Research paper

Seed Storability of *Maackia taiwanensis* Hoshi & Ohashi

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

【Summary】

Maackia taiwanensis is an endemic tree species and an endangered species of Taiwan. The purpose of this study was to examine the germination characteristics and storability of seeds of *M. taiwanensis*. The effects of the seed moisture content (MC) and storage temperature on germination were investigated to determine the seed storage behavior and evalute the feasibility of long-term seed storage for ex situ conservation of this rare species. Results showed that seeds of *M. taiwanensis* were non-dormant. Fresh mature seeds of 2 seedlots of *M. taiwanensis* completely germinated within 4 wk under fluctuating temperatures of 30/20℃ with 8 h of light. Moreover, stratification at 4℃ did not efficiently improve the germination percentage but significantly decreased the mean germination times (MGTs). Though the MGT of fresh mature seeds was significantly reduced from about 15 d to about 8 d after only 1-mo stratification at 4℃, pretreatment with stratification at 4℃ for germination of *M. taiwanensis* is not practical for nursery operations due to this species' quick germination characteristic. In addition, seeds began to germinate after stratification at 4℃ for 6 mo, and almost all viable seeds also germinated under a moist dark condition within 6~14 mo. Therefore, moist stratification at 4℃ is not workable to store seeds of this species. However, seeds of *M. taiwanensis* dried to 2.5~7.8% MCs (on a fresh-weight basis) still maintained viability after 2 yr of storage at -20, 4, and 15℃. From the results, seeds of *M. taiwanensis* can tolerate desiccation and sub-zero temperatures, which qualifies them as orthodox. In short, seeds of *M. taiwanensis* can be stored over a long-term period to ensure future seed supplies. If seed storage of *M. taiwanensis* is used for plant germplasm collection and long-term conservation, we recommend that seeds of *M. taiwanensis* be dehydrated to 3~7% MCs and then hermetically sealed before storage at -20℃.

Key words: *Maackia taiwanensis* Hoshi & Ohashi, germination, orthodox, seed storability, stratification.

Yang JC, Kuo SR. 2018. Seed storability of *Maackia taiwanensis* Hoshi & Ohashi. Taiwan J For Sci 33(3):231-44.

1) Botanical Garden Division, Taiwan Forestry Research Institute, 53 Nanhai Rd., Taipei 10066, Taiwan. 林業試驗所植物園組，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。

Received March 2018, Accepted May 2018. 2018年3月送審 2018年5月通過。

研究報告

臺灣馬鞍樹的種子可儲性

楊正釧[1,3] 郭幸榮[2]

摘 要

臺灣馬鞍樹是臺灣列為瀕臨絕滅等級的特有珍稀樹種，本研究探討臺灣馬鞍樹種子的發芽特性，並以種子含水率與儲藏溫度對發芽率之影響來判定其種子儲藏性質，以評估將種子儲藏作為本種區外保育方法之可行性。結果顯示臺灣馬鞍樹種子不具休眠性，當新鮮的成熟種子以30/20℃變溫發芽時，可在4週內全部完成發芽。4℃層積無法有效提升臺灣馬鞍樹種子的發芽率，雖只要1個月就可以將其平均發芽日數從約15日顯著下降至約8日，但在實際的育苗作業上，4℃層積處理顯然對快速發芽的本種不具實用價值，而且當4℃濕藏時間超過6個月後，種子會開始在黑暗潮濕的環境下自行發芽，故4℃濕藏並非儲藏本種種子的可行方法。當臺灣馬鞍樹種子被乾燥至含水率2.5~7.8%，在-20、4與15℃經2年儲藏後活力仍未有下降趨勢，顯示其能耐乾燥且耐零下低溫環境，故判定其屬長壽命的正儲型，亦即可將種子進行長期儲藏以供日後利用，建議未來以儲存種子來進行本種種源的收集與長期區外保育時，應先將種子含水率乾燥到3~7%後密封儲藏於-20℃環境中。

關鍵詞：臺灣馬鞍樹、發芽、正儲型、種子可儲性、層積。

楊正釧、郭幸榮。2018。臺灣馬鞍樹的種子可儲性。台灣林業科學33(3):231-44。

INTRODUCTION

Seed storage behaviors are divided into orthodox, recalcitrant, and intermediate according to the degree of desiccation tolerance (Hong and Ellis 1996). Orthodox seeds can tolerate desiccation and survive moisture contents (MCs) of < 5% (on a fresh-weight basis; all MC values in this study are on a fresh-weight basis). The longevity of orthodox seeds is prolonged with concurrent decreases in the MC and storage temperature, and relationships can be inferred from mathematical approaches (Roberts 1973).

In contrast to orthodox seeds, recalcitrant seeds have a low degree of desiccation tolerance (Tompsett 1987, 1989). When recalcitrant seeds are dried to < 12~31% MCs, 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 of close to 0℃ and MCs of 35~50%. Tropical-recalcitrant seeds have a weak tolerance of desiccation and low temperatures. They are almost unable to survive a temperature close to 0℃ and are even sensitive to 15~20℃ (Hong and Ellis 1996).

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 a storage temperature 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.

