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Research paper

Seed Germination and Storage Behavior of *Meliosma rhoifolia* Maxim.

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ABSTRACT

Meliosma rhoifolia is a deciduous tree species native to Taiwan, belonging to the family Sabiaceae. With its annual blooming, abundant crown floral coverage, and rich nectar and pollen attractive to insects and bees, *M. rhoifolia* has great potential as a honey plant for afforestation in Taiwan. This study investigated the germination characteristics and seed storability of *M. rhoifolia*. The effects of seed moisture content (MC) and storage temperature on germination were evaluated to determine the species' seed storage behavior and to assess the feasibility of long-term conservation storage of seeds. Results showed that fresh mature seeds of *M. rhoifolia* were deeply dormant. No seeds germinated within 12 weeks under alternating temperatures of 30/20℃ with 8 hours of light. However, moist stratification at 4℃ effectively promoted seed germination and significantly reduced mean germination time. When the chilling period was extended to 3 months, seed dormancy was completely broken, allowing viable seeds to fully germinate within 4 weeks. In addition, seeds began to germinate in dark cold conditions after 4 months of stratification at 4℃. Therefore, moist stratification at 4℃ is not suitable to store seeds of this species for a long time. Seeds of *M. rhoifolia* dehydrated to MC of 2.7-11.7% retained viability after 2 years of storage at -196℃ and -20℃. Based on these results, seeds of *M. rhoifolia* are classified as having orthodox storage behavior due to their dehydration tolerance and high viability at sub-zero temperatures. Thus, for long-term plant germplasm conservation, it is recommended that seeds of *M. rhoifolia* be dehydrated to 5-7% MC, hermetically sealed, and stored at sub-zero temperatures. However, for short-term storage of up to 2 years, seed MC can be reduced to 5-11%, if hermetically sealed, and stored at 4℃.

Keywords: *Meliosma rhoifolia* Maxim., germination, orthodox, seed storage behavior, cold moist stratification

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INTRODUCTION

Seed storage behavior is classified as orthodox, recalcitrant, or intermediate according to the degree of desiccation tolerance (Hong and Ellis 1996). Recalcitrant seeds have a low degree of desiccation tolerance (Tompsett 1987, 1989). When recalcitrant seeds are dried to below 12-31% moisture content (MC; all MC values in this study are on a fresh-weight basis), their viability decreases as drying duration increases (Roberts 1973). Many recalcitrant seeds are extremely sensitive to low temperatures, and at temperatures below 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 their level of temperature tolerance. Temperate-recalcitrant seeds cannot tolerate desiccation but can survive a temperature of close to 0℃ and MC of 35-50%. Tropical-recalcitrant seeds have a weak tolerance to desiccation and low temperatures. They are almost unable to survive a temperature near 0℃ and are sensitive even to 15-20℃ (Hong and Ellis 1996).

In contrast to recalcitrant, orthodox seeds can tolerate desiccation and survive moisture content below 5%. The longevity of orthodox seeds increases with decreasing MC and storage temperature, and these relationships can be described using mathematical models (Roberts 1973).

In addition to orthodox and recalcitrant seeds, Ellis et al. (1990a) found that *Coffea arabica* L. seeds survived desiccation to 5-10% MC but failed to survive at storage temperatures below10℃. Such seeds are classified as intermediate; unlike orthodox seeds, they do not exhibit strong desiccation tolerance and are vulnerable to low-temperature damage. According to Hong and Ellis (1996), most intermediate seeds can survive desiccation down to 10-12% MC, but their viability declines when MC falls below this range.

Meliosma rhoifolia Maxim., is a deciduous tree reaching up to 15 m high, in the family Sabiaceae. This species is distributed in the Ryukyus and on Taiwan. *Meliosma rhoifolia* is scattered in forests at elevations of 300-1,800 m in the northern and central parts of the main island of Taiwan (Li 1993). With its annual blooming, extensive floral coverage on the tree crown, and abundant nectar and pollen favored by insects and bees, *M. rhoifolia* has great potential as honey plant for afforestation in Taiwan. *Meliosma rhoifolia* usually blooms from July to August. The maturation period of fruits extends from mid October to early November, and this species consistently produces abundant yields every year in Taiwan. The globose drupes of this species are about 5-6 mm in diameter, and turn from green to red when mature (Figure 1). Each drupe produces 1 seed. Fresh mature seeds of *M. rhoifolia* are sphere shaped with an irregularly rugulose surface. Seeds are about 4 mm long, and 3.5 mm in diameter. The seeds turn brown or dark brown when mature (Figure 1).

There are no studies investigating the germination and seed storage of *M. rhoifolia*. Consequently, a factorial combination of different MCs and storage temperatures was used to investigate the seed storage behavior of *M. rhoifolia* and to determine optimal storage conditions. The results provide valuable information for nursery operations working on restoration, as well as for *ex situ* conservation programs of this Taiwanese native tree species, which has high potential as a locally produced honey source.

