

Research paper

Germination and Storage Behavior of Seeds of *Litsea coreana* Levl.

Jeng-Chuann Yang,^{1,3)} Shing-Rong Kuo,²⁾ Chiung-Mei Lee¹⁾

[Summary]

The objective of this study was to understand the germination characteristics of *Litsea coreana* seeds and to determine the seed storage behavior to suggest the most appropriate treatment for seed storage. Fresh samples from seedlot 1 reached a germination percentage of 6.3%, while no germination was observed in seedlot 2 after incubation under alternating temperatures of 30/20°C (day/night) for 12 wk. However, 6 and 7 mo of 4°C stratification broke the seed dormancy, leading to 63.4 and 81.9% germination rates for seedlots 1 and 2, respectively. Emergence was able to occur within 4 wk with mean germination times of 15.0 and 18.2 d, respectively. Moreover, a combined 2-mo warm (30/20°C) and 2-mo cold (4°C) stratification was another effective pretreatment for dormancy breaking, with the germination percentages of these 2 seedlots reaching 82.5 and 78.6%, while the germination period was decreased to 3 wk and the mean germination times were 13.8 and 21.9 d, respectively. According to our results, most seeds of *L. coreana* can survive desiccation to 6~14% moisture contents, whereas they were extremely sensitive to -20°C as evidenced by the fact that the viability of the seeds at 2~25% moisture contents were totally lost within 4 h. At a moisture content of about 6%, *L. coreana* seeds were most capable of remaining viable when stored at 1, 4, and 15°C; in comparison, the viability of those at moisture contents of < 3% or > 10% substantially declined at the same temperatures. Furthermore, seed viability was best maintained at a temperature of 4°C rather than 1 or 15°C. Therefore, *L. coreana* seeds were considered to express an intermediate storage behavior due to their moderate tolerance of desiccation and sensitivity to freezing temperatures. Additionally, the optimal approach for *L. coreana* seeds is moist storage at 4°C, which enabled 65% of initially germinable seeds to remain viable after 2 yr.

Key words: *Litsea coreana*, germination, stratification, seed storage behavior, intermediate seeds.

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¹⁾ Division of Silviculture, Taiwan Forestry Research Institute, 53 Nanhai Rd., Taipei 10066, Taiwan. 林業試驗所育林組，10066台北市南海路53號。

²⁾ Department of Forestry and Resource Conservation, National Taiwan University, 1 Roosevelt Rd., Sec. 4, Taipei 10617, Taiwan. 國立台灣大學森林環境暨資源學系，10617台北市羅斯福路四段1號。

³⁾ Corresponding author, e-mail: yjc@tfri.gov.tw 通訊作者。

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研究報告

鹿皮斑木薑子種子的發芽與儲藏性質

楊正釗^{1,3)} 郭幸榮²⁾ 李瓊美¹⁾

摘要

本研究目的在明瞭鹿皮斑木薑子種子的發芽特性與探討其儲藏行為，並決定其最適當的儲藏方法。供試的2批鹿皮斑木薑子新鮮種子以30/20°C(day/night)變溫經12週後之發芽率為6.3與0%，然經4°C層積6與7個月後可完全解除其深度休眠，使此2批種子的發芽率分別提升至63.4與81.9%，且可使種子集中在4週內發芽，平均發芽日數分別為15.0與18.2日。另2+2組合層積(先經2個月的30/20°C(day/night)暖溫層積再轉入4°C層積2個月)的發芽前處理亦能完全解除其休眠，使此2批種子的發芽率分別提升至82.5與78.6%，且可使種子集中在3週內發芽，平均發芽日數分別為13.8與21.9日。鹿皮斑木薑子種子能耐乾旱，當含水率被降至6~14%後，大部分種子仍能存活，然種子非常不耐-20°C環境，含水率2~25%之種子在儲藏4小時內就全部死亡；當儲藏在1、4與15°C時，均以含水率約6%的種子最能維持活力，含水率低至3%或高於10%之種子在短期內活力就會顯著下降。乾藏種子之最適溫度以4°C明顯優於1與15°C。因此，以其能忍受乾燥但卻對零下低溫敏感之特性，判斷屬中間型種子。儲存鹿皮斑木薑子種子之最佳條件為4°C濕藏，2年後約仍有65%之種子具有發芽能力。

關鍵詞：鹿皮斑木薑子、發芽、層積、種子儲藏行為、中間型種子。

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INTRODUCTION

Seed storage behavior can be classified into 3 main categories based on the degree of desiccation tolerance of mature seeds: orthodox, recalcitrant, and intermediate (Roberts 1973, Hong and Ellis 1996). Among them, orthodox seeds are most capable of tolerating dehydration, as they can maintain viability at moisture contents (MCs) of < 5% (on a fresh-weight basis; all MC values mentioned in this study are on a fresh-weight basis), and relationships derived from mathematical approaches indicate that seed longevity is prolonged with reductions in MC and storage temperature (Roberts 1973). In contrast to orthodox seeds, recalcitrant seeds are particularly incapable of withstanding desiccation, and the viability of seeds at 12~31% MCs decline as the drying process continues.

