Lan, Q.Y., Luo, Y.L., Qiu, Y.P., Jin, B.Q., He, H.Y. and Yin, S.H. (2014), *Seed Sci. & Technol.,* **42**, 97-100, http://doi.org/10.15258/sst.2014.42.1.11

Research Note

Storage of recalcitrant seeds of *Antiaris toxicaria* (Moraceae)

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(Accepted February 2014)

Summary

Seeds of the tropical tree *Antiaris toxicaria* have typical recalcitrant storage behaviour. The purpose of this work was to examine the effects of temperature and moisture content (MC) on germination during storage. Germination of seeds with a MC of 1.14, 0.90, 0.76 or 0.68 g H_2O g^{-1} DW was not significantly reduced when stored at 15°C for one year. However, seeds with a MC of 0.90 or 0.68 g g^{-1} DW stored at -20°C for one month lost viability, as did approximately 50% of those at 4°C. After one year, seeds with a MC of 0.90 g g^{-1} DW stored at 15°C had significantly higher germination percentage than those stored at -20, 4, 10 and 25°C; seeds with a MC of 0.68 g g^{-1} DW stored at 25°C had significantly higher germination percentage than those stored at -20, 4, 10 and 15°C. Thus, the lower the MC (0.68 g g^{-1} DW) of seeds, the higher the storage temperature (25°C) required to ensure seed survival.

Experimental and discussion

Antiaris toxicaria Leschenault (Moraceae) is a monoecious evergreen tree endemic to the tropical seasonal rainforest of Xishuangbanna, Yunnan Province, China. The rainforest in Xishuangbanna is at the altitudinal and latitudinal limits of tropical rainforests in the northern hemisphere, and the distribution of *A. toxicaria* is highly fragmented by the continued conversion of rainforest to rubber plantations (Zhu *et al*., 2006). Thus, this species is endangered in China and has been given protection by the Chinese Government (Fu, 1989). Since this is an endangered species, there is a need to conserve germplasm and to be able to germinate seeds for restoration projects.

Seeds of *A. toxicaria* are desiccation-sensitive (Yu *et al.*, 2008) and have been described as recalcitrant (Cheng and Song, 2008). Because recalcitrant seeds are shed at high moisture content (MC) and are sensitive to dehydration, they are difficult to store. In addition, recalcitrant seeds are intolerant of chilling (Konstantinidou *et al.*, 2008). The objective of our work on *A. toxicaria* was to determine appropriate seed MC and temperature conditions to maintain high seed viability during storage.

To obtain uniformly high-quality seeds, fruits were collected from a single tree growing in the Xishuangbanna Tropical Botanical Garden (21°41'N, 101°25'E; altitude, 600–700 m a.s.l.) in Menglun, Mengla, Yunnan, China. Seeds were removed from the fruits, rinsed with clean water and immediately used for experiments.

Before and after drying at 103°C for 17 hours (ISTA, 2005), five individual seeds were weighed to determine seed MC expressed on a dry weight basis (g H_2O g^{-1} DW).

Samples of fresh seeds were dried at 15°C and 7% RH for 0.5, 1 or 4 days. Fresh seeds with an initial MC of 1.14 g g^{-1} DW (95% germination) and those dried for one day (0.76 g g^{-1} DW; 95% germination) were placed in sealed containers and stored at 15°C for 0, 1, 3, 5, 7, 9 and 12 months. Three replicates of 10 seeds each were tested for germination on 1% agar in closed Petri dishes in the dark at 30°C for 40 days. Seeds dried for 12 hours (MC of 0.90 g g^{-1} DW; 95% germination) or for four days (0.68 g g^{-1} DW; 93% germination) were stored at -20, 4, 10, 15 and 25°C in sealed containers for 1, 3, 5, 7, 9 and 12 months. The germination of stored seeds was tested at 30°C, as previously described.

The effects of temperature and MC on germination of *A. toxicaria* seeds were analysed by fitting generalised linear models (GLM) in R version 3.0.2. Duncan's multiple range test was used to compare means after fitting the GLM.

There was 93-100% germination of seeds with MC of 1.14, 0.90, 0.76 or 0.68 g g^{-1} DW stored at 15°C for 12 months (figure 1). Seeds with a MC of 0.90 or 0.68 g g^{-1} DW stored at -20°C lost their germination ability within one month (figure 2). Storage at 4°C significantly reduced germination and no seeds with a MC of 0.90 or 0.68 g g^{-1} DW were alive after 5-7 months storage at this temperature. The germination of seeds with a MC of 0.90 g g^{-1} DW stored at 10, 15 and 25°C ranged from 80 to 100% and was significantly higher when stored at 25 and 15°C than when stored at 10°C ($P < 0.05$).

Germination of seeds with a MC of 0.68 g g^{-1} DW stored at 10, 15 and 25°C for one year was 43.3, 93.3 and 86.7%, respectively, and was significantly higher at 15 and 25°C than at 10°C ($P < 0.001$). Germination of seeds with MC of 1.14, 0.90, 0.76 or 0.68 g g^{-1} DW was not significantly reduced when stored at 15°C for one year ($P > 0.05$). Seed viability can be maintained in the short-term (at least one year) at relatively high MC (close to harvest MC).

