Research paper

Intermediate Seed Storage Behavior of *Goniothalamus amuyon* (Blanco) Merrill

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【Summary】

Goniothalamus amuyon is a nationally critically endangered species in Taiwan. The purpose of this study was to understand the germination characteristics of *G. amuyon* seeds and determine its seed storage behavior. The optimum seed storage conditions are also given for *ex situ* conservation. Under fluctuating temperatures of 30/20℃ with 8 hours of light for 16 weeks, the germination percentages of fresh mature seeds of the 2 seedlots were 91.7±2.4 and 91.7±4.7%, and the mean germination times were 54.4±2.6 and 59.9±2.0 days, respectively. They mainly emerged during 6~10 weeks, and all viable seeds of the 2 seedlots had completely germinated within 13 weeks. Results show that fresh mature seeds of *G. amuyon* of the 2 seedlots exhibited dormancy and had a slow germination speed. Moreover, 4℃ stratification could not break seed dormancy, and obvious seed deterioration emerged when the stratification period exceeded 2 months, and they all died after 6 months in a moist, low-temperature condition. Thus, a longer incubation period at 30/20℃ for up to 3 months is recommended for germination of seeds of *G. amuyon* before breaking dormancy can be effectively accomplished. Seeds of *G. amuyon* can tolerate desiccation to about a 6% moisture content (MC) and did not lose any germinability, and 89% (seedlot 1) and 67% (seedlot 2) of seeds at an MC of about 6% maintained their viability after 2 years of storage at 4℃. However, all seeds died when they were dried to < 3.5% MC. Moreover, seeds at MCs of 6~13% rapidly lost their viability when stored at -196 and -20℃. These characteristics of moderate tolerance of desiccation and sensitivity to freezing temperatures while being preserved well at 4℃ show that *G. amuyon* seeds can be classified as having temperate intermediate storage behavior. Seed viability of *G. amuyon* is not easy to maintain, so sowing of ordinary cultivators immediately after being harvested is suggested because it is possible for the seeds to seriously deteriorate in a short time. It is difficult to preserve *G. amuyon* seeds for long-term *ex situ* conservation because of their intermediate storage behavior with a short lifespan. We recommend that fresh mature seeds of *G. amuyon* be controlled to 6~9% MCs and then hermetically sealed immediately before storage at 4℃. Moreover, we suggest that stored seeds of *G. amuyon* be renewed about every 2~3 years.

Key words: *Goniothalamus amuyon*, germination, seed storage behavior, intermediate, *ex situ* conservation.

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研究報告

恆春哥納香種子的儲藏性質為中間型

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摘　要

恆春哥納香為臺灣「國家極危」等級的植物種，本研究目的在明瞭其種子發芽特性與儲藏性質，並探究其最佳儲藏條件，希能提供精確的種子儲藏條件與方法以進行本種的區外保育。供試的二批新鮮成熟種子立即以30℃ (8小時光照) /20℃ (16小時黑暗) 變溫發芽，經16週後之發芽率分別為91.7±2.4與91.7±4.7%，平均發芽日數分別是54.4±2.6與59.9±2.0天，種子主要在第6~10週中零散發芽，且在13週內發芽完畢，故恆春哥納香種子具休眠性且發芽速度緩慢。4℃層積無法解除其種子休眠，且當層積時間超過2個月後種子會顯著衰敗，在此潮濕低溫環境6個月後即死亡殆盡。因此，在有效的解除休眠方法被找到之前，建議用3個月的長時間來完成恆春哥納香的種子發芽。恆春哥納香種子可被乾燥到含水率6%而完全不會減損其發芽能力，且這些被乾燥到約6%含水率的種子在4℃儲藏2年後仍有89% (第1批)與67% (第2批)保有活力，但當種子再被乾燥到含水率3.5%以下時就會立刻全部死亡。此外，當含水率6~13%的種子儲藏在-196及-20℃時，其活力會快速下降。上述結果顯示恆春哥納香種子具有中等耐旱，及對零下低溫敏感但能在4℃儲藏良好的特性，故將其歸類為溫帶中間型種子。恆春哥納香種子活力不易維持，建議一般育苗者在種子採收後應將其立即播種，以免因難以掌握其儲藏技術而使種子在短期內就大量死亡。因恆春哥納香種子屬短壽命的中間型，故難以用儲存種子方式來進行其長期區外保育工作，建議在儲存前應先將其種子含水率控制在6~9%，然後立刻密封儲藏在4℃的環境中，且庫存種子應約每2~3年進行更新。