Moreover, tropical recalcitrant seeds are sensitive to low-temperature environments, and their viability tends to deteriorate under subsequent storage at 10~15°C (Bonner 1990). Regarding the level of temperature sensitivity in recalcitrant seeds, Hong and Ellis (1996) differentiated desiccation-intolerant seeds into temperate and tropical sub-types. Moreover, Ellis et al. (1990a) found that *Coffea arabica* L. seeds could be desiccated to 5~10% MCs without losing viability; however, the seeds failed to remain viable when stored at ≤ 10°C, so they specified such seeds as “intermediate”, which are considered to be moderately tolerant to desiccation but vulnerable to freezing temperatures. Subsequently, they found that *Elaeis guineensis* Jacq. seeds and some *Citrus* spp. seeds exhibit the similar storage

behavior; such findings confirmed the identification of intermediate seeds. According to Hong and Ellis (1996), most intermediate seeds can survive desiccation to 10~12% MCs, but their viability declines with a reduction in water content. Furthermore, whether or not intermediate seeds are tolerant of sub-zero temperatures depends on the climatic conditions within their natural distribution range.

Litsea coreana Levl., a small evergreen tree species of the Lauraceae, is distributed in Japan including the Ryukyus, and southern Korea, and is scattered over the lowlands of Taiwan (Liao 1996). Male individuals comprise a greater proportion within the wild population of this dioecious species. Only females growing in adequate sunlight are able to yield a considerable quantity of fruit. Adult individuals normally produce good seed crops at intervals of 2~3 yr. In northern Taiwan, the harvest time of fleshy fruit of *L. coreana* is around late July, when the 1-seed drupes gradually turn bright red when ripe. There

is no previous study on *L. coreana* seeds, so the objectives of this paper were to determine effective dormancy-breaking treatments and investigate the storage behavior of *L. coreana* seeds. Furthermore, the results can provide several practical techniques for nursery work and contribute some efficient processes for seed storage and advanced utilization of *L. coreana*.

MATERIALS AND METHODS

Seed collection and preparation

Detailed collection information and seed characteristics of the 2 seedlots are given in Table 1. About 15% of the fruit appeared bright red when harvested. Most green-colored fruit turned red when kept moist during a 10-d after-ripening period in a greenhouse. Seeds were extracted and cleaned as soon as the pulp had rotted. No floating seeds were found after soaking in water, and the physiological quality of seeds of the 2 seedlots were fine. Then, depulped cleaned seeds (Fig. 1)

Table 1. Information on the location, collection, seed characteristics, and germination of the 2 seedlots studied

Seedlot no.	Seedlot 1	Seedlot 2
Location	Datunshan, Taipei ¹⁾	
Latitude, Longitude	25°11'N, 121°30'E	
Elevation (m)	675	
Collection date	22 June 1998	13 July 2001
Moisture content (% FW basis)	26.8±2.1	25.8±0.7
Number of seeds /L	5340±82	4860±64
TSW ²⁾ (g)	107.4±2.4	115.3±2.2
Germination of mature seeds ³⁾ (%)	6.3±4.1	0
Initial viability percentage ⁴⁾ (%)	82.5±9.0	78.6±4.4
Initial mean germination time ⁴⁾ (d)	13.8±1.1	16.0±1.0

¹⁾ Seedlot 1 and 2 were collected from the same individual.

²⁾ TSW (thousand-seed weight) was estimated at the moisture content shown.

³⁾ Seeds were incubated at alternating temperatures of 30/20°C (day/night) and 8 h of light for 12 wk.

⁴⁾ The initial viability percentage and the mean germination time were obtained after the pretreatment of a combined 2-mo warm (30/20°C) and 2-mo 4°C stratification.

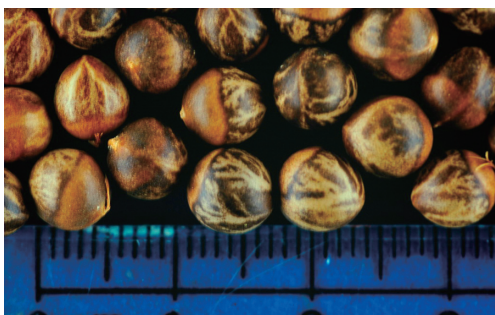


Fig. 1. Seeds of *Litsea coreana*.

were air-dried for 2 h by electric fans, and manually selected to eliminate deficient seeds with a disproportionately small size or a damaged shell, followed immediately by germination tests and determination of MCs.

Determination of MC

The MCs of seeds were determined gravimetrically with 4 replicates using the low-constant-temperature oven method ($103 \pm 2^\circ\text{C}$ for 17 ± 1 h) (ISTA 1999). All MCs are presented on a percentage fresh-weight basis. For each replicate, 5 seeds at 0.5–0.6 g each were randomly selected and cut into pieces of < 4 mm in length before dehydration. Initial MCs of mature fresh seeds of the 2 seedlots are shown in Table 1.

