Research paper

Seed Germination and Storage of *Pasania glabra* (Thunb. *ex* Murray) Oerst.

Jeng-Chuann Yang,[1,3] Shing-Rong Kuo[2]

【Summary】

Pasania glabra is a native tree species of Taiwan and is estimated to be a near-threatened plant. The purpose of this study was to examine the germination characteristics and seed storage behavior of *P. glabra* so as to determine efficient pretreatment for germination and an appropriate seed storage method. Results showed that seeds of *P. glabra* were dormant, and the dormancy could be broken by low-temperature stratification. Fresh mature seeds of *P. glabra* germinated slowly and took about 20 wk to completely germinate with a mean germination time (MGT) of about 70 d under fluctuating temperatures of 30/20℃ with 8 h of light. However, stratification at 1 and 4℃ was unable to efficiently improve germination percentages but significantly decreased the MGTs in a germination period of 20 wk, and 1 and 4℃ stratification for 2~12 mo completely broke the dormancy of *P. glabra* seeds. The MGT of fresh mature seeds significantly decreased from about 70 d to about 30 d after 4 mo of stratification at 4℃. Therefore, pretreatment with 4℃ chilling for the germination of *P. glabra* seeds is practical for nursery operations. We suggest that pre-chilling at 4℃ for 4 mo should be applied to fresh mature seeds of *P. glabra*. Furthermore, fresh mature seeds of *P. glabra* were extremely sensitive to desiccation. Seeds completely lost viability when the moisture content (MC) dropped below 25%. However, *P. glabra* seeds maintained their initial viability for up to 2 yr when stored at 1~4℃ with moist sphagnum. Thus, seeds of *P. glabra* are defined as having temperate-recalcitrant storage behavior due to their extreme intolerance of dehydration and high viability with low-temperature moist storage. The optimal seed storage conditions of *P. glabra* are moist storage at 1~4℃ for fresh mature seeds, and the initially germinable seeds still had germinability for up to 2 yr.

Key words: *Pasania glabra* (Thunb. *ex* Murray) Oerst, germination, temperate-recalcitrant, seed storage, stratification.

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研究報告

子彈石櫟種子的發芽與貯藏

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摘 要

子彈石櫟(*Pasania glabra* (Thunb. *ex* Murray) Oerst.)是臺灣列為接近威脅等級的原生樹種，本研究目的在瞭解子彈石櫟種子發芽特性及種子儲藏性質，並確定其有效的發芽前處理及適當的貯存方法。結果顯示子彈石櫟種子具休眠性，低溫層積可以解除其休眠。鮮採的子彈石櫟成熟種子在30/20℃變溫條件下發芽緩慢，約需20週才能全部完成發芽，平均發芽日數約70日。在20週的發芽期計算基礎下，1與4℃層積無法有效提升子彈石櫟種子的發芽率，但能顯著降低其平均發芽日數，且在1與4℃層積經2～12個月後能完全解除種子休眠。經4℃層積4個月後，子彈石櫟種子的平均發芽日數從新鮮種子的約70日顯著下降至約30日，因此，4℃層積發芽前處理對本種的育苗作業甚具實用價值，我們建議應該採用經4個月的4℃層積來解除其種子休眠。子彈石櫟種子對乾燥非常敏感，當種子含水率被降到25%以下時就死亡殆盡，但當其以水草濕藏在1~4℃時，於2年內仍能保有原來活力，以其非常不耐旱及能在低溫濕藏時維持活力之特性，故將其歸類為溫帶異儲型。儲藏子彈石櫟種子的最好方法是將新鮮種子以1~4℃濕藏之，於2年內仍能保持原有活力。

關鍵詞：子彈石櫟、發芽、溫帶異儲型、種子貯藏、層積。

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INTRODUCTION

Seed storage behaviors are divided into orthodox, recalcitrant, and intermediate according to the degree of desiccation tolerance (Hong and Ellis 1996). Recalcitrant seeds have a low desiccation tolerance (Tompsett 1987, 1989). When recalcitrant seeds are dried to < 12~31% moisture contents (MCs, on a fresh-weight (FW) basis; all MC values in this study are on an FW basis), the seed viability decreases with concurrent prolongation of the drying duration (Roberts 1973). Many recalcitrant seeds are extremely sensitive to low temperatures, and at temperatures of < 10~15℃, their viability often deteriorates, especially seeds from tropical areas (Bonner 1990). Hong and Ellis (1996), therefore, differentiated recalcitrant seeds into temperate and tropical subtypes according to the level of temperature tolerance of recalcitrant seeds. Temperate-recalcitrant seeds cannot tolerate desiccation but can survive a temperature close to 0℃ and MCs of 35~50%. For example, seeds of *Keteleeria davidiana* (Franchet) Beissner var. *formosana* Hayata (Yang et al. 2006) and *Camellia brevistyla* (Hay.) Coh.-Stuart (Yang and Kuo 2017) exhibit temperate-recalcitrant seed storage behavior. Tropical-recalcitrant seeds, such as large seeds of *Litsea garciae*, *Diospyros philippensis*, and *Myristica ceylanica* var. *cagayanensis* (Yang et al. 2008b), are weakly tolerant of desiccation and low temperatures. They are almost unable to survive temperatures close to 0℃ and are even sensitive to 15~20℃ (Wang et al. 1995, Hong and Ellis 1996). In contrast to recalcitrant seeds, orthodox seeds can tolerate desiccation and survive MCs of < 5%. The longevity of orthodox seeds is prolonged with concurrent decreases in the MC and storage temperature; the relationships can be inferred from mathematical approaches (Roberts 1973). In addition to orthodox and recalcitrant seeds, Ellis et al. (1990a) found that *Coffea arabica* L. seeds survived desiccation to 5~10% MCs but failed to survive at storage temperatures of < 10℃. Such seeds are considered intermediate; that is, intermediate seeds are not like orthodox seeds in that they show strong desiccation tolerance and are vulnerable to low-temperature damage. According to Hong and Ellis (1996), most intermediate seeds can survive desiccation to 10~12% MCs, but their viability declines with a concurrent reduction below those MCs. For example, seeds of Taiwanese *Acer* spp. (Yang and Lin 1999), *Zelkova serrata* (Thunb.) Makino (Yang et al. 2007), and *Litsea coreana* Levl. (Yang et al. 2008a) showed intermediate seed storage behavior.

