Lan, Q.Y., Luo, Y.L., Ma, S.M., Lu, X., Yang, M.Z., Tan, Y.H., Jiang, X.N., Tan, Y.P., Wang, X.F. and Li, Z.Y. (2012), *Seed Sci. & Technol.,* **40**, 200-208

Development and storage of recalcitrant seeds of *Hopea hainanensis*

Q.Y. LAN[1], Y.L. LUO[2], S.M. MA[1,2], X. LU[1,2], M.Z. YANG[2], Y.H. TAN[1], X.N. JIANG[3], Y.P. TAN[3], X.F. WANG[3] AND Z.Y. LI[4]

[1] Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Germplasm Bank, Mengla, 666303 Yunnan, China (E-mail: lqy@xtbg.org.cn)
[2] Department of Biology, Yunnan University, Yunnan Province 650091, PR China
[3] Beijing Forestry University, Beijing 100083, China
[4] Institute of Tropical Crop Genetic Resources, Chinese Academy of Tropical Agricultural Sciences / Key Laboratory of Tropical Crops Germplasm Resources Utilization, Ministry of Agriculture, Hainan Danzhou 571737, P.R. China

(Accepted April 2012)

Summary

Seed storage of *Hopea hainanensis* is problematic because its seeds are sensitive to desiccation and low temperature. In this study, we aimed to determine the best developmental stage for seed collection and the best moisture content (MC) and temperature for seed storage. Mass maturity occurred at about 173 days after anthesis (DAA); seeds harvested at this stage and later (183 DAA) were more desiccation tolerant than seeds harvested 14 d earlier (159 DAA). The freshly-collected pre-mass maturity, mass maturity and post-mass maturity seeds with initial MC of 44.7, 39.8 and 38.2%, respectively, remained viable during 14 months storage at 15 or 20°C with 87-91% germination. However, slightly dried post-mass maturity seeds (dried to MC of 33.9 and 31.5%) had higher viability than fresh seeds after 26 months storage at 20°C; the most favourable storage temperatures were 15 and 20°C. There was higher germination of seeds harvested at or after mass maturity (fresh or dried) than of seeds harvested before mass maturity, during storage at 4 or 10°C.

Introduction

Based on their storage behaviour, seeds can be divided into three groups: orthodox, intermediate and recalcitrant. Orthodox seeds which can be shed from the mother plant at low or high moisture content (MC) are desiccation tolerant (to 3-7% MC) and low temperature tolerant (< 0°C). Recalcitrant seeds are shed from the mother plant with high MC and are desiccation and chilling sensitive. Seeds with intermediate storage behaviour are tolerant of partial drying (to 6-12% MC), and their optimum storage temperature is 10-15°C (Roberts, 1973; Ellis *et al.*, 1990, 1991). Because many economically important tropical tree species have recalcitrant seeds, a prevailing problem is how to successfully store them (Daws *et al.,* 2004; Finch-Savage *et al.,* 1996; Pammenter and Berjak, 1999; Tommasi *et al.,* 1999; Tompsett, 1992). Further, since many recalcitrant seeds are damaged at storage temperatures < 15°C, their storage lifespan is quite short, varying from 2 weeks to several months (Chin and Roberts, 1980; Tompsett and Kemp, 1996; King and Roberts, 1982).

Both ecologically and economically, the Dipterocarpaceae is the most important tree family in tropical Asia. The genus *Hopea* occurs in the tropical forests of south and southeast Asia and comprises more than 100 species of evergreen trees (Tompsett, 1987). The wood of *H. hainanensis* Merr. et Chun is hard and heavy and is very good for indoor construction and for making furniture. In recent decades, many dipterocarps, including *Hopea* species, have become threatened due to logging and other human activities, resulting in critically reduced numbers of individuals and populations (Naito *et al.,* 2008). *H. hainanensis*, the subject of this study, is critically endangered due to overexploitation and is listed in the IUCN Red List of Threatened Species (IUCN, 2002). Thus, there is an urgent need to conserve germplasm of this species.

