Research paper

Seed Germination and Storage of *Cyclobalanopsis gilva* (Bl.) Oerst. (Fagaceae)

Jeng-Chuann Yang[1,2)]

【Summary】

Cyclobalanopsis gilva is a native large broadleaf tree species that has potential for afforestation in Taiwan. The purpose of this study was to examine the germination characteristics and seed storage behavior of *C. gilva* so as to determine efficient pretreatment for germination and an appropriate seed storage method. Results showed that some mature seeds of *C. gilva* had shallow dormancy, and the dormancy could be broken by 1~15℃ stratification. Fresh mature seeds of *C. gilva* had a little bit slow germination and took about 12 wk to completely germinate with a mean germination time (MGT) of about 42 d under fluctuating temperatures of 30/20℃ with 8 h of light. Stratification at 1~15℃ for longer than 1 mo completely broke the dormancy of *C. gilva* seeds, and significantly decreased the MGT in a germination period of 12 wk. The MGT of fresh mature seeds significantly decreased from about 42 d to 15 d after 4 mo of stratification at 4℃. Thus, we consider that a proportion of *C. gilva* seeds may have physiological dormancy. Therefore, pretreatment with 4℃ stratification for the germination of *C. gilva* seeds is practical for nursery operations. We suggest that pre-chilling at 4℃ for 4 mo should be applied to fresh mature seeds of *C. gilva*. Furthermore, fresh mature seeds of *C. gilva* were extremely sensitive to desiccation. Seeds completely lost viability when the moisture content dropped below 24%. However, *C. gilva* seeds maintained their total initial viability for up to 1 yr when stored at 1~4℃ with moist sphagnum. Thus, seeds of *C. gilva* 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 *C. gilva* are moist storage at 1℃ for fresh mature seeds, and the initially living seeds still had germinability for up to 1.5 yr.

Key words: *Cyclobalanopsis gilva* (Bl.) Oerst, germination, temperate-recalcitrant, seed storage, stratification.

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研究報告

赤皮種子的發芽與儲藏

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摘 要

赤皮(*Cyclobalanopsis gilva* (Bl.) Oerst.)是臺灣具有造林潛力的原生闊葉大喬木。本研究目的在瞭解赤皮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。結果顯示赤皮種子有些稍具休眠性，以1~15℃低溫層積可以解除其休眠。鮮採的赤皮成熟種子在30/20℃變溫條件下發芽有些緩慢，約需12週才能全部完成發芽，平均發芽日數約42日。在12週的發芽期計算基礎下，1~15℃層積經1個月後就能完全解除種子休眠，顯著降低其平均發芽日數。經4℃層積4個月後，赤皮種子的平均發芽日數從新鮮種子的約42日顯著下降至約15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。赤皮種子對乾燥非常敏感，當種子含水率被降到24%以下時就死亡殆盡，但當其以水草濕藏在1~4℃ 時，於1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。儲藏赤皮種子的最好方法是將新鮮種子以1℃濕藏之，於1.5年內仍能保持原有的種子活力。

關鍵詞：赤皮、發芽、溫帶異儲型、種子儲藏、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). Orthodox seeds can tolerate desiccation and survive moisture contents (MCs) (all MC values in this study are on a fresh-weight (FW) basis) 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 contrast to orthodox seeds, recalcitrant seeds have a low desiccation tolerance (Tompsett 1987, 1989). When recalcitrant seeds are dried to < 12~31% MCs, 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 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.

Cyclobalanopsis gilva (Bl.) Oerst, is an evergreen tree up to 40 m high in the Fagaceae family. This species is distributed in Japan, China, and Taiwan. *Cyclobalanopsis gilva* is scattered in forests at elevations of 250~1500 m in the northern and central parts of the main island of Taiwan (Liao 1996). Having the advantage of large straight timber and high wood density, *C. gilva* is used to produce valuable hardwood for making furniture; thus, it could become a potential broadleaf tree species for afforestation in Taiwan. *Cyclobalanopsis gilva* usually blooms in March to May. The maturation period of nuts is from mid-October to late-November, and there is an abundant harvest about every 3~4 yr. The ovoid nuts of this species are about 1.7 cm long and 1.2 cm in diameter. The seeds turn from green to brown when mature (Fig. 1).