Figure 1. Mature fruits and seeds of *Meliosma rhoifolia*

MATERIALS AND METHODS

Seed collection and processing

Table 1 presents detailed collection information of the two seedlots used in this study, which were collected from single wild individuals in 2018 and 2019. Only mature fruits, ranging from red to dark red were collected for the two seedlots. Because the outer skins and sarcocarp in the middle layer of most mature fleshy fruits are still hard at harvest, collected fruits were placed in a greenhouse and kept moist for 10-14 days to allow the fruit flesh to ripen and decompose. Once softened, the fruits were washed and the seeds extracted. Seed selection was performed by soaking them in water a second time. Heavy sinking seeds were retained for research. Light, floating seeds in seedlot 2 comprised about 2%. From the cleaned seeds, small-sized and damaged seeds were manually removed. About 11,200 high quality seeds from seedlot 1 were washed and extracted on 12 November, 2018, and about 35,800 high quality seeds from seedlot 2 were processed on 14 November, 2019. Seed characteristics of fresh mature seeds from the two seedlots are presented in Table 1.

Determination of MC

Seed moisture content was determined gravimetrically with four replicates. For each replicate, 5 filled seeds of each seedlot were randomly selected and cut into pieces of less than 4 mm in length. The minced seeds were then dehydrated. Seed MC was determined using the low-constant-temperature oven method (103 ± 2℃ for 17 ± 1 h) (International Seed Testing Association 1999). All MC values are expressed as percentages on a fresh-weight basis.

Germination assay

To avoid imbibition damage from rapid rehydration (Ellis et al. 1990b), seeds with different treatments were placed above water in a sealed container for 1 day before the germination test, allowing them to take up water at ambient temperature. The imbibed seeds were then thoroughly mixed with clean, moist sphagnum moss in sealable polyethylene (PE) bags (14 × 10 cm, and 0.04 mm thick), with approximately two-thirds of the volume left as air space for normal respiration. Excess water in the moss was squeezed out until water content reached about 450% of dry mass (Lin and Chen 1993). To estimate the germination capacity of fresh mature seeds as well as

Table 1. Information on fruit collection dates and sources, seed characteristics, and germination of fresh mature seeds of the two seedlots of *Meliosma rhoifolia*

	Seedlot 1	Seedlot 2
Collection date	30 Oct. 2018	29 Oct. 2019
Collection location	Shilin District, Taipei City	Jianshih Township, Hsinchu County
Latitude, longitude	25°09'N, 121°32'E	24°40'N, 121°16'E
Elevation (m)	598	1,073
Moisture content (%, FW[1] basis)	15.1 ± 0.4	14.2 ± 0.6
Seed length (mm)	3.99 ± 0.27	3.86 ± 0.21
Seed width (mm)	3.58 ± 0.18	3.57 ± 0.24
Seed thickness (mm)	3.32 ± 0.17	2.93 ± 0.25
Number of seeds L^{-1}	24,645 ± 115	31,285 ± 345
Thousand-seed weight[2] (g)	25.34 ± 0.06	20.22 ± 0.05
Germination percentage[3] (%)	48.9 ± 19.3	31.3 ± 5.3
Mean germination time[3] (days)	327.1 ± 76.3	350.7 ± 14.1

[1] FW, fresh-weight.
[2] Thousand-seed weight was estimated at the moisture content shown.
[3] Fresh mature seeds were incubated at alternating night/day temperatures of 20/30℃ and 8 hours of light per day for 90 weeks.

seeds in moist and dry storage, 30 seeds from seedlot 1 and 100 seeds from seedlot 2 were randomly sampled in 3 replicates per treatment. Germination tests were conducted at fluctuating temperatures of 30/20℃ (day/night) with 8 hours of light (about 5,000 lux) per day. During the 90-week (fresh mature seeds) and 20-week (cold moist stratification and dry stored seeds) test period, the number of protruding seeds was counted once a week. Seeds with a radicle reaching 5 mm were counted as having 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

Mean germination time (MGT) was calculated using the equation:

$$MGT = \Sigma (n \times d) / N$$

where n is the number of seeds germinated after each period of incubation in days (d), and N is the total number of seeds germinated by the end of the test (Hartmann et al. 1989).

Moist cold storage treatment

The durations of 4℃ moist storage for fresh seeds from the two seedlots are given in Table 2. In this study, moist cold storage refers to the same process as cold stratification, as storing seeds in moist sphagnum at low temperatures is functionally equivalent to stratification. These results were used to assess the effects of low-temperature stratification on seed germination and to evaluate the impact of low-temperature moist storage on the seed viability of *M. rhoifolia*. 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 stored at 4℃. About two-thirds of

Table 2. Durations of stratification at 4℃ and dry storage at 5 moisture content (MC) levels of *Meliosma rhoifolia* seeds stored at 3 (seedlot 1) or 4 (seedlot 2) different temperatures

	Seedlot 1	Seedlot 2
4℃ stratification duration (months)	0, 1, 2, 3, 4, 6, 9, 12, 18, 24	0, 1, 2, 3, 4, 6, 9, 12, 18, 24
MC of dry storage (FW[1] basis, %)	2.7 ± 0.2, 5.2 ± 0.2, 8.6 ± 0.2, 11.7 ± 0.2, 15.1 ± 0.4	3.0 ± 0.2, 5.4 ± 0.2, 9.0 ± 0.1, 11.0 ± 0.1, 14.4 ± 0.2
Dry storage temperature (℃)	-20, 4, 15	-196, -20, 4, 15
Dry storage duration (months)	0, 3, 6, 12, 18, 24	0, 3, 6, 12, 18, 24

[1] FW, fresh-weight.

the volume of each bag was filled with air to allow seed respiration, and during storage, water was added and the air was refreshed every 2 weeks.