Moist storage treatment

Freshly collected seeds of the 2 seedlots

were thoroughly mixed with wet sphagnum moss in a polyethylene (PE) bag (14×10 cm, and 0.04 mm thick) and then placed at 4°C for storage, which was the same procedure used for stratification. The extra water of the moss was removed until a water content of about 390% by mass (on a dry-weight basis) was achieved. Seeds of seedlots 1 and 2 were stored for up to 12 mo for 6 different periods and 24 mo for 16 periods (Table 2), and seeds were germinated in an incubator at temperatures of $30/20^\circ\text{C}$ (day/night) with 8 h of light ($50\text{--}80 \mu\text{E s}^{-1} \text{m}^{-2}$).

Combined stratification

A series of combined stratifications for different periods was applied to seedlot 2 for dormancy breaking. The first step was to carry out warm stratification in which seeds were mixed with moist sphagnum moss and placed at alternating day/night temperatures of $30/20^\circ\text{C}$ (8 h with light) for 2–6 mo, followed by cold stratification at 4°C for 2–6 mo (Table 3). Thereafter, the seeds were transferred to temperatures within the range of $30/20^\circ\text{C}$ (day/night) for a germination test.

Storage treatment and determination of the moisture level after desiccation

To obtain seeds with different MC levels

Table 2. Information on seed storage conditions and durations of wet and dry storage for the 2 seedlots

	Moisture contents (%, fresh-weight basis)	Storage temperature ($^\circ\text{C}$)	Storage duration
Seedlot 1	Wet storage 26.4–32.5	4	2, 4, 6, 8, 10, 12 mo
	Dry storage 2.9 ± 0.3 , 13.0 ± 0.5 , 26.9 ± 1.3	-20, 4, 15	0, 1, 3, 6, 12 mo
Seedlot 2	Wet storage 28.6–33.8	4	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, 24 mo
	Dry storage 2.6 ± 0.4 , 6.0 ± 0.2 , 10.1 ± 0.4 , 14.0 ± 0.4 , 24.7 ± 0.8	-20	0, 2, 4, 6, 8, 12, 24, 48, 72 h
		1	0, 0.1, 1, 2, 3, 6, 9, 12 mo
		4	0, 1, 2, 4, 6, 8, 10, 12, 18, 24 mo
		15	0, 1, 2, 4, 6, 8, 10, 12, 18 mo

Table 3. Effects of 25 different periods of combined stratification on germination (%) of seedlot 2. Seeds were incubated at alternating day/night temperatures of 30/20°C with 8 h of light for 12 wk. The germination percentages of 25 pretreated combinations did not show significant differences ($p < 0.05$) as determined by Tukey's test

4°C prechilling (mo)	20/30°C warm stratification (mo)				
	2	3	4	5 ¹⁾	6 ¹⁾
2	78.6±4.4	79.4±2.7	82.9±5.3	87.5±5.9	85.7±11.2
3	80.6±2.9	81.3±3.8	79.3±6.8	88.1±6.5	88.6±9.3
4	86.4±7.7	79.4±5.7	81.4±4.3	80.0±9.2	91.4±2.3
5	92.1±4.2	93.1±3.7	81.9±2.7	86.3±1.3	65.7±12.6
6	80.7±4.2	81.9±5.4	90.0±3.2	70.6±11.9	74.3±8.3

¹⁾ Germination occurred after 5 mo of warm stratification.

between about 2% and the initial MC value of mature fresh seeds, the selected seeds from seedlots 1 and 2 were respectively divided into 3 and 5 sub-lots. Seeds of each sub-lot were desiccated with a relative humidity of about 3~5% in a hermetically sealed acrylic box containing silica gel at 15°C. A small fan was installed in the box to circulate the air. After dehydration for different periods, seeds were immediately wrapped in an aluminium foil bag and stored at 4°C for 5 d to allow the moisture to equilibrate among the seeds. The seeds were sealed in double-layered foil bags and stored separately at different temperatures as soon as the moisture contents of each sub-lot were established. Finally the 3 MC levels obtained for seedlot 1 were 2.9 (5 d of desiccation), 13.0 (3 h of desiccation), and 26.9% (untreated) while the 5 MC levels for seedlot 2 were 2.6 (7 d of desiccation), 6.0 (1 d of desiccation), 10.1 (3 h of desiccation), 14.0 (2 h of desiccation), and 24.7% (untreated). Changes in seed moisture contents of seedlots 1 and 2 during storage at different temperatures are shown in Fig. 2. A factorial design was used to interpret the responses of *L. coreana* seed survival to the MC, temperature, and storage period. Thus, 3 or 4 temperatures (-20, 1, 4, and 15°C) were combined with 3 or 5 MCs (from 2.6 to 26.9%), and with storage

periods of 0 to 24 mo (Table 2).