Over the years, very few reports have researched the germination and storage of *C. gilva* seeds. For seeds of *C. gilva* with a short life expectancy, recalcitrant seed storage behavior is noticeable, and they can maintain 1-yr lifespan during wet storage at low temperature (Lin, 1995). Seeds of *C. gilva* begin to germinate when new proteins are made after imbibition, and unsuccessful germination is mainly due to a lack of energy (Zaynab et al. 2017, 2021). Consequently,

Fig. 1. Mature fruits and seeds of *Cyclobalanopsis gilva*.

a careful research design was used to investigate the germination and storage behaviors of *C. gilva* seeds in this study. Furthermore, the results extend the earlier work offering useful information about nursery operations for restoration and seed storage for conservation programs of this important native Taiwanese tree species.

MATERIALS AND METHODS

Seed collection and processing

Table 1 gives detailed collection information of the seedlot used in this study. About 9800 seeds were collected from a single mother tree in 2017, a year of seed abundance. Mature nuts of the seedlot 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 this seedlot comprised < 1%. Seeds were extracted and washed on 25 November 2017. Among pure seeds with no debris left, small-sized and damaged seeds were manually removed. Seed characteristics of fresh mature seeds of the seedlot are also given in Table 1.

Determination of the MC

Seed MCs were determined gravimetrically with 4 replicates. For each replicate, 2 filled seeds were randomly selected and cut into pieces of < 4 mm in length, and 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 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

Table 1. Information on fruit collection date and source, seed characteristic, and germination of the seedlot of fresh mature seeds of *Cyclobalanopsis gilva*

Collection date	23 Nov. 2017
Collection location	Jiamshih Township, Hsinchu County
Latitude, longitude	24°40’N, 121°17’E
Elevation (m)	1494
Moisture content (%, FW[1)] basis)	42.8±0.9
Seed length (mm)	17.4±1.0
Seed width (mm)	12.4±0.6
Seed thickness (mm)	12.3±0.6
Number of seed L^{-1}	498±6
Thousand-sees-weight[2)] (g)	1475.9±23.4
Germination percentage (%)	97.8±1.6
Mean germination time (d)	42.5±0.2

[1)] FW, Fresh-weight.

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

and seeds for storage, 30 seeds 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 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 = 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 and naked storage treatments of fresh seeds at low temperatures

Fresh seeds of *C. gilva* were stored in a moist and naked condition at low-temperatures (1, 4, 10, and 15℃) with storage durations of 1, 2, 3, 4, 6, 9, 12, 18, 24, and 36 mo. Seeds being stored in moist sphagnum at a low temperature is essentially the same as cold stratification. These results were used to understand the effects of low-temperature moist storage on seed viability and the impact of low-temperature naked dry storage on fresh seeds at the initial MC (42.8%) of *C. gilva*. 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, 10 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.

Determination of MCs of dehydrated seeds

Sub-seedlots exhibited different MC levels, and the ranges were about 18~43% for the seedlot studied. When a sub-seedlot reached the desired MC during the dehydration process, seeds were placed in an aluminum foil bag and stored at 4℃ 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 seedlot 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. The MC of fresh mature seeds was 42.8±0.9%. After dehydration treatment, the 5 MC levels were controlled to 18.4±0.8% (6.1 d of desiccation), 24.3±0.6% (4.2 d of desiccation), 30.9±0.9% (1.7 d of desiccation), 37.3±0.3% (0.5 d of desiccation), and 42.8±0.9% (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 seedlot were statistically analyzed by variables implemented using Tukey's test of the general linear model (GLM) procedure of R statistical software (The R foundation).

RESULTS

Germination of fresh mature seeds

Seeds of the seedlot of *C. gilva* exhibited kind of dormancy. When fresh seeds were incubated at fluctuating temperatures of 30/20°C for 12 wk, their germination percentage was 97.8±1.6% and MGT was 42.5±0.2 d (Table 1). Seeds mainly germinated during 4~7 wk, and there was no germination after 12 wk (Fig. 2). In addition, ungerminated seeds of the seedlot were found to have totally decayed when cut open after 12 wk.

