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Desiccation tolerance and storability of seeds in *Hopea mollissima* C.Y. Wu

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Summary

Freshly harvested mature seeds of *Hopea mollissima* were used in the present study to examine the effect of temperature (10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 20/30°C) on germination, the desiccation tolerance of seed under slow and rapid dehydration, and the effects of storage temperature (4°C, 10°C and 15°C) and moisture content (0.79 g H_2Og^{-1} DW, 0.62 g H_2Og^{-1} DW, 0.57 g H_2Og^{-1} DW) on storage behaviour. The results showed that seeds of *H. mollissima* had a higher germination and shorter germination time at 15°C than at other temperatures. Germination decreased during dehydration as seed moisture content decreased, but the hazardous effect of slow drying on germination was more pronounced than that of fast drying. The higher seed moisture content (0.79 g H_2Og^{-1} DW) combined with the storage temperature of 15°C was most suitable for the conservation of seeds in *H. mollissima*.

Introduction

Hopea mollissima, belonging to Dipterocarpaceae, is distributed in the tropical forests of south China and northern Vietnam (Tompsett, 1987). This species has great value in the chemical and construction industries, and can provide the material of dammar resin for varnishes. Mature trees generally can grow over 35 m in height and the trunk, characterized by finely grained and very durable wood, is used for making boats, bridges and furniture. As a result of its high economic value, the wild populations of *H. mollissima* have suffered an alarming decline due to excessive collection. It is urgent to conserve this valuable germplasm resource. However, there is little information available for effective conservation of H. *mollissima.*

For most plant species, the most economical method of conserving germplasm is to store their seeds in a seed-gene bank. In order to store the seeds successfully, it is necessary to understand the environmental requirements for seed germination, desiccation

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tolerance and storage condition. Seeds have traditionally been grouped into two main groups, namely recalcitrant and orthodox seed (Roberts 1973). Orthodox seed can be dried to low moisture content (2-5%) and stored at low temperature. The seeds of recalcitrant species have a high moisture content (often > 30-50%) at maturity, and are sensitive to the desiccation below moisture contents of 12-30%, depending on the species. Recalcitrant seed has a short storage potential and can rapidly lose viability under any kinds of storage conditions (King and Roberts, 1979). It is believed that desiccation tolerance depends not only on the inherent characteristics of the species, but also on the developmental status of the seeds, and the environmental conditions in which they are dried, particularly the rate of dehydration (Pammenter and Berjak, 1999). It has been found that the more rapidly dehydration can be achieved, the lower the water content to which the seeds can be dried before viability is lost (Pammenter *et al.*, 1991, 2000; Berjak *et al.*, 1993).

Regardless of the storage type or species of seeds, the most critical factors affecting seed storage are seed moisture and temperature. Seeds can lose their viability during the dehydration and storage. The loss of seed viability is mainly due to the physical, chemical and metabolic changes during the dehydration and storage which can damage both the cytoplasm and cell membranes (Priestley, 1986; Bewley and Black, 1994). Deterioration of membranes is a primary indicator of cell death (Priestley, 1986; Hoekstra and Golovina, 1999). The objectives of the present study were to determine the moisture content that result in loss of seed viability in *H. mollissima* during desiccation at different drying rates, and to determine a suitable seed moisture content and temperature for the seed storage of *H. mollissima*.

Materials and methods

Plant materials

Mature seeds of *H. mollissima* were collected from the trees grown in Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Sciences, Yunnan, Southwest China, in early March. The fruit maturity was determined by the colour of the wing, which becomes dark yellow when the seeds ripen.

Germination test

Four replications, each of fifteen seeds were sown on moist filter paper in closed Petri dishes and placed in an incubator in darkness at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 20/30°C. The number of seeds germinated was recorded every 2 days up to 30 days. The seeds were considered as germinated when the radicle length was more than 2 mm (ISTA, 2005).

Desiccation treatment

Seed drying- rapid dehydration

The seeds were removed from the fruit by removing the pericarp. Rapid dehydration was achieved by placing 200 seeds without pericarp on a small filter paper boat over activated silica gel in a closed glass container at 25°C.

Seed with pericarp - slow dehydration
Slow dehydration was achieved by mixing 200 seeds with the pericarp still intact with activated silica gel in a closed glass container at 25°C. The ratio of silica gel to fruit was 15:1 (v/v). The silica gel was regenerated every 24 h.

Water content determination
Water content was determined using the low temperature method (ISTA, 2005). After drying at 103°C for 17 h, the seeds and their embryonic axes were weighed to determine the dry weight and moisture content (MC) on 5 replications of each treatment (1 seed). MC was expressed as the water content per unit seed dry weight (g H_2Og^{-1}DW).