Germination assay

Before the germination test, to avoid imbibition damage by rapid rehydration (Ellis et al. 1990b), seeds with different treatments were placed above water in a sealed container for 1 d to allow the uptake of water at ambient temperature. The imbibed seeds were then thoroughly mixed with clean sphagnum moss in sealable PE bags (14×10 cm, and 0.04 mm thick) with adequate air inside. The excess water of the moss was squeezed out until a water content of about 450% by mass (on a dry-weight basis) was reached (Lin and Chen 1993). To estimate the germination of mature fresh seeds and dry-stored seeds, 20 seeds from seedlot 1 and 50 from seedlot 2 were randomly sampled in 4 replicates per treatment for the germination test at alternating temperatures of 30/20°C (day/night) with 8 h of light (50~80 $\mu\text{E s}^{-1} \text{m}^{-2}$). During the 12-wk test period, the sum of the protruding seeds was counted once per week with about 3 ml of water added to the moss at the same time. Seeds with a radicle reaching 5 mm were counted as having germinated.

Data analysis

The equation for calculating the mean

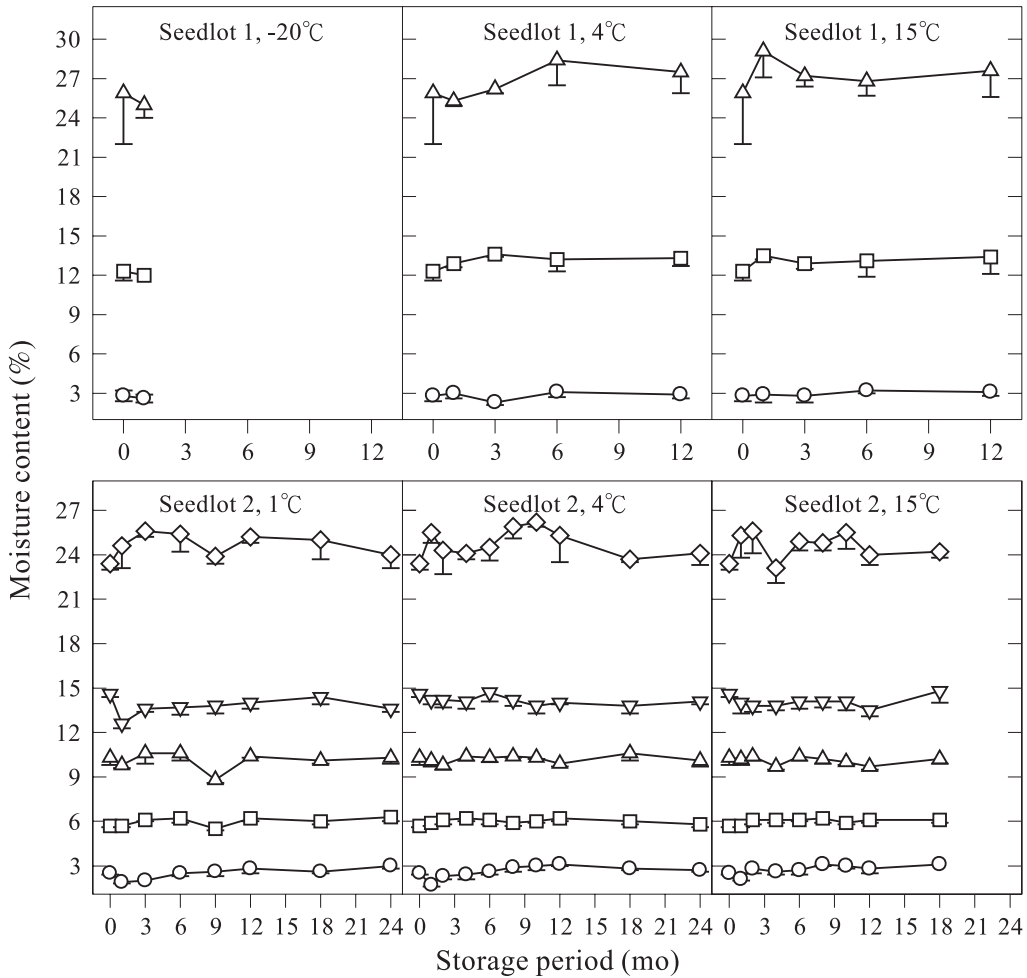


Fig. 2. Changes in moisture contents of seedlot 1 (○, $2.9 \pm 0.3\%$; □, $13.0 \pm 0.5\%$; △, $26.9 \pm 1.3\%$) and seedlot 2 (○, $2.6 \pm 0.4\%$; □, $6.0 \pm 0.2\%$; △, $10.1 \pm 0.4\%$; ▽, $14.0 \pm 0.4\%$; ◇, $24.7 \pm 0.8\%$) during storage at different temperatures. Vertical bars represent the mean \pm standard error.

germination time (MGT) was: $MGT = \Sigma (n \times d) / N$, where n is the number of germinated seeds after each period of incubation in days (d), and N is the total number of emerged seeds recorded by the end of the test (Hartmann et al. 1989).