Maackia taiwanensis Hoshi & Ohashi, also known as Taiwan Maackia, is a deciduous tree in the family Leguminosae. This species is endemic to the island of Taiwan; mature individuals usually grow up to 10 m in height, and its distribution is only in open forests or forest margins of Yangmingshan National Park located in northern Taipei City at elevations of 500~1000 m (Huang and Ohashi 1993). This species is estimated to be endangered by the Council of Agriculture (Lu and Lin 1996). Taiwan Maackia usually blooms from early to late August (Chen et al. 2009). The maturation period of pods is from late October to early November, and there is an abundant harvest about every 3 yr. The elongated shape pods of this species are about 3.5~7 cm in length and 1.5 cm in width. When mature, the outer skin of the pods changes from green to brown (Fig. 1). Fresh mature seeds of *M. taiwanensis* are long-elliptical in shape; the length of the seeds is about 7 mm, and the diameter is about 4 mm. The seeds turn brown or dark brown in color when mature (Fig. 1).

The genus *Maackia* includes about 12 species worldwide, and all of them are distributed in eastern Asia. In Taiwan, there is only 1 species. There were very few studies on seeds of *M. taiwanensis* in the past. According to Young and Young (1992), because *Maackia* seeds have hard seedcoats, the seeds require scarification before they germinate, and dry seeds can be stored well at low temperatures. Furtheremore, seeds of *M. amurensis* distributed in northeastern China, Korea, and Russia can be dehydrated and stored well at low temperatures. Because of the hard-seedcoat feature, seeds of *M. amurensis* without pretreatment had only 5% germination. However, after the seeds were soaked in hot water for 24 h or subjected to acid treatment for 1 h, seeds showed 94 and 96% germination, respectively.

Fig. 1. Mature pod and seeds of *Maackia taiwanensis*.

Moreover, *M. chinensis* seeds produced in China also need the same pretreatment (Dirr and Heuser 1987). Consequently, a more-careful research design was used to investigate germination and storability of seeds of *M. taiwanensis* in this study. The results can offer useful information about nursery operations for restoration and seed storage for ex situ conservation programs of this Taiwanese native endangered tree species.

MATERIALS AND METHODS

Seed collection and processing

Table 1 gives detailed collection information of the 2 seedlots used in this study. Only mature pods were collected at the time of seed collection of the 2 seedlots. Once the pods were desiccant and became brittle, the seeds were immediately extracted and washed to obtain cleaned seeds. Seeds of seedlot 1 were extracted and washed on November 15, 2004, and seeds of seedlot 2 were retrieved on November 10, 2013. Among pure seeds with no debris left, small-sized and damaged seeds were removed manually. 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, 4 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 fresh-weight basis.

Germination assay

To avoid imbibition damage by rapid rehydration, seeds with different treatments were placed above water in a sealed container for 1 d before the germination test so that the seeds could take up water at ambient temperature

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

	Seedlot 1	Seedlot 2
Collection date	11 Nov. 2004	5 Nov. 2013
Collection location	Taipei	Taipei
Latitude, longitude	25°10′N, 121°30′E	25°10′N, 121°33′E
Elevation (m)	780	450
Moisture content (%, FW[1)] basis)	35.4±0.7	39.6±0.6
Seed length (mm)	7.20±0.66	7.40±0.61
Seed width (mm)	3.98±0.47	4.16±0.46
Seed thickness (mm)	3.01±0.37	3.26±0.33
Number of seeds L^{-1}	9,225±114	8,675±155
Thousand-seed-weight[2)] (g)	73.4±1.3	80.3±2.4
Germination percentage (%)	93.3±5.3	94.4±4.2
Mean germination time (d)	14.8±0.7	14.6±1.1

1) FW, fresh-weight.

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

(Ellis et al. 1990b). Then, the imbibed seeds were thoroughly mixed with clean sphagnum moss in sealable polyethylene (PE) bags (14×10 cm and 0.04 mm thick) with adequate air inside. 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 capacity of fresh mature seeds and seeds for dry storage, 15 seeds from seedlot 1 and 30 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 12-wk test period, the number of protruding seeds was counted once a week. Seeds with a radicle reaching 8 mm were counted as having germinated. Meanwhile, about 5 ml of water was added to the moss each week.

Germination speed

The equation for calculating the mean germination time (MGT) is: MGT = Σ(n×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 storage treatment

Table 2 shows durations of stratification at 4℃ for which seeds of the 2 seedlots were treated. The results were used to understand the effects of low-temperature stratification on seed germination of *M. taiwanensis* and the impact of low-temperature moist storage on this species. The stratification method was to thoroughly mix seeds with wet sphagnum moss in a PE bag (14×10 cm, and 0.04 mm thick) and then place it at 4℃ 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 2 wk.

Dry storage and determination of MCs of dehydrated seeds

Sub-seedlots exhibited different MCs, and the ranges were about 2~10% for seedlot 1 and 2~15% for seedlot 2. 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 35.4% (Table 1). After dehydration treatment, the 3 MCs were controlled to 2.6% (11.6 d of desiccation), 5.3% (5.6 d of desiccation), and 10.3% (1.2 d of desiccation) (Table 2). In seedlot 2, the MC of fresh mature seeds was 39.6%. After dehydration treatment, the 5 MCs were 2.5% (10.6 d of desiccation), 4.9% (4.8 d of desiccation), 7.8% (2.8 d of desiccation), 11.2% (1.0 d of desiccation), and 14.5% (0.6 d of desiccation) (Table 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 MCs of each sub-seedlot were achieved, seeds were sealed in double-layered aluminum foil bags to stabilize the MCs. Table 2 shows storage temperatures and durations of dehydrated seeds of the 2 seedlots.