Determination of electrolyte leakage from seeds
Five replications of each treatment (5 × 1 seed) were each placed in a vial containing 20 ml of de-ionized water. The vials were shaken and the conductivity was measured both immediately after shaking and after 4 hours at room temperature using a conductivity meter (DDS-307, Leici, Shanghai, China). The vials containing seeds were then put in boiling water for 1 h, cooled to room temperature (about 20 ± 2°C), shaken and the total conductivity was measured. Electrolyte leakage was expressed as the percentage of net conductivity of the solution with seeds immersed for 4 h to the total conductivity after boiling.

Storage test
The fresh seeds with pericarp were desiccated for 24 h and 48 h in a closed glass container over activated silica gel at 25°C. The fresh fruits and desiccated fruits with 0.79 g H_2Og^{-1} DW, 0.62 g H_2Og^{-1} DW, 0.57 g H_2Og^{-1} DW were packed in the aluminum foil bags, and stored at 4°C, 10°C and 15°C with an air relative humidity of 60%. Seed water content and germination were evaluated after 15, 30, 60, 150, 270 and 360 days using the methods as described above.

Results

Seed germination at different temperatures
The germinations of the freshly harvested seeds differed from the germinations of seed with pericarp when they were placed at 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 20/30°C. The germination percentages of the seeds (minus pericarp) at all temperatures were over 95% (figure 1). However the time from the beginning of germination to its completion was longer at 10°C and 20/30°C than at the other temperatures. Thus the duration of seed germination was 4 days at 10°C and 7 days at 20/30°C, while at other temperatures, the germination was complete after 2 days. In contrast, the germinations of seeds with pericarp were significantly different at different temperatures (figure 1). The germination of the seeds with pericarp increased from 89% at 10°C to 96% at 15°C but decreased sharply at 20°C and above and was only 43% at 35°C. The optimal germination temperature for both the seeds and the seeds with pericarp was 15°C (figure 1).

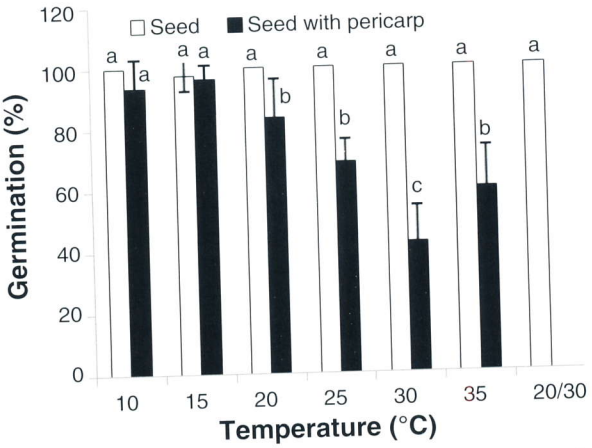

Figure 1. Effects of temperature on seed germination of *H. mollissima*. Error bars indicate ±1SE of the means: each data point is the mean of 4 replications of 15 seeds or seeds with pericarp. The same letters indicate that there are no significant differences between data; different letters indicate a statistically significant difference (ANOVA, $P < 0.01$).

Desiccation tolerance of H. mollissima seeds

The water content of fresh seed at the phase of physiological maturation was 0.68 g H_2Og^{-1} DW. The decrease in water content of seeds was clearly more rapid during the rapid dehydration treatment than during slow dehydration (figure 2). The survival of seeds did not decrease following both the rapid and slow dehydration from 0.68 to 0.49 g H_2Og^{-1} DW (figure 3A). However the ability to germinate was markedly affected by the drying rate when the moisture content was below 0.49 g H_2Og^{-1} DW. After rapid desiccation seeds were able to germinate at relatively lower moisture contents than after slow desiccation. For example, 48h of rapid drying or 6d of slow drying reduced the moisture content of seeds minus the pericarp to 0.31g H_2Og^{-1}DW. However the germination of rapidly desiccated seeds had only decreased to 70%, while the slowly desiccated seeds failed to germinate (figure 3A).

Increased electrolyte leakage was also used as an indicator of desiccation damage (figure 3B). In all cases, the electrolyte leakage of seeds increased with the decreasing moisture content. However, electrolyte leakage increased more rapidly during slow desiccation than rapid desiccation, particularly during the initial stages of drying. Thus, during slow dehydration, the electrolyte leakage increased from 4.05% to 16.52% when water content fell from 0.68 g H_2Og^{-1} DW to 0.56 g H_2Og^{-1} DW (figure 3B). In contrast leakage from rapidly dehydrated seeds increased only from 1.13% to 2.78% as the water content decreased from 0.68 to 0.51 g H_2Og^{-1} DW.