Pasania glabra (Thunb. *ex* Murray) Oerst, a synonym of *Lithocarpus glaber* (Thunb.) Nakai, is an evergreen tree in the Fagaceae family. This species is distributed in Japan, China, and Taiwan. In Taiwan, *P. glabra* is separately scattered in forests at elevations of 500~1600 m (Liao 1996). This species was estimated to be near-endangered by the Council of Agriculture (Lu and Chiou 1998). *Pasania glabra* usually blooms in March to May. The maturation period of nuts is from mid-October to mid-November, and there is an abundant harvest about every 3 yr. The ovoid nuts of this species are about 2 cm long and 1.3 cm in diameter. The Chinese name of *P. glabra* is "bullet tanoak" which is derived from its bullet-shaped nuts. The seeds turn from green to brown when mature (Fig. 1).

There are no research reports on the germination and storage of *P. glabra* seeds. Consequently, a careful research design was used to investigate the germination and storage behavior of *P. glabra* seeds in this study. The results can offer useful information about

Fig. 1. Mature fruits and seeds of *Pasania glabra*.

nursery operations for restoration and seed storage for ex situ conservation programs of this rare native Taiwanese tree species.

MATERIALS AND METHODS

Seed collection and processing

Table 1 gives detailed collection information of the 2 seedlots used in this study. About 8200 and 7450 seeds were collected from the 2 population in 2 yr (2010 and 2013) of abundance. Mature nuts of the 2 seedlots were not collected until they had become greenish-brown. Then, after seed selection by soaking in water, heavy sinking seeds were acquired for research. Light, floating seeds in the 2 seedlots comprised 24 and 31%. This was because seeds of *Pasania* spp. in the wild in Taiwan can easily be gnawed on by larvae of some species of the Curculionidae (unpublished data). Seeds of seedlot 1 were extracted and washed on 15 November 2010, and those of seedlot 2 were processed on 24 November 2013. Among pure seeds with no debris left, small-sized and damaged seeds were manually removed. Seed characteristics of fresh mature seeds of the 2 seedlots are also given in Table 1. There were no significant differences in the MC, seed length, seed width, seed thickness, number of seeds L^{-1}, or thousand-seed weight between seedlots 1 and 2.

Determination of the MC

Seed MCs were determined gravimetrically with 4 replicates. For each replicate, 2 filled seeds of each seedlot were randomly selected and cut into pieces of < 4 mm in length. Then, the selected seeds were dehydrated. MCs of the seeds were determined using a low-constant-temperature oven method (103 ± 2℃ for 17 ± 1 h) (International Seed Testing Association 1999). All MCs are presented on a percentage FW basis.

Germination assay

To avoid imbibition damage by rapid rehydration, seeds with different treatments were placed above water in a sealed container

Table 1. Information on fruit collection dates and sources, seed characteristics, and germination of the 2 seedlots of fresh mature seeds of *Pasania glabra*

	Seedlot 1	Seedlot 2
Collection date	11 Nov. 2010	16 Nov. 2013
Collection location	Jianshih Township, Hsinchu County	Fuxing District, Taoyuan City
Latitude, longitude	24°41’N, 121°20’E	24°40’N, 121°23’E
Elevation (m)	980~1060	700~930
Moisture content (%, FW[1)] basis)	51.9±4.1	50.6±4.3
Seed length (mm)	20.2±1.2	20.3±1.2
Seed width (mm)	12.8±1.3	13.1±1.2
Seed thickness (mm)	12.5±1.1	12.7±1.2
Number of seeds L^{-1}	370±15	363±6
Thousand-seed-weight[2)] (g)	1634.9±90.0	1646.7±59.2
Germination percentage (%)	65.7±10.2	59.0±8.8
Mean germination time (d)	66.9±2.9	68.9±3.2

[1)] FW, Fresh-weight.

