Research paper

Germination and Storage Behavior of Seeds of *Garcinia subelliptica* (Guttiferae), *Drypetes littoralis* (Euphorbiaceae), and *Premna serratifolia* (Verbenaceae)

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【Summary】

Seed germination characteristics of 3 native coastal forest species in Taiwan were examined in this study. Effects of seed moisture contents (MCs) and storage temperatures on germination were investigated to determine their seed storage behavior. Results showed that seeds of *Drypetes littoralis* and *Premna serratifolia* exhibited no dormancy and germinated within 4 wk under fluctuating temperatures of 30/20℃ with 8 h of light. In contrast, seeds of *Garcinia subelliptica* germinated slowly, and viable seeds took 22 wk for complete germination. At MCs of 2.3~11.3% (on a fresh-weight basis), *P. serratifolia* seeds maintained their viability after 24 mo of storage at -20~15℃. The results suggest that *P. serratifolia* seeds show orthodox seed storage behavior. The larger fresh mature seeds of *G. subelliptica* and *D. littoralis* were extremely sensitive to desiccation and low temperatures. They lost viability when the MC dropped to about 30%, and seed germinability decreased rapidly at storage temperatures of < 4℃. Moreover, the maximum seed longevities of the 2 species were 28 and 60 d with 4℃ wet storage. In addition, *G. subelliptica* seeds maintained their initial germinability for 0.5 yr when stored at 15℃ with moist sphagnum. Thus, seeds of *G. subelliptica* and *D. littoralis* are defined as having tropical-recalcitrant storage behavior. Based on the above findings, we suggest that tropical-recalcitrant seeds with no dormancy should be sown immediately instead of being stored after depulping.

Key words: germination, stratification, seed storage behavior, orthodox, tropical recalcitrant.

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研究報告

福木、鐵色與臭娘子種子的發芽與儲藏性質

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摘　要

本研究探討3種台灣原生海岸林樹種種子的發芽特性，並以種子含水率與儲藏溫度對發芽率之影響來判定各種種子的儲藏行為。結果顯示鐵色(*Drypetes littoralis*)及臭娘子(*Premna serratifolia*)等種子不具休眠性，以30/20℃變溫經4週後就幾能發芽完畢，而福木(*Garcinia subelliptica*)種子則有些不易在短期內發芽，具有活力之種子可在22週內完成發芽，但發芽零散。臭娘子種子乾燥至含水率2.3~11.3%(鮮重)，經24個月的-20~15℃儲藏後種子均仍能維持活力，故判定其屬正儲型。福木與鐵色此二大型種子非常不耐乾燥及低溫，當種子含水率被乾燥至約30%時則完全喪失活力，且無法在4℃以下之低溫存活，新鮮種子在4℃濕藏時，壽命之維持分別不超過28與60日，而福木種子以水草濕藏在15℃時，半年後活力並未下降，故判斷此二種種子屬熱帶異儲型。依據上述結果，建議此類不具休眠的熱帶異儲型種子不應儲藏，洗淨後應即播。

關鍵詞：發芽、層積、種子儲藏行為、正儲型、熱帶異儲型。

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INTRODUCTION

According to the degree of desiccation tolerance of mature seeds, seed storage behavior was initially classified into 2 categories: orthodox and recalcitrant (Roberts 1973). Orthodox seeds tolerate desiccation and remain viable when the seed moisture content (MC) decreases to 5% (on a fresh-weight basis; all MC values in this study are on a fresh-weight basis). As the MC and storage temperature decrease, the longevity of orthodox seeds is prolonged, and relationships among MC, storage temperature, and longevity can be inferred from mathematical approaches (Roberts 1973).

Unlike orthodox seeds, recalcitrant seeds are particularly intolerant of desiccation. When they are dried to < 12~31% MCs, seed viability declines with concurrent prolongation of the drying duration (Roberts 1973). Furthermore, many recalcitrant seeds are extremely sensitive to temperature, and at temperatures below 10~15℃, their viability often deteriorates, especially tropical-recalcitrant seeds (Bonner 1990). Tropical-recalcitrant seeds are more intolerant of dehydration and low temperatures than are temperate-recalcitrant seeds. Thus, they are almost unable to survive a temperature of close to 0℃ and are even sensitive to 15~20℃. Low temperatures are disadvantageous to tropical-recalcitrant seed storage, even when seeds are stored at high MCs with good air circulation. Moreover, viable seeds begin to germinate shortly after storage at high temperatures. However, a low-temperature approach may inhibit germination for some tropical-recalcitrant seeds, but the optimum storage temperature is difficult to determine. Moreover, relative to the longevity of temperate-recalcitrant seeds, that of tropical-recalcitrant seeds is shorter. Take the Dipterocarpaceae for example. Among 79 species of these tropical-recalcitrant seeds, the maximum seed longevity is 1 yr, and the minimum is only 14 d (Tompsett 1987, 1989). These large, high-moisture seeds are almost all from trees such as the Dipterocarpaceae and Araucariaceae in tropical rainforests, which constitute the most economically important tropical trees e.g., *Hevea brasiliensis* Muell. Arg., *Theobroma cacao* L., *Artocarpus heterophullus* Lam., *Cocos nucifera* L., *Persea americana* Miller, *Dimocarpus longan* Lour., *Mangifera indica* L., and *Durio zibethinus* L. (Chin 1988, Chin and Roberts 1980). Hong and Ellis (1996), therefore, subdivided desiccation-intolerant seeds into temperate and tropical subtypes according to the level of temperature tolerance of recalcitrant seeds. Temperate-recalcitrant seeds are intolerant of dehydration but capable of being stored at a temperature close to 0℃ and MCs of 35~50%. Temperate-recalcitrant seeds need to have air circulation during storage; i.e., seed longevity is more likely to reach 1~3 yr after storage at high MCs with appropriate air exchange (Wang et al. 1995). Temperate-recalcitrant seeds mainly include seeds of the Fagaceae (Lin 1995) and Lauraceae in Taiwan such as *Litsea acuminata* (Bl.) kurata (Lin and Wu 1991), *Machilus* spp. (Lin and Chen 1993, Lin et al. 1993, Lin and Chien 1995), and *Keteleeria davidiana* var. *formosana* (Yang et al. 2006).