Analysis of variance (ANOVA) was used for the seed germination percentages and MGT to evaluate the effects of the stratification period on germination. Additionally, the germination results at different storage

temperatures, seed MCs, and storage periods were statistically analyzed by variables implemented in Tukey’s test of the PROC GLM procedure of the SAS statistical package (Enterprise Guide 4.1, SAS Institute, Cary, NC, USA).

RESULTS

Germination of mature fresh seeds

The acquired germination percentages of

mature fresh seeds of seedlots 1 and 2 were only 6.3 and 0%, respectively, after a 12-wk germination period; nevertheless, all ungerminated seeds appeared healthy and firm when we cut them open, hence we considered the seeds to still be viable but dormant. Furthermore, a combined 2-mo warm and 2-mo cold stratification was sufficient to completely overcome dormancy. The germination percentages increased to 82.5 and 78.6%, respectively, representing initial viabilities for seedlots 1 and 2 (Table 1).

Effects of moist storage on germination

A dormancy-breaking effect in 4°C wet storage as the prechilling temperature was revealed at which seeds of seedlot 1 exhibited a significant increase in germinability from 10.8 to 63.4% over 2~6 mo (Fig. 3). Insignifi-

cant differences in germination (62.0~63.4%) were exhibited during the following 6~10 mo; however, germination was significantly promoted to 80.2% ($p < 0.05$) after 12 mo. Moreover, the MGT declined from 42.0 to 15.0 d within 2~6 mo, but no further differences were found in the MGT (11.7~19.6 d) during 6~12 mo of storage.

Within 7 mo of 4°C moist storage, the germination percentage of seedlot 2 considerably increased with extension of the storage duration. It grew from 0.6 to 81.9% during 1~7 mo of storage while the seed germinability changed insignificantly over 8~21 mo (with the germination percentage fluctuating between 72.5 and 90.6%); however, a reduction to 54.4% in germination occurred after 24 mo of storage ($p < 0.05$) with 33% of initial vital seeds having deteriorated (Fig. 3). The MGT

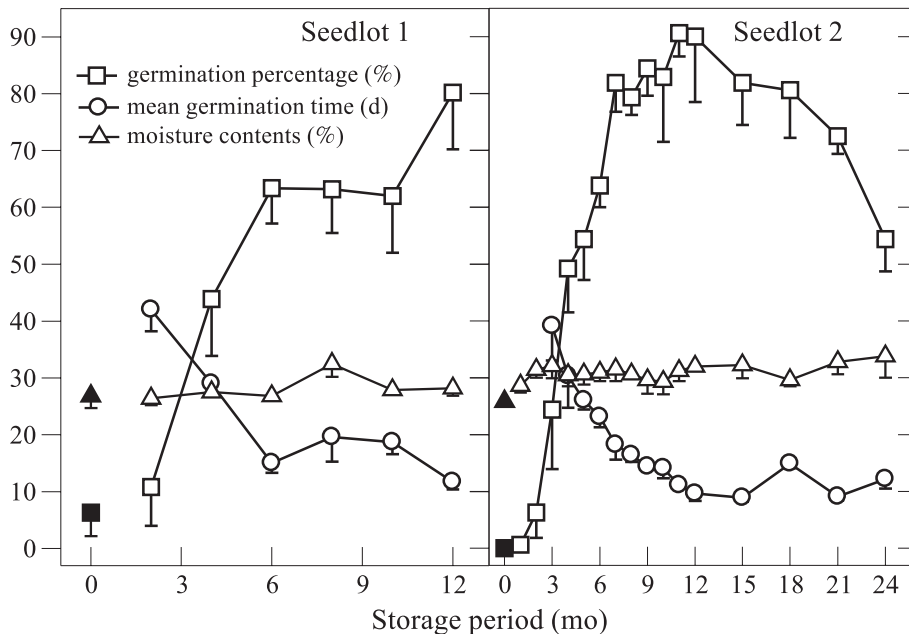


Fig. 3. Effects of 4°C stratification for 2~12 and 1~24 mo on the mean germination time (○), germination percentage (□), and changes in seed moisture content (△) of the 2 seedlots studied. Filled circles (●), squares (■), and triangles (▲) represent the mean germination time, germination percentage, and seed moisture content of mature fresh seeds, respectively. Vertical bars represent the mean ± standard error.

of seedlot 2 declined with an increase in the duration of 4°C wet storage; it lapsed from 39.1 to 18.2% as the storage period increased from 3 to 7 mo. However, these seeds showed insignificant differences in MGTs over the following 8–24 mo (8.9–16.4 d) (Fig. 3).

To sum up, these findings demonstrate that moist storage preceded by prechilling was sufficient to promote seed germination; nonetheless, *L. coreana* seeds still require 7 mo of wet storage at 4°C to break dormancy.