[2)] Thousand-seed weight was estimated at the moisture content shown.

for 1 d before the germination test so that those seeds could take up water at ambient temperature (Ellis et al. 1990b). Then, the imbibed seeds were thoroughly mixed with clean sphagnum moss in sealable polyethylene (PE) bags (17×12 cm and 0.04 mm thick) with adequate air inside. Excess water in 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 capacity of fresh mature seeds and seeds for storage, 50 seeds from seedlot 1 and 35 seeds from seedlot 2 were randomly sampled in 3 replicates per treatment for the germination test at fluctuating temperatures of 30/20℃ (day/night) with 8 h of light (50~80 μmol m^{-2} s^{-1}). During the 24-wk test period, the number of protruding seeds was counted once a week. Seeds with a normal radicle reaching 2 cm were also counted as having germinated. Meanwhile, about 10 ml of water was added to the moss each week.

Germination speed

The equation for calculating the mean germination time (MGT) is: MGT = $\Sigma(n \times \mathrm{d}) / N$, 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).

Moist and naked storage treatments of fresh seeds at low temperatures

Table 2 shows the low-temperature (1, 4, and 15℃) moist and naked storage durations of fresh seeds at which seeds of the 2 seedlots were stored. These results were used to understand the effects of low-temperature stratification on seed germination and the impact of low-temperature storage on fresh seeds at their initial MCs (51.9% for seedlot 1 and 50.6% for seedlot 2) of *P. glabra*. The wet storage method was to thoroughly mix seeds with moist sphagnum moss in a PE bag (17×12 cm, and 0.04 mm thick) and then place it at 1, 4, and 15℃ for storage. In each bag, about 2/3 of the volume of air was left for seed respiration, and during storage, water was added and air was refreshed every month. However, fresh seeds with naked storage were directly placed in a 4-L glass bottle and sealed with no medium. In each bottle, about 1/2 of the volume of air was left for seed respiration, and during storage, air was refreshed every month.

Dry storage and determination of MCs of dehydrated seeds

Sub-seedlots exhibited different MC levels, and the ranges were about 20~56% for seedlot 1 and 21~55% for seedlot 2. When a sub-seedlot reached the desired MC during the dehydration process, seeds were placed in an aluminum foil bag and stored at 15℃ for about 3~5 d before the average equilibrium MC was determined. As soon as the desired MC of each sub-seedlot was achieved, seeds were sealed in double-layered aluminum foil bags to stabilize the MC. In this study, fresh mature seeds of the 2 seedlots were placed in a hermetically sealed acrylic box with a small fan installed to circulate the air, and molecular sieves were used to dry the seeds. In seedlot 1, the MC of fresh mature seeds was 51.9±4.1%. After dehydration treatment, the 5 MC levels were controlled to 21.8±1.8% (10.2 d of desiccation), 25.6±2.0% (8.1 d of desiccation), 32.6±2.6% (5.6 d of desiccation), 42.4±2.2% (0.3 d of desiccation), and 51.9±4.1% (without desiccation). In seedlot 2, the MC of fresh mature seeds was 50.6±4.3%. After dehydration treatment, the 6 MC levels were controlled to 22.5±1.5% (9.5 d of desiccation), 25.4±2.8% (8.7 d of desiccation), 30.0±1.5% (5.2 d of desic-

Table 2. Durations of low-temperature (1, 4, and 15℃) stratification and naked storage of the 2 seedlots of *Pasania glabra* seeds

	Seedlot 1	Seedlot 2
1℃ stratification (mo)	1, 2, 4, 6, 9, 12, 18, 24	1, 2, 3, 4, 6, 9, 12, 18, 24, 36
4℃ stratification (mo)	1, 2, 4, 6, 9, 12, 18, 24	1, 2, 3, 4, 6, 9, 12, 18, 24, 36
15℃ stratification (mo)	-	1, 2, 3, 4, 6, 9, 12, 18
1℃ naked storage (mo)	1, 2, 4, 6, 9, 12, 18, 24	1, 2, 3, 4, 6, 9, 12, 18, 24, 36
4℃ naked storage (mo)	1, 2, 4, 6, 9, 12, 18, 24	1, 2, 3, 4, 6, 9, 12, 18, 24, 36
15℃ naked storage (mo)	-	1, 2, 3, 4, 6, 9, 12, 18

cation), 35.2±1.4% (1.2 d of desiccation), 41.2±1.6% (0.3 d of desiccation), and 50.6±4.3% (without desiccation).

Data analysis

An analysis of variance (ANOVA) was used to analyze the seed germination percentage and MGT to evaluate the effects of stratification and the naked storage period on germination. Additionally, germination results of the different seed MCs of the 2 seedlots were statistically analyzed by variables implemented in Tukey's test of the GLM procedure of the R statistical software (The R foundation).

RESULTS

Germination of fresh mature seeds

Seeds of *P. glabra* exhibited dormancy. When fresh seeds of seedlot 1 were incubated at fluctuating temperatures of 30/20℃ for 24 wk, their germination percentage was 65.7% and MGT was 66.9 d (Table 1). Seeds irregularly germinated during 4~19 wk, and there was no germination after 19 wk (Fig. 2). In seedlot 2, the germination percentage and MGT of fresh seeds under the same germination conditions as seedlot 1 were 59.0% and 68.9 d, respectively (Table 1). Seeds irregularly emerged during 4~20 wk (Fig. 2). In addition, ungerminated seeds of the 2 seedlots were found to have totally decayed when cut open after 24 wk.