關鍵詞：恆春哥納香、發芽、種子儲藏性質、中間型、區外保育。

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INTRODUCTION

Goniothalamus amuyon (Blanco) Merrill is an evergreen shrub to small tree species in the family Annonaceae. This species is distributed in the Philippines and Taiwan. In Taiwan, *G. amuyon* is only scattered in tropical rainforests of the Hengchun Peninsula, which is the extreme southern part of the island (Liao 1996). Because the number of mature individuals of *G. amuyon* is fewer than 250 and they are seriously separated, the Council of Agriculture has designated *G. amuyon* a critically endangered species (Lu and Lin 1996). Subsequently, *G. amuyon* was judged to be an NCR (nationally critically endangered) species again by the Editorial Committee of the *Red List of Taiwan Plants* in 2017.

Goniothalamus amuyon usually blooms in May to July. The maturation period of fruit is from late October to late November. The pericarp changes from green to orangish-red when mature (Fig. 1). The oblong or ovoid drupes are about 1~2 cm in length and about 1.3 cm in diameter. Each drupe produces 1~3, but usually 2 seeds. Fresh mature seeds of *G. amuyon* are elliptical in shape; the length of the seeds is about 7 mm, and the diameter is about 5.5 mm. The seeds turn brown or dark-brown in color when mature

Fig. 1. Fruits and seeds of *Goniothalamus amuyon*.

(Fig. 1). Seeds are very hard to collect due to the rare populations and being eaten by wildlife.

According to the degree of desiccation tolerance of mature seeds, the seed storage behavior was initially classified into 2 categories: orthodox and recalcitrant (Roberts 1973). Orthodox seeds can tolerate desiccation and remain viable when the moisture content (MC) decreases to < 5% (on a fresh-weight basis; all MC values in this study are on a fresh-weight basis). Unlike orthodox seeds, recalcitrant seeds are particularly intolerant of desiccation. When they are dried to < 12~31% MCs, seed viability declines with concurrent prolongation of the drying duration (Roberts 1973). Furthermore, many recalcitrant seeds are extremely sensitive to low temperatures, and at temperatures of < 10~15℃, their viability often deteriorates, which is especially true for tropical recalcitrant seeds (Bonner 1990). In addition to orthodox and recalcitrant, Ellis et al. (1990a) found that seeds of *Coffea arabica* L. survived desiccation to 5~10% MCs but did not survive at storage temperatures of ≤ 10℃. They considered such seeds to be "intermediate", i.e., they are moderately tolerant of desiccation but vulnerable to freezing temperatures. Subsequently, they also found that seeds of *Elaeis guineensis* (Ellis et al. 1991b) and *Citrus* spp. (Hong and Ellis 1995) showed similar storage behaviors. Most intermediate seeds survive desiccation to 10~12% MCs, but their viability declines with a concurrent decrease in MCs. Optimal storage temperatures of intermediate seeds are mainly determined by the ecological environment of their native habitats. Therefore, some intermediate seeds, such as Taiwan *Acer* spp. (Yang and Lin 1999), can survive sub-zero temperatures, while others cannot, such as *Zelkova serrata* (Yang et al. 2007) and *Litsea coreana* (Yang et al. 2008).

There are about 50 species in the genus of *Goniothalamus* worldwide, and all of them are distributed in tropical southern and southeastern Asia. In Taiwan, there is only 1 species. There are no previous studies of the seed storage behavior of *Goniothalamus* species. Consequently, a factorial combination of different MCs and storage temperatures was used to accurately investigate the seed storage behavior of *G. amuyon* in this study and determine optimal storage conditions. Results can offer useful information on nursery operations for restoration and seed storage for *ex situ* conservation programs of this native Taiwanese endangered species.

MATERIALS AND METHODS

Seed collection and extraction

Table 1 gives detailed collection information of the 2 seedlots used in this study which were collected from several of the same cultivated individuals in 2003 and 2004. Only mature fruits of an orangish-red color were collected from the 2 seedlots. Because the outer skins and sarcocarp in the middle layer of mature fleshy fruits were still hard when harvested, the collected fruits of these 2 seedlots were placed in a greenhouse and kept moist for 12~26 days, which caused the fruit flesh to ripen and decompose. Once the fruits had become softer, they were immediately washed and extracted to obtain cleaned seeds. About 2500 excellent seeds in seedlot 1 were washed and extracted on 11 November 2003, and about 5600 excellent seeds of seedlot 2 were processed on 2 December 2004. Among the 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.