Effects of stratification at 1, 4, 10, and 15℃ on germination and seed MCs

Seed germination percentages were 87.8, 74.4, 87.8, 93.3, 94.4, 98.9, 91.1, and 94.4% after stratification at 1℃ for 1, 2, 3, 4, 6, 9, 12, and 18 mo, respectively, while there were no significant differences in germination percentages ($p > 0.1$) compared to fresh seeds (97.8%), and a significant decrease in germination percentage (72.2%) was found after 24 mo ($p = 0.02$). Moreover, after wet storage at 1℃ for 36 mo, the germination percentage of seeds had continually declined to 11.1% ($p < 0.0001$). (Fig. 3).

After stratification at 4°C for 3, 4, 6, 9, and 12 mo, seed germination percentages were 97.8, 98.9, 100.0, 96.7, and 96.7%, respectively, while there were no significant differences in germination percentages ($p > 0.1$) compared to fresh seeds (97.8%) (Fig. 4). Seeds began to germinate in the dark after stratification at 4℃ for 12 mo, and almost all viable seeds had germinated under moist, dark conditions within 12~32 mo (Fig. 4). However, the total germination percentage within 36 mo of stratification at 4℃ showed no significant differences in germination ($p > 0.05$) compared to fresh seeds. For example, the total germination percentages of seeds with 18, 24, and 36 mo of stratification at 4℃ were 86.7, 90.0, and 85.0%, respectively, which showed no significant differences ($p = 0.1$, 0.07, and 0.14) compared to fresh seeds (Fig. 4). In addition, seed germination percentages were 86.7 and 85.6% after stratification at 4℃ for 1 and 2 mo, respectively, while these data

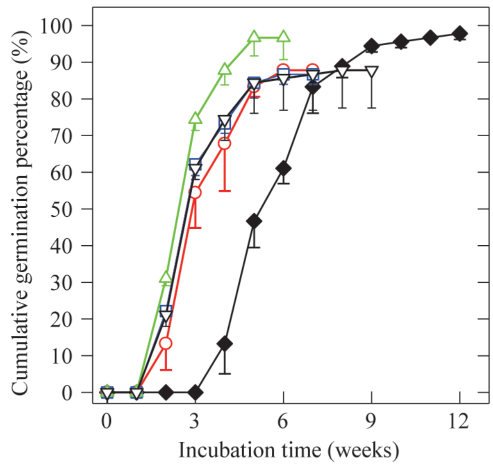

Fig. 2. Cumulative germination percentages of fresh mature seeds (◆), and seeds of *Cyclobalanopsis gilva* after stratification pretreatments at 1 (○), 4 (□), 10 (△), and 15°C (▽) for 1 mo.

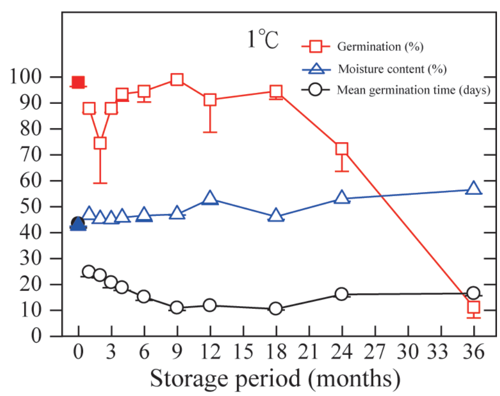

Fig. 3. Effects of moist storage at 1℃ for 1~36 mo on the germination percentage (□), mean germination time (○), and moisture content (△) of seeds of *Cyclobalanopsis gilva*. Filled squares (■), circles (●), and triangles (▲) respectively represent the germination percentage, mean germination time, and moisture content of fresh mature seeds. Vertical bars represent the standard error of the mean.

showed significant differences in germination percentages (p = 0.007 and 0.04). This might have been due to the higher heterogeneity of this seedlot and lower sampling density of the experimental design in this study.

The outcome of the germination at 10℃ stratification was similar to that at 4℃ The slight difference, however, was when stratified at 10℃, seeds germinated with 4 mo of stratification, which was earlier than with stratification at 4℃. Germination was 96.7~100.0% after stratification at 10℃ for 1~4 mo, while there were no significant differences in germination percentages (p > 0.1) compared to fresh seeds (97.8%) (Fig. 5). All viable seeds had germinated under moist, dark conditions within 4~22 mo (Fig. 5). However, the total germination percentage within 24 mo of stratification at 10℃ showed no significant differences in germination (p > 0.1) compared to fresh seeds. For example, the total germination percentages of seeds with 12, 18, and 24 mo of stratification at 10℃ were 98.9, 100.0, and 99.4%, respectively, which showed no significant differences (p = 0.52, 0.12, and 0.25) compared to fresh seeds (Fig. 5).