Dry storage treatment and determination of moisture content levels

Seeds were adjusted to different moisture content (MC) levels, ranging from 3-15% for the two seedlots. Fresh mature seeds were placed in hermetically sealed acrylic boxes equipped with a small fan installed to circulate the air. Molecular sieves were used to gradually dry the seeds at temperatures of 15-20℃. When a sub-seedlot reached the desired MC during this controlled process, seeds were placed in an aluminum foil bag and temporarily stored at 4℃ for about 3-5 days to stabilize the water content before average equilibrium MC was measured. In seedlot 1, the MC of fresh mature seeds was 15.1 ± 0.4% (Table 1). After dehydration, five distinct MC levels were adjusted to 2.7 ± 0.2% (18.3 hours of desiccation), 5.2 ± 0.2% (6.8 hours of desiccation), 8.6 ± 0.2% (2.3 hours of desiccation), 11.7 ± 0.2% (0.9 hour of desiccation), and 15.1 ± 0.4% (without desiccation). In seedlot 2, the MC of fresh mature seeds was 14.2 ± 0.6%. Five distinct MC levels were adjusted to 3.0 ± 0.2% (20.3 hours of desiccation), 5.4 ± 0.2% (4.2 hours of desiccation), 9.0 ± 0.1% (1.8 hours of desiccation), 11.0 ± 0.1% (0.6 hour of desiccation), and 14.4 ± 0.2% (without desiccation) (Table 2). As soon as the desired MC of each sub-seedlot was achieved, seeds were sealed in double-layered aluminum foil bags before storage at different temperatures. Table 2 shows storage temperatures and durations of the 5 MC levels of seeds of the 2 seedlots.

Data analysis

An analysis of variance (ANOVA) was conducted to analyze seed germination percentage and MGT in relation to the stratification period on germination. Additionally, effects of storage temperatures, seed MC, and storage duration periods on germination were statistically analyzed using a general linear model (GLM) with Tukey's test in R statistical software (The R Foundation).

RESULTS

Germination of fresh mature seeds

When fresh mature seeds of the 2 seedlots were incubated at fluctuating temperatures of 30/20℃ for 20 weeks, germination percentages were only 5.6 and 0.7%, respectively. Furthermore, the germination rates for the two seedlots seeds

gradually increased to 48.9 and 31.3% with MGTs of 327.1 and 350.7 days when the incubation period was extended to 90 weeks, respectively (Figure 2 and Table 1). However, most of the ungerminated seeds of the 2 seedlots had not rotted when cut open after the 90-weeks germination period. The above findings show that seeds of the two seedlots of *M. rhoifolia* exhibited deep dormancy and had slow germination rates (Figure 2).

Effects of moist stratification at 4℃ on germination

Germination percentage and germination speed were measured to investigate the effects of low-temperature stratification on breaking seed dormancy in seeds of the two seedlots of *M. rhoifolia*. In seedlot 1, seed germination percentages were 72.2, 95.6, 98.9, and 100.0% after stratification at 4℃ for 1, 2, 3, and 4 months, respectively, which all showed significant differences ($p < 0.000001$) in germination percentages compared to fresh mature seeds (5.6%) when seeds were incubated at fluctuating temperatures of 30/20℃ for 20 weeks (Figure 3). Seeds began to germinate after stratification at 4℃ for 4 months, and all viable seeds had germinated under moist dark conditions within 4-6 months (Figure 4). Furthermore, after stratification at 4℃ for 1, 2, 3, and 4 months, the MGTs of seeds decreased progressively to 37.7, 21.3, 8.3, and 7.1 days, respectively (Figure 3). These reductions in MGT were significant ($p < 0.00001$) compared to fresh mature seeds (327.1 days).