Storage of seeds is an important tool in ex situ conservation of plant species. However, it is not easy to store *Hopea* seeds due to the high MC of their fleshy cotyledons and desiccation sensitivity. For example, seeds of *H. parviflora* Beddome and *H. pomga* (Dennst.) Mabb. lose germinability when dried to 26 or 28% MC (wet mass basis), respectively (Rajeeswari *et al.,* 2000). When the MC of *H. hainanensis* seeds was decreased from 34.9 to 25.6%, the germination percentage declined from 65 to 20%, and various types of ultrastructural damage were observed (Song *et al.*, 1983). For storage of *H. hainanensis* seeds, relatively high MCs and temperatures are recommended: 33–38% MC and 15-20°C. After storage at 18°C for 1 year, 80% of seeds with 36–38% moisture still were viable (Song *et al.,* 1984). Previous studies on *H. hainanensis* seeds have also revealed that (1) immature seeds (i.e. before physiological maturity) were more desiccation tolerant than mature seeds, and (2) rapid drying was less harmful to seeds than slow drying (Lan *et al.,* 2007). Thus, we reasoned that it may be possible to achieve long term storage of *H. hainanensis* germplasm by subjecting immature seeds to rapid drying.

The aim of our research was therefore to determine the optimum MC and temperatures for storage of *H. hainanensis* seeds harvested at three developmental stages and subjected to rapid drying for various periods of time.

Materials and methods

Study site

The study was conducted from February to April, 2008. Seeds were collected in the Dipterocarp Park of Xishuangbanna Tropical Botanical Garden (21°55'N, 101°15'E 570 m a.s.l.) in southern Yunnan Province China. The climate is dominated by a southwest monsoon, with most of the rain (85%) falling between May and October. Xishuangbanna is cooler and has less rainfall than a typical equatorial rainforest. The annual mean temperature is 21.7°C. The coldest month is January, and the warmest month is June, with mean temperatures of 15.5°C and 25.3°C, respectively. Annual precipitation is 1221 mm; however, foggy days during the dry season increase the humidity, which help to compensate for the low rainfall (Zhang and Cao, 1995). The soil is a sandy alluvium, containing 0.875 g N kg^{-1}, 0.329 g P kg^{-1} and 9.693 g K kg^{-1} at 0–20 cm depth (Zhang *et al.,* 2009).

Field measurements

Fruits of *H. hainanensis* at different developmental stages were collected from ten neighbouring 30-year old trees (25-27 m tall, trunk diameter about 25 cm at 1.5 m height). Fruit development was monitored by tagging flowers at anthesis and harvesting at 2-week intervals from 103 to 173 days after anthesis (DAA). A final collection was made at 183 DAA, when most fruits were naturally being shed from the tree. At each collection, fruit samples from all ten trees were pooled into a single lot.

Measurement of seed fresh mass, dry mass and moisture content

The collected fruits at each developmental stage were dewinged and removed from the pericarp. The seeds then were weighed, dried at 103°C for 17 hours and reweighed to determine fresh mass, dry mass and MC. There were five replicates of one seed each for seed MC determination. The MC was calculated on a fresh mass basis. In addition fresh mass, dry mass and MC were determined for five replicate samples each of three excised axes.

Effect of dehydration on germination

The purpose of this experiment was to determine the effect of rapid partial desiccation on germination of seeds in three stages of development (159, 173 and 183 DAA). Seeds were dried in a closed glass container (26 cm in diameter and 17 cm in height) over activated silica gel at 25°C for 0, 1, 1.5 and 2 days; the ratio of silica gel to fruit was 10:1 (v/v) and the silica gel was regenerated every 24 hours.

Germination tests were performed in darkness at 30 ± 1°C using four replicates of 25 seeds each on moist filter paper in 12 cm diameter closed Petri dishes in an incubator for 30 days. Seeds were considered to be germinated when radicle length was at least 2 mm. At the end of the germination test, soft non-germinated seeds and those with black axes and cotyledons were considered to be non-viable; non-germinated seeds with white axes and cotyledons were considered to be viable.

Effect of dehydration on seed storage at different temperatures

A factorial design was used to study the survival of *H. hainanensis* seeds in different development stages at different storage MC and temperature conditions. Seeds in three development stages (159, 173 and 183 DAA) were dried at 25°C for 0-2 days resulting in three moisture levels for each stage of development. After the appropriate MC was reached, seeds were stored at four temperatures (4, 10, 15 and 20°C) for 0 to 26 months. For each treatment combination, 120 seeds were placed in laminated aluminium foil packets (11.5 × 7.5 cm). Samples were removed from storage periodically and tested for germination and MC (as above).