In addition to MC, temperature is also an important factor in determining seed viability during storage. Not surprisingly, all *A. toxicaria* seeds stored at the highest MC (0.90 g g^{-1} DW) lost viability after one month at -20°C; approximately 50% of the seeds stored at 4°C died over this period. At a MC of 0.90 g g^{-1} DW, survival was 80-100% at 10, 15 and 25°C with significant differences in survival at the three temperatures. When MC of seeds was 0.68 g g^{-1} DW, the optimum temperatures for seed survival were 15 and 25°C, where 80% of the seeds were viable after one year. Pammenter *et al.* (1994) reported that it is generally difficult to maintain seeds in a mild water loss condition for extended periods and suggested that the deleterious events associated with a long period of water loss ultimately leads to death of the seeds. However, when seeds of *Hopea mollissima* C.Y. Wu were dehydrated slightly to 0.62 or 0.57 g g^{-1} DW, they became sensitive to low temperature and lost germination ability after only a short period of storage (Ma *et al.*, 2011). Thus, the more seeds are dehydrated prior to storage, the higher the storage

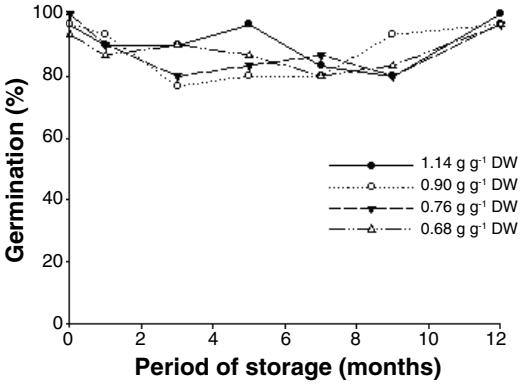

Figure 1. Changes in germination of *Antiaris toxicaria* seeds with different moisture contents (g H_2O g^{-1} DW, as indicated) during storage at 15°C. Seeds we re tested for germination at 30°C.

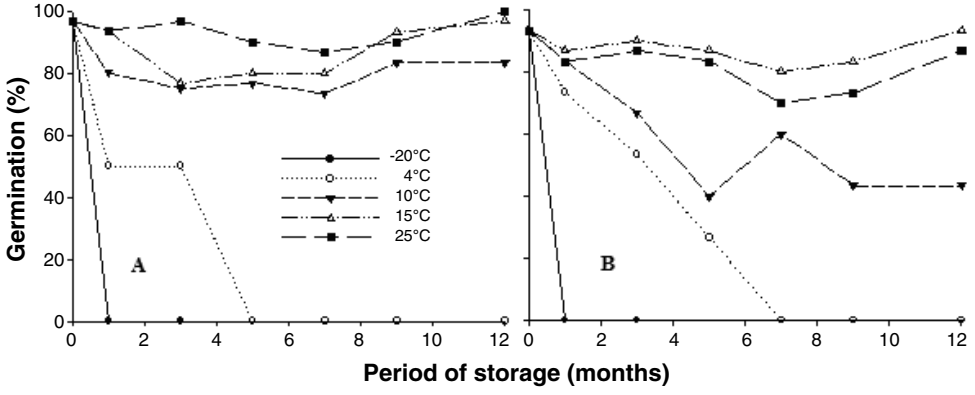

Figure 2. Changes in germination of seeds stored at different temperatures, as indicated. (A) Seeds with MC of 0.90 g H_2O g^{-1} DW. (B) Seeds with MC of 0.68 g g^{-1} DW. Seeds were tested for germination at 30°C.

temperature needs to be to ensure seed survival. Pritchard *et al.* (2004) recommended a storage temperature of 15°C for recalcitrant seeds of most tropical lowland species. Low temperatures (e.g. 4°C) can block metabolism and facilitate storage of orthodox seeds. However, such a low temperature could cause chilling injury and/or death of recalcitrant seeds, especially if they are well hydrated.

Potential chilling damage is also closely connected to MC. Generally, high MC is most likely to induce low temperature damage to seeds, which in turn means that the lower the MC the less the risk of chilling injury (Hong and Ellis, 2002). However, in our study, *A. toxicaria* seeds with a MC of 0.90 rather than of 0.68 g g^{-1} DW MC were more tolerant of storage at 10 and 15°C. Thus, if low temperature seed storage facilities are not available, the best procedure for seeds of *A. toxicaria* would be to store them at 25°C at MC 0.90 g g^{-1} DW. However, if seeds are dehydrated to 0.68 g g^{-1} DW viability will be decreased if seeds are stored at 15°C or a lower temperature. In the storage of recalcitrant seeds, it is desirable to reduce the MC as low as possible without exceeding the critical MC.

In conclusion, the recalcitrant seeds of *A. toxicaria* can be stored at 10, 15 and 25°C if the seeds are not dehydrated (1.14 g g^{-1} DW) or only slightly dehydrated (0.90 g g^{-1} DW). However, if seeds are dehydrated further (0.68 g g^{-1} DW) they need to be stored at 15 or 25°C.

Acknowledgements

Authors wish to thank the Knowledge Innovation Project of Chinese Academy of Sciences (KSCXZ-SW-117), the Supporting System Program of Strategic Biological Resources of the Chinese Academy of Sciences (08ZK121B01) and the Ministry of Science and Technology of China (2005DKA21006) for providing financial support for the research. The authors are grateful to Professors Carol C. Baskin and Jerry M, Baskin (University of Kentucky) for their help in revising this manuscript.

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