Data analysis

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

Table 2. Durations of stratification at 4℃ and dry storage durations of seeds at 3 (seedlot 1) or 5 (seedlot 2) moisture contents (MCs) stored at 3 temperatures of 2 seedlots of *Maackia taiwanensis*

	Seedlot 1	Seedlot 2
4℃ stratification duration (mo)	0, 1, 2, 3, 4, 6, 9, 12, 18, 24	0, 1, 2, 4, 6, 9, 12
MCs of dry storage (FW[1)] basis, %)	2.6±0.0, 5.3±0.3, 10.3±0.4	2.5±0.1, 4.9±0.1, 7.8±0.2, 11.2±0.1, 14.5±0.2
Dry storage temperature (℃)	-196, -20, 4	-20, 4, 15
Dry storage duration (mo)	0, 3, 6, 12, 18	0, 3, 6, 9, 12, 15, 18, 24

[1)] FW, fresh-weight.

RESULTS

Germination of fresh mature seeds

Seeds of *M. taiwanensis* exhibited no dormancy. In seedlot 1, when fresh mature seeds were incubated at fluctuating temperatures of 30/20℃ for 12 wk, their germination percentage was 93.3% and the MGT was 14.8 d (Table 1). Seeds mainly emerged during 1~3 wk, and had totally germinated within 4 wk (Fig. 2). In seedlot 2, the germination percentage and MGT of fresh mature seeds under the same germination conditions as seedlot 1 were 94.4% and 14.6 d, respectively (Table 1). Seeds mainly emerged during 1~3 wk, and there was no germination after 4 wk (Fig. 2).

Effects of stratification at 4℃ on germination

Stratification at 4℃ did not increase the germination percentage of seeds of *M. taiwanensis* but significantly decreased the MGTs. In seedlot 1, seed germination percentages were 86.7~100.0% after stratification at 4℃ within 6 mo, while there were no significant differences in germination percentages ($p > 0.05$) compared to fresh mature seeds (93.3%) (Fig. 3). Seeds began to germinate after stratification at 4℃ for 6 mo, and almost all viable seeds had germinated under moist dark conditions within 6~14 mo (Fig. 4). However, the total germination percentage within 18 mo of stratification at 4℃ showed no significant differences ($p > 0.05$) compared to fresh mature seeds. The total germination percentage of seeds with 12 and 18 mo of stratification at 4℃ were 80.0 and 60.0%, respectively, which showed no significant differences ($p = 0.13$ and 0.07) compared to fresh mature seeds. When the stratification period was extended to 24 mo, the total germination percentage significantly decreased to 55.6% ($p = 0.02$), and seeds completely germinated under moist dark conditions within 6~14 mo (Figs. 3, 4). Moreover, after stratification at 4℃ for 1 mo, the MGT of seeds was reduced to 7.6 d. Clearly, a significant decrease in MGTs ($p < 0.001$) was found compared to fresh mature seeds (14.8 d). The MGTs of the seeds were 7.0~7.2 d after stratification at 4℃ for the following 2~12 mo. Significant decreases in MGTs ($p < 0.001$) were found after the above stratification duration compared to fresh mature seeds (14.8 d) (Fig. 3).

Seeds of seedlot 2 within 6-mo stratification at 4℃ still maintained higher viability. Germination percentages were 87.8~98.9%, and there were no significant differences ($p > 0.5$) in germination compared to fresh mature seeds (94.4%) (Fig. 3). Seeds began to germinate after stratification at 4℃ for 6 mo, and almost all viable seeds had germinated under moist dark conditions within 6~16 mo (Fig. 5). However, total germination percentages of seeds with 9, 12, and 18 mo of stratification at 4℃ were 87.8, 83.3, and 67.8%, respectively,

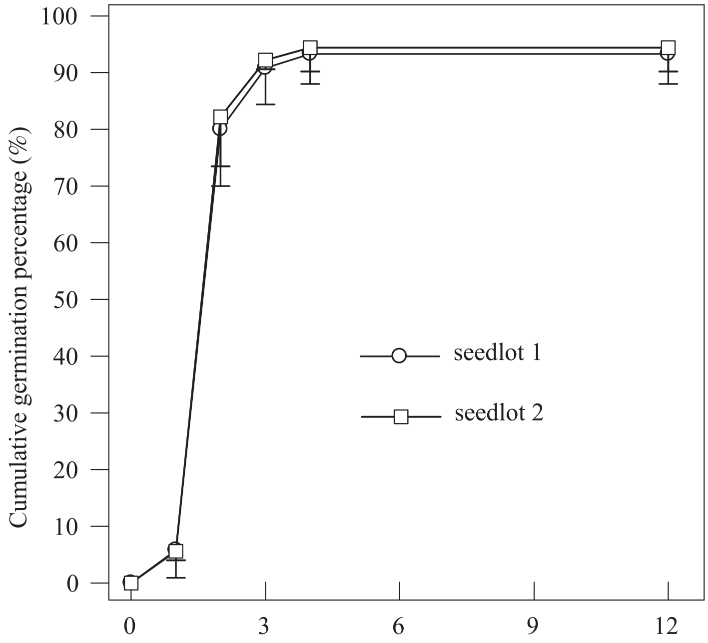

Fig. 2. Cumulative germination percentage of fresh mature seeds of 2 seedlots of *Maackia taiwanensis*.