Effect of storage on germination

The seeds with pericarp in *H. mollissima* (0.79 g H_2Og^{-1} DW, 0.62 g H_2Og^{-1}DW, 0.57 g H_2Og^{-1} DW) were stored at three storage temperatures viz. 4°C, 10°C and 15°C. All seeds retained a high germination after 30 days storage at 4°C, but after 60 days storage, the

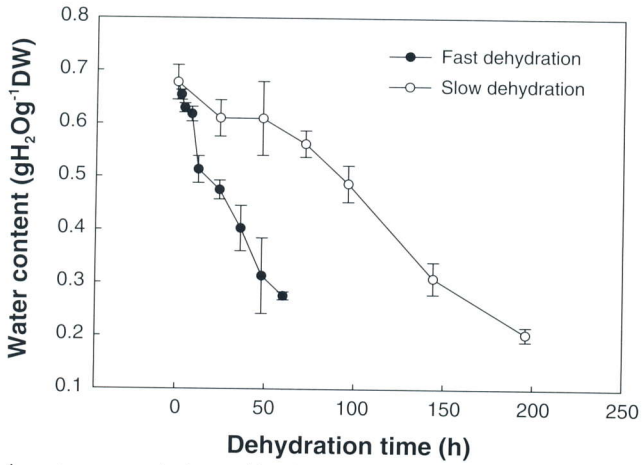

Figure 2. Changes in water content during rapid and slow dehydration of *H. mollissima* seeds. Error bars indicate ±SE of the means: each data point is the mean of 5 replications of 1 seed.

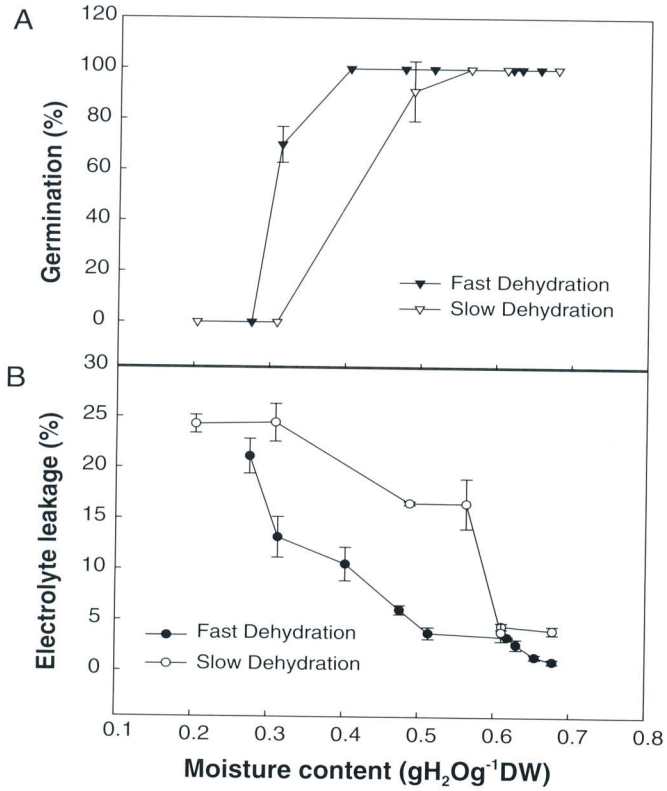

Figure 3. Changes in germination percentage (A) and electrolyte leakage (B) of *H. mollissima* seeds during fast and slow dehydration. Error bars indicate ±1SE of the means: each data point is the mean of 4 replications of 15 seeds (germination) or 5 replications of 1 seed (electrolyte leakage).

germination decreased, and was zero after 150 days storage. In contrast seed stored at 10°C and 15°C retained a higher germination after 150 days storage. For example, the germination of fresh (undried) seeds was 100% and that for seeds with 0.57 g H_2Og^{-1} DW water content was 70%. The germination of fresh seed was not significantly reduced throughout the storage period when the seeds were stored at 10°C or 15°C. However seeds dried to 0.62 g H_2Og^{-1} DW and 0.57 g H_2Og^{-1} DW showed a fall in germination after 150 days storage, with a greater decline in germination seen for seeds having the lower water content.

