Fan, Liu, Hu, Ji and Lan (2020).
Seed Science and Technology, **48**, 2, 201-207.
https://doi.org/10.15258/sst.2020.48.2.07

Research Note

Storage of seeds of *Calamus palustris* var. cochinchinensis

Y.K. Fan[1,2,3], M. Liu[4], J.X. Hu[4], M.Y. Ji[1,5] and Q.Y. Lan[1,2,3]*

[1] Germplasm Bank, CAS Key Laboratory of Tropical Plant Resources and Sustainable Use, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China
[2] Center for Economic Botany, Core Botanical Gardens, Chinese Academy of Sciences, Xishuangbanna 666303, China
[3] Center of Conservation Biology, Core Botanical Gardens, Chinese Academy of Sciences, Xishuangbanna 666303, China
[4] Department of Gardening and Horticulture, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla 666303, China
[5] Bureau of Human Resources and Social Security, Changshan County, Quzhou City, Zhejiang Province, 324200, China
* Author for correspondence (E-mail: lqy@xtbg.org.cn)

(Submitted November 2019; Accepted March 2020; Published online April 2020)

Abstract

The present study examined the effect of temperature (15, 20, 25, 30 and 20/30°C) on germination and the storage behaviour of freshly harvested mature seeds of *Calamus palustris* var. cochinchinensis. Seed desiccation tolerance and the effects of storage temperature (4 and 15°C), perlite water content (120, 180 and 240%) and seed moisture content (27.8, 38.2 and 49.2%) on viability were observed. Seeds had a higher germination at 25°C (88.3%) than at the other tested temperatures. Germination decreased as the seed moisture content decreased during desiccation. The germination of seeds stored at 15°C was higher than that of seeds stored at 4°C. Germination of seeds stored at 15 and 4°C was < 65% and with extension of storage time, the germination decreased, indicating that neither temperature can be used for long-term conservation. For short-term storage, the seeds can be stored at 15°C with perlite with 180% water content in plastic bottles or at 15°C with 49.2% moisture content sealed inside aluminum foil bags.

Keywords: *Calamus palustris*, germination, rattan, seed desiccation, seed storage behaviour

Experimental and discussion

The genus *Calamus* is the largest genus in the *Palmae* family (approximately 370 species (Dransfield and Manokaran, 1993)) and is mainly distributed in tropical and subtropical Asia (Jiang *et al*., 2007; An *et al*., 2010). Its milky white scabbard stem (cane) has good flexibility and tensile strength that makes it an excellent material for traditional weaving and furniture production (Cai *et al*., 2003). Due to intense human disturbance and natural environmental degradation, wild resources have been severely reduced with some *Calamus* species becoming extinct. Therefore, it is particularly important to conserve the genetic resources of *Calamus*. For most plant species, the most economical method of conserving germplasm is to store their seeds in a seed genebank (Ma *et al*., 2011). In order to store the seeds successfully, it is necessary to know something about their storage behaviour. Seeds have traditionally been classified into two main groups, recalcitrant and orthodox (Roberts, 1973). Orthodox seeds have desiccation tolerance and can be dried to low moisture contents (2-5%) (MC; % fresh weight basis) and stored for predictable periods under controlled conditions (Ellis and Roberts, 1980; Ma *et al*., 2011; Pammenter and Berjak, 2014). Recalcitrant seeds are shed at high moisture contents and are sensitive to desiccation and can neither be stored at low moisture contents nor at sub-zero temperatures because of freezing damage caused by ice formation (Roberts, 1973; Konstantinidou *et al*., 2008; Pammenter and Berjak, 2014). They are therefore difficult to store, rapidly losing viability under any kinds of storage conditions (King and Roberts, 1979; Lan *et al*., 2014). A third category, intermediate between orthodox and recalcitrant has also been recognised (Ellis *et al*., 1990). The main feature is that seeds are able to tolerate desiccation to moisture contents in equilibrium with about 40-50% relative humidity (r.h.), i.e., about 7-10% moisture content depending upon species, but further drying results in more rapid loss in viability of stored seeds and sometimes immediate damage occurs on further desiccation (Ellis *et al*., 1990; Hong and Ellis, 1996). In this study, the effects of temperature, desiccation and storage on seed germination of *Calamus palustris* var. cochinchinensis were investigated. This plant is distributed in the south and southeast of Yunnan, China and also found in Cambodia and Vietnam.