Seeds at 15℃ stratification began to germinate under moist, dark conditions much earlier than those at 4 and 10℃. All viable seeds had germinated in the dark within 2~12 mo (Fig. 6). Germination was 87.8 and 93.3% after stratification at 15℃ for 1, 2, and 3 mo, while there were no significant differences in germination percentages (p = 0.25, 0.73, and 1.0) compared to fresh seeds (97.8%) (Fig. 6). However, the total germination percentage within 12 mo of stratification at 15℃ showed no significant differences in germination (p > 0.1) compared to fresh seeds. For example, the total germination percentages of seeds with 4, 6, and 12 mo were 98.9, 97.8, and 99.7%, respectively,

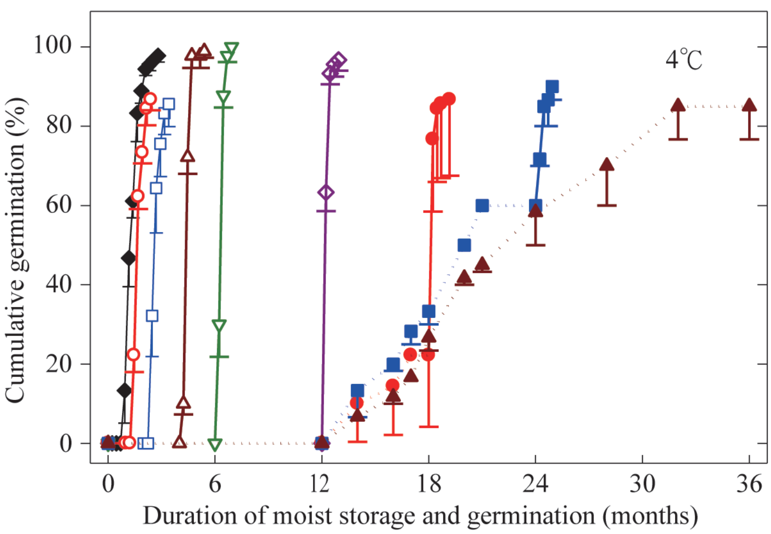

Fig. 4. Cumulative germination percentages of seeds of *Cyclobalanopsis gilva* at 4℃ moist storage for 1~36 mo (○, 1 mo; □, 2 mo; △, 4 mo; ▽, 6 mo; ◇, 12 mo; ●, 18 mo; ■, 24 mo; ▲, 36 mo). Dotted lines (- - -) represent the cumulative germination percentages in the dark with 4℃ moist storage, while solid lines (—) represent the cumulative germination percentages at fluctuating temperatures of 30/20℃ with 8 h of light after moist storage. Filled diamonds (◆) represent the cumulative germination percentage of fresh mature seeds. Vertical bars represent the standard error of the mean.

which showed no significant differences (p = 0.52, 1.0, and 0.16) compared to fresh seeds (Fig. 6).

In addition, stratification at 1, 4, 10, and 15℃ did not increase the germination percentage of seeds of *C. gilva* but significantly decreased the MGTs in the germination period of 12 wk. After stratification at 1, 4, 10 and 15℃ for 1 mo, the MGTs of seeds were reduced to 24.6, 22.4, 21.0, and 22.9 d, respectively. Clearly, significant decreases in MGTs ($p < 0.0002$) were found compared to fresh seeds (42.5 d). The MGTs of seeds were 10.4~23.3, 9.6~21.2, 13.8~20.5, and 15.5~21.6 d after stratification at 1, 4, 10, and 15℃, respectively, in the following 2~36, 2~12, 2~4, and 2~3 mo. Significant decreases in MGTs ($p < 0.0001$) were found after the all above stratification durations compared to fresh seeds (42.5 d). Moreover, MCs of seeds were 45.0~56.4, 45.8~57.1, 44.4~46.5, and 44.2~45.8% after stratification at 1, 4, 10, and 15℃ for 1~18, 1~12, 1~4, and 1~3 mo, respectively. No significant differences in MCs ($p > 0.05$) were found after the above stratification durations compared to fresh seeds (42.8%).