Effects of combined stratification on germination

The germination percentage and MGT of seeds with the 2+2 combined stratification pretreatment (a combined 2-mo warm (30/20°C) and 2-mo cold (4°C) stratification), were 78.6% and 21.9 d, respectively (Tables 3, 4), and about 82% of the initially viable seeds germinated within only 3 wk. When the 2+3 combined stratification was applied, the derived germination percentage and MGT were 80.6% and 16.7 d, respectively (Tables 3, 4), and 92% of the initially viable seeds germinated within 3 wk. Seeds without protrusion were found to have decayed internally after 12 wk of the test period. None of the 25 combination treatments showed significant differences in germination using Tukey's test; however, a trend of germination reduc-

tion was observed in the 5+6, 6+5, and 6+6 combination treatments (Table 3). In addition, the MGTs gradually declined with the accumulation of combined stratification time and even dropped to < 10 d for stratification periods exceeding 9 mo (Table 4). Based on this information, we assumed that the 2+2 combined stratification was suitable for *L. coreana* seeds to overcome their dormancy and adequately stimulate germination as well.

Effects of storage temperature, seed MC, and storage period on germination

The effects of different MCs and storage temperatures on the germination percentages for seedlot 1 during 12 mo of storage are shown in Fig. 4. After the drying process, seeds at 3 levels of MCs, of 2.9, 13.0, and 26.9%, were pretreated with the 2+2 combined stratification, and the acquired germination percentages were 76.3, 81.3, and 82.5%. Additionally, these values were not found to differ from the initial viability rate of 78.6% ($p > 0.05$). Seeds at 2.9–26.9% MCs totally lost their viability within 1 mo at -20°C, which implies an extreme intolerance of *L. coreana* seeds to freezing temperatures. When stored at 4°C, seeds at a 2.9% MC did not remain viable, and the germination percentage dropped to 20.8% after 6 mo of storage, whereas no further loss in viability was detected after

Table 4. Effects of different periods of combined stratification on the mean germination time (d) of seedlot 2. Seeds were incubated at alternating temperatures of 30/20°C (day/night) with 8 h of light for 12 wk

4°C prechilling (mo)	20/30°C warm stratification (mo)				
	2	3	4	5	6
2	21.9±3.8 ^{a1}	16.7±1.3 ^b	14.8±0.5 ^{ab}	13.6±0.3 ^{cd}	11.0±1.1 ^{de}
3	16.7±0.5 ^b	15.5±1.0 ^{ab}	14.2±0.3 ^{ab}	10.8±0.9 ^{de}	7.9±0.9 ^{ef}
4	15.2±0.6 ^{ab}	15.6±0.8 ^{ab}	10.4±0.6 ^{de}	7.6±0.4 ^{ef}	9.1±0.4 ^{ef}
5	15.5±0.7 ^{ab}	12.9±0.4 ^{cd}	7.7±0.3 ^{ef}	7.6±0.4 ^{ef}	7.1±0.1 ^f
6	13.0±0.5 ^{cd}	9.1±0.9 ^{ef}	7.5±0.3 ^{ef}	7.5±0.8 ^{ef}	7.1±0.2 ^f

¹⁾ Numbers by the same letter do not significantly differ ($p < 0.05$) as determined by Tukey's test.

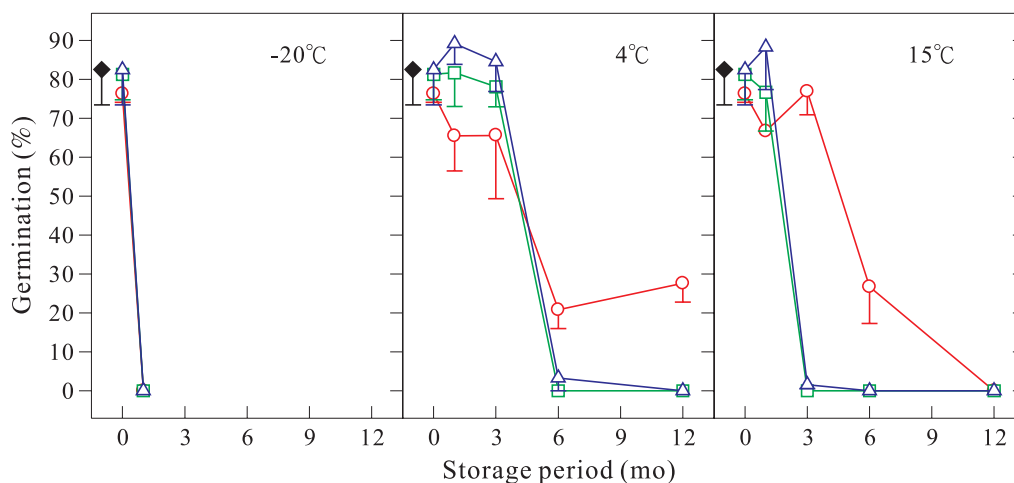


Fig. 4. Effects of storage temperature (-20, 4, and 15°C) and moisture content (○, 2.9 ± 0.3%; □, 13.0 ± 0.5%; and △, 26.9 ± 1.3%) on the germination percentage of seedlot 1. The initial viability percentage of mature fresh seeds (◆) was 82.5%. Vertical bars represent the mean ± standard error.

subsequent storage for 12 mo. At 13.0 and 26.9% MCs, no loss in seed viability was observed during the first 3 mo, but the loss was complete within 6 mo of storage. With 15°C storage, seeds at a MC of 2.9% had a tendency to lose viability after 3 mo, and they had completely lost it within 12 mo. Moreover, seeds at 13.0 and 26.9% MCs appeared to maintain their viability only within the first month but exhibited a nearly complete loss of germination after 3 mo of storage (Fig. 4).