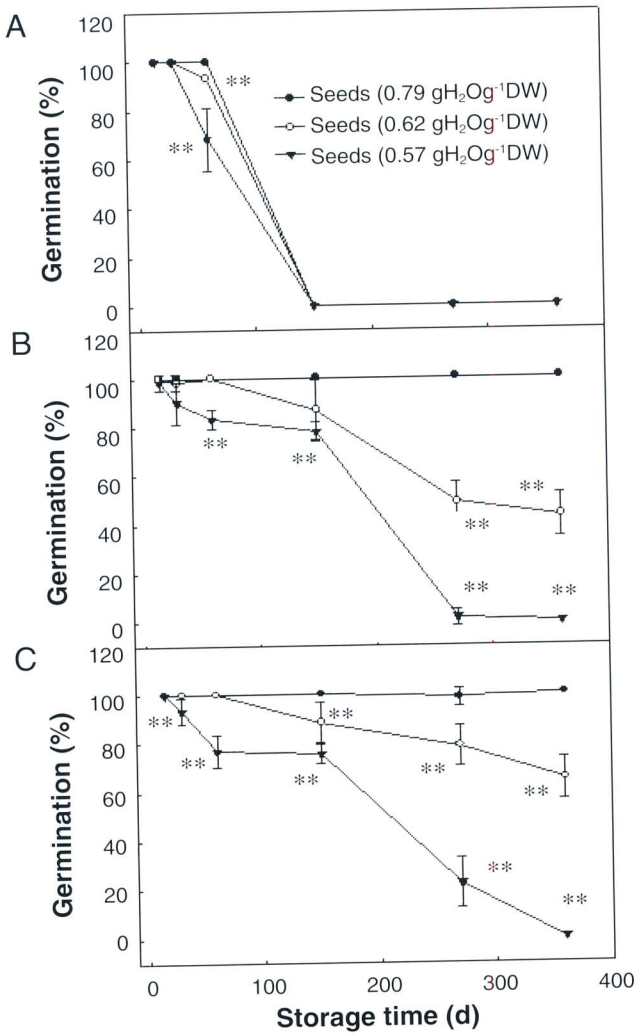

Figure 4. Effects of storage temperature and water content on seed germination of *H. mollissima*. (A) storage at 4°C; (B) 10°C; (C) 15°C. Error bars indicate ±1SE of the means: 4 replications of 15 seeds. Double asterisks indicate a statistically significant difference ($P < 0.01$) between desiccated seeds (0.62 g H_2Og^{-1} DW, 0.57 g H_2Og^{-1} DW) and fresh seeds (0.79 g H_2Og^{-1} DW).

Discussion

The seeds of *H. mollissima* germinated at a wide range of temperatures from 10 to 30°C, which reflected its natural ecological adaptation to the growing environments. In the natural habitat, the plants of *H. mollissima* grow in the tropical rain forest in Xishuangbanna. Most seeds germinate immediately after shedding from the trees in March. The day and night temperature in March is about 33°C and 12°C, respectively. However seed germination is affected by the microclimate in the understory, which is characterized by lower temperature and larger temperature fluctuations. So the optimal temperature for the seed germination in *H. mollissima* was 15°C.

Dehydration at different rates markedly influenced the desiccation response of *H. mollissima* seeds, with slow drying having a more hazardous effect on germination of than fast drying. These results are in accordance with Sun (1999), who suggested that fast drying of *Quercus rubra* seeds resulted in a higher germination than slow drying. The longer the drying time, the lower was germination. When the seed moisture content was reduced to 0.31 g H_2Og^{-1} DW using both desiccation methods, the germination of rapidly dehydrated seeds was 70%, while that after slow drying was zero. Thus the decrease in survival rate was not only a direct response to dehydration, but also involved the drying time of seeds. From this perspective, desiccation damage is a time-dependent process and might be considered to be an ageing phenomenon (Walters *et al.*, 2002). Therefore, dehydration time cannot be ignored in the dehydration experiments, and it can be very difficult to determine 'critical water content' for viability loss of seeds.

Seed longevity was related to the storage temperature. The seeds of *H. mollissima* stored at 10°C and 15°C retained their ability to germinate for a longer period compared with those stored at 4°C. This may be because tropical recalcitrant seeds are normally sensitive to low temperature. Chilling damage often occurs at 15–20°C for most tropical plants (Sacande *et al.*, 2004). For example, when storage temperature was below 10°C, neem seeds died (Sacande 2000). However, the recalcitrant seeds of *Cordia* and *Vitex* in Kenya can tolerate the storage temperatures of 2°C (Schaefer 1991). Potential chilling damage is closely related to moisture content. The seed with high moisture content is most prone to low temperature damage, indicating that the lower the moisture content is, the less the risk of chilling injury is (Hong and Ellis, 2002). In addition, the reduction in moisture content has a dual effect, viz. to reduce metabolism and to prevent germination. However when the seeds of *H. mollissima* were dehydrated slightly to 0.62 g H_2Og^{-1} DW and 0.57 g H_2Og^{-1} DW, they became sensitive to low temperature and lost the germination ability at the early stage of storage. Thus storage at 15°C with 0.79 g H_2Og^{-1} DW moisture content was proved to be the most successful way of storage.

Based on the desiccation tolerance and the storage behaviour, we can conclude that the seeds of *H. mollissima* were typically recalcitrant. In order to preserve the germplasm, the undried seeds stored at 10°C and 15°C maintain their germination for at least a year.

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