In addition to orthodox and recalcitrant seeds, Ellis et al. (1990) found that *Coffea arabica* L. seeds survived desiccation to 5~10% MCs but did not survive at a storage temperature of < 10℃. Thus, the seeds were defined as "intermediate," which are moderately tolerant of desiccation but vulnerable to freezing temperatures. Subsequently, they also found that *Elaeis guineensis* Jacq and some *Citrus* spp. seeds showed the same intermediate storage behavior (Ellis et al. 1991, Hong and Ellis 1995). According to Hong and Ellis (1996), most intermediate seeds survive desiccation to 10~12% MCs, and their viability declines with a concurrent reduction in the MC. The optimal storage temperature of intermediate seeds is mainly determined by the ecological environment of their native habitats. Therefore, some intermediate seeds can survive sub-zero temperatures, while others cannot (Hong and Ellis 1996).

Garcinia subelliptica Merr., a small dioecious evergreen tree species of the Guttiferae, is distributed in the Philippines, Taiwan, and the Ryukyus of Japan (Robson 1996). In Taiwan, it grows in coastal forests of Lanyu (Orchid Island) and Ludao (Green Island). In addition, the depressed-globose berries are about 3~5 cm in diameter, and the fruits usually produce 1~4 seeds. Mature fruits are yellow. The maturation period for *G. subelliptica* is from August to September in southern Taiwan.

Drypetes littoralis (C. B. Rob.) Merr., a small evergreen tree species of the Euphorbiaceae, grows in the Philippines and Taiwan. In Taiwan, this species is only found on the Hengchun Peninsula and Lanyu (Hseih et al. 1993). The populations are sparse, and the habitats are narrow (less than 100 km) so this species was designated a vulnerable species by the Council of Agriculture (Lu and Chiou 1998). Moreover, its ellipsoid-ovoid drupes are about 1.5 cm long, and the fruits are red when mature. The fruit maturation period is in July and August. Each drupe has a single seed which is about 1.2 cm long and 0.9 cm wide.

Premna serratifolia Linn., a small tree species of the Verbenaceae, is distributed in tropical Asia and Australia. In Taiwan, it mainly grows along coastal lowlands (Yang et al. 1998). The single-seed globose drupe is about 4~5 mm wide. Fruits are dark purple when mature, and the fruit maturation period is in August and September. The globose seeds are about 3~4 mm in diameter.

Seed germination and storage behaviors of these 3 native coastal forest species in Taiwan have not been studied. The present study is expected to provide insights into the germination and storage behaviors of these 3 species. The purpose of this study was to offer useful information for nursery operations of afforestation programs of native coastal forest species in the future.

MATERIALS AND METHODS

Seed collection and processing

Detailed information about collection of the 3 seedlots in this study is given in Table 1. First, the mature fleshy fruits of these 3 species were placed in a greenhouse for 7~10 d and kept moist to cause the fruit flesh to decompose. Once the fruits had become softer, they were immediately washed or extracted to obtain cleaned seeds with no debris left. Flotation and manual selection were used to remove empty and damaged seeds. Tables 2 and 3 respectively show detailed information on the characteristics and germination percentages of each species.

Determination of the MC, germination assay, and data analysis

Information on determining the MC, germination, and germination period is respectively given in Tables 2, 3, and 4. The MC, germination conditions, germination speed, and data analyses were determined as described by Yang et al. (2006). In addition to the germination temperatures described in Yang et al. (2006), we further used a temperature of 35℃ (8 h with light)/25℃ (16 h in the dark) for germination of *G. subelliptica* seeds.

Moist storage treatment

Table 4 presents the moist storage temperature and duration of each seedlot. Moist storage at different temperatures revealed the effects on the seed viability of these 3 seedlots. The moist storage method and conditions were the same as those described in Yang et al. (2006).