Effects of stratification at 1, 4, and 15℃ on germination and seed MCs

Stratification at 1, 4 and 15℃ did not increase the germination percentage of seeds of *P. glabra* but significantly decreased the MGTs in the germination period of 20 wk. Seeds of seedlot 1 at 1℃ still maintained higher viability for up to 12 mo of stratification. Germination percentages were 66.0~79.3%, and there were no significant differences ($p > 0.1$) in germination compared to fresh seeds (65.7%) (Fig. 3). Although the germination percentage respectively dropped to 49.3 and 52.0% after 18 and 24 mo of stratification at 1℃, there were still no significant differences ($p = 0.15$ and 0.23). In addition, the germination percentages at 4℃ stratification showed the same declining trend as those at 1℃. Germination was 58.0~73.3% after stratification at 4℃ within 12 mo, while there was no significant difference in germination percentages ($p > 0.4$). The germination percentage respectively dropped to 37.3 and 38.7% after 18 and 24 mo of stratification at 4℃, but no significant differences were found ($p = 0.051$ and 0.07). Moreover, after stratification at 1 and 4℃ for 2 and 1 mo, MGTs of seeds had significantly decreased to 42.6 and 52.4 d, respectively ($p = 0.002$ and 0.02). MGTs of seeds were 23.5~46.8 and 17.6~36.5 d after stratification at 1 and 4℃ for the following 4~24 mo, respectively. Significant decreases in MGTs ($p < 0.005$) were found after

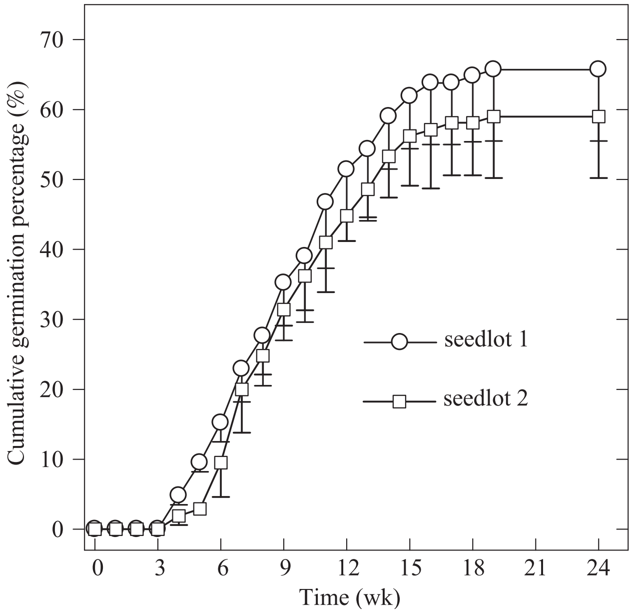

Fig. 2. Cumulative germination percentages of the 2 seedlots of fresh mature seeds of *Pasania glabra*.

the above stratification duration compared to fresh seeds (66.9 d) (Fig. 3). Furthermore, seedlot 1 seeds maintained a stable water content when they were at 1 and 4℃ moist storage for up to 24 mo. MCs of seeds were 52.6~57.2 and 51.3~57.4% after stratification at 1 and 4℃ for 1~24 mo, respectively. No significant differences in MCs ($p > 0.1$) were found after the above stratification duration compared to fresh seeds (51.9%) (Fig. 3).

In seedlot 2, seed germination percentages were 41.2~71.4% after stratification at 1℃ for up to 24 mo, while there were no significant differences in germination percentages ($p > 0.1$) compared to fresh seeds (59.0%), and a significant decrease in germination percentage (17.1%) was found after 36 mo ($p = 0.006$) (Fig. 4). In addition, the germination percentages at 4℃ stratification showed the same deteriorating trend as those at 1℃. Germination was 41.0~68.6% after stratification at 4℃ for up to 24 mo, while there were no significant differences in germination percentages ($p > 0.1$). However, there was a significant decrease in the germination percentage (21.9%) after 36 mo ($p = 0.008$) (Fig. 4). Moreover, seed germination percentages were 60.8~61.0% after stratification at 15℃ for up to 2 mo, while there were no significant differences in germination percentages ($p > 0.8$) compared to fresh seeds (59.0%) (Fig. 5). Seeds began to germinate after stratification at 15℃ for 3 mo, and almost all viable seeds had germinated under the moist dark conditions within 3~18 mo (Fig. 5). However, the total germination percentage within 18 mo of stratification at 15℃ showed no significant differences in germination ($p > 0.05$) compared to fresh seeds. For example, the total germination percentages of seeds with 12 and 18 mo of stratification at 15℃ were 79.0 and 64.8%, respectively, which showed no significant differences ($p = 0.05$ and 0.44) compared to fresh seeds (Fig. 5). In addition, after stratification at 1 and 4℃ for 2 mo, the MGTs of seeds were reduced to 41.8 and 48.4 d, respectively. Clearly, significant decreases