The seeds used in the experiment were collected from Menglang town, Lancang County, Yunnan, China on 20 October 2009. Prior to experimentation, the seeds were removed from the fruits, rinsed with clean water and dried (in the shade until seed surface was dry). Seed MC (% fresh weight basis) was determined by weighing five replicates (one individual seed for each replicate) before and after drying at 103°C for 17 hours (ISTA, 2005).

Three replicates of 20 seeds each were tested for germination on 0.8% agar in closed Petri dishes at different temperatures (constant 15, 20, 25 and 30°C and alternating 20/30°C) for one month. Temperature had a significant effect on seed germination (figure 1; $P < 0.05$). SPSS 16.0 was used for one-way ANOVA of the data. As temperature increased, so did germination, reaching a maximum at 25°C (88.3%) and then declining. Seed germination was lowest at 15°C, with just 21.7%; at all other tested temperatures, germination was higher than 50%. At 20/30°C, the germination was 60%, lower than that of 20, 25 and 30°C. This indicated that seed germination was sensitive to the low temperature (15°C) and that constant temperature (20, 25 and 30°C) is more beneficial to improve seed germination than alternating temperature (20/30°C). The necessity for these higher temperatures for germination is consistent with the temperature conditions in its area of distribution.

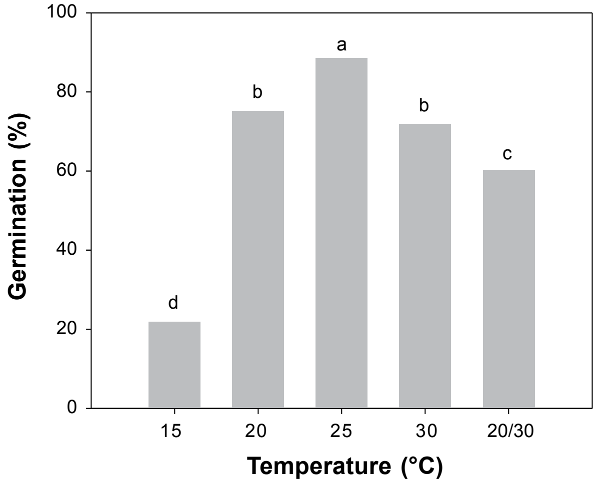

Figure 1. Effects of temperature (15, 20, 25, 30 and 20/30°C) on seed germination of *Calamus palustris* var. cochinchinensis (1-month germination test). Different letters indicate a statistically significant difference at $P = 0.05$.

Fresh seeds were desiccated with activated silica gel at room temperature (25 to 28°C). Seeds were desiccated for 0, 1, 3, 6, 12, 24 and 48 hours, and seed MC and germinability at 25°C determined as described previously. Desiccation had a significant effect on the seed MC and seed germination (figure 2; $P < 0.05$). Both the seed MC and germination gradually decreased, from 49.2% and 88.3%, respectively (control, 0 hours seeds), with increasing desiccation time. After 48 hours, seed MC and germination had been reduced to 9.6% and 10%, respectively. The seeds of recalcitrant species have a high MC (often > 30-50%) at maturity and are sensitive to desiccation below moisture contents of 12-30% (Roberts, 1973; Ma *et al*., 2011). When the seed MC of *Hopea hainanensis* was 38.3%, the germination percentage was 100% but when the MC was reduced to less than 20%, the germination capacity was completely lost (Song *et al*., 1986). For *Vatica astrotricha* seeds, when MC was reduced from 41.6 to 31%, germination similarly decreased significantly (Song *et al*., 1986; Tang *et al*., 2004). Our results are inconsistent with these data: although with the seed MC declining, the germination of *C. palustris* var. cochinchinensis decreased significantly, some seeds survived (10%) at a MC of 9.6%. Collectively these data suggest that the seeds of *C. palustris* var. cochinchinensis were sensitive to desiccation.