In seedlot 2, seed germination percentages were 14.0, 73.3, 91.0, and 90.0% after stratification at 4℃ for 1, 2, 3, and 4 months, respectively, which all showed significant differences ($p < 0.005$) in germination percentages compared to fresh mature seeds (0.7%) when seeds were incubated at fluctuating temperatures of 30/20℃ for 20 weeks (Figure 3). Seeds began to germinate after stratification at 4℃ for 4 months, and all viable seeds had germinated under moist dark conditions within 4-8 months (Figure 5). Furthermore, after stratification at 4℃ for 1, 2, 3, and 4 months, the MGTs of seeds

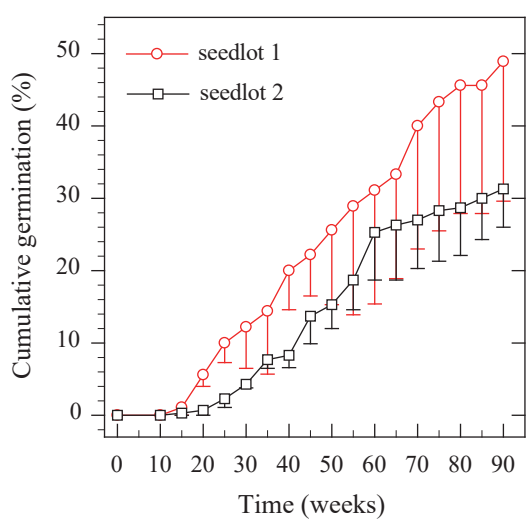

Figure 2. Cumulative germination percentages of fresh mature seeds of the two seedlots of *Meliosma rhoifolia*. Seeds were incubated at fluctuating temperatures of 30/20℃ with 8 hours of light daily for 90 weeks.

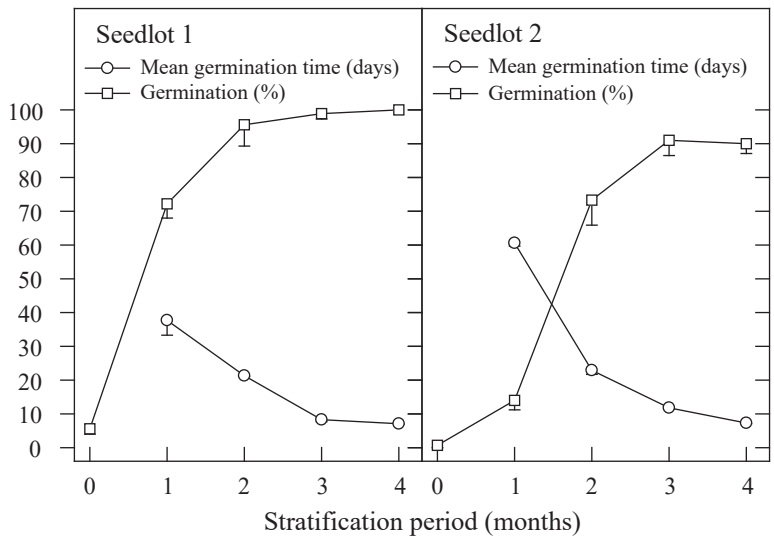

Figure 3. Effects of stratification at 4℃ for 0-4 months on the germination percentage (□) and mean germination time (○) of the two seedlots seeds of *Meliosma rhoifolia*. Vertical bars represent the standard error of the mean. Seeds were incubated at fluctuating temperatures of 30/20℃ with 8 hours of light daily for 20 weeks after stratification.

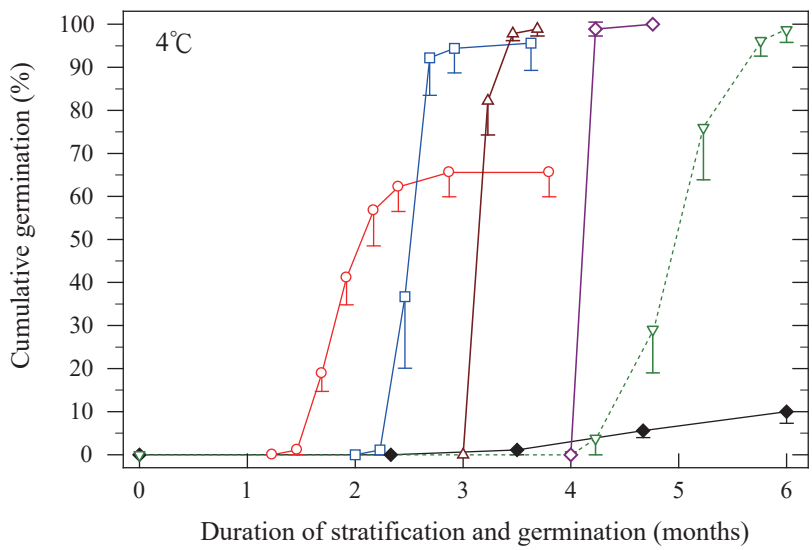

Figure 4. Cumulative germination percentages of the seedlot 1 seeds of *Meliosma rhoifolia* at 4℃ moist stratification for 0-6 months (○, 1 month; □, 2 months; △, 3 months; ◇, 4 months; ▽, 6 months). Each symbol corresponds to a treatment represented by both a dotted line (---) and a solid line (—). Dotted lines represent cumulative germination in darkness of moist stratification at 4℃, while solid lines represent cumulative germination at alternating temperatures of 30/20℃ with 8 hours of light following the same stratification. Filled diamonds (◆) represent the cumulative germination percentage of fresh mature seeds without any pretreatment. Vertical bars represent the standard error of the mean.

