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Research Note

Seed storage behaviour of *Magnolia odoratissima*

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Summary

Magnolia odoratissima is endemic to Yunnan Province in southwest China. The flowers and leaves contain approximately 0.2% aromatic oil, making it an excellent perfume plant. However, the species is now facing an extremely high risk of extinction in the wild and has been proposed as a critically endangered tree. To effectively preserve its germplasm via seeds, we studied the effects of moisture content and storage conditions on seed germination. The 1000-seed weight, initial moisture content and germination of fresh seeds were 184.0 g, 33.2% and 98%, respectively. Most of the seeds tolerated desiccation to 21.6% moisture content, while more than half of the seeds lost viability when dried to 14.1% and only a few seeds (21-28%) survived when the moisture content was reduced to 9.9%. Therefore, *M. odoratissima* seeds showed intermediate storage behaviour. Non-desiccated seeds retained viability at storage temperatures of 4, 8 and 20°C. However, the viability of both air-dried and pre-chilled seeds stored at 4, 8 and 20°C was reduced with prolonged storage time. The seeds stored for all treatments at -20°C lost viability. The data consequently suggest that *M. odoratissima* seeds are chilling-tolerant intermediate seeds which can be stored at 4°C for up to one year.

Experimental and discussion

Magnolia odoratissima Law and Z. Zhou is a small evergreen tree with white fragrant flowers (Liu, 2004). Due to deforestation and habitat destruction, its natural population size has been greatly reduced. Based on IUCN criteria, *M. odoratissima* has been categorised as a critically endangered species (Cicuzza *et al.*, 2007). In nature, less than 400 individuals of *M. odoratissima* are distributed in the counties of Guangnan, Qiubei, Xichou and Malipo of Yunnan Province in southwest China. The trees are scattered in shrubby woods and evergreen or deciduous broad-leaf forest on sunny east- or southwest-facing mountain slopes at altitudes of 1100-1500 m a.s.l. (Yang, 2008). The region is characterised by a warm and damp climate with a lot of fog in the dry season, mean annual rainfall of 1294 mm, mean annual humidity 85% and mean temperature 15-19°C (Qi *et al.*, 2010). At present, little is known about the seed dormancy, germination and storage behaviour of *M. odoratissima*. Yang (2008) found that in freshly collected

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M. odoratissima arillate seeds, only 15-18% contained embryos. The 1000-seed weight and germination percentage of those seeds were 240 g and 85%, respectively. The seeds required cold stratification to break dormancy and increase germination (Yang, 2008). However, the sensitivity to desiccation and storability of *M. odoratissima* seeds has not been studied in detail. Consequently, we have examined the characteristics of seed storage and desiccation tolerance of this species, as well as the influence of pre-chilling on seed storage behaviour, in order to prolong the seed longevity and to preserve the germplasm via seeds.

Fruits of *M. odoratissima* were harvested from County Malipo of Yunnan Province of China in October 2009 and 2010. The immature bottle-green fruits turn fulvous or amaranthine at maturity, and the vermeil arillate seeds that were collected were suspended on filiform pseudofuniculi of the dehiscent fruits. After immersing in water at room temperature (20-23°C) for 4-5 days, the arils were rubbed off manually and the seeds air-dried in a shady, cool place. The dry seeds were stored at 4°C prior to experimentation.

Fresh seeds were dried to different moisture contents in sealed containers with silica gel at room temperature (20-23°C). After each of 0, 2, 4, 6, 8, 12, 18 and 24 hours drying, 100 seeds were removed and set to germinate. The moisture content of 20 seeds was determined on a fresh weight basis after oven drying for 17 hours at 103°C (International Seed Testing Association, 1999). To determine the storability, after desiccation, samples of 100 seeds were put into sealed polyethylene bags and stored at 8, 4 and -20°C for two and four months. In addition, to determine the effects of pre-chilling on storage behaviour of *M. odoratissima* seeds, air-dried seeds were pre-chilled in moist perlite at 4°C for one year before desiccation and storage experiments. After drying, desiccated pre-chilled seeds were stored at 20, 4 and -20°C for four months.

All seeds were surface and decontaminated in chlorox (0.2% NaOCl) solution for 20 minutes, and then rinsed with distilled water. For germination, four replicates of 25 seeds for each treatment were sown on wet sands in Petri dishes at 25°C with a 12-hour photoperiod. Seeds were recorded as germinated when the protruding radicle was ≥ 1mm in length. Germinated seeds were counted and removed every three days for 40 days.

The 1000-seed weight, initial moisture content and germination of fresh *M. odoratissima* seeds were 184.0 g, 33.2% and 98%, respectively. Seed water content decreased sharply with drying time (table 1). At 30.0% moisture content, 92% of *M. odoratissima* seeds germinated (figure 1a). After rapid drying to 9.9%, the germination declined significantly to 28%. With further desiccation to 4.6% the seed germination percentage was only 11%. At storage temperatures of 8 and 4°C, non-dried seeds remained viable, whereas most of the dried seeds lost their germinability after two months. After four months storage, there was almost no germination of seeds with moisture content less than 14.1%. At -20°C, no seeds survived at all.