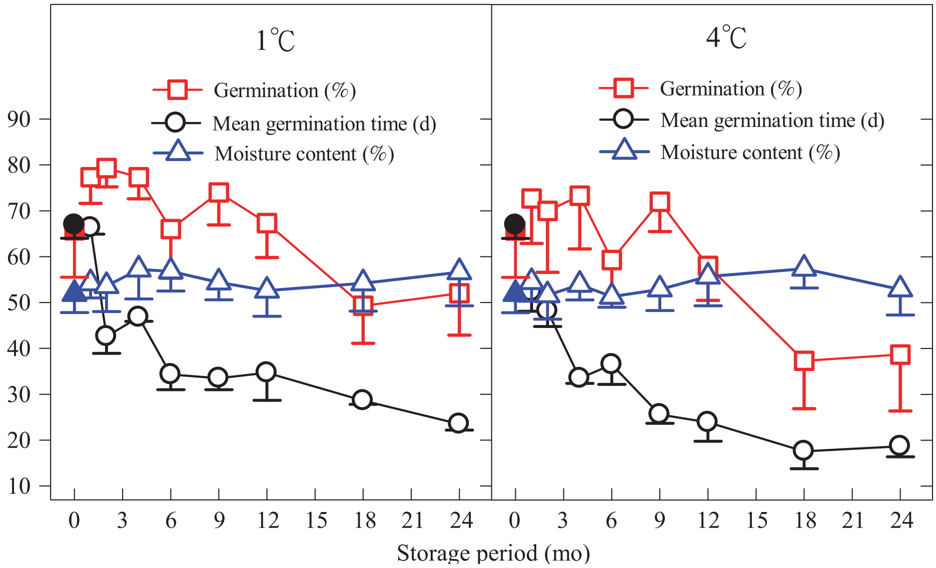

Fig. 3. Effects of stratification at 1 and 4℃ for 1~24 mo on the germination percentage (□), mean germination time (○), and moisture content (△) of seedlot 1 seeds of *Pasania glabra*. Filled squares (■), circles (●), and triangles (▲) respectively represent the germination percentage, mean germination time, and moisture content of fresh mature seeds. Vertical bars represent the standard error of the mean.

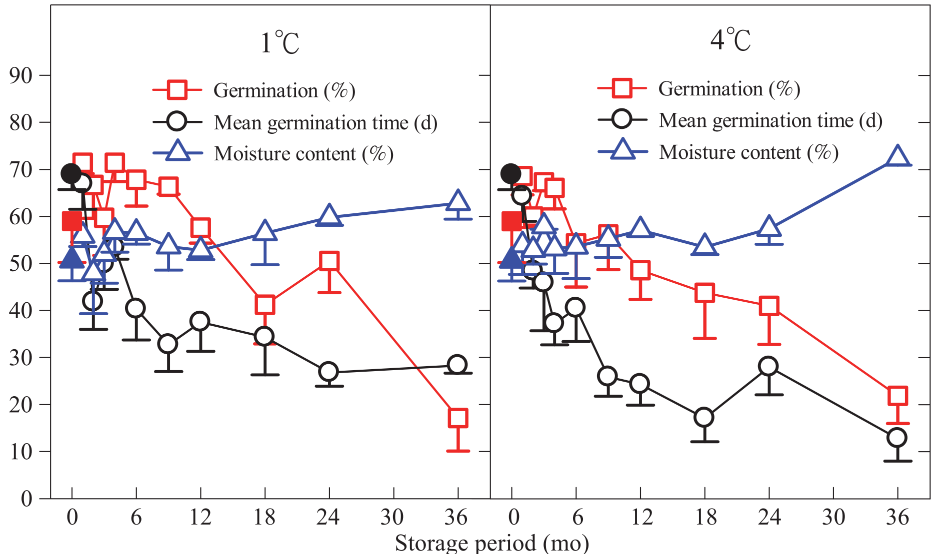

Fig. 4. Effects of stratification at 1 and 4℃ for 1~36 mo on the germination percentage (□), mean germination time (○), and moisture content (△) of seedlot 2 seeds of *Pasania glabra*. Filled squares (■), circles (●), and triangles (▲) respectively represent the germination percentage, mean germination time, and moisture content of fresh mature seeds. Vertical bars represent the standard error of the mean.

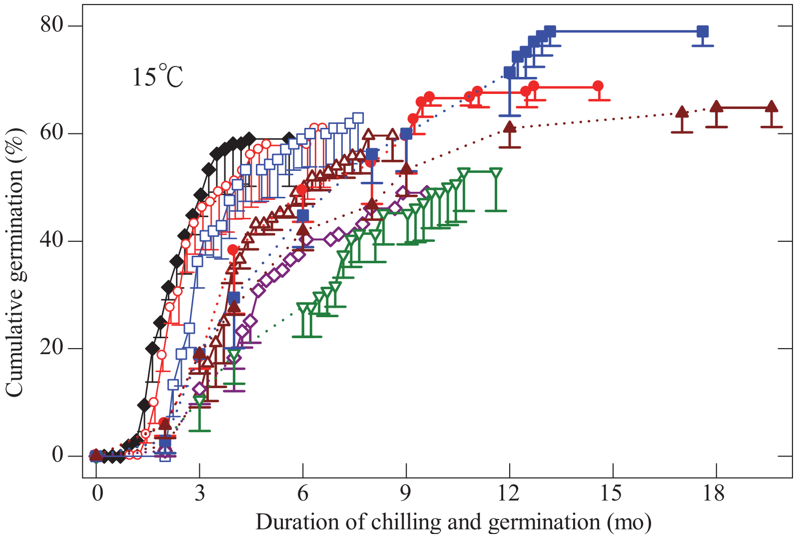

Fig. 5. Cumulative germination percentages of seedlot 2 seeds of *Pasania glabra* at 15℃ moist stratification for 1~18 mo (○, 1 mo; □, 2 mo; △, 3 mo; ◇, 4 mo; ▽, 6 mo; ●, 9 mo; ■, 12 mo; ▲, 18 mo). Dotted lines (- - -) represent the cumulative germination percentages in the dark with 15℃ moist stratification, while solid lines (—) represent the cumulative germination percentages at fluctuating temperatures of 30/20℃ with 8 h of light after moist stratification. Filled diamonds (◆) represent the cumulative germination percentage of fresh mature seeds. Vertical bars represent the standard error of the mean.