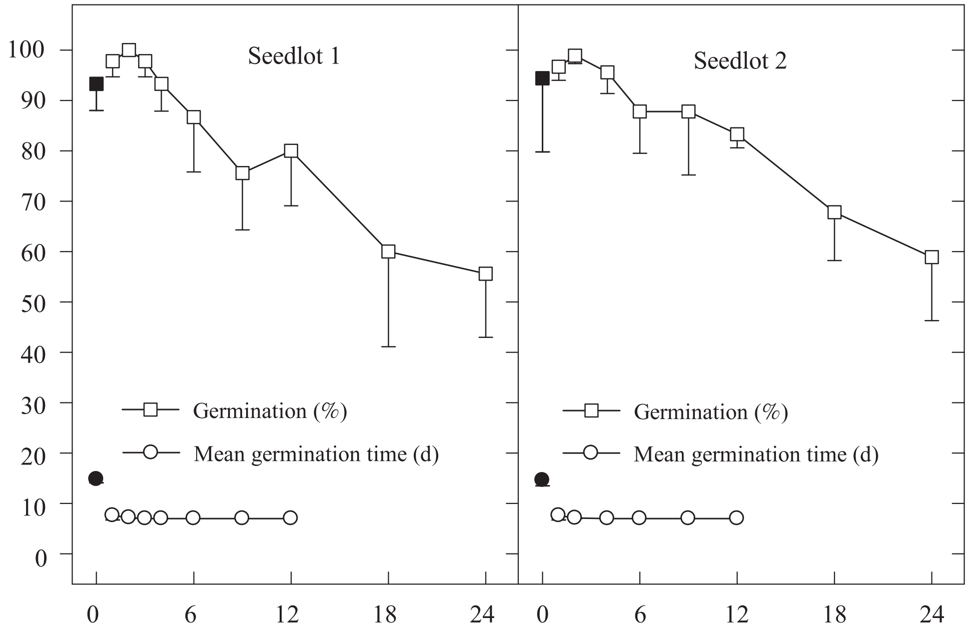

Fig. 3. Effects of stratification at 4℃ for 1~24 mo on the germination percentage (□) and mean germination time (○) of 2 seedlots of seeds of *Maackia taiwanensis*. Filled squares (■) and circles (●) respectively represent the germination percentage and mean germination time of fresh mature seeds. Vertical bars represent the standard error of the mean.

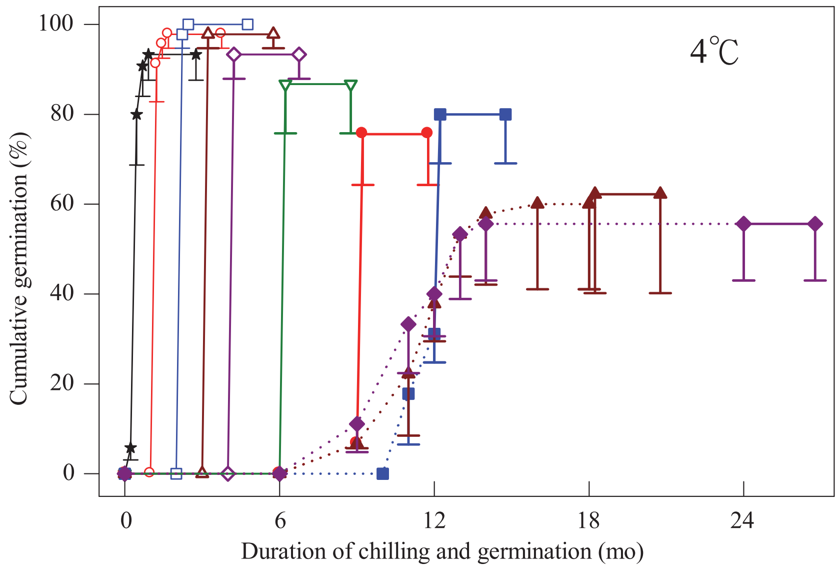

Fig. 4. Cumulative germination percentages of seedlot 1 seeds of *Maackia taiwanensis* at 4℃ moist stratification for 0~24 mo (○, 1 mo; □, 2 mo; △, 3 mo; ◇, 4 mo; ▽, 6 mo; ●, 9 mo; ■, 12 mo; ▲, 18 mo; ◆, 24 mo). Dotted lines (---) represent the cumulative germination percentages in the dark with 4℃ moist stratification, while solid lines (—) represent the cumulative germination percentages at fluctuating temperatures of 30/20℃ with 8 h of light after moist stratification. Filled stars (★) represent the cumulative germination percentage of fresh mature seeds. Vertical bars represent the standard error of the mean.

which showed no significant differences (p = 0.52, 0.08, and 0.053) compared to fresh mature seeds. The germination percentage continued to decrease to 58.9% and presented a significant difference ($p = 0.02$) after 24 mo of stratification, and seeds completely germinated under moist dark conditions during 6~14 mo (Figs. 3, 5). Moreover, the MGTs of the seeds were 7.0~7.6 d after stratification at 4℃ for 1~12 mo. Significant decreases in MGTs ($p < 0.001$) were found after the above stratification duration compared to fresh mature seeds (14.8 d) (Fig. 3).