Following Pritchard *et al*. (1995), seeds were rapidly desiccated for 0, 1 and 3 hours with activated silica gel and seed MC determined after each drying period. Then the dried seeds were sealed in aluminum foil bags (60 seeds in each bag) and stored at 4 and 15°C for 0.5, 1 and 3 months. After each storage time, germination was tested at 25°C, as previously described. After desiccation for 0, 1 and 3 hours, seed MC was 49.2, 38.2 and 27.8%, respectively. Germination of seeds stored at 4 and 15°C decreased with the extension of storage time, and for all of the tested seed MC, germination was lower than 60% (figure 3). With decrease in seed MC, the germination percentages showed a decreasing trend. Temperature also had an impact as the germination percentage of seeds stored at 4°C was lower than that stored at 15°C. Indeed when seed MC was 27.8% and the storage time was three months, the seeds stored at 4°C had lost viability. Collectively the results showed that the seeds of *C. palustris* var. cochinchinensis were sensitive to low temperature, and as such were not suitable for long-term storage.

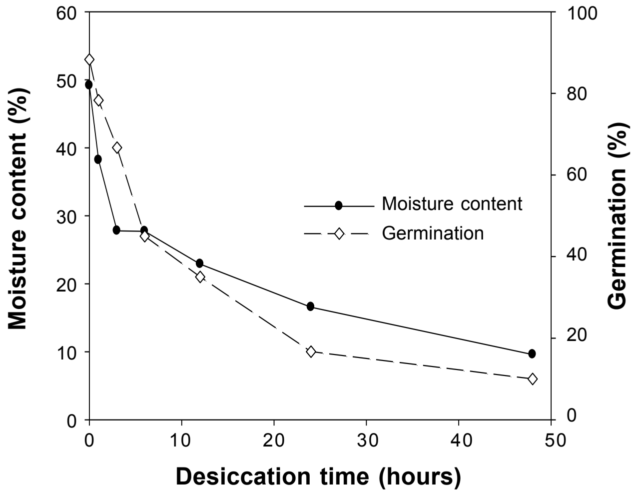

Figure 2. Effects of desiccation time (0, 1, 3, 6, 12, 24 and 48 hours) on seed moisture content and germination of *Calamus palustris* var. cochinchinensis.

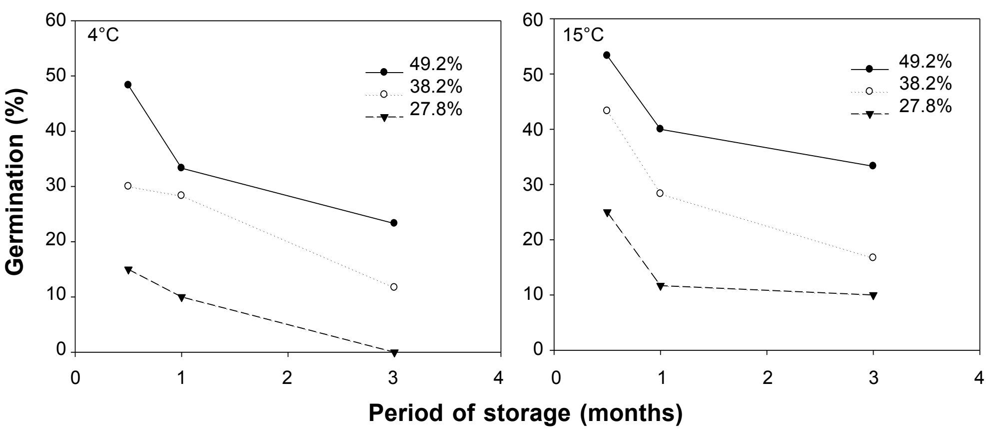

Figure 3. Effects of storage time (0.5, 1 and 3 months), storage temperature (4 and 15°C) and seed MC (49.2, 38.2 and 27.8%) on seed germination of *Calamus palustris* var. cochinchinensis.