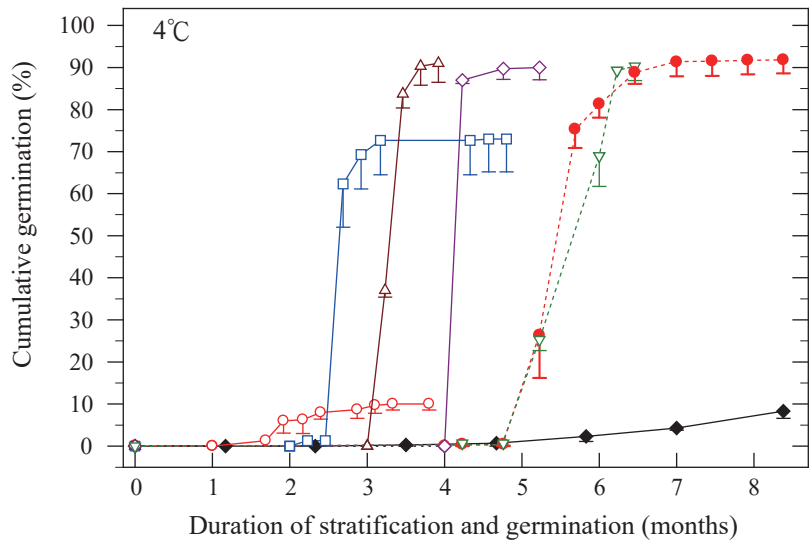

Figure 5. Cumulative germination percentages of the seedlot 2 seeds of *Meliosma rhoifolia* at 4℃ moist stratification for 0-9 months (○, 1 month; □, 2 months; △, 3 months; ◇, 4 months; ▽, 6 months; ●, 9 months). Each symbol corresponds to a treatment represented by both a dotted line (---) and a solid line (—). Dotted lines represent cumulative germination in darkness of moist stratification at 4℃, while solid lines represent cumulative germination at alternating temperatures of 30/20℃ with 8 hours of light following the same stratification. Filled diamonds (◆) represent the cumulative germination percentage of fresh mature seeds without any pretreatment. Vertical bars represent the standard error of the mean.

continuously declined to 60.6, 22.9, 11.8, and 7.3 days, respectively (Figure 3). Significant decreases in MGTs ($p < 0.00001$) were found after the above stratification duration compared to fresh mature seeds (350.7 days).

The above findings show that stratification at 4℃ effectively increases the germination percentage and reduces the MGT of seeds of *M. rhoifolia*, and the pretreatment of 4℃ stratification for about 2 months is sufficient to almost completely break the seed dormancy. The estimated initial germination percentages of the 2 seedlots were 93.3 ± 4.7 and 73.0 ± 7.8%, respectively, based on germination tests of the two seedlot seeds conducted after dormancy was broken.

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

Figures 6 and 7 show the effects of different MCs and storage temperatures on the germination percentage of *M. rhoifolia* seeds of seedlot 1 and 2 with dry storage for 0-24 months, respectively. All dry-storage seeds were pretreated with stratification at 4℃ for 2 months to break seed dormancy, and then incubated under fluctuating temperatures of 30/20℃ for 20 weeks to get the germination percentages.

In seedlot 1, once the five desired MC levels were reached, seeds were immediately pretreated with 2 months of stratification at 4℃ to break dormancy, and then incubated under fluctuating temperatures of 30/20℃ for

20 weeks. The germination percentages of these seeds at MC values of 2.7, 5.2, 8.6, 11.7, and 15.1% were 92.2 ± 1.6, 94.4 ± 3.1, 97.8 ± 1.6, 98.9 ± 1.6, and 94.4 ± 1.6%, respectively. There was no significant difference ($p > 0.3$) in germination compared to the estimated initial germination percentage of fresh mature seeds (93.3 ± 4.7%) (Figure 6).