Effects of MC, storage temperature, and storage duration on seed longevity

Seeds of *M. taiwanensis* can tolerate desiccation and sub-zero temperatures, so they show orthodox storage behavior. Figure 6 shows the effects of different MC levels and storage temperatures on the germination percentage of seeds of *M. taiwanensis* of seedlot 1 with dry storage for 0~18 mo. Once the 3 desired MC levels were reached, seeds were immediately incubated under fluctuating temperatures of 30/20℃ for 12 wk. Germination percentages of these seeds at MCs of 2.6, 5.3, and 10.3% were 97.8, 84.4, and 95.6%, respectively. There was no significant difference ($p > 0.2$) in germination compared to fresh mature seeds (93.3%). During storage at -196℃, seeds at MCs of 2.6, 5.3, and 10.3% maintained germination percentages of 80.0~97.8, 84.4~97.8, and 88.9~100% within 18 mo, respectively. There was no significant decline in germination compared to fresh mature seeds after 18 mo of storage ($p > 0.1$). During storage at -20℃, germination percentages were 93.3, 86.7, and 80.0% at MCs of 2.6, 5.3, and 10.3%

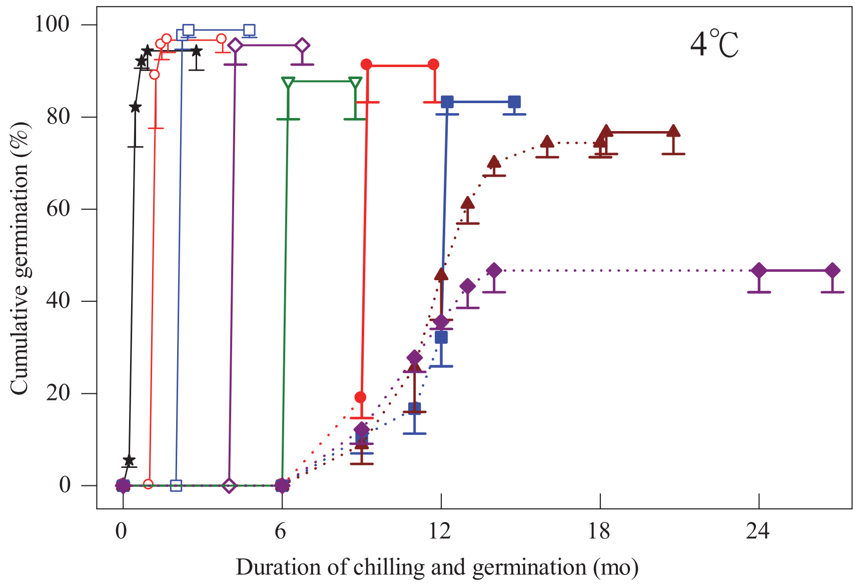

Fig. 5. Cumulative germination percentages of seedlot 2 seeds of *Maackia taiwanensis* at 4℃ moist stratification for 0~24 mo (○, 1 mo; □, 2 mo; ◇, 4 mo; ▽, 6 mo; ●, 9 mo; ■, 12 mo; ▲, 18 mo; ◆, 24 mo). Dotted lines (---) represent the cumulative germination percentages in the dark with 4℃ moist stratification, while solid lines (—) represent the cumulative germination percentages at fluctuating temperatures of 30/20℃ with 8 h of light after moist stratification. Filled stars (★) represent the cumulative germination percentage of fresh mature seeds. Vertical bars represent the standard error of the mean.

after 18 mo of storage, respectively. There was no significant difference in germination compared to fresh mature seeds ($p > 0.1$). During storage at 4℃, no significant difference in germination was revealed among seeds at the 3 MC levels compared to fresh mature seeds ($p > 0.1$), and germination percentages of these seeds at MCs of 2.6, 5.3, and 10.3% after 18 mo of storage were 93.3, 91.1, and 77.8%, respectively (Fig. 6). Survival of seeds at the 3 MCs exhibited no significant difference ($p > 0.05$) for the 3 storage temperatures (Fig. 6).

Figure 7 shows the effects of different MC levels and storage temperatures on germination percentages of seeds of *M. taiwanensis* of seedlot 2 after dry storage for 0~24 mo. After the drying process was completed, freshly dried seeds with 5 MC levels were incubated under fluctuating temperatures of 30/20℃ for 12 wk. Germination percentages of these seeds at MCs of 2.5, 4.9, 7.8, 11.2, and 14.5% were 96.7, 94.4, 94.4, 92.2, and 93.3%, respectively. No significant difference ($p > 0.5$) in germination was found compared to fresh mature seeds (94.4%). When stored at -20℃, seeds at an MC of 14.5% had a sharp decline in germination after 3 mo and had significantly decreased to 65.6% after 6 mo of storage ($p < 0.03$), and totally died after 15 mo of storage. In addition, germination percentages of the other 4 lower MC seeds at MCs of 2.5, 4.9, 7.8 and 11.2% with 24 mo of storage were 95.6, 92.2, 92.2, and 83.3%, respectively, and no significant decrease ($p > 0.05$) in germination was found (Fig. 7). After storage at 4℃ for 0~24 mo, viability of seeds at an MC of 14.5% began to decrease after 6