Effects of naked storage at 1, 4, 10, and 15℃ on germination and seed moisture contents of fresh seeds

Fresh seeds of *C. gilva* rapidly lost their viability when stored naked at 1, 4, 10, and 15℃. They all died within 6 mo. In addition, the higher the storage temperature at which they were maintained, the shorter was the seed longevity they exhibited (Fig. 7).

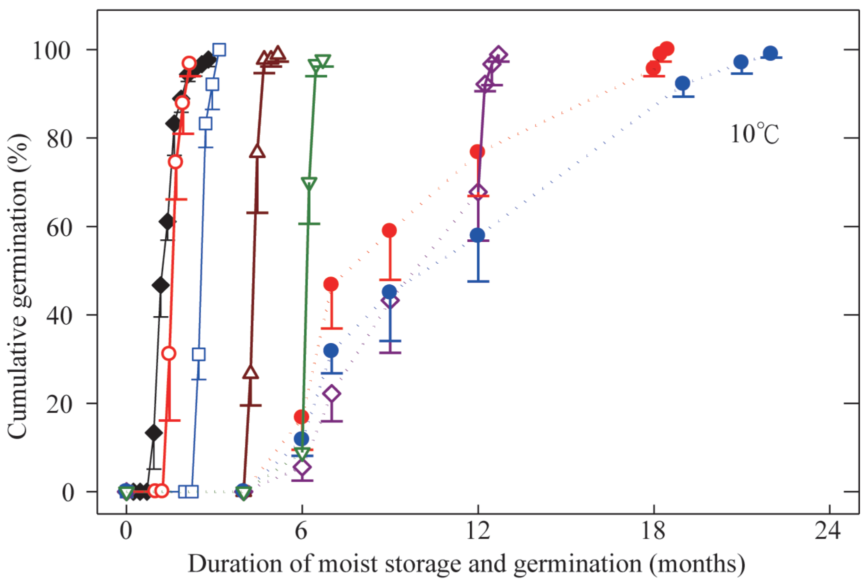

Fig. 5. Cumulative germination percentages of seeds of *Cyclobalanopsis gilva* at 10℃ moist storage for 1~24 mo (○, 1 mo; □, 2 mo; △, 4 mo; ▽, 6 mo; ◇, 12 mo; ●, 18 mo; ■, 24 mo). Dotted lines (- - -) represent the cumulative germination percentages in the dark with 10℃ moist storage, while solid lines (—) represent the cumulative germination percentages at fluctuating temperatures of 30/20℃ with 8 h of light after moist storage. Filled diamonds (◆) represent the cumulative germination percentage of fresh mature seeds. Vertical bars represent the standard error of the mean.

Seeds still maintained higher viability (91.1%, p = 0.1) within 1 mo of naked storage at 1℃. Nevertheless, after naked storage at 1℃ for 2 mo, the germination percentage of seeds had significantly declined to 50.0% (p = 0.006), and seeds totally died when the storage duration was extended to 4 mo. The decrease in the rate of seed viability with naked storage at 4℃ occurred more slowly than that of seeds stored at 1℃. The germination percentage dropped to 62.2% after 1 mo of naked storage at 4℃, and there was a significant difference in germination percentage (p = 0.000008). Besides, the seed germination percentage significantly decreased to 20.0% (p = 0.000003) after 2 mo of storage, and seeds had absolutely lost their viability after 6 mo (Fig. 7). In addition, MGTs with naked storage at 1℃ for up to 3 mo were 26.3~33.5 d while MGTs with naked storage at 4℃ were 26.2~33.2 d for up to 4 mo. Still, seeds did not completely all die, and a significant decrease in MGTs (p < 0.001) was found after the above storage duration compared to fresh seeds (42.5 d) (Fig. 7). Moreover, seeds maintained a similar water content at 1 and 4℃ naked storage for up to 6 mo, even when seeds had completely deteriorated. MCs of the seeds were 43.2~45.0 and 42.5~45.9% after naked storage at 1 and 4℃ for 1~6 mo, respectively. No significant difference in MCs (p > 0.05) was found after the above storage duration compared to fresh seeds (42.5%) (Fig. 7). Moreover, there was a more-rapid decline in germination percentage with 10 and 15℃ naked storage than with storage at 1 and 4℃. The germination percentage suddenly decreased to 18.9% after storage at 10℃ for