Germination of the pre-chilled seeds was 89% with 30.8% moisture content (figure 1b). Seeds retained high germination potential (85.3%) as moisture content decreased to 19.8%. Thereafter, the germination of *M. odoratissima* seeds exhibited a significant decrease from 71 to 39% when seed moisture content declined from 14.6 to 5.0%. After four months storage at 20 and 4°C, non-dried seeds germinated to 87 and 84%, respectively. However, the germination of dried seeds decreased significantly during four

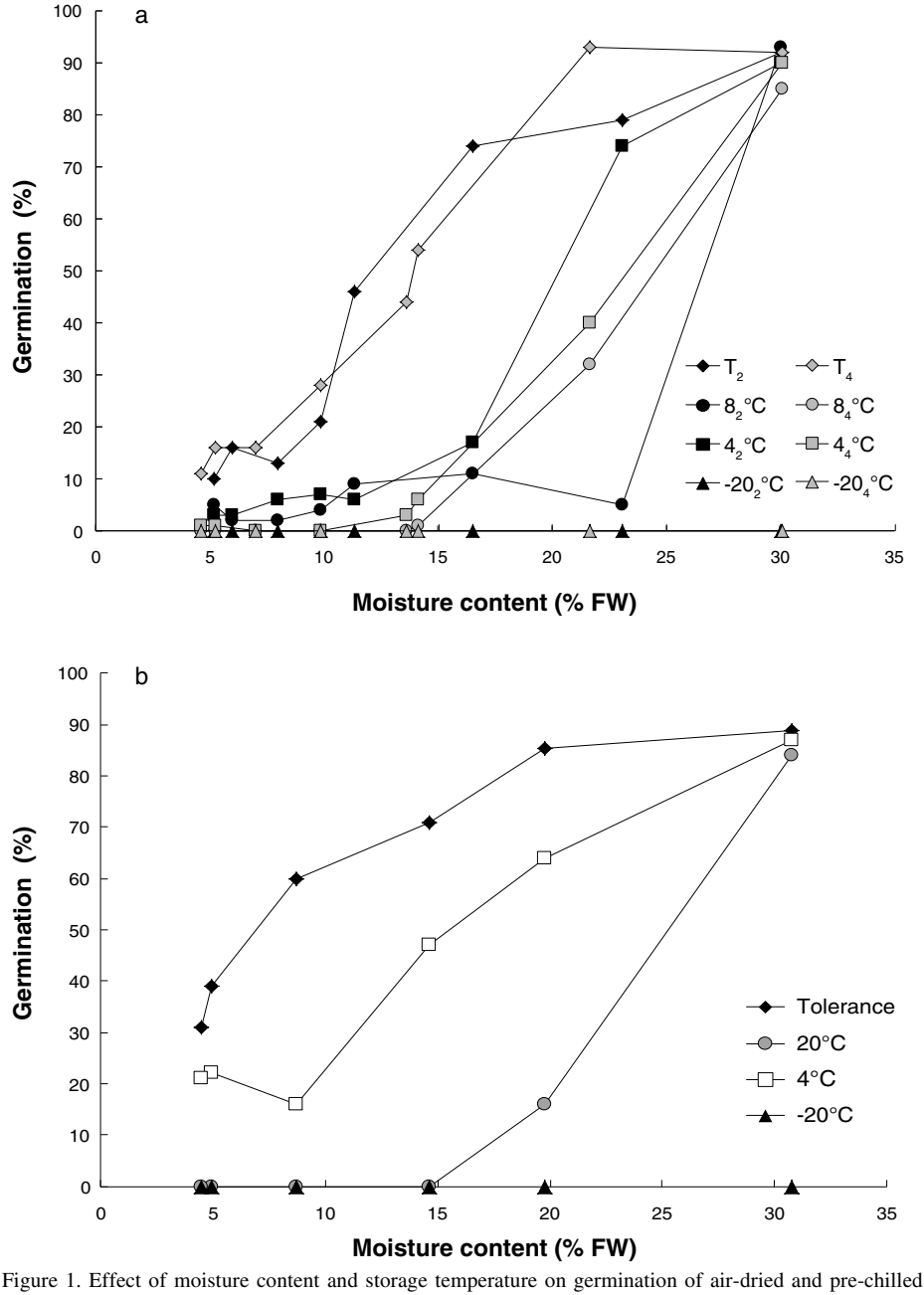

Figure 1. Effect of moisture content and storage temperature on germination of air-dried and pre-chilled seeds of *Magnolia odoratissima*. (a) Germination of non-stored air-dried seeds ('T_2' and 'T_4'; two replicates) and of air-dried seeds stored at 8, 4 and -20°C for two (8_2, 4_2 and -20_2°C) or 4 months (8_4, 4_4 and -20_4°C), respectively. (b) Germination of pre-chilled, non-stored air-dried seeds ('Tolerance') and of pre-chilled air-dried seeds stored 20, 4 and -20°C for four months.