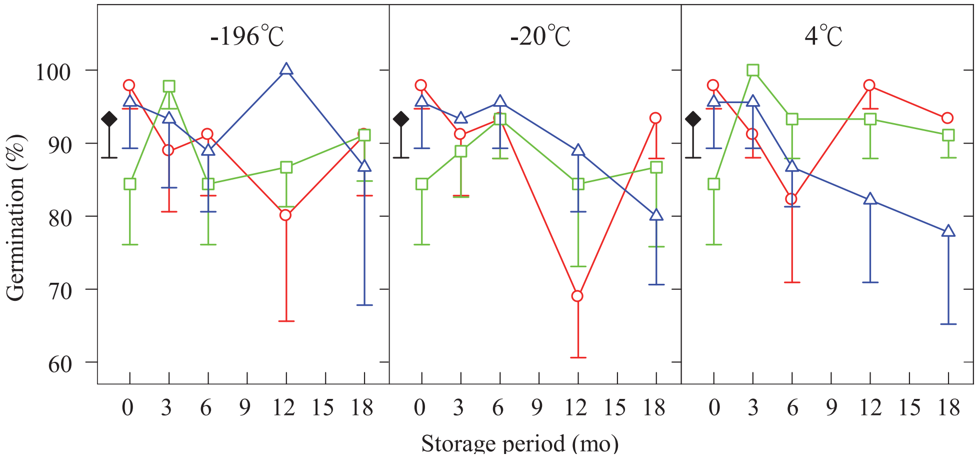

Fig. 6. Effects of storage temperatures (-196, -20, and 4℃) and moisture contents (MCs) (2.6~10.3%, on a fresh-weight basis) on germination percentages of seedlot 1 seeds of *Maackia taiwanensis* stored for up to 18 mo. No difference (p > 0.05) in seed survival at the 3 MC levels was observed for the 3 temperatures. The initial germination percentage of fresh mature seeds was 93.3±5.3% (◆). MCs of seeds: ○, 2.6±0.0%; □, 5.3±0.3%; △, 10.3±0.4%. Vertical bars represent the standard error of the mean.

mo, and had significantly decreased to 85.6% after 9 mo of storage (p = 0.048), and the germination percentage continued to decrease to 55.6% after 24 mo of storage. In addition, seeds at an MC of 11.2% maintained germination percentages of 86.7~92.2% within 18 mo and showed no significant difference (p > 0.3) with fresh mature seeds, and had significantly decreased to 85.6% after 24 mo of storage (p = 0.048). Moreover, there was no significant difference (p > 0.5) in germination of seeds at the other 3 lower MC levels compared to fresh mature seeds after 24 mo of storage, and their germination percentages at MCs of 2.5, 4.9, and 7.8% were 92.2±1.6, 92.2±6.3, and 92.2±1.6%, respectively (Fig. 7). The germination result of seeds stored at 15℃ was similar to that at 4℃, but the viability of the seeds at MCs of 14.5 and 11.2% decreased faster when stored at 15℃, germination percentages of seeds at an MC of 14.5% quickly decreased after 3 mo, and there was a significant reduction in germination of 72.2% (p < 0.03) after 6 mo of storage, which continued to decline to 36.7% after 24 mo of storage. In addition, seed germination at an MC of 11.2% exhibited a significant decrease to 72.2% (p = 0.02) after 12 mo of storage, and continued to decrease to 60.0% after 24 mo of storage. Survival of seeds at the 5 MCs significantly differed (p < 0.0001), but no significant difference (p > 0.05) was found if seeds of sub-seedlots at MCs of 11.2 and 14.5% were excluded from the analysis for the 3 storage temperatures (Fig. 7).

DISCUSSION

Hong and Ellis (1996) used 2 parameters, the thousand-seed weight and MC, to categorize seed storage behaviors of species they examined. Their samples were collected from mature seeds of 94 species of 5 families, including the Aceraceae, Araucariaceae, Dipterocarpaceae, Fagaceae, and Myrtaceae. The findings of their study clarified seed storage behaviors as follows. MCs of mature recalcitrant seeds range 36~90%. Mature seeds at

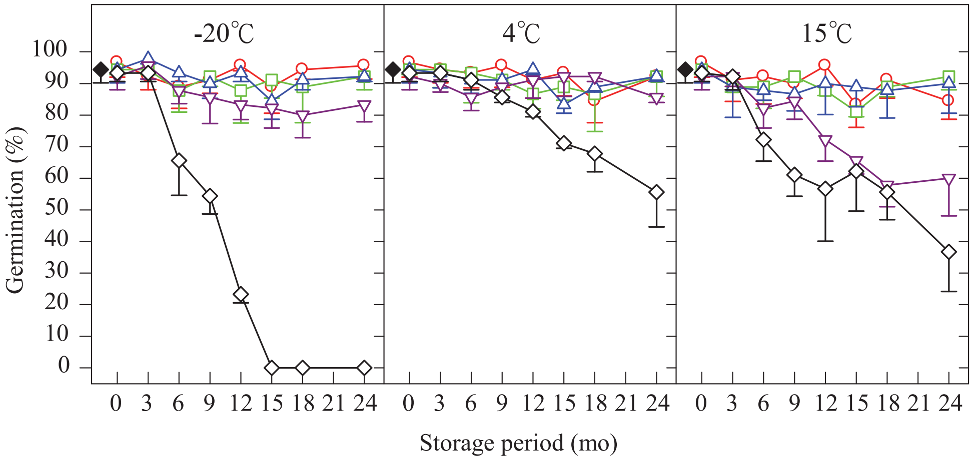

Fig. 7. Effects of storage temperatures (-20, 4, and 15℃) and moisture contents (MCs) (2.5~14.5%, on a fresh-weight basis) on germination percentages of seedlot 2 seeds of *Maackia taiwanensis* stored for up to 24 mo. Survival of seeds at the 5 MC levels significantly differed (p < 0.001), but no difference (p > 0.05) was observed when seeds of sub-seedlots at MCs of 11.2 and 14.5% were excluded from the analysis for all temperatures. The initial germination percentage of fresh mature seeds was 94.4±4.2% (◆). MCs of seeds: ○, 2.5±0.1%; □, 4.9±0.1%; △, 7.8±0.2%; ▽, 11.2±0.1%; ◇, 14.5±0.2%. Vertical bars represent the standard error of the mean.

an MC of < 35% are not seen as recalcitrant seeds, and those at an MC of < 23% are orthodox seeds. In addition, mature seeds at MCs of 23~55% are intermediate seeds. Furthermore, a thousand-seed weight of < 25 g is classified as orthodox, while a weight above 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 *M. taiwanensis* were considered orthodox seeds, e.g., the MC of fresh mature seeds was about 35~40%, and the thousand-seed weight was about 72~83 g (Table 1). For this species, the relation of the MC and thousand-seed weight with the seed storage behavior was not completely consistent with Hong and Ellis's criteria (1996). As far as seeds with orthodox storage behavior are concerned, mature seeds of *M. taiwanensis* had a still higher MC.