According to the method of Wen (2009), 50 g of dried and cooled perlite was put into three plastic bottles, then 60, 90 and 120 ml distilled water was added into each bottle resulting in perlite with water content of 120, 180 and 240% of starting weight, respectively. Samples of 65 seeds were placed into each bottle and the seeds distributed evenly through the perlite by shaking. Each bottle was loosely sealed and then stored at 4 and 15°C for 0.5, 1, 3 and 6 months. After each storage period, seed MC and germination were tested at 25°C, as previously described. The seed MC was affected by the storage conditions (figure 4A, C). With the extension of storage time, when the perlite water content was 120%, the seed MC decreased, but when the perlite water content was 180 or 240%, the seed MC first increased and then decreased. The associated germination percentages showed a decreasing trend with the extension of storage time (figure 4B, D). When the perlite water content and storage time were the same, the germination percentage of seeds stored at 4°C was lower than that of seeds stored at 15°C. At 4°C, the lower the storage water content, the lower the germination percentage with the storage time of 0.5 and 1 month, and at 15°C, the germination percentage of seeds was the highest under 180% perlite water content. When the storage time reached three months, germination percentages were the lowest for seeds stored in perlite with 240% water content at 4 and 15°C. These data indicate that seed germination was sensitive to the low temperature.

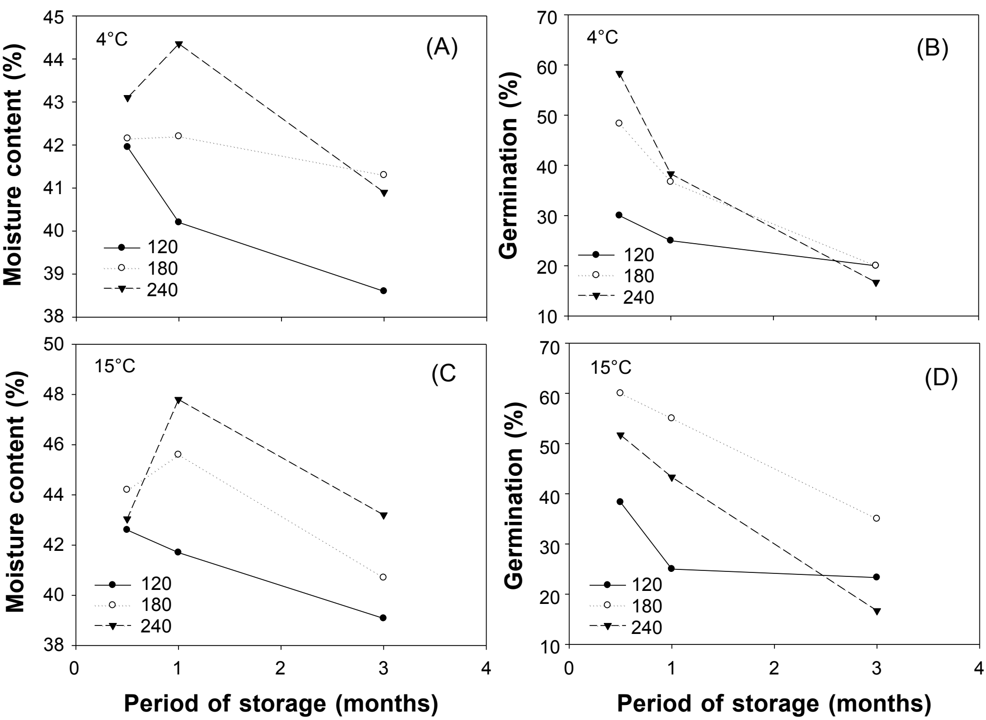

Figure 4. Effects of perlite water content (120, 180 and 240%), storage time (0.5, 1 and 3 months) and storage temperature (4 and 15°C) on seed MC and germination of *Calamus palustris* var. cochinchinensis.

In our study, after storage by two methods, the germination percentages both significantly decreased, and the germination percentage of seeds stored at 4°C was lower than that of seeds stored at 15°C. With a certain seed MC or storage water content, seeds of *C. palustris* var. cochinchinensis could be stored for a short time. However, the storage behaviour is inconsistent with that of orthodox or recalcitrant seeds. Seed germination was sensitive to low temperature (15°C), and temperatures of 20 to 30°C were suitable for the germination of the seeds, with the highest germination (88.3%) at 25°C. Although seed MC and seed germination declined significantly with the extension of desiccation time, the seeds could tolerate some desiccation: when the seed MC was 9.6%, there was still 10% germination. For short-term storage, the seeds can be stored at 15°C with 180% perlite water content in plastic bottles or at 15°C with seed MC of 49.2% sealed in aluminum foil bags.