Table 1. Effects of desiccation on the moisture content of air-dried and pre-chilled seeds of *Magnolia odoratissima.*

Drying time (hours)	Fs (mean ± s.e.)		PCs (mean ± s.e.)
	2 months	4 months	
0	30.0 ± 3.16	30.0 ± 1.00	30.8 ± 0.87
2	23.1 ± 0.76	21.6 ± 1.35	19.8 ± 0.5
4	16.5 ± 1.29	14.1 ± 0.95	14.6 ± 1.05
6	11.3 ± 0.77	13.6 ± 0.82	-
8	9.8 ± 0.80	9.9 ± 0.14	10.4 ± 0.45
12	7.9 ± 0.42	7.0 ± 0.28	8.7 ± 0.23
18	6.0 ± 0.14	5.3 ± 0.19	5.0 ± 0.02
24	5.1 ± 0.17	4.6 ± 1.11	4.5 ± 0.12

Fs = moisture content of *M. odoratissima* seeds dried for different lengths of time at room temperature (20-23°C) before storage for two or four months, respectively.
PCs = moisture contents of pre-chilled *M. odoratissima* seeds dried for different lengths of time at room temperature (20-23°C) before four months storage.

months storage. In the moisture content range 14.6-4.5%, no seeds were viable after four months storage at 20°C. Furthermore, all seeds completely lost viability when they were stored at -20°C.

Seed storage behaviour is classified into three kinds: orthodox, intermediate and recalcitrant (Hong and Ellis, 1996). Orthodox seeds can be dried to a moisture content below 5.0% without losing their viability and are best stored at temperatures below 0°C (Hong and Ellis, 1996). Most intermediate seeds survive drying to moisture contents in the region of 10-12.5%; however, further desiccation reduces viability (Hong and Ellis, 1996). Recalcitrant seeds, in contrast, tolerate only minor reductions in moisture content, and viability is usually reduced upon drying to 10-12% moisture content (Hong and Ellis, 1996). Consequently recalcitrant seeds cannot be stored under the conventional conditions of low water content and below-freezing temperatures (Pammenter and Berjak, 1999). In our study, the water content of *M. odoratissima* seeds decreased as drying time was extended, indicating that *M. odoratissima* seeds easily lose water under drying stress. The seeds were so sensitive to desiccation that only a few (21-28%) were able to survive drying to a water content of 9.9%. However, seeds germinated to 46% with a water content of 11%. For most intermediate seeds of tropical origin, the longevity of dry seeds (7-10% moisture content) is reduced with reduction in storage temperature below about 10°C (Hong and Ellis, 2003). Germination of the dried *M. odoratissima* seeds was significantly reduced and this was exacerbated by four months storage. Non-desiccated *M. odoratissima* seeds retained viability at the storage temperatures of 8 and 4°C. After drying to 22% moisture content and storage at 8 and 4°C for four months, germination of *M. odoratissima* seeds was significantly reduced to 32 and 40% (storage at 8 and 4°C, respectively). When the moisture content of the seeds had been reduced to 14.1%, most of the seeds failed to germinate after storage (figure 1a). All seeds lost viability at -20°C. Based on these results, *M. odoratissima* seeds showed intermediate seed storage

behaviour.

Nevertheless, intermediate seeds which require long periods of pre-chilling to overcome dormancy can be maintained at between -3°C and 5°C (Hong and Ellis, 2003). Fresh seeds of *M. odoratissima* required pre-chilling for several months to alleviate dormancy (Yang, 2008). After four months dry storage at 4°C, pre-chilled *M. odoratissima* seeds gave higher germination than air-dried seeds at different moisture contents (figure 1). Thus, *M. odoratissima* seeds belonged to chilling-tolerant intermediate category which could be stored for about one year at 4°C, but not at freezing temperatures.

The storage requirements of seeds vary from species to species and the storage requirements for pre-chilled seeds can be more complex as they have been reported as being more sensitive to desiccation (Yilmaz, 2008). The present data showed that both air-dried and pre-chilled seeds of *M. odoratissima* retain high germinability even when the moisture content was decreased to 21.6%. Germination of the freshly harvested seeds was dramatically reduced to 28% and 10% when seed moisture content was 9.9% and 5.0%, respectively (figure 1a). Whereas, the pre-chilled seeds germinated to 60% and 39% when dried to moisture contents of 8.7% and 5.0% respectively (figure 1b). The results of this investigation therefore showed that pre-chilling *M. odoratissima* seeds enhanced the tolerance to desiccation as well as overcame dormancy.

In conclusion, the data suggest that *M. odoratissima* produces intermediate seeds which can be stored at 4°C for about one year.

Acknowledgements

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