Because most *Maackia* seeds have hard seedcoats, pretreatments, such as scarification, soaking in hot water or acid treatment, are required to achieve germination in a short period (Young and Young 1992). Results of this study show that mature seeds of *M. taiwanensis* have no hardseedness so as to be non-dormant. No pretreatment is needed to make the seeds germinate within 4 wk. This result is very different from that of seeds with hard seedcoats, as demonstrated by *M. amurensis* and *M. chinensis* (Dirr and Heuser 1987). This is a new morphological evidence to prove that *M. taiwanensis* is a single isolated species in the genus *Maackia*.

Results of this study showed that stratification at 4℃ did not improve the germination percentage but significantly decreased the MGTs of seeds of *M. taiwanensis*. Although the MGT of the fresh mature seeds significantly dropped from about 15 d to about 8 d after only 1 mo of stratification at 4℃, pretreatment with stratification at 4℃ of *M. taiwanensis* is not practical for nursery operations due to this species quick germination characteristic. Moreover, under low-temperature moist storage for more than 6 mo, seeds of *M. taiwanensis* began to germinate under moist dark conditions, and all seeds germinated within 6~14 mo (Figs. 4, 5). Consequently, low-temperature moist storage is inefficient for storing seeds of *M. taiwanensis*.

In conclusion, effects of storage at 3 temperatures (-196, -20, and 4℃) and 3 different MC levels (2.6, 5.3, and 10.3%) within 18 mo and at 3 temperatures (-20, 4, and 15℃) and 5 different MC levels (2.5, 4.9, 7.8, 11.2, and 10.3%) within 24 mo on seed viability presented similar results for the 2 seedlots. Therefore, seeds of *M. taiwanensis* dried to 2.5~7.8% MCs still maintained viability after 24 mo of storage at -20, 4, and 15℃; i.e., seeds of this species can tolerate desiccation and sub-zero temperatures. Therefore, we confirm that seeds of *M. taiwanensis* have orthodox storage behavior (Hong and Ellis 1996). According to Young and Young (1992), dry seeds of *Maackia* store well at low temperatures, and their results were the same as ours.

Generally speaking, when seeds at an MC of > 15% are stored at sub-zero temperatures, their free water turns into ice crystals which causes freeze damage to seeds (Tompsett 1985). Before sub-zero storage, MCs should be decreased to < 14% in case the available water in cells freezes and causes membranes to break. This is linked to seed deterioration (Copeland and McDonald 1995). Thus, decreasing the MC keeps seeds from the risk of freeze damage. This study showed that seeds of *M. taiwanensis* are orthodox; however, when the seed MC exceeded a specific level, seeds in frozen storage showed obvious deterioration, and more quickly died than those stored at 4 and 15℃ (Fig. 7). We inferred that the value of the MC at which seeds of *M. taiwanensis* suffered freeze damage was about 14%. Apparently, the ability of *M. taiwanensis* seeds to protect against freeze damage was weaker than that of other orthodox seeds. In this study, seeds of seedlot 2 at an MC of 14.5% sharply deteriorated after 6 mo of storage at -20℃ (Fig. 7). Thus, we speculated that when seeds of *M. taiwanensis* at MCs of > 12% are stored at sub-zero temperatures, freeze damage will occur. As the MC increased, the harmful effect of freeze damage was simultaneously strengthened in a short time (Fig. 7). Many species have similar characteristics, including *Chionanthus retusus* Lindl. & Paxt. (Yang and Lin 2004), *Sapium discolor* Muell.-Arg. (Yang et al. 2006a), *Celtis sinensis* Pers. (Yang et al. 2006b), *Scolopia oldhamii* Hance (Yang et al. 2007), and *Scaevola taccada* (Gaertner) Roxb. (Yang and Kuo 2018). When the MCs of these 5 species stored at -20℃ exceeded 8, 9, 9, 11, and 11%, respectively, they suffered freeze damage and rapidly died. In this study, seeds of *M. taiwanensis* at MCs of 2.5~7.8% maintained good viability after storage at -20℃ for 2 yr. One reason might be that the period of our test was not long enough, such that the effects of freeze damage on seeds at slightly higher MCs of 10.3% (seedlot 1) and 7.8% (seedlot 2) did not emerge within 2 yr. In conclusion, when seeds of *M. taiwanensis* need to be stored at sub-zero temperatures for a long time, we recommend following the rules of FAO/IPGRI (1994). The MC of orthodox seeds should be 3~7% to avoid excess moisture causing seed deterioration. If seeds of *M. taiwanensis* need short-term storage within 2 yr, decreasing the MC to < 11% and storing seeds at 4℃ are suggested, which will not decrease the germination percentage (Figs. 6, 7).