Table 1. Information on fruit collection dates and sources of the 3 species

Species	Collection date	Collection location	Latitude, longitude	Elevation (m)
Garcinia multiflora Champ.	2 Oct. 2007	Hengchuen	21°58'N, 120°48'E	285
Drypetes littoralis (C. B. Rob.) Merr.	12 Dec. 2007	Hengchuen	21°58'N, 120°48'E	245
Premna serratifolia Linn.	2 Sept. 2006	Hengchuen	21°58'N, 120°48'E	270

Table 2. Information on seed characteristics of the 3 species

Species	Determination of MCs	Moisture content (%, FW basis)	Number of seeds/L	Thousand seed weight[1)] (g)
Garcinia multiflora	1 seed×4 replicates	44.2±2.0	102±3	6,065±114
Drypetes littoralis	3 seeds×4 replicates	42.9±1.4	955±9	604±3
Premna serratifolia	10 seed×4 replicates	11.3±0.7	30,118±309	12.5±0.6

[1)] The thousand seed weight was estimated at the moisture content (mc) shown.

Table 3. Information on the germination of fresh mature seeds of the 3 species

Species	Determination of germination (seeds×replicates)	Germination period (wk)	Germination of fresh mature seeds (%)	Mean germination time of fresh mature seeds (d)
Garcinia multiflora	15×3	24	64.4±6.3	88.4±11.1
Drypetes littoralis	25×3	12	90.7±1.9	12.1±0.7
Premna serratifolia	40×4	12	79.4±8.7	18.1±0.9

Table 4. Treatment and germination assay of wet storage of the 3 species used in this study

Species	Temperature (℃)	Moist storage duration	Determination of germination (seeds×replicates)	Germination period after moist storage (wk)
Garcinia multiflora	1	3, 7, 14, 21 (d)	15×3	20
	4	7, 14, 21, 28, 35, 42 (d)	15×3	20
	15	14, 28, 56, 84, 112, 168 (d)	15×3	20
	20	28, 56, 84, 112, 140, 168, 224 (d)	15×3	20
Drypetes littoralis	4	1, 2, 4, 6, 9 (mo)	25×3	12
	15	1, 2, 4, 6, 9, 12, 18, 24 (mo)	25×3	12
Premna serratifolia	4	1, 2, 4, 6, 8, 10 (mo)	40×4	12

Storage treatment and determination of the moisture level of dehydrated seeds

Sub-seedlots exhibited different moisture levels, and the range was between approximately 2% and the MC of fresh mature seeds. When a sub-seedlot reached the desired moisture level, seeds were placed in an aluminum foil bag and stored at 25~28℃ for 3~5 d before the average equilibrium MC was determined. Once the desired MC of each sub-seedlot was achieved, seeds were sealed in double-layered foil bags to stabilize the MCs. The dry storage temperature and storage duration for each sub-seedlot are given in Table 5. The MC levels of the 3 seedlots were described and determined as follows:

1. *Garcinia subelliptica*

Seeds were either desiccated at 25±2℃ in a hermetically sealed acrylic box with a molecular sieve inside or dehydrated at 25±2℃ and a 36~38% relative humidity (RH) with an electric fan. Table 5 shows that 4 MC levels were controlled to 29.1% (19 d of desiccation with a molecular sieve), 31.7% (13 d of desiccation with an electric fan), 38.6% (8 d of desiccation with an electric fan), and 44.2% (untreated fresh mature seeds).

2. *Drypetes littoralis*

Mature *D. littoralis* seeds contained 42.9% moisture (untreated). Fresh mature seeds were placed at 25±2℃ and 36~38% RH followed by drying with an electric fan. Average MCs of the seeds dried for 8 and 14 h were 36.3 and 29.0%, respectively (Table 5).

3. *Premna serratifolia*

Mature seeds were placed in a hermetically sealed acrylic box with a small fan installed to circulate the air at 20±2℃, and molecular sieves and pure water were used to dry and moisten the seeds, respectively. The MC of fresh mature seeds was 11.3%. After seed treatment, MCs of the seeds were 2.3% (4 d of desiccation), 5.0% (1 d of desiccation), 8.0% (5.5 h of desiccation), and 14.8% (7.5 h of moisture) (Table 5).

Table 5. Dry storage assays of the 3 species. The determinations and periods of germination are the same as those shown in Table 4

Species	Moisture contents of dry storage (%, on a fresh-weight basis)	Storage temperature (℃)	Dry storage duration
Garcinia multiflora	29.1±3.1, 31.7±2.5, 38.6±0.5, 44.2±2.0	—	0
Drypetes littoralis	29.0±0.4, 36.3±1.7, 42.9±1.4	-20	0, 3 (d)
		4, 15	0, 1, 3, 6 (mo)
Premna serratifolia	2.3±0.2, 5.0±0.1, 8.0±0.3, 11.3±0.7, 14.8±0.4	-20, 4, 15	0, 3, 6, 9, 12, 18, 24 (mo)

RESULTS

Germination of fresh mature seeds

Information on the germination of fresh mature seeds of the 3 species is given in Table 3. First, after incubation at the fluctuating temperatures of 35/25℃ for 24 wk, the germination percentage and mean germination time (MGT) of fresh *G. subelliptica* seeds were 64.4% and 88.4 d, respectively. The seeds irregularly germinated during 2~22 wk, and there was no germination after 22 wk. In addition, ungerminated seeds were found to have decayed when cut open after 24 wk. Second, the germination percentage and MGT of fresh *D. littoralis* seeds were respectively 90.7% and 12.1 d after being incubated at the fluctuating temperatures of 30/20℃ for 12 wk (Table 3). Seeds mainly emerged during 1~2 wk, and all seeds had germinated within 3 wk. The germination percentage and MGT of *P. serratifolia* seeds were respectively 79.4% and 18.1 d (Table 3). Almost all seeds germinated during 2~4 wk, and there was no germination after the 5th wk.