Determination of the MC

Seed MCs of the 2 seedlots were determined gravimetrically with 4 replicates. For each replicate, 3 filled seeds from the 2 seedlots were randomly selected and cut into pieces of < 4 mm in length. Then, the minced seeds were dehydrated. MCs of the seeds were determined using a low-constant-temperature oven method (103±2℃ for 17±1 hour) (International Seed Testing Association 1999). All MCs are presented on a percentage fresh-weight basis.

Germination assay

To avoid imbibition damage by rapid rehydration (Ellis et al. 1990b), seeds undergoing different treatments were placed above water in a sealed container for 1 day before the germination test so that the seeds could take up water at ambient temperature. Then,

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

Seedlot no.	Seedlot 1	Seedlot 2
Collection date	October 28, 2003	November 4, 2004
Collection location	Hengchuen Township, Pingtung County	Hengchuen Township, Pingtung County
Latitude, longitude	21°58′N, 120°48′E	21°58′N, 120°48′E
Elevation (m)	260	260
Moisture content (%, FW[1)] basis)	24.8±0.4	23.9±0.5
Seed length (mm)	7.23±0.52	7.28±0.43
Seed width (mm)	5.77±0.33	6.02±0.30
Seed thickness (mm)	5.16±0.24	5.23±0.21
Number of seeds L^{-1}	4520±51	3935±38
TSW[2)] (Thousand-seed-weight, g)	130.1±1.7	141.1±0.2
Germination percentage[3)] (%)	91.7±2.4	91.7±4.7
Mean germination time[3)] (days)	54.4±2.6	59.9±2.0

[1)] FW, fresh-weight.

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

[3)] Seeds were incubated at alternating night/day temperatures of 20/30℃ and 8 hours of light for 16 weeks.

the imbibed seeds were thoroughly mixed with clean sphagnum moss in sealable polyethylene (PE) bags (14 × 10 cm, and 0.04 mm thick) with about 2/3 volume of adequate air inside for normal respiration. 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 undergoing moist and dry storage, 20 seeds from the 2 seedlots were each randomly sampled in 3 replicates per treatment for the germination test at fluctuating temperatures of 30/20℃ (day/night) with 8 hours of light (50~80 μmol m^{-2} s^{-1}). During the 16-weeks test period, the number of protruding seeds was counted once a week. Seeds with a radicle reaching 8 mm were considered to have germinated. Meanwhile, about 3~5 ml of water was added to the moss and the air in the PE bags was renewed 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).

Low-temperature moist storage treatments

The 4℃ moist storage durations of fresh seeds of seedlot 2 are given in Table 2. 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 stratification on seed germination and the impact of low-temperature moist storage on the seed viability of *G. amuyon.* Freshly collected seeds of each seedlot were thoroughly mixed with moist sphagnum moss in PE bags (14 × 10 cm, and 0.04 mm thick) and then placed 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 the air was refreshed monthly.

Dry storage treatments and determination of moisture levels

Sub-seedlots exhibited different MCs, and the ranges were about 5~14% for the 2 seedlots. 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 at temperatures of 15~20℃. When a sub-seedlot reached the desired MC during the controlled process, seeds were placed in an aluminum foil bag and temporarily stored at 4℃ for about 3 days to stabilize the water contents before the average equilibrium MC was determined. In seedlot 1, the MC of fresh mature seeds was 24.8±0.4% (Table 1). After dehydration, 3 MC levels were controlled to 2.2±0.1% (13.2 days of desiccation), 5.8±0.3% (4.4 days of desiccation), and 9.3±0.4% (0.9 day of desiccation). In seedlot 2, the MC of fresh mature seeds was 23.9±0.5%. The 4 MC levels were controlled to 3.4±0.2% (16.6 days of desiccation), 5.9±0.2% (1.7 days of desiccation), 10.1±0.5% (1 day of desiccation), and 13.0±0.8% (0.8 day of desiccation) after dehydration treatments (Table 2). As soon as the desired MCs of each sub-seedlot were achieved, seeds were sealed in double-layered aluminum foil bags before storage at different temperatures. Table 2 shows storage temperatures and durations of the various MC levels of seeds of the 2 seedlots.

Data analysis

An analysis of variance (ANOVA) was

used to analyze seed germination percentages and MGTs to evaluate the effects of the stratification period on germination. Additionally, germination results of combinations 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).