Results

Seed development and maturation

Seed fresh and dry mass increased gradually from 103 to 159 DAA and then rapidly from 159 to 173 DAA, reaching maximum values at 173 DAA (figure 1A). The mass of axes was very slight at 103 DAA, and then it increased until the maximum mass occurred at 173 DAA (figure 1B). Between 173 and 183 DAA (when the fruits were about to be shed), fresh and embryonic masses decreased, but dry masses of both were stable.

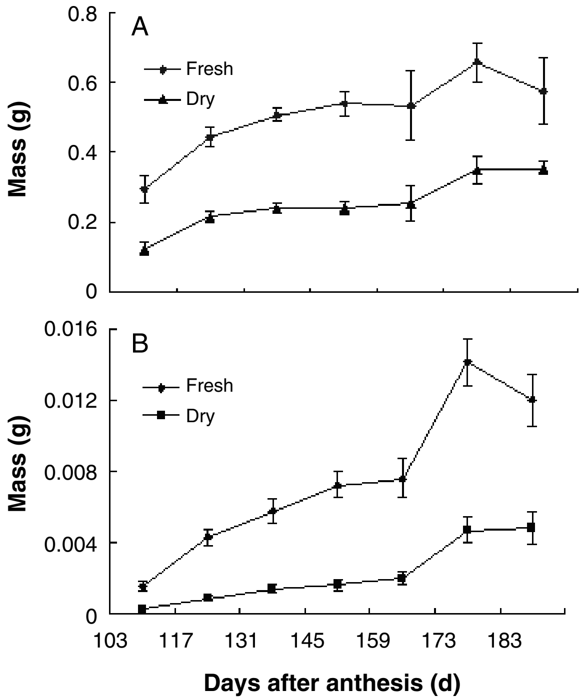

Figure 1. Changes in fresh and dry mass of *Hopea hainanensis* seeds (A) and embryonic axes (B) during development. Bars indicate s.e. of the mean.

Seeds were viable but did not germinate from 103 to 131 DAA; 85% germination was observed at 145 DAA and almost all (98%) seeds were able to germinate by 173 DAA (figure 2).

Effect of partial desiccation on germination

After 0, 1 and 1.5 days of rapid drying, the MC of the 159 DAA seeds was 44.7, 38.8 and 35.6%, respectively, and they germinated to 95, 73 and 53%, respectively. After 0, 1 and 2 days of rapid drying, the MC of the 173 DAA seeds was 39.8, 34.2 and 29.4%, respectively, and they germinated to 94, 90 and 60%, respectively. After 0, 1 and 1.5 days of rapid drying, the MC of the 183 DAA seeds was 38.2, 33.9 and 31.5%, respectively, and they all germinated to 100%.

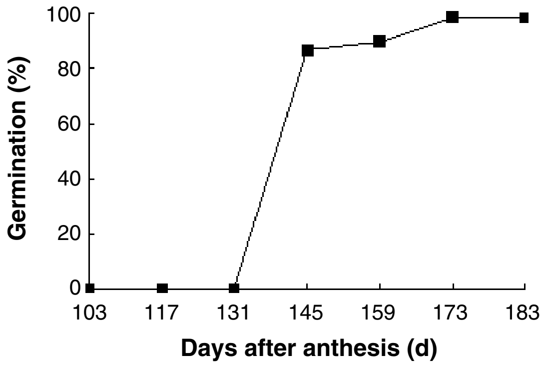

Figure 2. Acquisition of germinability of *Hopea hainanensis* seeds (without pericarp) during development.

Effect of dehydration on storage at different temperature

Fresh seeds of the three stages were all dead after four months storage at 4°C (figure 3A, D, G). After drying, the 159 and 173 DAA seeds lost viability after four months storage at 4°C (figure 3B, C, E, F). Dried 183 DAA seeds had higher viability than 159 and 173 DAA seeds. The 183 DAA seeds dried for 1 or 1.5 days germinated to 62.5 and 52.5%, respectively after storage at 4°C for four months (figure 3H, I). No seeds survived storage at 4°C for 6 months, regardless of MC or developmental stage (figure 3).