Effects of dry and moist storage on seed longevity

1. *Garcinia subelliptica*

Figure 1 shows the effects of different levels of desiccation on the germination percentage of *G. subelliptica* seeds. The seeds were dehydrated to 4 MCs, and subsequent germination percentages of the freshly dried seeds at MCs of 44.2, 38.6, 31.7, and 29.1% were 64.4±6.3, 64.4±19.1, 8.9±3.1, and 0%, respectively. The results indicate that *G. subelliptica* seeds are extremely sensitive to

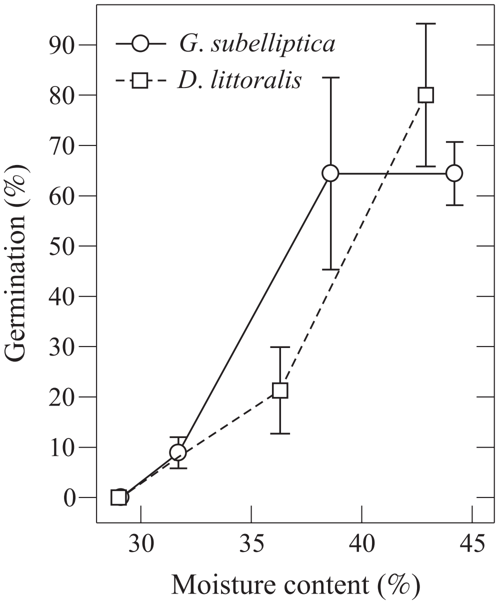

Fig. 1. Effects of moisture contents (%, on a fresh-weight basis) on the germination percentage of seeds of *Garcinia subelliptica* and *Drypetes littoralis*. Vertical bars represent the standard error of the mean.

dehydration. Moreover, seeds at an MC of about 32% almost all died, and desiccants were unable to reduce the seed MC to < 25% within a short time.

Fresh mature seeds were mixed with wet sphagnum moss and stored at 1, 4, 15, and 20℃. The effects of storage temperature on seed longevity are given in Figs. 2 and 3. After moist storage at 1℃ for 3 d, a significant decrease in the germination percentage (2.2%) was observed, and seeds had completely lost germinability after 7 d. After storage at 4℃ for 7 d, a significant decrease in the germination percentage (11.1%) was observed, and seeds had totally lost viability after 28 d. After moist storage at 15℃ for 14~168 d, an insignificant difference ($p > 0.05$) in germination percentages was found between stored seeds (48.9~68.9%) and fresh mature seeds (64.4%) (Fig. 2). After moist storage at 20℃ in the dark, seeds germinated after about 56 d. When seeds were stored for 84, 140, 168, and 224 d, there were 7, 39, 42, and 79% viable seeds which germinated in the dark, respectively (Fig. 3).

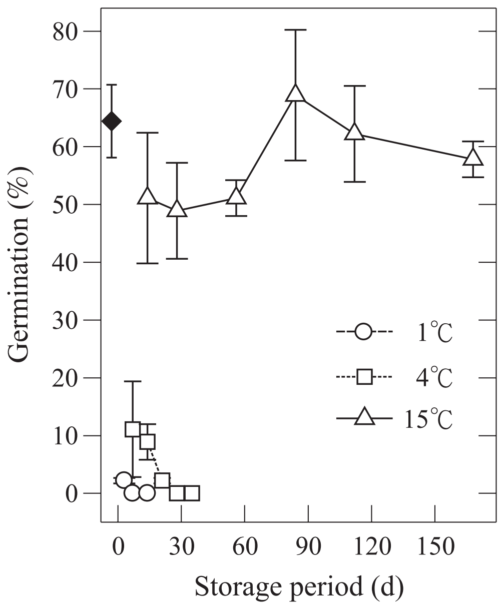

Fig. 2. Effects of 1, 4, and 15℃ storage temperatures with moist sphagnum for 0~168 d on the germination percentage of *Garcinia subelliptica* seeds. The filled diamond (◆) represents germination of fresh mature seeds. Vertical bars represent the standard error of the mean.

2. *Drypetes littoralis*

Figure 1 shows the effects of different MCs on *D. littoralis* seeds. Of seeds dehydrated to 3 MC levels, germination percentages at MCs of 42.9, 36.3, and 29.0% were 80.0±14.2, 33.3±11.5, and 0%, respectively.

Results showed that all *D. littoralis* seeds died at 3 MCs of 42.9, 36.3, and 29.0% after 3-d storage at -20℃. The germination of seeds at a 36.3% MC was 32.0±10.0% after storage at 4℃ for 1 mo, and no significant difference ($p > 0.05$) in germination was found compared to freshly dehydrated seeds (33.3±11.5%) at the same MC. All seeds of *D. littoralis* had died after 3-mo storage. Similarly, after storage at 4℃ for 1 mo, there was no significant difference ($p > 0.05$) in the germination of seeds at a 42.9% MC (96.0±3.3%) compared to freshly dehydrated seeds (80.0±11.5%). Moreover, all seeds had totally lost viability after storage for 3 mo (Fig. 4). After storage at 15℃ for 1 mo, all seeds at a 36.3% MC had died. Germination of seeds at a 42.9% MC decreased to 54.7±14.0% after 3 mo of storage, but no significant difference ($p > 0.05$) was observed compared to freshly dehydrated seeds. Moreover, they had totally lost viability after storage for 6 mo.