RESULTS

Germination of fresh mature seeds

In seedlot 1, when fresh mature seeds were incubated at fluctuating temperatures of 30/20℃ for 16 weeks, their germination percentage was 91.7% and the MGT was 54.4 days. In seedlot 2, the germination percentage of fresh mature seeds under the same germination conditions as seedlot 1 was 91.7%, and the MGT was 59.9 days (Table 1). Of the 2 seedlot seeds, they mainly emerged during 6~10 weeks, about 94% viable seeds had germinated within 5 weeks, and no germination appeared after 13 weeks (Fig. 2). Ungerminated seeds of the 2 seedlots had all rotted when cut open after the 16-weeks germination period. The above findings show that fresh mature seeds of *G. amuyon* of the 2 seedlots exhibited dormancy and had a slow germination speed compared to seeds of most woody plants.

Effects of moist storage at 4℃ on germination of fresh mature seeds

The germination percentage and germination speed of the seedlot 2 seeds were used to investigate the effects of low-temperature stratification on seed germination of *G. amuyon*. The seed germination percentage was 70.0±18.7% after stratification at 4℃ for 2 months, which showed no significant difference (p = 0.18) compared to fresh mature seeds (91.7±4.7%) (Fig. 3). However, when the stratification duration was extended to 4 months, the germination percentage decreased to 61.7±8.5%, a significant difference in germination (p = 0.01) compared to fresh mature seeds (Fig. 3). Afterwards all seeds had died by 6 months of stratification at 4℃ In addition, the MGTs of the seeds decreased to 40.9±4.4 and 42.6±4.7 days after stratification at 4°C for 2 and 4 months, respectively, and significant differences in MGTs (p = 0.005 and 0.009) were found compared to fresh mature seeds (59.9±2.0 days) (Fig. 3).

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

Figures 4 and 5 show the effects of different MCs and storage temperatures on the germination percentages of *G. amuyon* seeds of seedlots 1 and 2, respectively, with dry storage for 0~24 months. In seedlot 1, after the drying process was completed, freshly dried seeds with 3 MCs were immediately

Table 2. Durations of moist storage at 4℃ and dry storage durations at 3 or 4 moisture contents (MCs) stored at 3 or 4 temperatures of the 2 seedlots seeds of *Goniothalamus amuyon* in this study

Seedlot no.	Seedlot 1	Seedlot 2
4℃ moist storage duration (months)	-	0, 2, 4, 6, 9, 12
MCs of dry storage (FW[1)] basis, %)	2.2±0.1, 5.8±0.3, 9.3±0.4	3.4±0.2, 5.9±0.2, 10.1±0.5, 13.0±0.8
Dry storage temperature (℃)	-196, -20, 4	-196, -20, 4, 15
Dry storage duration (months)	0, 3, 9, 15, 24	0, 3, 6, 12, 18, 24

1) FW, fresh-weight.

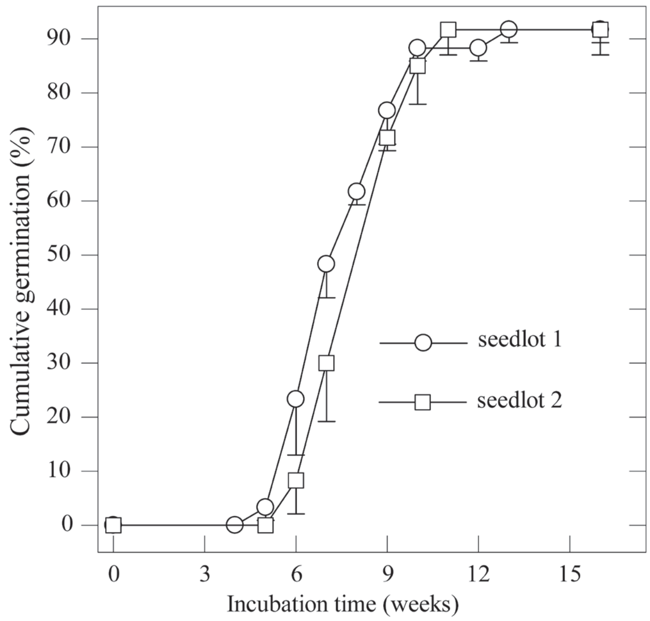

Fig. 2. Cumulative germination percentages of fresh mature seeds of the 2 seedlots of *Goniothalamus amuyon*.

incubated under fluctuating temperatures of 30/20℃ for 16 weeks. Seeds at an MC of 2.2% had a sharp decline in germination to 1.7%. The respective germination percentages of seeds at MCs of 5.8 and 9.3% were 86.0±8.9 and 83.3±10.3% while there were no significant differences (p = 0.4 and 0.3) in germination compared to fresh mature seeds (91.7%) (Fig. 4).