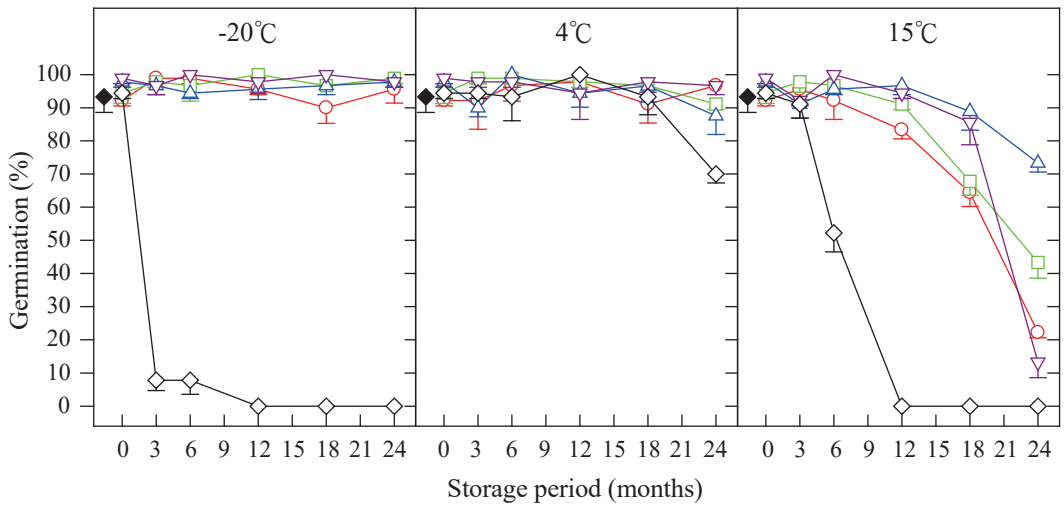

Figure 6. Effects of storage temperatures (-20, 4, and 15℃) and moisture content (MC) levels of 2.7-15.1% (on a fresh-weight basis) on germination percentages of seedlot 1 seeds of *Meliosma rhoifolia* stored for up to 24 months. The initial germination percentage of fresh mature seeds was 93.3 ± 4.7% (◆). MC of seeds: ○, 2.7 ± 0.2%; □, 5.2 ± 0.2%; △, 8.6 ± 0.2%; ▽, 11.7 ± 0.2%; ◇, 15.1 ± 0.4%. Vertical bars represent the standard error of the mean.

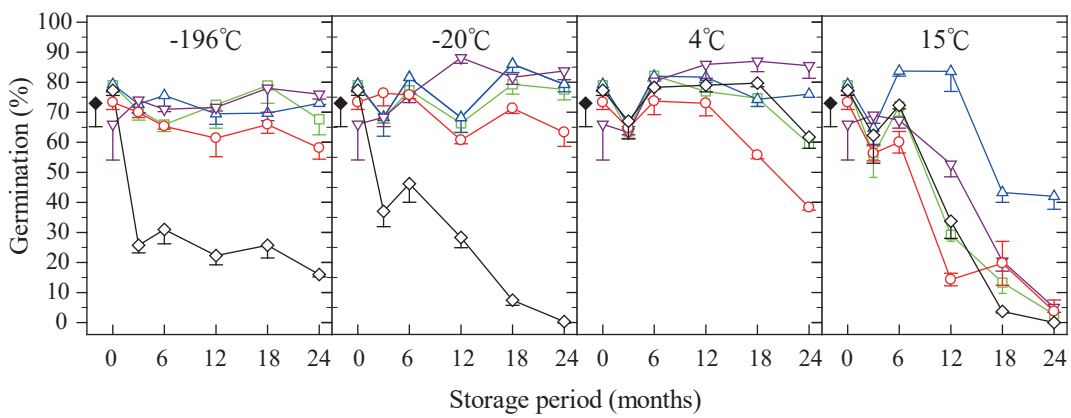

Figure 7. Effects of storage temperatures (-196, -20, 4, and 15℃) and moisture content (MC) levels (3.0-14.4%, on a fresh-weight basis) on germination percentages of seedlot 2 seeds of *Meliosma rhoifolia* stored for up to 24 months. The initial germination percentage of fresh mature seeds was 73.0 ± 7.8% (◆). MCs of seeds: ○, 3.0 ± 0.2%; □, 5.4 ± 0.2%; △, 9.0 ± 0.1%; ▽, 11.0 ± 0.1%; ◇, 14.4 ± 0.2%. Vertical bars represent the standard error of the mean.

During storage at -20℃, seeds of seedlot 1 at MC levels of 2.7, 5.2, 8.6, and 11.7% maintained germination percentages of 90.0-98.9, 94.4-100, 94.4-97.8, and 96.7-100% within 24 months, respectively. There was no significant reduction in germination compared to fresh mature seeds (93.3%) after 24 months of storage ($p > 0.3$). However, seeds at an MC of 15.1% showed a sharp decline in germination, and there was a significant reduction in germination of 7.8% after 3 months of storage ($p = 0.0000003$), which continued to decline to 0% after 12 months of storage (Figure 6).

During storage at 4℃, the germination result of seedlot 1 was similar to that at -20℃. The seeds at MC levels of 2.7-11.7% maintained high viability for 24 months, germination percentages were 96.7, 91.1, 87.6, and 96.7% at MCs of 2.7, 5.2, 8.6, and 11.7% after 24 months of storage, respectively. There was no significant difference in germination compared to fresh mature seeds (93.3%) ($p > 0.5$). However, seeds at an MC of 15.1% maintained high germination within 0-18 months of storage, but later showed marked decline in viability and the germination percentage significantly decreased to 70.0% after 24 months of storage ($p = 0.0005$) (Figure 6).

When the seeds of seedlot 1 were stored at 15℃, seeds at a MC of 15.1% lost their germinability quickly and died after 12 months of storage. Furthermore, the viability of seeds at the other four MC values declined more rapidly than those stored at -20 and 4℃, and germination percentages significantly decreased to 22.2, 43.3, 73.3, and 13.3% ($p = 0.000006$, 0.00002, 0.001, and 0.000001) at MC levels of 2.7, 5.2, 8.6, and 11.7% after 24 months of storage, respectively (Figure 6).