Effects of moist storage at 4 and 15℃ on seed germinability are respectively shown in Figs. 5 and 6. After storage at 4℃ for 1 mo, a significant decrease ($p < 0.05$) in the germination percentage (70.7±10.5%) was observed, while a significant increase in the MGT (21.4 d) was seen. Moreover, seeds suddenly lost

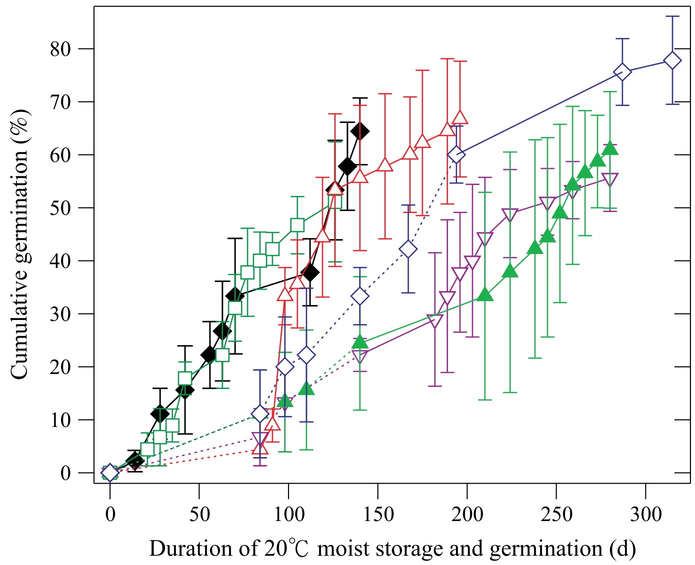

Fig. 3. Cumulative germination percentages of *Garcinia subelliptica* seeds at 20℃ moist storage for 0~224 d (□, 56 d; △, 84 d; ▽, 140 d; ▲, 168 d; ◇, 224 d). Dotted lines (---) represent the cumulative germination percentages in the dark with 20℃ wet storage, while solid lines (—) represent the cumulative germination percentages at fluctuating temperatures of 30/20℃ with 8 h of light after moist storage. Filled diamonds (◆) represent the cumulative germination of fresh mature seeds. Vertical bars represent the standard error of the mean.

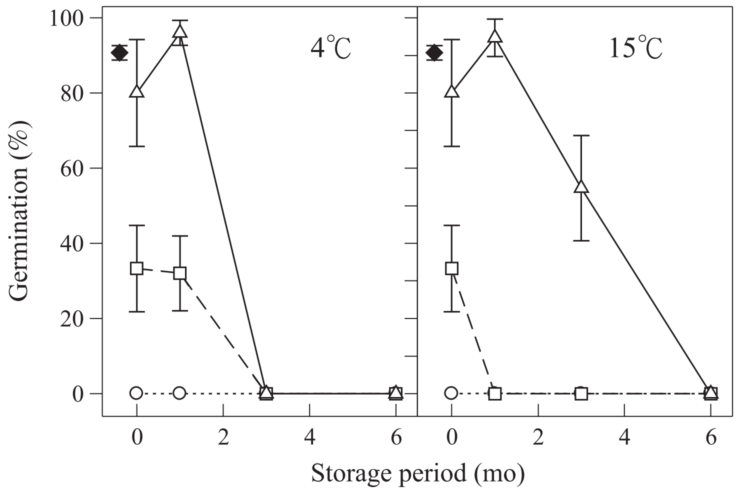

Fig. 4. Effects of storage temperatures (4 and 15℃) and moisture contents (29.0~42.9%, on a fresh-weight basis) on the germination percentage of *Drypetes littoralis* seeds stored for up to 6 mo. Survival of seeds at 3 moisture contents significantly differed ($p < 0.001$). The germination percentage of fresh mature seeds was 90.7±1.9% (◆). Moisture contents of seeds: ○, 29.0±0.4%; □, 36.3±1.7%; △, 42.9±1.4%. Vertical bars represent the standard error of the mean.