Seeds stored at 15 and 20°C had higher germination percentages than those stored at 4 and 10°C, regardless of developmental stage or MC (figure 3). Before 14 months, fresh seeds had stable germination percentage at 15 and 20°C but a sharp decrease at 4 and 10°C. Maximum survival of fresh seeds stored for 14 months was observed when stored at 15°C with 96.3% for seeds harvested at 159 DAA (figure 3 A); 96.3% for seeds harvested at 173 DAA (figure 3D); and 97% for seeds harvested at 183 DAA (figure 3G). Partially desiccated seed showed a gradual decrease in germination with the storage time, but seeds stored at 15 and 20°C had higher viability than those stored at 4 and 10°C (figure 3B-C, E-F, H-I). Among the four storage temperatures, 15 and 20°C were better than 10 or 4°C (figure 3).

Discussion

The development of techniques to store germplasm of recalcitrant species has received considerable attention from researchers; seed developmental stage, MC and storage conditions (temperature and relative humidity) have been investigated for various species (Berjak *et al.,* 1992). Seeds of *H. hainanensis* are generally considered to have a short period of longevity. Based on seed desiccation and storage behaviour, the storage physiology of *H. hainanensis* seeds was classified as true typical tropical recalcitrant (Song *et al.,* 1983, 1984). Lan (2007) reported that embryonic axes of this species are better able to tolerate a small loss of MC if the seeds are harvested before they have reached maximum dry mass (150 DAA) rather than at mass maturity (157 DAA).

However, in the present study, seeds were more tolerant at mass maturity (173 DAA) than at earlier harvests. In fact, the seeds collected in 2008 were not tolerant of drying prior to 173 DAA. The reasons for differences between 2004 and 2008 in ability to tolerate small decreases in MC in *H. hainanensis* seeds may be due to differences in climate, especially soil moisture during the seed maturation period. In 2004 and 2008, seeds matured in April and early May, respectively. The reason for annual differences in time of seed maturation