Acknowledgements

This study was funded by a Special Grant for Basic Work of Science and Technology from the Ministry of Science and Technology, China (2012FY110300), the Supporting System Program of Strategic Biological Resources of the Chinese Academy of Sciences (CZBZX-1), and the Ministry of Science and Technology of China (2005DKA21006).

References

An, Q., Zhang, G.X., He, C.Z., Wang, J. and Yang, Y.M. (2010). Analysis of phylogenetic relationship on 11 *Calamus* species (*Palmae*) by AFLP markers. *Acta Botanica Boreali-Occidentalia Sinica*, **30**, 1117-1122.

Cai, Z.M., Xu, H.C., Yin, G.T., Yang, J.C. and Li, R.S. (2003). Research and progress on Rattan utilization. *Forest Research*, **16**, 479-487.

Dransfield, J. and Manokaran, N. (1993). *Plant Resources of South-East Asia 6. Rattans*, Pudoc Scientific Publishers, Wageningen, Netherlands.

Ellis, R.H. and Roberts, E.H. (1980). Improved equations for the prediction of seed longevity. *Annals of Botany*, **45**, 13-30.

Ellis, R.H., Hong, T.D. and Roberts E.H. (1990). An intermediate category of seed storage behaviour? I. Coffee. *Journal of Experimental Botany*, **41**, 1167-1174.

Hong, T.D. and Ellis, R.H. (1996). A protocol to determine seed storage behaviour. *IPGRI Technical Bulletin No. 1*, International Plant Genetic Resources Institute, Rome.

ISTA (2005). *International Rules for Seed Testing*, International Seed Testing Association, Bassersdorf, Switzerland.

Jiang, Z.H., Fan, S.H., Zhang, C.S. and Guan, F.Y. (2007). Advance in rattan research. *Acta Agriculturae Universitatis Jiangxiensis*, **29**, 957-964.

King, M.W. and Roberts, E.H. (1979). *The Storage of Recalcitrant Seeds: Achievements and Possible Approaches*, International Board for Plant Genetic Resources, Rome.

Konstantinidou, E., Takos, I. and Merou, T. (2008). Desiccation and storage behavior of bay laurel (*Laurus nobilis* L.) seeds. *European Journal of Forest Research*, **127**, 125-131.

Lan, Q.Y., Luo, Y.L., Qiu, Y.P., Jin, B.Q., He, H.Y. and Yin, S.H. (2014). Storage of recalcitrant seeds of *Antiaris toxicaria* (Moraceae). *Seed Science and Technology*, **42**, 97-100.

Ma, S.M., Lan, Q.Y., Tan,Y.H., Yu, L. and Yang, M.Z. (2011). Desiccation tolerance and storability of seeds in *Hopea mollissima* C.Y. Wu. *Seed Science and Technology*, **39**, 435-442. <https://doi.org/10.15258/sst.2011.39.2.15>

Pammenter, N.W. and Berjak, P. (2014). Physiology of desiccation-sensitive (recalcitrant) seeds and the implications for cryopreservation. *International Journal of Plant Sciences*, **175**, 21-28.

Pritchard, H.W., Tompsett, P.B., Manger, K. and Smidt, W.J. (1995). The effect of moisture content on the low temperature responses of *Araucaria hunsteinii* seed and embryos. *Annals of Botany*, **76**, 79-88.

Roberts, E.H. (1973). Predicting the storage life of seeds. *Seed Science and Technology*, **1**, 499-514.

Song, X.Z., Chen, Q.D., Wang, D.F. and Yang, J. (1986). Further study on ultrastructural changes of root tip cells in *Hopea hainanensis* seeds dewatering deterioration. *Science and Technology of Tropical Forestry*, **4**, 1- 6.

Tang, A.J., Long, C.L. and Dao, Z.L. (2004). Molecular mechanisms and storage technologies of recalcitrant seeds. *Acta Botanica. Boreali-Occidentalia Sinica*, **24**, 2170-2176.

Wen, B. (2009). Storage of recalcitrant seeds: a case study of the chinese fan palm, *Livistona chinensis*. *Seed Science and Technology*, **37**, 167-179. <https://doi.org/10.15258/sst.2009.37.1.19>