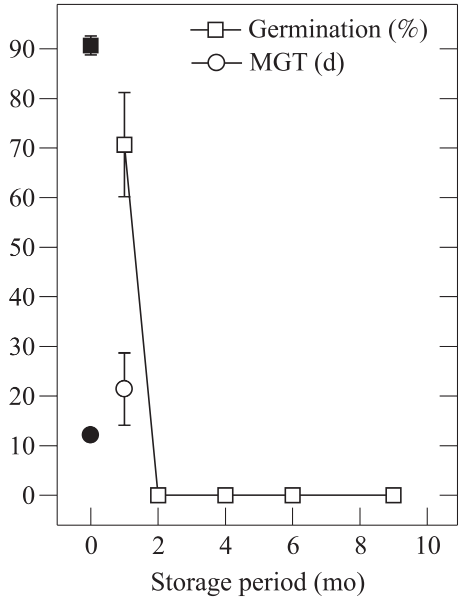

Fig. 5. Effects of 4℃ storage with moist sphagnum for 1~9 mo on the mean germination time (MGT) (○) and germination percentage (□) of *Drypetes littoralis* seeds. The filled circle (●) and square (■) represent the MGT and germination percentage of fresh mature seeds. Vertical bars represent the standard error of the mean.

viability after storage for 2 mo (Fig. 5). After moist storage at 15℃ for 1 mo, seeds germinated in the dark, and 51% germination of viable seeds was recorded. In addition, under moist dark storage conditions, all seeds had germinated within 2 mo (Fig. 6).

3. *Premna serratifolia*

Figure 7 shows the effects of different MC levels and storage temperatures on the germination of *P. serratifolia* seeds after dry storage for 0~24 mo. When seeds were dehydrated to 5 MCs, freshly dehydrated seeds germinated at fluctuating temperatures of 30/20℃ for 12 wk. Of these seeds, germination percentages were 77.5 (at a 14.8% MC), 84.1 (at an 11.3% MC), 78.8 (at an 8.0% MC), 83.2 (at a 5.0% MC), and 78.8% (at a 2.3% MC). An insignificant difference ($p > 0.05$) was observed among germination percentages at these 5 moisture levels of dried seeds and the fresh sample (79.4%).

Of these seeds at 5 moisture levels, only seeds at a 14.8% MC had totally lost viability after 3-mo storage at -20℃. The germination percentages of the other 4 lower-MC seeds with 24 mo of storage were 75.0 (at an 11.3% MC), 78.8 (at an 8.0% MC), 75.6 (at a 5.0% MC), and 83.8% (at a 2.3% MC) (Fig. 7). There was no significant difference compared to the germination percentage of the fresh sample ($p > 0.1$). However, after storage at 4℃, a significant decrease in germination was revealed in seeds at a 14.8% MC stored for 3 mo (46.2%), and they had almost all lost their viability after 12 mo. The germination percentages of the other 4 MC levels of seeds with 24 mo of storage were 82.5 (at an 11.3% MC), 76.9 (at an 8.0% MC), 75.0 (at a 5.0% MC), and 85.6% (at a 2.3% MC). There was no significant difference ($p > 0.1$) compared to the germination percentage of fresh seeds (Fig. 7). Seeds stored at 15℃ were similar to seeds stored at -20 and 4℃. Seeds with a 14.8% MC died quickly after 6-mo storage, whereas other seeds at the 4 lower moisture levels still remained viable after 24 mo (Fig. 7).

Premna serratifolia seeds displayed intolerance to moist storage at 4℃. Statistically, an insignificant difference ($p > 0.05$) was observed after storage for 1 mo, although those seeds showed a substantially decreased germination percentage (59.4±13.5%) compared to fresh seeds (79.4%). However, after storage for 2 mo, the germination percentage had decreased to 25.6±8.2%, and the seeds had almost totally lost their viability after 4 mo (Fig. 8). After 4℃ moist storage for 1 and

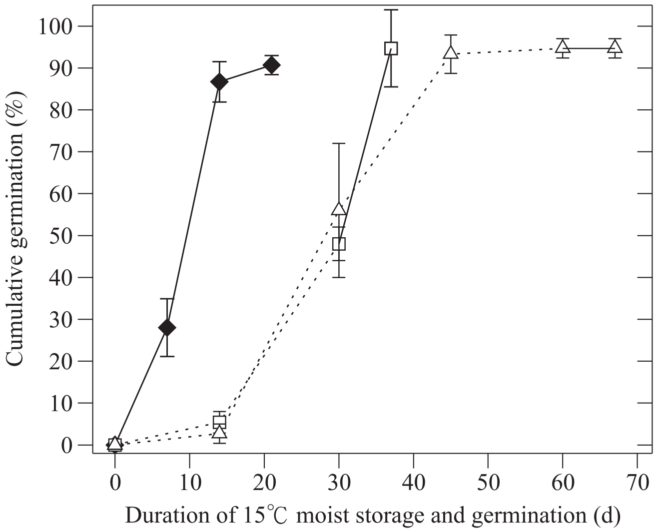

Fig. 6. Cumulative germination percentages of *Drypetes littoralis* seeds at 15℃ moist storage for 1 (□) and 2 mo (△). Dotted lines (---) and solid lines (—) respectively represent the cumulative germination percentages in the dark at 20℃ moist storage, and at fluctuating temperatures of 30/20℃ with 8 h of light after the periods of moist storage as indicated. The filled diamonds (◆) represent the cumulative germination of fresh mature seeds. Vertical bars represent the standard error of the mean.