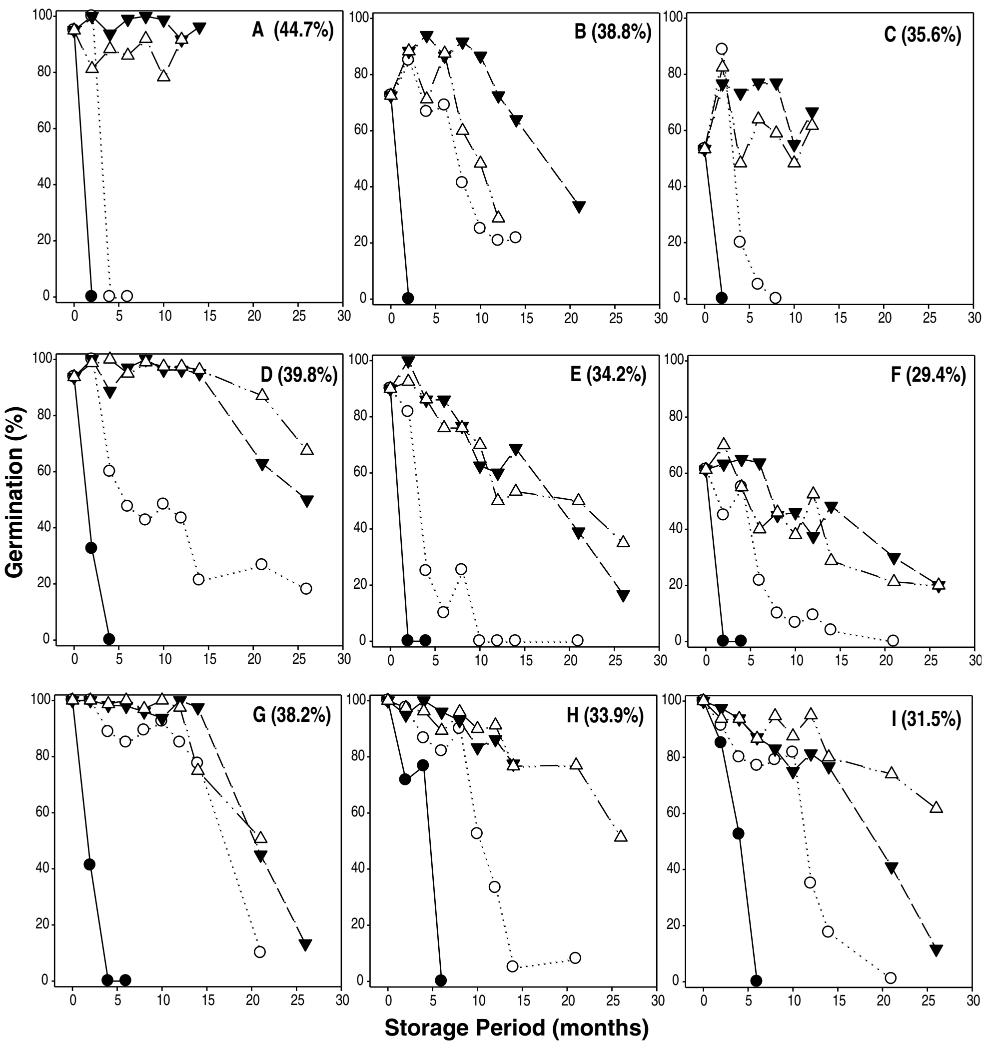

Figure 3. Germination of *Hopea hainanensis* seeds before mass maturity (159 DAA; A-C), at mass maturity (173 DAA; D-F) and post-mass maturity (183 DAA; G-I) after hermetic storage at 4°C (●), 10°C (○), 15°C (▼) or 20°C (△). The values in parenthesis are MCs for seeds stored without drying (A, D, G) or dried for 1 (B, E, H) or 1.5 days (C, F, I).

is not known, but it is possibly related to the amount of rainfall, with seeds maturing faster in dry years. Average rainfall from the time of anthesis until fruit collection in 2004 and 2008 was 23.7 and 32.7 mm, respectively. Thus, seeds that matured in early April 2004 developed under lower rainfall than those that matured in early May 2008. Dussert *et al.* (2000) found a negative correlation between desiccation sensitivity and the mean number of dry months in nine species of *Coffea* after seed-shed in various habitats.

Storage temperature plays an important role in conservation of recalcitrant seeds. Tamari *et al.* (1988) reported that seeds of *Hopea* species can tolerate 4°C for three weeks to three months, after which time viability rapidly declines. *H. hainanensis* seeds at 183 DAA with 33.9% MC had 77% germination after four months storage at 4°C. Fresh and partially dried seeds (159, 173 and 183 DAA) were also damaged at 10°C, whereas those stored at 15 and 20°C were not (figure 3). Similarly, in *H. odorata* Roxb. seed viability decreased during storage at 10°C (Tang and Tamari, 1973). However, seeds of *H. quisumbingiana* Gutierrez, *H. beccariana* Burck, *H. Montana* Symington, *H. dyeri* F. Heim, *H. altocollina* P.S. Ashton and *H. pterygota* P.S. Ashton, which are adapted to high (1000~1600 m a.s.l.) altitudes, can tolerate storage temperatures lower than 15 and 20°C, and storage at 10°C may help prolong their longevity (Hong *et al.,* 1998). *H. mollissima* C.Y. Wu seeds were highly sensitive to high temperatures and desiccation, and storage at temperatures ≥ 30°C or relative humidity ≤ 60% significantly decreased germination (Wen, 2011). Several reports indicate that optimum storage temperatures of *Hopea* seeds are in the range of 15-21°C; however, optimum storage MC is high, e.g. 35-38% for seeds of *H. hainanensis* (Song *et al.,* 1983, 1984); 47% for seeds of *H. helferi* Brandis (Tamari, 1976); and 43-50% for seeds of *H. nervosa* King (Sasaki, 1980).

In working with recalcitrant seeds, it is useful to know the lowest safe MC below which dehydration damage occurs; seed storage MC can be reduced to as close to that level as possible. Our results demonstrated that fresh seeds harvested pre- (159 DAA) or at mass maturity (173 DAA) and not subjected to a drying treatment have much higher germination and production of normal seedlings than seeds that have been dried for 1-2 days and stored at 4-20°C (figure 3 B-C, E-F). Similarly, there are suggestions that partial drying is not beneficial in extending the storage lifespan of other recalcitrant seeds (King and Roberts, 1982; Xia *et al.,* 1992; Pritchard *et al.,* 1995; Tompsett and Pritchard, 1998). However, post-mature seeds (183 DAA) of *H. hainanensis* can be stored at 4-20°C when they are partially dried (1-2 days) (figure 3H-I).