During storage at -196℃, all seeds of seedlot 1 at an MC of 2.2% had died after 3 months of storage. Seeds at MCs of 5.8 and 9.3% exhibited sharp declines in germination and had significantly decreased to 16.4 and 47.8% after 3 months of storage (p = 0.00003 and 0.01), respectively, which continued to decrease to 1.7 and 8.7% after 24 months of storage (Fig. 4).

When stored at -20℃, seedlot 1 seeds at MCs of 5.8 and 9.3% maintained respective germination percentages of 88.3 and 91.3% after 3 months of storage and showed no significant differences (p = 0.4 and 0.9) with fresh mature seeds. However, germination percentages of seeds at MCs of 5.8 and 9.3% significantly declined to 41.7 and 50.0% after 9 months of storage (p = 0.00003 and 0.0002), respectively, and had further decreased to 23.1 and 36.1% after 24 months of storage (Fig. 4).

When stored at 4℃, all seeds of seedlot 1 at an MC of 2.2% lost their viability after 3 months of storage. Seeds at MCs of 5.8 and 9.3% maintained germination percentages of 81.7~86.7 and 83.3~91.5%, respectively, within 24 months. Separate germination percentages of seeds at MCs of 5.8 and 9.3% were 81.7 and 91.5% after 24 months of storage, while there were no significant differences in germination percentages (p = 0.06 and 0.9) compared to fresh mature seeds (91.7%) (Fig. 4).

In seedlot 2, once the 4 desired MC levels were reached, seeds were immediately incubated under fluctuating temperatures of 30/20℃ for 16 weeks. Germination percent-

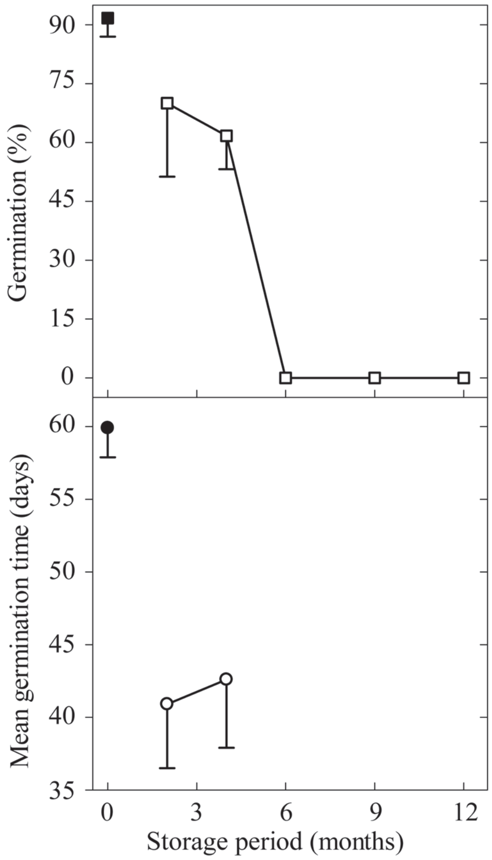

Fig. 3. Effects of moist storage at 4℃ for 0~12 months on the germination percentage (□) and mean germination time (○) of seedlot 2 seeds of *Goniothalamus amuyon*. Filled squares (■) and circles (●) respectively represent the germination percentage and mean germination time of fresh mature seeds. Vertical bars represent the standard error of the mean.

ages of seeds at MCs of 3.4, 5.9, 10.1, and 13.0% were 0, 91.7±2.4, 88.3±6.2, and 96.7±2.4%, respectively. No significant difference (*p* = 1.0, 0.6, and 0.3) in germination at the higher 3 MCs was found compared to fresh mature seeds (91.7%) (Fig. 5).

All seeds of seedlot 2 at 4 MCs had died after 3 months of storage when stored at -196℃ (Fig. 5). During storage at -20℃, seeds at MCs of 5.9, 10.1, and 13.0% exhibited sharp declines in germination, with respective germination percentages of 10.0, 0, and 6.7% after 3 months of storage, which had germination rates of 15.0, 1.7, and 0% after 24 months of storage (Fig. 5).