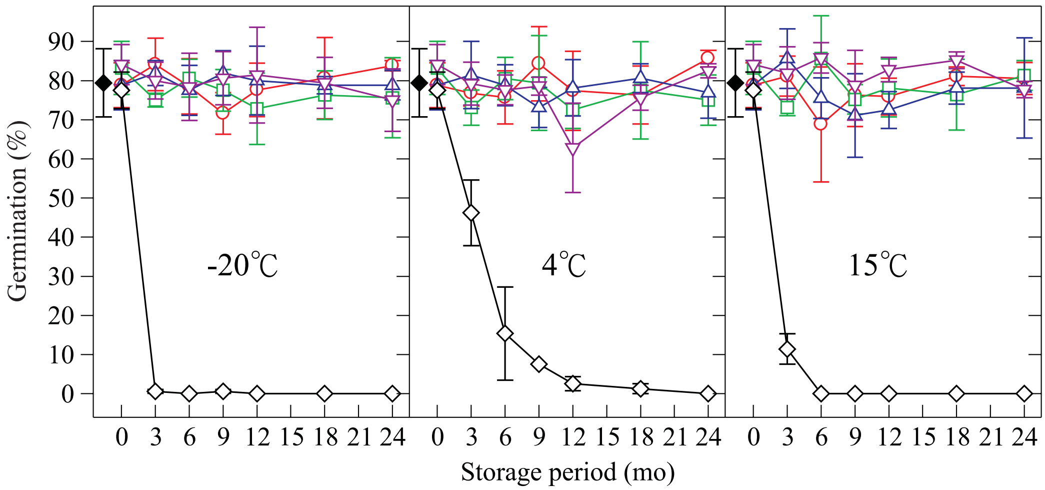

Fig. 7. Effects of storage temperatures (-20, 4, and 15℃) and moisture contents (MCs) (2.3~14.8%, on a fresh-weight basis) on the germination percentage of seeds of *Premna serratifolia* stored for up to 24 mo. Survival of seeds at the 5 MCs significantly differed ($p < 0.0001$), but no significant difference was found if the 2.3, 5.0, 8.0, and 11.3% MC groups were excluded from the analysis at all temperatures. The estimated initial germination percentage of fresh mature seeds was 79.4±8.7% (◆). MCs of seeds: ○, 2.3±0.2%; □, 5.0±0.1%; △, 8.0±0.3%; ▽, 11.3±0.7%; ◇, 14.8±0.4%. Vertical bars represent the standard error of the mean.

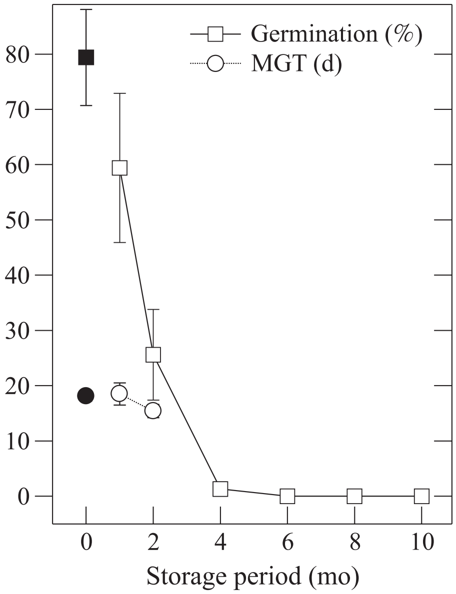

Fig. 8. Effects of 4℃ storage with moist sphagnum for 1~10 mo on the mean germination time (MGT) (○) and germination percentage (□) of seeds of *Premna serratifolia*. The filled circle (●) and square (■) represent the MGT and germination percentage of fresh mature seeds. Vertical bars represent the standard error of the mean.

2 mo, seeds of this seedlot showed an insignificant difference ($p > 0.05$) in MGTs (18.5 and 15.4 d, respectively) compared to the fresh sample (18.1 d).

DISCUSSION

Results of this study showed that *D. littoralis* and *P. serratifolia* seeds are non-dormant and display a high germination capability within 30 d, which is similar to most tree species in humid tropical areas. Viable seeds of *G. subelliptica* were able to germinate in about 20 wk when they were provided with optimal germination conditions (Fig. 3).

Garcinia subelliptica seeds were extremely intolerant to desiccation and low temperatures, and thus showed tropical-recalcitrant storage behavior. Therefore, it was impossible to apply the method of dry storage to *G. subelliptica* seeds. After moist storage at 1 and 4℃, the seeds were found to have decayed and rapidly died, while seeds remained viable for at least 0.5 yr under 15℃ moist storage (Fig. 2). Thus, moist storage at 15℃ is efficient for the short-term storage of *G. subelliptica* seeds.

Drypetes littoralis seeds rapidly deteriorated when the MC of the fresh seeds slightly decreased (Fig. 4). Therefore, *D. littoralis* seeds are tropical-recalcitrant seeds due to their extreme intolerance of dehydration and low temperatures. Thus, dry storage is not suitable for this species. In addition, *D. littoralis* seeds failed to survive a temperature of ≦ 4℃ and quickly germinated under moist storage at 15℃ (Fig. 6). Seed viability was not maintained for more than 0.5 yr when seeds were stored at 15℃ (Fig. 4). In other words, even when providing optimal storage conditions, the viability of *D. littoralis* seeds could not be maintained for 6 mo (Fig. 4). Therefore, it is impossible to use such a method to preserve the germplasm of intact *D. littoralis* seeds.