Similar to the results of seedlot 1, seedlot 2 also clearly exhibited different germination outcomes for the various MCs and storage temperatures within 24 mo of storage (Fig. 5). After pretreatment with 2+2 stratification, the germination percentages were 33.3, 83.3, 84.4, 81.1, and 86.7% for seeds dried to 2.6, 6.0, 10.1, 14.0, and 24.7% MCs, respectively. In a comparison with the initial viability percentage of 83.5%, only seeds at a 2.6% MC presented a significant reduction in viability ($p < 0.0001$), while the others did not ($p > 0.1$). Since all seeds with 5 MCs entirely lost their germinability within 4 h of storage at -20°C

(data not shown), these seeds were determined to be extremely intolerant of a freezing environment. When stored at 1°C, the seeds at 2.6% MC showed a rapid decrease in germination and lost all viability after 3 mo of storage; the germination percentage of seeds significantly decreased to 43.9% after 12 mo of storage at a 6.0% MC ($p < 0.001$). Seeds at 10.1% MC exhibited an even greater reduction in viability, completely losing germinability after 12 mo of storage. Moreover, the seed viability diminished even more rapidly and significantly at 14.0 and 24.7% MCs, as no germination occurred in these seeds after 9 mo of storage (Fig. 5). At a storage temperature of 4°C, a rapid loss of germination percentage to 3.8% took place during 2 mo of storage at a 2.6% MC, and the complete loss was observed after 8 mo of storage; seeds at a 6.0% MC revealed insignificant differences in germination during 24 mo of storage ($p > 0.05$). Seeds lost significant germinability after 6 mo of storage (55.1% germination) at a 10.1% MC and showed a total lapse in germination within 12 mo. Moreover, 8 mo of

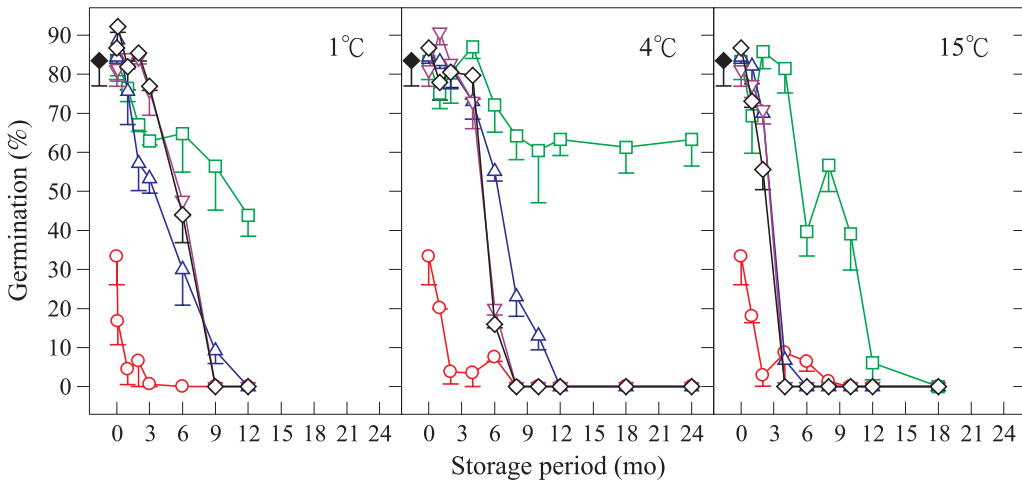


Fig. 5. Effects of storage temperature (1, 4, and 15°C) and moisture content (MC) (○, $2.6 \pm 0.4\%$; □, $6.0 \pm 0.2\%$; △, $10.1 \pm 0.4\%$; ▽, $14.0 \pm 0.4\%$; and ◇, $24.7 \pm 0.8\%$) on the germination percentage of seedlot 2. The germination percentages of seeds at the 5 MCs showed a significant difference ($p < 0.001$) at each temperature. The initial viability percentage of mature fresh seeds (◆) was 83.5%. Vertical bars represent the mean \pm standard error.

storage terminated seed viability at 14.0 and 24.7% MCs (Fig. 5). When seeds were stored at 15°C, the germination percentage of those at a 2.6% MC sharply dropped to 2.9% after 2 mo of storage; seed germination percentages remained below 10% and the ability was entirely lost after 10 mo. Seeds at 10.1, 14.0, and 24.7% MCs lost a significant proportion of their germinability and completely deteriorated after 6, 4, and 4 mo, respectively (Fig. 5), while the germination of seeds at a 6.0% MC declined to 39.7% with 6 mo of storage and totally died after 18 mo.