When stored at 4℃, the germination percentage of seedlot 2 seeds at an MC of 5.9% significantly decreased to 55.0% after 3 months of storage ($p = 0.001$), and seeds maintained germination percentages of 58.3~71.7% within 6~24 months, exhibiting significant differences ($p < 0.05$) compared to fresh mature seeds. Moreover, viability of seeds at the MCs of 10.1 and 13.1% began to decrease after 3 months and had significantly decreased to 73.3 and 70.0% after 6 months of storage (p = 0.02 and 0.04), and germination percentages continued to decrease to 30.0 and 0%, respectively, after 24 months of storage (Fig. 5).

When stored at 15℃, germination percentages of seedlot 2 seeds at MCs of 5.9, 10.1, and 13.0% quickly decreased, and almost all of them had decayed after 12 months of storage (Fig. 5).

DISCUSSION

Most seeds of *G. amuyon* exhibited morphological dormancy, but a proportion of them had morphophysiological dormancy. So the seed population of *G. amuyon* consisted of a mixture of morphological dormancy and morphophysiological dormancy (Chen et al. 2014). Results of this study also showed that fresh mature seeds of *G. amuyon* have dormancy. However, 4℃ stratification could not break the seed dormancy, and seed deterioration emerged when the stratification period exceeded 2 months (Fig. 3). Thus, longer incubation periods at 30/20℃ for up to 3 months are recommended for germination of seeds of *G. amuyon* before the effective method of breaking dormancy is accomplished (Fig. 2).

The above results show that seeds of both *G. amuyon* seedlots could tolerate desiccation to about 5.5~13.0% MCs and lost no germinability, and 89% (seedlot 1) and 67% (seedlot 2) of seeds at an MC of about 6% had maintained their viability after 2 years of storage at 4℃ (Figs. 4, 5). However, all seeds died when they were dried to < 3.5% MCs (Figs. 4, 5). Moreover, seeds of the 2 seedlots at 6~13% MCs rapidly lost their viability when stored at -196 and -20℃ (Figs. 4, 5). These characteristics of quickly losing seed viability at low MCs and intolerance of sub-zero temperatures show differences from orthodox seeds. Based on these findings, seeds of *G. amuyon* are considered to show temperate intermediate seed storage behavior due to their moderate tolerance of desiccation and sensitivity to freezing temperatures, while they preserved well at 4℃. In addition, we recommend that fresh mature seeds of *G. amuyon* be controlled to 6~9% MCs and then hermetically sealed immediately before storage at 4℃. It is difficult to preserve *G. amuyon* seeds for long-term *ex situ* conservation because of their intermediate storage behavior with a short lifespan. Thus, it is suggested that stored seeds of *G. amuyon* be renewed about every 2~3 years. Besides, seed viability of *G. amuyon* is not easily maintained for ordinary cultivators because of insufficiency and difficulty of seed storage techniques. Thus, we suggest that seeds of *G. amuyon* be sowed immediately after being harvested, because it is possible for the seeds to seriously deteriorate in a short time.

Most intermediate seeds can survive desiccation within a moisture content range of 10~12%, but the germinability tends to decline with a reduction in the MC (Hong and Ellis, 1996). Furthermore, the optimal water contents of some intermediate seeds were revealed to obviously be below the above-described range (10~12%), and such intermediate seeds with better desiccation tolerance include *Khaya senegalensis* (Desr.) A. Juss., *Swietenia macrophylla* King (Hong and Ellis 1998), *Coffea canephora* Pierre *ex*