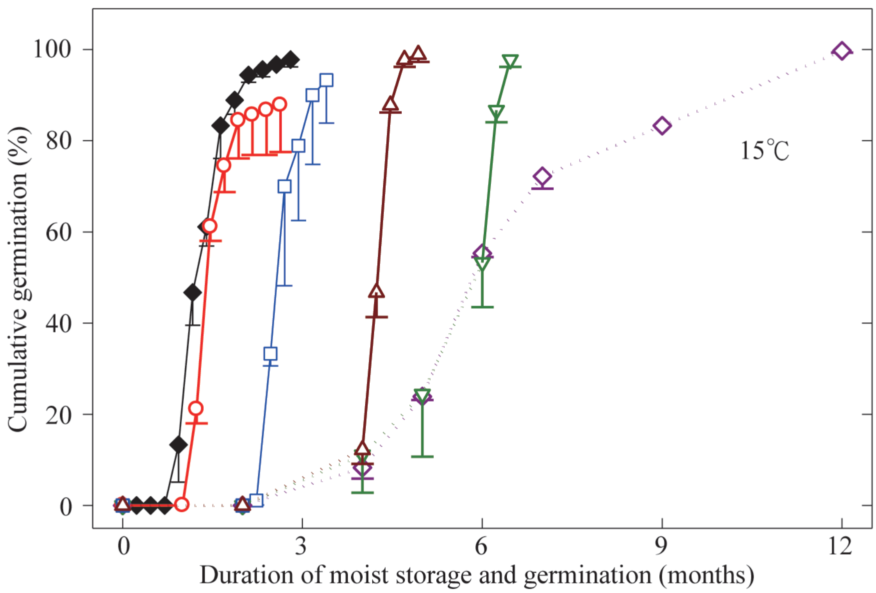

Fig. 6. Cumulative germination percentages of seeds of *Cyclobalanopsis gilva* at 15℃ moist storage for 1~12 mo (○, 1 mo; □, 2 mo; △, 4 mo; ▽, 6 mo; ◇, 12 mo). Dotted lines (- - -) represent the cumulative germination percentages in the dark with 15℃ moist storage, while solid lines (—) represent the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

1 mo, and all seeds immediately died the next month (Fig. 7). In addition, seeds had completely died within 1 mo when stored naked at 15℃ (Fig. 7).

Effects of seed moisture on viability

Seeds of *C. gilva* cannot tolerate desiccation. Most seeds died when the MC dropped to about 31%, and they completely lost viability when the MC was < 24%, so they show recalcitrant storage behavior. Figure 6 shows the effects of different MC levels on germination percentages of fresh mature seeds of *C. gilva*. Once the 5 desired MC levels of the seeds were reached, seeds were immediately incubated under fluctuating temperatures of 30/20℃ for 12 wk. Germination percentages of seeds at MCs of 18.4, 24.3, 30.9, 37.3, and 42.8% were 0, 1.1, 31.1 (p = 0.006), 95.6 (p = 0.5), and 95.6% (p = 0.5), respectively. The seed viability rapidly dropped when they dried out (Fig. 8).