CONCLUSIONS

Seeds of *M. taiwanensis* exhibited no dormancy. Under fluctuating temperatures of 30/20℃ with 8 h of light, all viable seeds germinated completely within 4 wk. Stratification at 4℃ did not efficiently improve the germination percentage but significantly decreased the mean germination times (MGTs). Pretreatment by stratification at 4℃ of *M. taiwanensis* is not practical for nursery operations due to this species' quick germination characteristic, although MGTs were obviously reduced. Seeds of *M. taiwanensis* dehydrated to 2.5~7.8% MCs still maintained viability after storage at -20, 4, and 15℃ for 2 yr. Results showed that seeds of *M. taiwanensis* have orthodox storage behavior, and a long-term seed storage strategy can be adopted for ex situ conservation and future restoration demands of this endemic and endangered tree species of Taiwan. We recommend that seeds of M. taiwanensis be dehydrated to 3~7% MCs and then hermetically sealed before long-term sub-zero storage. When seeds of *M. taiwanensis* need short-term storage within 2 yr, the MC of seeds should be decreased to < 11% and then stored at 4℃ after being hermetically sealed.

ACKNOWLEDGEMENTS

Thanks go to Chiung-Mei Lee, Feng-Yi Tseng, Hui-E Shen, Jie-Ru Jiang, Mei-Hua Lin, Shu-Mei Li, Wan-Zhen Li, Yi-Ying Tseng, and Ze-Yi Jiang for technical assistance.

LITERATURE CITED

Bonner FT. 1990. Storage of seeds: potential and limitations for germplasm conservation. For Ecol Manage 35:35-43.

Chen KW, Tsou CC, Liou JY, Lo HC. 2009. Embryogenesis in *Maackia taiwanensis* Hoshi et Ohashi. J Exp For Nati Taiwan Univ. 23:47-69. [in Chinese with English summary].

Copeland LO, McDonald MB. 1995. Seed science and technology. 3rd ed. New York: Kluwer Academic Publishers. p 189.

Dirr MA, Heuser CW. 1987. The reference manual of woody plant propagation. Athens, GA: Varsity Press. p 149.

Ellis RH, Hong TD, Roberts EH. 1990a. An intermediate category of seed storage behaviour? J Exp Bot 41:1167-74.

Ellis RH, Hong TD, Roberts EH. 1990b. Effect of moisture content and method of rehydration on the susceptibility of pea seeds to imbibition damage. Seed Sci Technol 18:131-7.

FAO/IPGRI. 1994. Genebank standards. Rome: Food and Agricultural Organization of the United Nations (FAO)/International Plant Genetic Resources Institute (IPGRI). p 141.

Hartmann HT, Kester DE, Devies ET. 1989. Plant propagation: principals and practices. 5th ed. Englewood Cliffs, NJ: Prentice-Hall. 647 p.

Hong TD, Ellis RH. 1996. A protocol to determine seed storage behavior. IPGRI Technical Bulletin no. 1. Rome: International Plant Genetic Resources Institute (IPGRI). 62 p.

Huang TC, Ohashi H. 1993. Leguminosae. In: Editorial Committee of the Flora of Taiwan. Flora of Taiwan. 2nd ed, Vol. 3, Taipei, Taiwan: Department of Botany, National Taiwan University. p 160-396.

International Seed Testing Association. 1999. International rules for seed testing. Rules 1999. Seed Sci Technol 27(Suppl):1-333.

Lin TP, Chen MC. 1993. Desiccation intolerance in seeds of *Machilus kusanoi* Hay. Taiwan For Res Inst New Series 8(2):143-7. [in Chinese with English summary].

Lu SU, Lin MJ. 1996. Rare and endangered plants in Taiwan (I). Taipei, Taiwan: Council of Agriculture. 163 p. [in Chinese].

Roberts EH. 1973. Predicting the storage life of seeds. Seed Sci Technol 1:499-514.

Tompsett PB. 1985. The influence of moisture content and temperature on the viability of *Shorea almon*, *S. robusta* and *S. roxburghii* seeds. Can J For Res 15:1074-9.

Tompsett PB. 1987. A review of literature on storage of dipterocarp seeds. Forest Seed Problems in Africa. Umea, Sweden: Department of Forest Genetics, Swedish Univ. Agricultural Science. p 348-65.

Tompsett PB. 1989. Predicting the storage life of orthodox tropical forest tree seeds. Tropical tree seed research. ACIAR proceeding no. 28. Canberra, Australia: Australian Centre for International Agricultural Research (ACIAR). p 93-8.

Yang JC, Kuo SR. 2018. Germination and seed storage behavior of *Scaevola taccada* (Gaertner) Roxb. Taiwan J For Sci 33(1):1-14.

Yang JC, Kuo SR, Lin TP. 2006b. Seed storage behavior and germination of fragrant manjack (*Cordia dichotoma* G. Forst.) and Chinese hackberry (*Celtis sinensis* Pers.). Taiwan J For Sci 21(4):447-59. [in Chinese with English summary].

Yang JC, Kuo SR, Lin TP. 2007. Germination and storage behavior of seeds of *Litsea garciae*, *Diospyros philippensis*, *Myristica ceylanica* var. *cagayanensis*, and *Scolopia oldhamii*. In a collection of papers for the 2nd Symposium of Forest Management for Environmental Protection of Chungpu Research Center. p 51-68. [in Chinese with English summary].

Yang JC, Lin TP. 2004. Effects of seed moisture content and storage temperature on the storage behavior of seeds of Chinese fringetree (*Chionanthus retusus* Lindl. & Paxt.). Taiwan J For Sci 19(3):247-58. [in Chinese with English summary].

Yang JC, Lin TP, Kuo SR. 2006a. Seed storage behavior of *Sapium discolor* Muell.-Arg. and *Bischofia javanica* Blume. Taiwan J For Sci 21(4):433-45. [in Chinese with English summary].

Young JA, Young CG. 1992. Seeds of woody plants in North America. Portland, OR: Dioscorides Press. p 360.