In seedlot 2, after the drying process was completed, freshly dried seeds at five different MC levels were immediately pretreated with 2 months of stratification at 4℃ then incubated under fluctuating temperatures of 30/20℃ for 20 weeks. Germination percentages of these seeds at MC values of 3.0, 5.4, 9.0, 11.0, and 14.4% were 73.3 ± 2.4, 79.0 ± 2.4, 79.3 ± 2.4, 66.0 ± 11.9, and 77.3 ± 1.7%, respectively. No significant difference ($p > 0.2$) in germination was found compared to the estimated initial germination percentage of fresh mature seeds (73.0 ± 7.8%) (Figure 7).

When stored at -196℃, seeds of seedlot 2, at an MC of 14.4% showed a sharp decline in germination, significantly decreasing to 25.7% ($p = 0.001$) after 3 months of storage. They maintained germination percentages of 22.3-31.0% within 6-18 months of storage, and the germination rate steadily dropped to 16.0% after 24 months. In addition, germination percentages of seeds at MC levels of 3.0, 5.4, 9.0 and 11.0% after 24 months of storage were 58.1, 67.7, 73.0, and 76.0%, respectively, and no significant decrease ($p > 0.07$) in germination was found (Figure 7).

After storage at -20℃, viability of seedlot 2 seeds at an MC of 14.4% significantly decreased to 37.0% after 3 months of storage ($p = 0.005$), and the germination percentage continued to decrease to 7.4% after 18 months, and totally lost germinability after 24 months of storage. Furthermore, there was no significant difference in germination of seedlot 2 seeds at the other 4 lower MC levels compared to fresh mature seeds (73.0%) after 24 months of storage, and their germination percentages at MC levels of 3.0, 5.4, 9.0, and 11.0% were 63.3% ($p = 0.2$), 77.6% ($p = 0.5$), 79.3% ($p = 0.3$), and 83.7% ($p = 0.1$), respectively (Figure 7).

During storage at 4℃, seeds of seedlot 2 at an MC of 3.0% maintained good germination percentages (64.7-73.7%) within 3-12 months of storage while there were no

significant differences ($p > 0.2$) in germination percentages compared to fresh mature seeds (73.0%). Furthermore, they showed no significant decrease ($p = 0.05$) in germination percentage of 55.7% after 18 months of storage, but significantly declined to 38.3% after 24 months of storage ($p = 0.003$) (Figure 7). In addition, the germination percentages of seeds at MC levels of 5.4, 9.0, 11.0, and 14.4% were 59.5, 76.0, 85.5, and 61.7% after 24 months of storage, which showed no significant differences in germination percentages ($p = 0.07$, 0.6, 0.1, and 0.1) compared to fresh mature seeds (73.0%), respectively (Figure 7).

Seedlot 2 seeds stored at 15℃, and an MC of 9.0% maintained high germination percentages (64.7-83.7%) within 12 months of storage, gradually dropping to 42.0% after 24 months of storage, showing significant difference ($p = 0.008$) in germination compared to fresh mature seeds. In addition, germination of the seeds at MC values of 3.0, 5.4, 11.0, and 14.4% declined sharply and these seeds almost died after 24 months of storage (Figure 7).

DISCUSSION

Results of this study showed that fresh mature seeds of *M. rhoifolia* exhibited deep dormancy indicated by very slow germination; no seeds germinated during 12 weeks incubation (Figure 2). However, stratification at 4℃ completely broke seed dormancy and enabled viable seeds to fully germinate within 4 weeks when the chilling period extended to 2 months for seedlot 1 or 3 months for seedlot 2 (Figure 3, 4, 5). Therefore, seeds of *M. rhoifolia* are considered to exhibit deep dormancy, which can be broken by low-temperature stratification. Fresh mature seeds of *M. rhoifolia* may possess physiological dormancy with a deep level (Baskin and Baskin 2014). Moreover, under moist storage conditions at 4℃ for over 4 months, seeds of *M. rhoifolia* initiated germination in moist, dark environments, with complete germination of all viable seeds occurring within 4 to 8 months under dark conditions (Figure 4, 5). Consequently, moist storage at 4℃ over 4 months is not appropriate for storing seeds of *M. rhoifolia*.

Stratification at 4℃ successfully promoted germination rates and significantly decreased the germination period of *M. rhoifolia* seeds. The MGT of fresh mature seeds of *M. rhoifolia* significantly decreased to about 7 days after 4 months of stratification at 4℃ (Figure 3). Therefore, pretreatment with about 4℃ stratification is a practical approach to enhance *M. rhoifolia* seed germination for nursery operations. We recommend applying cold stratification at 4℃ for 4 months to fresh mature seeds of *M. rhoifolia* collected in October to break seed dormancy. This allows for short-term storage of up to 4 months before sowing. March is an optimal time for nursery operations, as rising temperatures promote uniform germination and rapid seedling growth.