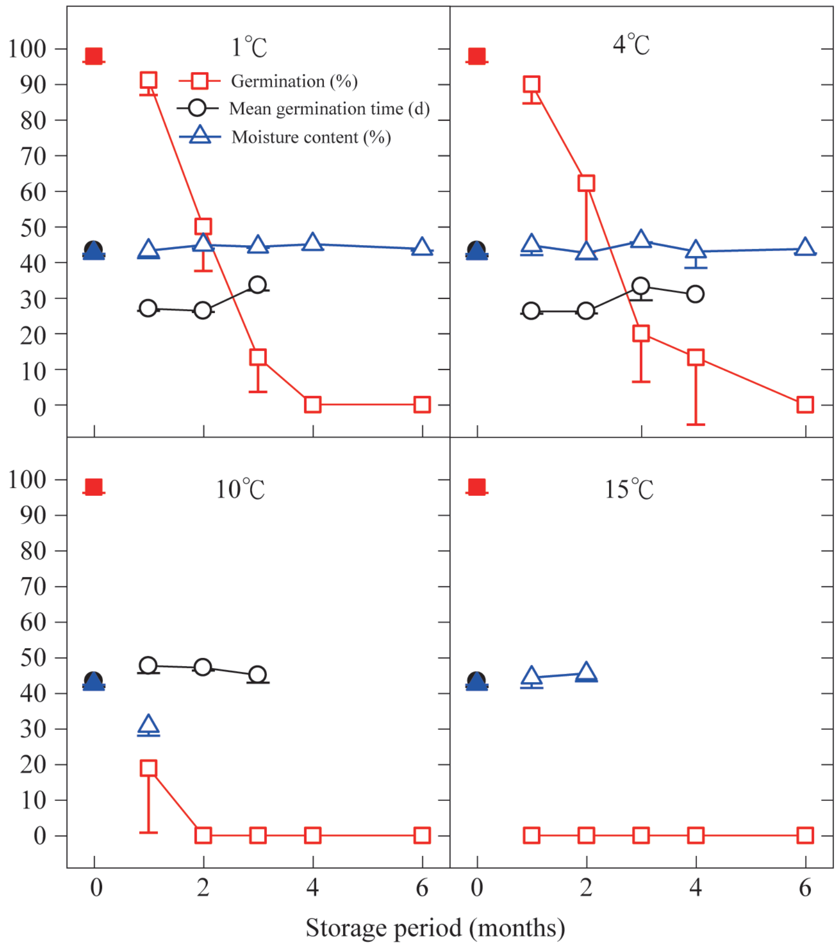

Fig. 7. Effects of 1~6 mo of naked storage at 1, 4, 10, and 15℃ on the germination percentage (□), mean germination time (○), and moisture content (△) of seeds of *Cyclobalanopsis gilva* at an initial moisture content of 42.8±0.9%. Filled squares (■), circles (●), and triangles (▲) respectively represent the germination percentage, mean germination time, and moisture content of fresh mature seeds. Vertical bars represent the standard error of the mean.

DISCUSSION

Results of this study showed that some fresh mature seeds of *C. gilva* had shallow dormancy because they had slightly slow germination, all viable seeds could not germinate during 30 days, and all viable seeds emerged during 3~12 wk, but took about 12 wk to complete germination with an MGT of about 42.5 d (Fig. 2). Above all, after stratification at 1, 4, 10, and 15℃ for more than 1 mo, significant decreases in the MGTs (< 25 d) were observed (Figs. 2). Therefore, some seeds of *C. gilva* are considered to exhibit slight dormancy, which can be broken by low-temperature stratification. We consider that a proportion of *C. gilva* seeds may have physiological dormancy with a non-deep level (Baskin and Baskin, 2014).

Stratification at 1~15℃ did not improve germination percentages but significantly decreased the MGTs of *C. gilva* seeds with a germination period of 12 wk. Furthermore, the MGT of fresh mature seeds significantly decreased from 42 d to about 15 d after 4 mo of stratification at 4℃ (Fig. 3). Therefore, pretreatment with about 4℃ stratification for germination of *C. gilva* seeds is practical for nursery operations. We suggest that prechilling at 4℃ for 4 mo should be applied to fresh mature seeds of *C. gilva* collected in November in order to break seed dormancy and maintain short-term storage. This is a good process for nursery operations the following March when the temperature is rising.