Premna serratifolia seeds dried to 2.3~11.3% MCs still maintained their viability after storage at -20~15℃ for 24 mo. Thus, *P. serratifolia* seeds are orthodox seeds based on their tolerance of desiccation and sub-zero temperatures (Hong and Ellis 1996).

Hong and Ellis (1996) collected mature seeds of 94 species, including of the Aceraceae, Araucariaceae, Dipterocarpaceae, Fagaceae, and Myrtaceae. Two parameters, the thousand seed weight and MC, were used to categorize seed storage behaviors of the species examined. MCs of recalcitrant mature seeds were 36~90%. Mature seeds with an MC of < 35% are not seen as recalcitrant seeds, and those with an MC of < 23% are considered orthodox seeds. In addition, mature seeds at MCs of 23~55% are considered intermediate seeds. A thousand seed weight of < 25 g is classified as orthodox, while a weight of > 13,000 g is seen as recalcitrant. If the weight is between 30 and 13,000 g, the seeds could be orthodox, recalcitrant, or intermediate. In this study, seeds of *G. subelliptica* and *D. littoralis* were determined to be tropical-recalcitrant seeds. MCs of mature seeds of these 2 species were respectively 44 and 43%; moreover, thousand seed weights were respectively about 6000 and 600 g (Table 2). However, *P. serratifolia* seeds were orthodox seeds; in addition, mature seeds contained 11% moisture and a thousand seed weight was only about 12 g (Table 2). Of the 3 species examined, the relations of MC and thousand seed weight to seed storage behavior were consistent with Hong and Ellis's results (1996).

Large tropical-recalcitrant seeds growing in Taiwan do not survive a low temperature of < 4℃ even if the seed moisture is maintained and good air circulation is provided, e.g., *Litsea garciae* Vidal, *Diospyros philippensis* (Desr.) Gurke, *Myristica ceylanica* A. DC. var. *cagayanensis* (Merr.) J. Sinclair, and so on (Yang et al. 2008). In this study, seeds of *G. subelliptica* and *D. littoralis* were also included. The lowest temperature tolerated by *G. subelliptica* and *D. littoralis* seeds was 10~15℃, and moist storage at higher temperatures of 15~20℃ induced these tropical-recalcitrant seeds to rapidly germinate when the seed moisture was maintained at that temperature. Thus, long-term storage of intact seeds is not effective in these species. However, *G. subelliptica* seeds of this study still maintained their viability for at least 0.5 yr after moist storage at 15℃ with good air circulation. In this view, dormancy in *G. subelliptica* seeds contributes to the control and maintenance of seed longevity for storage. In short, when tropical-recalcitrant seeds show no dormancy or their storage behavior is unknown, they should be immediately cleaned and sown after collection if the goal is just to produce seedlings. If there is no alternative but to store the seeds for a short period, mix up loose, coarse sphagnum moss as a medium and furthermore offer oxygen for moist storage at about 15~20℃ to maintain their viability. However, this method is not without its flaws. Some seeds still germinated in a few days, and for some tropical-recalcitrant seeds sensitive to low temperature, this method could risk destroying seed viability.

Plants which bear tropical-recalcitrant seeds usually grow in high-temperature, humid environments. Moreover, it is the sensitivity to desiccation and their short longevity that limit their distribution and rate of successful colonization. Therefore, these plants with large seeds intolerant to desiccation are mainly distributed in humid tropical forests. Still, non-pioneer evergreen rainforests are generally composed of taxa with recalcitrant seeds in tropical areas (Tweddle et al. 2003). Large tropical-recalcitrant seeds are usually non-dormant, so they immediately germinate after ripening with high vigor during the fall season. Therefore, with limited photosynthetic ability, the growth of unreleased seedlings is hampered because the only nutrition they can absorb is from the seed itself. Tropical species possessing recalcitrant seeds are hence classified as climax species which are sustained in forests in the form of seedlings. Take recalcitrant seeds in the tropical forests of Panama in Central America as an example. Their seed size is clearly large, and the average weight of each seed is about 12.5 times as heavy as orthodox seeds. Moreover, ripe recalcitrant seeds rapidly spread in the rainy season and promptly germinate to avoid destruction by other organisms. Clearly, this characteristic helps tropical-recalcitrant seeds reduce the risk of desiccation damage (Daws et al. 2005). Seeds of *G. subelliptica* and *D. littoralis* of this study are tropical-recalcitrant seeds, and their characteristics are similar to those of seeds of *Diospyros philippensis*, *Litsea garciae*, and *Myristica ceylanica* var. *cagayanensis* (Yang et al. 2008). The above seeds possess these survival strategies. Although *P. serratifolia* seeds of this study are orthodox seeds which can tolerate desiccation, they still adopt the survival strategy of rapid germination in humid environments after ripening. However, when *P. serratifolia* seeds were moistened, they only maintained their viability for 4 mo at a low temperature (Fig. 6). The results revealed that they are not like temperate seeds which are able to maintain viability for at least 2~3 yr in low-temperature environments, and they are not like tropical seeds which readily die in a few days after storage at a low temperature. In fact, their germination and desiccation tolerance are very similar to seeds of *Scolopia oldhamii* Hance (Yang et al. 2008). Obviously, such seed characteristics of these species have gradually evolved from the humid subtropical surroundings in which they grow.

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