The developmental stages at which seeds are collected influences the desiccation response of recalcitrant seeds (reviewed by Pammenter and Berjak, 1999). Desiccation sensitivity has been shown to increase with storage in *Camellia sinensis* L. (Berjak *et al.,* 1993), *Quercus robur* L. (Finch-Savage *et al.,* 1996) and *Aesculus hippocastanum* L. (chilled storage; Tompsett and Pritchard, 1998). Results from our study demonstrated that seeds of *H. hainanensis* at mass maturity (173 DAA) and post-mass maturity (183 DAA) are better able to tolerate loss of MC than pre-mass maturity (159 DAA) seeds. Seeds harvested at and after mass maturity also maintained higher germination than less mature seeds (159 DAA) during storage at 4 and 10°C (figure 3).

In conclusion, our data suggest that for *H. hainanensis*:

1. For seeds harvested before (159 DAA) and at mass maturity (173 DAA), it is better to store them without drying (i.e. at harvest MC) at 4, 10, 15 and 20°C; seeds harvested after mass maturity (183 DAA) will be useful for storage at 4, 10, 15 and 20°C if seeds are subject to rapid drying for 1-1.5 days, to 33.9-31.5% MC.
2. More mature seeds can tolerate low temperatures better than immature ones.
3. Seeds harvested at and after mass maturity maintain higher percentages of germination than pre-mass maturity seeds when stored at 4, 10, 15 and 20°C.

Acknowledgements

This study was funded by National Forest Department 948 Project (Grant No. 2011-4-54), the Open Fund of Key Laboratory of Tropical Crops Germplasm Resources Utilization, Ministry of Agriculture (KFKT-2010-09) and the Supporting System Program of Strategic Biological Resources of the Chinese Academy of Sciences (08ZK121B01). The authors thank the National Forest Ecosystem Research Station at Xishuangbanna provided the climatic data.The authors are grateful to Professors Carol C. Baskin and Jerry M. Baskin (University of Kentucky) for revising the English in this manuscript.

References

Berjak, P., Pammenter, N.W. and Vertucci C. (1992). Homoiohydrous (recalcitrant) seeds: Developmental status, desiccation sensitivity and the state of water in axes of *Landolphia kirkii* Dyer. *Planta*, **186**, 249-261.

Berjak, P., Vertucci, C.W. and Pammenter, N.W. (1993). Effects of developmental status and dehydration rate on characteristics of water and desiccation-sensitivity in recalcitrant seeds of *Camellia sinensis*. *Seed Science Research*, **3**, 155-166.

Chin, H.F. and Roberts, E.H. (1980). *Recalcitrant Crop Seeds,* Tropical Press, SDN BDH, Kuala Lampur. Malaysia.

Daws, M.I., Lydall, E., Chmierlaz, P., Leprince, O., Matthews, S., Thanos, C.A. and Pritchard, H.W. (2004). Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytologist*, **162**, 157-166.

Dussert, S., Chabrillange, N., Engelmann F., Anthony F., Louarn, J. and Hamon, S. (2000). Relationship between seed desiccation sensitivity, seed water content at maturity and climatic characteristics of native environments of nine *Coffea* L. species. *Seed Science Research*, **10**, 293-300

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.

Ellis R.H., Hong T.D. and Roberts, E.H. (1991). Effect of storage temperature and moisture on the germination of papaya seeds. *Seed Science Research*, **1**, 69-72.

Finch-Savage, W.E., Blake, P.S. and Clay, H.A. (1996). Desiccation stress in recalcitrant *Quercus robur* L. seeds results in lipid peroxidation and increased synthesis of jasmonates and abscisic acid. *Journal of Experimental Botany*, **47**, 661-667.

Hong, T.D., Linington, S. and Ellis, R.H. (1998). *Compendium of information on seed storage behavior,* Royal Botanic Gardens, Kew, Richmond, Surrey. Volume 1 A-H, 287-288.

IUCN (2002). 2002 IUCN Red List of Threatened Species [see also at http://www.redlist.org]

King, M.W. and Roberts, E.H. (1979). *The storage of recalcitrant seeds-achievements and possible approaches,* International Board for Plant Genetic Resources, Rome.