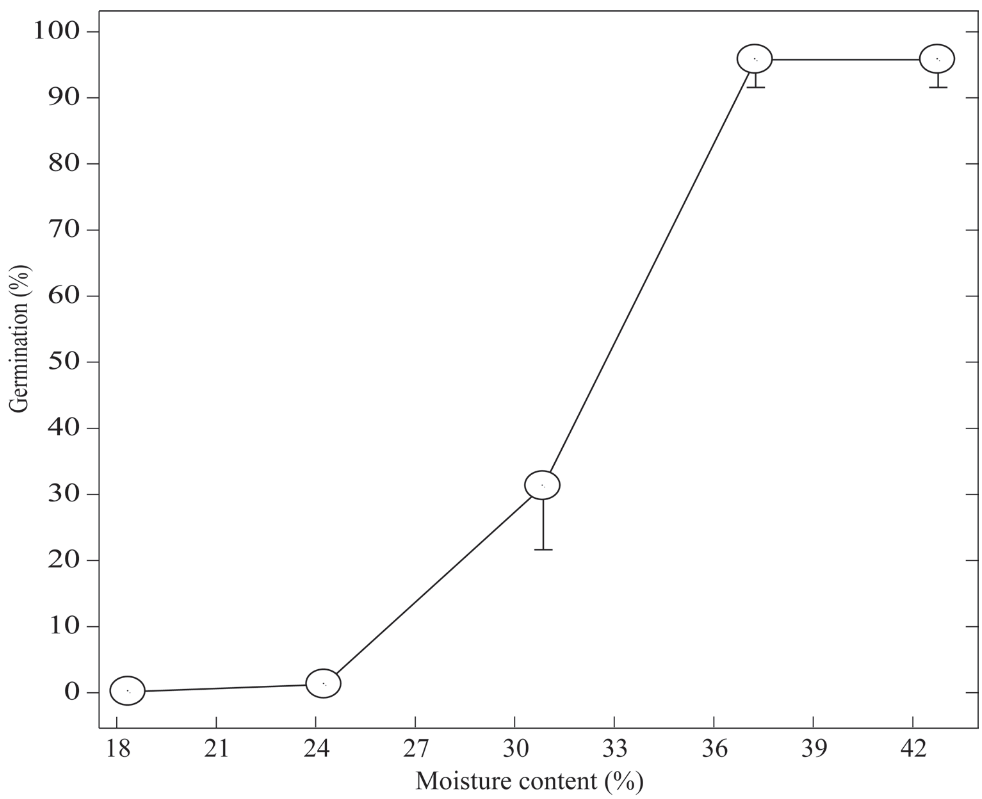

Fig. 8. Effects of moisture contents (%, on a fresh-weight basis) on the germination percentage of seeds of the seedlot of *Cyclobalanopsis gilva*. Vertical bars represent the standard error of the mean.

In this way, the best regular germination can be obtained, and seedlings will immediately continue growing after germinating.

Cyclobalanopsis gilva seeds showed the same desiccation intolerance as most of the Fagaceae in Taiwan such as seeds of *Cyclobalanopsis glauca* (Thunb.) Oerst., *C. morii* (Hay.) Schott., *Quercus spinosa* A. David (Lin 1995), and *Pasania glabra* (Thunb. *ex* Murray) Oerst (Yang and Kuo 2018). They all rapidly deteriorated when the MC of fresh mature seeds slightly decreased. However, seeds of *C. gilva* completely maintained their initial germinability when stored moist at 1~4℃ for 1 yr (Figs. 3, 4). Therefore, *C. gilva* seeds showed temperate-recalcitrant storage behavior due to their extreme intolerance of dehydration and high viability with low-temperature storage, so that it is impossible to apply long-term dry storage to *C. gilv*a seeds. In addition, seeds were found to have decayed and rapidly died after naked storage at 1~15℃ (Fig. 7), while seeds remained viable under 1 and 4℃ moist storage. However, seed viability was not well maintained for more than 1.5 and 1 yr when seeds were stored moist at 1 and 4℃, respectively (Figs. 3, 4). In other words, even when providing optimal storage conditions, the viability of *C. gilva* seeds could not be maintained beyond 1.5 yr. Therefore, it is unworkable to preserve the germplasm of *C. gilva* by storing intact seeds. Consequently, moist storage at 1℃ is efficient for the short-term storage of *C. gilva* seeds for up to 1.5 yr.

CONCLUSIONS

Some seeds of *C. gilva* exhibited shallow physiological dormancy. Fresh mature seeds of *C. gilva* could emerge during 3~12 wk, and viable seeds took 12 wk for complete germination under fluctuating temperatures of 30/20℃ with 8 h of light. Stratification at 4℃ did not efficiently improve the germination percentage but significantly decreased the MGT from about 42 to 15 d after 4 mo. Therefore, pretreatment with 4℃ chilling for germination of *C. gilva* is practical for nursery operations. We suggest that pre-chilling at 4℃ for 4 mo should be applied to fresh mature seeds of *C. gilva*. Furthermore, seeds of *C. gilva* were extremely sensitive to desiccation. Most seeds died when the MC dropped to about 31%; moreover, they completely lost viability when the MC dropped below 24%. However, *C. gilva* seeds maintained their initial viability when stored at 1℃ with moist sphagnum for up to 1.5 yr. Thus, seeds of *C. gilva* are defined as having temperate-recalcitrant storage behavior due to their extreme intolerance of dehydration and high viability with low-temperature storage, and a long-term seed storage strategy cannot be adopted for this species. Additionally, the optimal seed storage conditions of *C. gilva* are moist storage at 1℃ for fresh mature seeds, and the initially germinable seeds still had germinability for up to 1.5 yr.

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