in MGTs ($p < 0.005$) were found compared to fresh seeds (68.9 d). The MGTs of seeds were 26.8~53.4 and 12.8~45.9 d after stratification at 1 and 4℃, respectively, in the following 3~36 mo. Significant decreases in MGTs ($p < 0.02$) were found after the above stratification duration compared to fresh seeds (68.9 d) (Fig. 4). Furthermore, after stratification at 15℃ for 1 and 2 mo, MGTs of seeds had significantly declined to 53.4 and 42.9 d ($p = 0.04$ and < 0.001), respectively, and seeds began to germinate under the moist dark conditions. MCs of seeds were 47.9~56.7, 52.9~57.9, and 49.2~55.7% after stratification at 1, 4, and 15℃ for 1~18, 1~24, and 1~6 mo, respectively. No significant differences in MCs ($p > 0.1$) were found after the above stratification durations compared to fresh seeds (50.6%). MCs of seeds significantly increased to 59.8 and 62.8% ($p = 0.03$ and 0.02) after stratification at 1℃ for 24 and 36 mo, respectively; and those seeds after stratification at 4℃ for 36 mo showed an increase in MC (72.4%) ($p < 0.001$) (Fig. 4). The significant increase in MC implied that seeds had begun to deteriorate.

Effects of naked storage at 1, 4, and 15℃ on germination and seed moisture contents of fresh seeds

Fresh seeds of *P. glabra* rapidly lost their viability when stored naked at 1, 4, and 15℃. In addition, the higher the storage temperature at which they were maintained, the shorter was the seed longevity they exhibited. Seeds of seedlot 1 still maintained higher viability for up to 4 mo of naked storage at 1℃. Although the germination percentage dropped to 39.3% after 4 mo of storage at 1℃, there was still no significant difference ($p = 0.14$) in germination compared to fresh seeds (65.7%)

(Fig. 6). Nevertheless, after naked storage at 1℃ for 6 mo, the germination percentage of seeds had significantly declined to 17.3% (p = 0.006), and seeds totally died when the storage duration was extended to 9 mo. The decrease in the rate of seed viability with naked storage at 4℃ occurred more quickly than that of seeds stored at 1℃. The germination percentage dropped to 42.0% after 1 mo of naked storage at 4℃, and there was no significant difference in germination percentages (p = 0.054). However, seed germination percentage significantly decreased to 34.0% (p = 0.03) after 2 mo of storage, and they had almost all died after 6 mo (Fig. 6). In addition, MGTs with naked storage at 1℃ for up to 6 mo were 64.4~77.9 d while MGTs with naked storage at 4℃ were 71.1~82.1 d for up to 2 mo. Still, seeds did not completely all die, and no significant decrease in MGTs (p < 0.005) was found after the above storage duration compared to fresh seeds (66.9 d) (Fig. 6). Moreover, seedlot 1 seeds maintained a similar water content at 1 and 4℃ naked storage for up to 12 mo, even when seeds had completely deteriorated. MCs of the seeds were 43.5~48.9 and 46.7~49.6% after naked storage at 1 and 4℃ for 1~12 mo, respectively. No significant difference in MCs (p > 0.3) was found after the above storage duration compared to fresh seeds (51.9%) (Fig. 6).

In seedlot 2, seed germination percentages were 28.9~56.2% after naked storage at 1℃ for up to 4 mo, while there was no significant difference in the germination percentage (p > 0.05) compared to fresh seeds (59.0%), but a significant decrease in germination percentage (15.2%) was found after 6 mo (p = 0.04) (Fig. 7). In addition, germination percentages with naked storage at 4℃ showed a sharper dete-