DISCUSSION

Due to the fact that almost no germination was observed in mature seeds of *L. coreana* after 12 wk, and even 1~6 mo of prechilling failed to completely initiate germination, the seeds are considered to exhibit dormancy. We suggest 2 pretreatments for dormancy breaking: (1) a 2+2 combined strat-

ification or (2) 4°C stratification for at least 7 mo; however, a considerable loss in seed viability can occur if stored at 4°C stratification for over 21 mo (Fig. 3).

Seeds of *L. coreana* exhibited no loss in viability within the range of 6~14% MCs; but a temperature of -20°C killed all seeds. Based on these results, we consider *L. coreana* to be intermediate seeds, characterized as being tolerant to desiccation but sensitive to freezing (Hong and Ellis 1996). Furthermore, feasible storage conditions for *L. coreana* seeds are a combination of a 6% MC with a temperature of 4°C (Figs. 4, 5).

Most intermediate seeds can survive desiccation within a moisture range of 10~12%, but the germinability tends to decrease with a reduction in MC (Hong and Ellis 1996). Furthermore, the optimal water content for our investigated species, *L. coreana* seeds, was obviously below the range as previously described, and such intermediate seeds with better desiccation tolerance include *Coffea*

canephora Pierre ex A. Froehner, *C. congensis* Froehner, *C. racemosa* Lour. (Dussert et al. 1999, Eira et al. 1999), *Citrus limon* (L.) Burm. f. (Hong et al. 2001), *Khaya senegalensis* (Desr.) A. Juss., *Swietenia macrophylla* King (Hong and Ellis 1998), *Fagus crenata* Blume (Pedro and Ellis 2002), *Acer morrisonense* (Yang and Lin 1999), *Bischofia javanica* Blume (Yang et al. 2006), and *Zelkova serrata* (Thunb.) Makino (Yang et al. 2007).

In general, intermediate seeds have the ability to adapt to different ecological environments, and sub-zero temperatures normally deteriorate the viability of tropical intermediate seeds more rapidly than temperate intermediate seeds; thus the optimal temperature for storing tropical intermediate seeds should be $> 10^{\circ}\text{C}$ (Hong and Ellis 1996). Such species include seeds of *Elaeis guineensis* Jacq. (Ellis et al. 1991b), *Coffea* spp. (Ellis et al. 1990a, 1991a, Hong and Ellis 1992, 1995, Dussert et al. 1999, Eira et al. 1999), *Azadirachta indica* A. Juss. (Gamene et al. 1996, Hong and Ellis 1998, Nayal et al. 2000), *K. senegalensis*, *S. macrophylla* (Hong and Ellis 1998), and *Phoenix reclinata* Jacquin (von Fintel et al. 2004). On the other hand, temperate-intermediate seeds are more likely to tolerate freezing temperatures, and some can even survive for about 2~3 yr, so the ideal storage temperature for those seeds should be $< 5^{\circ}\text{C}$ (Hong and Ellis 1996), such as seeds of *Araucaria columnaris* (Forst.) Hook. (Tompsett 1984), *Cit. limon* (Hong et al. 2001), *Fagus sylvatica* L., *F. crenata* Blume (Pedro and Ellis 2002), and *Zizania palustris* L. (Berjak et al. 1994, Vertucci et al. 1994, 1995, Hong and Ellis 1996, White and Jayas 1996, Ntuli et al. 1997). Based on the protocol suggested by Hong and Ellis (1996), all seeds of the Aceraceae and some Lauraceae in Taiwan are considered to be temperate intermediates, especially dry-fruit seeds of

the Aceraceae which can adapt to sub-zero temperatures very well (Yang and Lin 1999). However, intermediate seeds with a fleshy fruit of a larger size, such as members of the Lauraceae, are normally short-lived, such as *Cinnamomum camphora* (L.) Presl. (Chien and Lin 1999), *Neolitsea parvigemma* (Hay.) (Kanehira & Sasaki) (Lin 1996), *C. subavennium* (Miq.) (Lin 1996), *Lindera communis* Hemsl. (Chien et al. 2004), *Lin. megaphylla* Hemsl. (Lin 1996, Chien et al. 2004), and *Lin. coreana*, which are all extremely sensitive to freezing temperatures. According to various references, the most appropriate storage temperature for such intermediate seeds of the Lauraceae is about 4°C (Lin 1996, Chien et al. 2004).

Based on the above-described results, the best condition for the dry storage of *L. coreana* seeds is at 4°C with about a 6% moisture content. However, when comparing the longevity of seeds under dry and wet storage, the latter method was more successful for maintaining viability (Figs. 3, 4). Moreover, it is difficult to precisely control the seed water content to 6%, and over-desiccation may kill a great proportion of seeds, so wet storage seems more appropriate after considering such practical issues. Additionally, low-temperature moist storage was sufficient to break seed dormancy with no pretreatment. To sum up, the application of wet storage at 4°C for storing intermediate seeds of the Taiwanese Lauraceae is highly recommended.

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