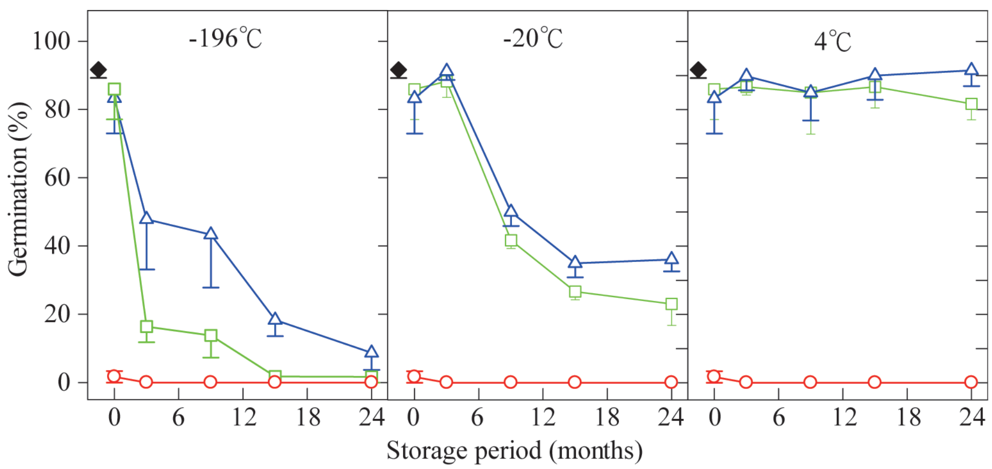

Fig. 4. Effects of storage temperatures (-196, -20, and 4℃) and moisture contents (MCs, on a fresh-weight basis) on germination percentages of *Goniothalamus amuyon* seeds of seedlot 1 stored for up to 24 months. The initial germination percentage of fresh mature seeds of seedlot 1 was 91.7±2.4% (◆). MCs of seedlot 1 seeds: ○, 2.2±0.1%; □, 5.8±0.3%; △, 9.3±0.4%. Vertical bars represent the standard error of the mean.

A. Froehner, *C. congensis* Froehner, *C. racemosa* Lour. (Dussert et al. 1999, Eira et al. 1999), *Acer morrisonense* Hayata (Yang and Lin 1999), *Citrus limon* (L.) Burm. f. (Hong et al. 2001), *Fagus crenata* Blume (Pedro and Ellis 2002), *Bischofia javanica Blume* (Yang et al. 2006), *Zelkova serrata* (Thunb.) Makino (Yang et al. 2007), and *Litsea coreana* Levl. (Yang et al. 2008). However, the estimated optimal water content for storage of *G. amuyon* seeds is about 6~9%, which is much lower than that of most intermediate seeds. It shows that *G. amuyon* seeds have greater desiccation tolerance than most intermediate seeds.

Seeds potentially adapt to their ecological environments, and temperate seeds can often survive sub-zero temperatures and maintain much longer viability than tropical seeds. Based on the protocol suggested by Hong and Ellis (1996), the optimal temperature for storing tropical intermediate seeds should be ≥ 10℃. Species with tropical intermediate seed storage behavior include *Elaeis guineensis* Jacq. (Ellis et al. 1991b), *Coffea* spp. (Ellis et al. 1990a, 1991a, Hong and Ellis 1992, 1995, Dussert et al. 1999, Eira et al. 1999), *Azadirachta indica* A. Juss. (Gamene et al. 1996, Hong and Ellis 1998, Nayal et al. 2000), *Khaya senegalensis* (Desr.) A. Juss., *Swietenia macrophylla* King (Hong and Ellis 1998), *Phoenix reclinata* Jacquin (von Fintel et al. 2004), and *Zelkova serrata* (Thunb.) Makino (Yang et al. 2007). On the other hand, the optimal temperature for storing temperate intermediate seeds should be ≤ 5℃ (Hong and Ellis 1996). Some temperate intermediate seeds are more likely to tolerate freezing temperatures, and they can survive for about 2~3 years and even longer, such as seeds of *Araucaria columnaris* (Forst.) Hook. (Tompsett 1984), *Citrus limon* (Hong et al. 2001), *Fagus sylvatica* L., *F. crenata* Blume (Pedro and Ellis 2002), and *Zizania palustris* L. (Berjak et al. 1994, Vertucci et al. 1994, 1995, Hong and Ellis 1996, White and Jayas 1996, Ntuli et al. 1997). All temperate intermediate seeds of the Aceraceae in Taiwan also can adapt to sub-zero temperatures very well (Yang and Lin 1999), and the estimated longevity of seeds of Taiwanese *Acer* spp. in proper storage conditions is about 10~20 years. In addition, some temperate intermediate seeds of the Taiwanese Lauraceae are usually short-lived and extremely sensitive to freezing