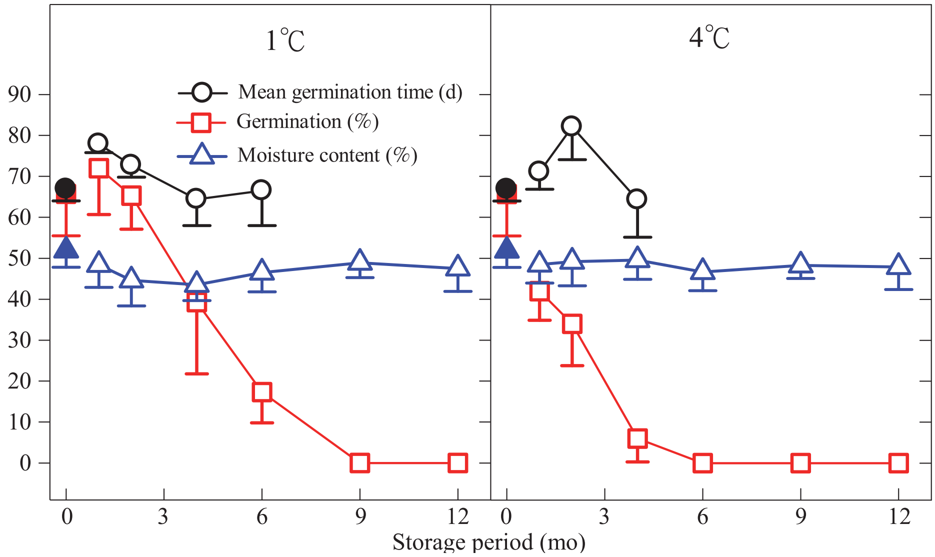

Fig. 6. Effects of 1~12 mo of naked storage at 1 and 4℃ on the germination percentage (□), mean germination time (○), and moisture content (△) of seedlot 1 seeds of *Pasania glabra* at an initial moisture content (▲) of 51.9±4.1%. Filled squares (■), circles (●), and triangles (▲) respectively represent the germination percentage, mean germination time, and moisture content of fresh mature seeds. Vertical bars represent the standard error of the mean.

riorating trend than that at 1℃. Germination percentages were 42.0 and 34.0% after storage at 4℃ for 1 and 2 mo, respectively, while there was no significant difference in germination percentages ($p > 0.1$). However, there was a significant decrease in the germination percentage (6.0%) after 4 mo ($p = 0.02$), and seeds had absolutely lost their viability after 6 mo (Fig. 7). Moreover, there was a more-rapid decline in germination percentage with 15℃ naked storage than with storage at 1 and 4℃. The germination percentage suddenly decreased to 3.8% after storage at 15℃ for 1 mo, and all seeds immediately died the next month (Fig. 7). In addition, the stability of MCs of seedlot 2 seeds stored naked at 1 and 4℃ was similar to that of seedlot 1 seeds. They maintained an approximate water content for up to 12 mo even when seeds had completely died (Fig. 7).

Effects of seed moisture on viability

Seeds of *P. glabra* cannot tolerate desiccation. Most seeds died when the MC dropped to about 35%, and they completely lost viability when the MC was < 25%, so they show recalcitrant storage behavior. Figure 8 shows the effects of different MC levels on germination percentages of fresh mature seeds of *P. glabra*. Once the 5 and 6 desired MC levels of seedlots 1 and 2 were reached, respectively, seeds were immediately incubated under fluctuating temperatures of 30/20℃ for 24 wk. Germination percentages of seedlot 1 seeds at MCs of 21.8, 25.6, 32.6, 42.4, and 51.9% were 0, 0, 24.0, 42.7, and 65.7%, while those of seedlot 2 seeds at MCs of 22.5, 25.4, 30.0, 35.2, 41.2, and 50.6% were 0, 0, 0, 20.0, 31.4, and 59.0%, respectively. The viability of both seedlots rapidly dropped when they dried out (Fig. 8).

DISCUSSION

Results of this study showed that fresh mature seeds of *P. glabra* germinate slowly, and viable seeds irregularly emerged during 4~20 wk, and took about 20 wk for complete

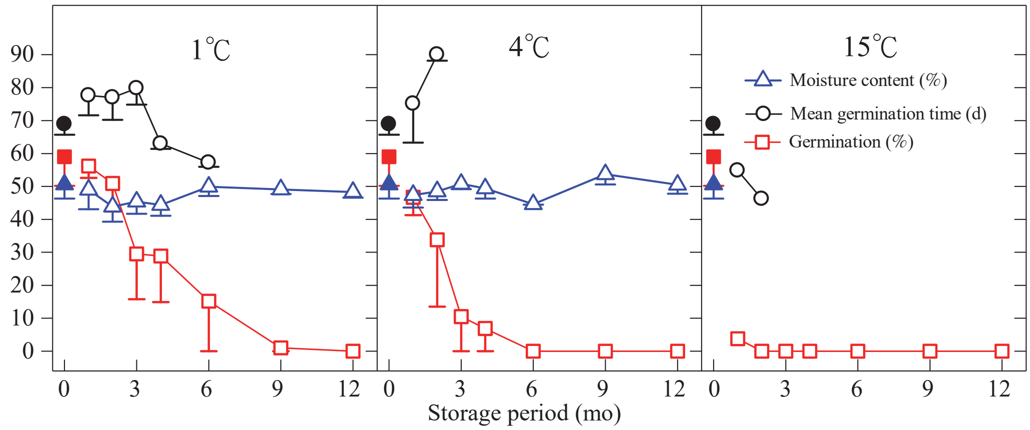

Fig. 7. Effects of 1~12 mo at 1, 4 and 15℃ of naked storage on the germination percentage (□), mean germination time (○), and moisture content (△) of seedlot 2 seeds of *Pasania glabra* at an initial moisture content (▲) of 50.6±4.3%. Filled squares (■), circles (●), and triangles (▲) respectively represent the germination percentage, mean germination time, and moisture content of fresh mature seeds. Vertical bars represent the standard error of the mean.

germination with an MGT of about 70 d (Fig. 2). Above all, after stratification at 1 and 4℃ for more than 2 mo, significant decreases in the MGTs (about 18~45 d) were observed (Figs. 3, 4). Therefore, *P. glabra* seeds are considered to exhibit dormancy, which can be broken by low-temperature stratification.