King, M.W. and Roberts, E.H. (1982). The imbibed storage of cocoa (*Theobroma cacao*) seeds. *Seed Science and Technology*, **10**, 535-540.

Lan, Q.Y., Jiang, X.C. and Song, S.Q. (2007). Changes in germinability and desiccation-sensitivity of recalcitrant *Hopea hainanensis* Merr. *et* Chun seeds during development. *Seed Science and Technology*, **35**, 21-31

Naito, Y., Kanzaki, M., Iwata, H. and Obayashi, K. (2008). Density-dependent selfing and its effects on seed performance in a tropical canopy tree species, *Shorea acuminata* (Dipterocarpaceae). *Forest Ecology and Management*, **256**, 375-383.

Pammenter, N.W. and Berjak, P. (1999). A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research*, **9**, 13-37.

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

Rajeeswari, D.B. and Kaveriappa, K.M. (2000). Effect of desiccation and temperature on germination and vigour of the seeds *Hopea parviflora* Beddome and *H. ponga* (Dennst.) Mabb. *Seed Science and Technology*, **28**, 497-506.

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

Sasaki, S. (1980) Storage and germination of dipterocarp seeds. *Malaysian Forester*, **43**, 290-308.

Song, X.Z., Chen, Q.D., Wang, D.F. and Yang, J. (1983). A study of ultrastructural changes in radicle-tip cells and seed vigor of Hopea and Vatica in losing water process. *Scientia Silvae Sinicae*, **19**, 121-125.

Song, X.Z., Chen, Q.D., Wang, D.F. and Yang, J. (1984). Studies on the storage of *Hopea hainanensis* seeds. *Scientia Silvae Sinicae*, **20**, 225-235.

Tamari, C. (1976). Phenology and seed storage trials of dipterocarps. *Research Pamphlet,* No. 69. Forest Department, Kuala Lumpur 73.

Tamari, C., Tang, H.T. and Domingo, I.L. (1988). Prospect for the storage of dipterocarp fruits. In: Proc. Int. Symp., Seed Quality of Tropical and Sub-Tropical Species, Bangkok, Thailand, May, 1984. IUFRO Project Group on Seed Problems.

Tang, H.T. and Tamari, C. (1973). Seed description and storage tests of some dipterocarps. *Malaysian Forester*, **36**, 112-128.

Tommasi, F., Paciolla, C. and Arrigoni, O. (1999). The ascorbate system in recalcitrant and orthodox seeds. *Physiologia Plantarum*, **105**, 193-198.

Tompsett, P.B. (1987). Desiccation and storage studies on Dipterocarpus seeds. *Annals of Applied Biology*, **110**, 371-379.

Tompsett, P.B. (1992). A review of the literature on storage of dipterocarp seeds. *Seed Science and Technology*, **20**, 251-267.

Tompsett, P.B. and Kemp, R. (1996). *Database of tropical tree seed research,* Royal Botanic Gardens, Kew, Richmond, Surrey.

Tompsett, P.B. and Pritchard, H.W. (1998). The effect of chilling and moisture status on the germination, desiccation tolerance and longevity of *Aesculus hippocastanum* L. seed. *Annals of Botany*, **82**, 249-261.

Wen, B. (2011). Changes in the moisture and germination of recalcitrant *Hopea mollissima* seeds (Dipterocarpaceae) in different desiccation regimes. *Seed Science and Technology*, **39**, 214-218

Xia, Q.H., Chen, R.Z. and Fu, J.R. (1992). Moist storage of lychee (*Litchi chinensis* Sonn.) and longan (*Euphorbia longan* Steud.) seeds. *Seed Science and Technology*, **20**, 269-279.

Zhang, J.H. and Cao, M. (1995). Tropical forest vegetation of Xishuangbanna, SW China and its secondary changes, with special reference to some problems in local nature conservation. *Biological Conservation*, **73**, 229-238

Zhang, J.L., Meng, L.Z. and Cao, K.F. (2009). Sustained diurnal photosynthetic depression in uppermost-canopy leaves of four dipterocarp species in the rainy and dry seasons: does photorespiration play a role in photoprotection? *Tree Physiology*, **29**, 217-228.