replications of 20 seeds, was found to be 43 ± 1.5% (fresh weight basis). Thousand seed weight (TSW) of fresh seeds was 291 ± 2.4 g, calculated from the mean weight of three replications of 500 seeds each.

Initial germination tests on fresh seeds were set up within four hours of extraction. To determine optimal germination conditions, samples of 20 fresh seeds were sown on 1% water agar held in 90 mm-diameter Petri dishes and incubated at 20, 25, 30 or 25/10°C. The photoperiod of the incubators was 12 hours light/12 hours dark; the light was cool white fluorescent light at 1000 lux. In case the seeds were dormant, additional tests at 25/10°C involved the addition of either 200 mg L^{-1} gibberellic acid (GA_3) or 100 mg L^{-1} KNO_3 to the agar. Germination was checked weekly; seeds with radical emergence more than 2 mm were considered germinated and the germination tests were stopped when there had been no further germination for 150 days. The remaining seeds were checked to determine whether they were fresh or mouldy. The speed of germination was estimated as the mean germination time (MGT) in days and calculated using the formula MGT = $\sum$ (days $\times$ newly germinated seeds) / $\sum$ germinated seeds (Ellis and Roberts, 1981).

There was 100% germination of seeds sown at 25°C by 20 days (table 1). No seeds germinated at 20°C after 150 days, but when they were transferred to 25°C, all seeds germinated within 20 days. At 30°C, most seeds germinated within 25 days, but many seedlings became mouldy. At 25/10°C, 60% of the seeds germinated within 150 days; nearly all the remaining seeds germinated upon transfer to 25°C. Addition of GA_3 promoted germination at this temperature regime, but KNO_3 had no effect. At the end of the tests, the ungerminated seeds in all treatments were all mouldy and dead. Seeds of

Table 1. Germination percentage and mean germination time (MGT) of fresh *F. shweliensis* seeds incubated under different conditions.

	Germination conditions (temperature, chemical)					
	20°C	25°C	30°C	25/10°C	25/10°C +GA_3	25/10°C +KNO_3
Germination (%)	0	100	78.9	60	90	60
MGT (days)	0	19.7	24.2	41.4	29.7	38.5
Germination (%) *	100	-	-	95	95	100

*Germination percentage reached after 150 days under the original conditions, followed by transfer of ungerminated seeds to 25°C.

F. shweliensis were not dormant and 25°C was the optimal germination temperature, for both total germination and rate of germination.

F. shweliensis grows in a primary moist subtropical evergreen broadleaf forest, where annual average precipitation is about 1500 mm, the highest monthly mean temperature is 20°C and average annual temperature is 15°C (Annals of Tengchong County Codification Committee, 1995). Since no seeds germinated at 20°C in our tests, this may explain why seedlings have not been observed *in situ* (Li *et al*., 2006; Dao *et al*., 2011). In addition, this tree does not appear to be able to propagate by other means; no saplings were observed beneath the trees in the natural population. This may be one of the reasons why the *F. shweliensis* population is so small and there is a risk of loss of this species from the wild.

Desiccation tolerance tests were carried out 20 days after harvest (stored as intact fruits at 15°C and 75% RH). Seeds were extracted from the fruits as before and 2250 seeds were placed at 15% RH and 15°C in the dry room of the Germplasm Bank of Wild Species in Kunming, China. Samples were removed after each of 1.5 hours, 1, 2, 3, 6, 7, 10, 14 and 55 days and tested for eRH and moisture content (MC), as described for fresh seeds. Germination tests were carried out using three replications of 50 seeds each sown on 1% water agar, at 25°C (the optimum germination temperature).

The rate of seed moisture loss was rapid, reaching 20% MC after the first three days (figure 1). A further halving in MC, to 10% took a further 10 days (figure 1). Seeds started to lose germination ability when dried to 16% MC (92% germination). Seeds dried to 12% MC had 73% germination. Further desiccation, to 7% MC and below, was fatal; none of the seeds survived. Based on the seed drying curve, the moisture content when germination was reduced to 50% was found to be at approximately 10%. Hence, seeds of *F. shweliensis* were partly desiccation-tolerant, consistent with the intermediate category of seed storage behaviour (Hong *et al*., 1998). The seeds cannot be stored under conventional long-term seed storage conditions (3-7% moisture content at -18°C; FAO/IPGRI, 1994) and thus their storage for use in reforestation and *ex situ* conservation programmes is problematic. It is necessary to explore alternative conservation methods for *F. shweliensis*, such as nursery garden conservation, cryopreservation and *in situ* conservation.

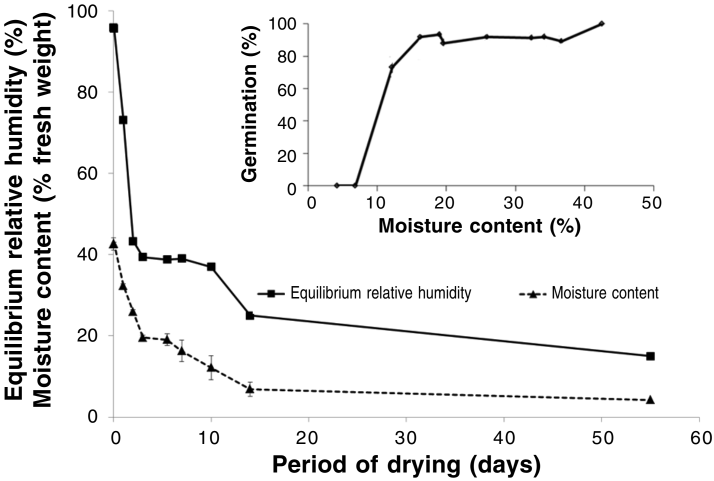

Figure 1. The seed drying curve of *F. shweliensis*. The inset graph shows the relationship between germination percentage and moisture content of *F. shweliensis* seeds.

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