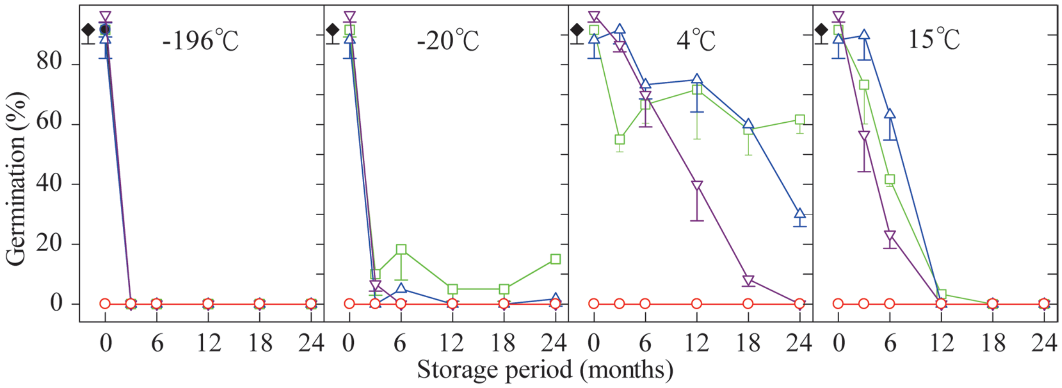

Fig. 5. Effects of storage temperatures (-196, -20, 4, and 15℃) and moisture contents (MCs, on a fresh-weight basis) on germination percentages of *Goniothalamus amuyon* seeds of seedlot 2 stored for up to 24 months. The initial germination percentage of fresh mature seeds of seedlot 2 was 91.7±4.7% (◆). MCs of seedlot 2 seeds: ○, 3.4±0.2%; □, 5.9±0.2%; △, 10.1±0.5%; ▽, 13.0±0.8%. Vertical bars represent the standard error of the mean.

temperatures, and the best condition for keeping their viability is moist storage at about 4℃, e.g., *Cinnamomum camphora* (L.) Presl. (Chien and Lin 1999), *Cinnamomum subavenium* (Miq.) (Lin 1996), *Neolitsea parvigemma* (Hay.) Kanehira & Sasaki (Lin 1996), *Lindera communis* Hemsl. (Chien et al. 2004), *Lindera megaphylla* Hemsl. (Lin 1996, Chien et al. 2004), and *Lindera coreana* Levl. (Yang et al. 2008). These mature seeds with intermediate storage behavior have high water contents (about 25~38%) in fleshy fruits and show a larger size (seed length > 5 mm). As stated above, in terms of intermediate seeds of Taiwan, the freeze tolerance of fleshy-fruit seeds of the Lauraceae is inferior to that of dry-fruit seeds of *Acer* spp.

Like the Lauraceae seeds above, the mature seeds from fleshy drupes of *G. amuyon* show a similar size, and the best storage temperature is about 4℃ instead of freezing temperatures. Therefore, seeds of *G. amuyon* are also defined as having temperate intermediate storage behavior. Our study reveals that *G. amuyon* seeds have a similar freeze tolerance to intermediate seeds of the Taiwanese Lauraceae. However, the optimum seed storage for *G. amuyon* is dehydration to 6~9% MCs and 4℃. That differs from intermediate seeds of the Taiwanese Lauraceae which should be under moist storage at about 4℃.

CONCLUSIONS

1. Fresh mature seeds of *G. amuyon* exhibited dormancy and had a slow germination speed. They mainly emerged during 6~10 weeks, all viable seeds had completely germinated within 13 weeks, and the mean germination times were about 55~60 days under fluctuating temperatures of 30/20℃ with 8 hours of light.
2. Stratification at 4℃ could not break the dormancy of *G. amuyon* seeds, seed deterioration emerged when the stratification period exceeded 2 months, and all had died after 6 months in a moist, low-temperature condition. A longer incubation period of up to 3 months is recommended for germination before the effective method of breaking dormancy is presented.
3. Seeds of *G. amuyon* dehydrated to about 6~13% MCs still maintained viability. However, all seeds died when they were dried to < 3.5% MCs. Moreover, seeds at 6~13% MCs rapidly lost their viability when stored at -196 and -20℃, while seeds at an MC of about 6% maintained high viability after 2 years of storage at 4℃. Thus, seeds of *G. amuyon* are considered to show temperate intermediate seed storage behavior due to their moderate desiccation tolerance and sensitivity to freezing temperatures.
4. Freshly collected seeds of *G. amuyon* should be sown as soon as possible, because seed viability is not easy to be maintained for ordinary cultivators.
5. The optimum seed storage for *G. amuyon* is dehydration to 6~9% MCs and storage at 4℃. It is difficult to preserve *G. amuyon* seeds for long-term *ex situ* conservation because of their intermediate storage behavior with a short lifespan. Thus, it is suggested that stored seeds of *G. amuyon* be renewed about every 2~3 years.

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