Stratification at 1 and 4℃ did not improve germination percentages but significantly decreased the MGTs of *P. glabra* seeds with a germination period of 20 wk. Furthermore, the MGT of fresh mature seeds significantly decreased from about 70 d to about 30 d after 4 mo of stratification at 4℃ (Figs. 3, 4). Therefore, pretreatment with 4℃ chilling for germination of *P. glabra* seeds is practical for nursery operations. We suggest that prechilling at 4℃ for 4 mo should be applied to fresh mature seeds of *P. glabra* collected in November in order to break dormancy. This is a good process for nursery operations the following March when the temperature is rising. In this way, the best regular germination can be obtained, and seedlings will continue growing after germinating.

Pasania glabra seeds showed the same desiccation intolerance as most of the Fagaceae in Taiwan such as seeds of *Cyclobalanopsis gilva* (Bl.) Oerst., *C. glauca* (Thunb.) Oerst., *C. morii* (Hay.) Schott., and *Quercus spinosa* A. David (Lin 1995). They all rapidly deteriorated when the MC of fresh mature seeds slightly decreased. However, seeds of *P. glabra* almost completely maintained their germinability when stored moist at 1~4℃ for 2 yr (Figs. 3, 4). Therefore, *P. glabra* seeds showed temperate-recalcitrant storage behavior due to their extreme intolerance of dehydration and high viability with low-temperature storage, so that it was impossible to apply long-term dry storage to *P. glabra* seeds. In addition, seeds were found to have decayed and rapidly died after naked storage at 1, 4, and 15℃ (Figs. 6, 7), while seeds remained viable under 1 and 4℃ moist storage. However, seed viability was not well maintained for more than 2 yr when seeds were stored moist at 1~4℃ (Fig. 4). In other words, even when providing

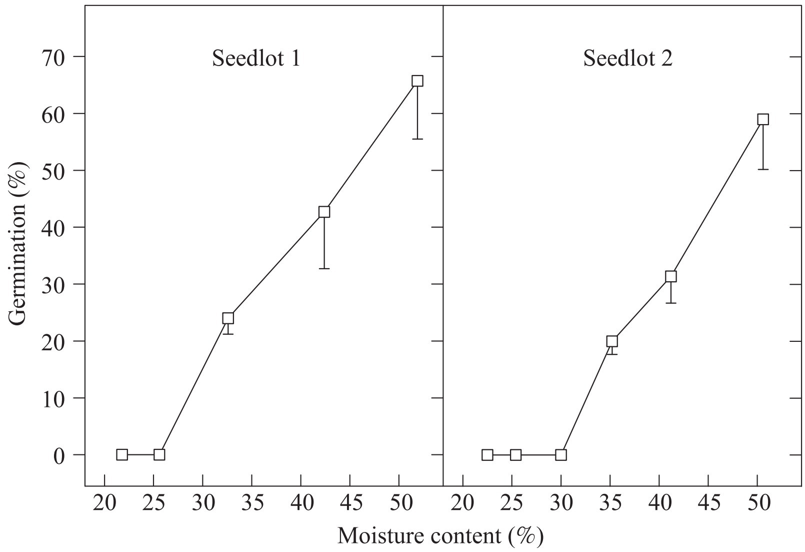

Fig. 8. Effects of moisture contents (%, on a fresh-weight basis) on the germination percentage of seeds of 2 seedlots of *Pasania glabra*. Vertical bars represent the standard error of the mean.

optimal storage conditions, the viability of *P. glabra* seeds could not be maintained beyond 2 yr. Therefore, it is unworkable to preserve the germplasm of *P. glabra* by storing intact seeds. Consequently, moist storage at 1~4℃ is efficient for the short-term storage of *P. glabra* seeds for up to 2 yr.

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 *P. glabra* were determined to be temperate-recalcitrant seeds. The MC of mature seeds of this species was about 50%; moreover, the thousand-seed weight was about 1600 g (Table 1). For this species, relationships of the MC and thousand-seed weight with the seed storage behavior were consistent with Hong and Ellis's criteria (1996).

CONCLUSIONS

Seeds of *P. glabra* exhibited dormancy. Fresh mature seeds of *P. glabra* germinated slowly, and viable seeds emerged irregularly and took about 20 wk for complete germination under fluctuating temperatures of 30/20℃ with 8 h of light. Stratification at 1 and 4℃ did not efficiently improve the germination percentage but significantly decreased the MGT from about 70 to 30 d after 4 mo. Therefore, pretreatment with 4℃ chilling for germination of *P. glabra* is practical for nursery operations. We suggest that pre-chilling at 4℃ for 4 mo should be applied to fresh mature seeds of *P. glabra*. Furthermore, seeds of *P. glabra* were extremely sensitive to desiccation. Most seeds died when the MC dropped to about 35%; moreover, they completely lost viability when the MC dropped below 25%. However, *P. glabra* seeds maintained their initial viability when stored at 1~4℃ with moist sphagnum for up to 2 yr. Thus, seeds of *P. glabra* are defined as having temperate-recalcitrant storage behavior due to their extreme intolerance of dehydration and high viability with low-temperatures storage, and a long-term seed storage strategy cannot be adopted for ex situ conservation of this near-threatened tree species of Taiwan. Additionally, the optimal seed storage conditions of *P. glabra* are moist storage at 1~4℃ for fresh mature seeds, and the initially germinable seeds still had germinability for up to 2 yr.

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