Effects of storage at three temperatures (-20, 4, and 15℃) and five different MC levels (2.7, 5.2, 8.6, 11.7, and 15.1%) and at four temperatures (-196, -20, 4, and 15℃) and five different MC levels (3.0, 5.4, 9.0, 11.0, and 14.4%) within 24 months on seed viability presented similar results for the two seedlots of *M. rhoifolia*. Consequently, seeds of *M. rhoifolia* dried to an MC of 2.7-11.7% maintained viability after 24 months of storage at -196 and -20℃ (Figure 6, 7). Therefore, seeds of *M. rhoifolia* are defined as having orthodox storage behavior due to their tolerance of dehydration and high viability at sub-zero temperatures. Moreover, the above results show a clear relationship

between viability and storage temperature of *M. rhoifolia* seeds. During storage at different temperatures (4, 15, -20, and -196℃), the rate of viability loss in *M. rhoifolia* seeds was in the order 15 > 4 > -20 and -196℃ (Figures 6, 7). In other words, lower storage temperatures are more effective for preserving *M. rhoifolia* seed viability.

Hong and Ellis (1996) used two parameters—the thousand-seed weight and MC—to categorize seed storage behavior 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. MC values of mature recalcitrant seeds ranged 36-90%. Mature seeds with an MC under 35% are not seen as recalcitrant seeds, and those at an MC under 23% are orthodox seeds. In addition, mature seeds at an MC of 23-55% are intermediate seeds. Furthermore, a thousand-seed weight below 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. rhoifolia* were considered orthodox seeds, e.g., the MC of fresh mature seeds is only about 14-15%, and the thousand-seed weight is about 20-25 g (Table 1). For this species, the relation of MC and thousand-seed weight is consistent with the orthodox seed storage behavior of Hong and Ellis's criteria (1996).

The above results show that *M. rhoifolia* seeds have orthodox storage behavior. However, after 24 months of storage, seeds from seedlot 1 with 8.6% MC showed the highest viability at 15℃. For seedlot 2, the best viability was maintained at 4℃ with 11.0% and 9.0% MC, and at 15℃ with 9.0% MC. This finding implies that seeds with moisture content of 3-7% do not exhibit the highest viability when stored at 4℃ or 15℃, suggesting a potential deviation from typical orthodox storage behavior. Nevertheless, we still consider *M. rhoifolia* seeds to be orthodox, as no intermediate seeds have been reported to retain full viability after 2 years of storage at -196℃ with moisture contents as low as 3.0-5.4% (Figure 7).

Generally speaking, when seeds at an MC below 15% are stored at sub-zero temperatures, their free water turns into ice crystals which cause freeze damage to seeds (Tompsett 1985). Before sub-zero storage, MC should be decreased to below 14% in case the available water in cells freezes and causes membranes to break. This is linked to seed deterioration (Copeland and McDonald 1995). Decreasing the MC of seeds reduces their risk of freeze damage. This study showed that seeds of *M. rhoifolia* 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℃ (Figure 6, 7). We inferred that the value of the MC at which seeds of *M. rhoifolia* suffered freeze damage was about 14%. Apparently, the ability of *M. rhoifolia* seeds to protect against freeze damage was weaker than that of other orthodox seeds. In this study, the viability of seeds from seedlot 1 at an MC of 15.1% sharply deteriorated after 3 months of storage at -20℃ (Figure 6) and seeds of seedlot 2 at an MC of 14.4% rapidly lost their viability after 3 months of storage at -196 and -20℃ (Figure 7). Thus, we cautiously speculated that when seeds of *M. rhoifolia* at an MC below 12% are stored at sub-zero temperatures, freeze damage will be likely to occur. As MC increased, the harmful effects of freeze damage were amplified

within a short time. Many tree species of Taiwan with orthodox seed storage behavior exhibit 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), *Scaevola taccada* (Gaertner) Roxb. (Yang and Kuo 2018a), and *Maackia taiwanensis* Hoshi & Ohashi (Yang and Kuo 2018b). When the MC of these 6 species stored at -20℃ exceeded 8, 9, 9, 11, 11, and 12%, respectively, they suffered freeze damage and rapidly died.

Whether exhibiting true orthodox seed storage behavior or intermediate seed storage behavior with good desiccation and sub-zero temperature tolerance, seeds of *M. rhoifolia* at MC levels of 2.7-11.7% retained their initial viability after storage at -20℃ for 2 years. One reason might be that the period of our test was not long enough, such that the effects of freeze damage on seeds at higher MC values of 11.7% (seedlot 1) and 11.0% (seedlot 2) did not emerge within 2 years. In conclusion, when seeds of *M. rhoifolia* need to be stored at sub-zero temperatures for long-term conservation, we recommend that fresh mature seeds of *M. rhoifolia* should be quickly dried to 5-7% MC and then hermetically sealed immediately before storage at -20℃. If seeds of *M. rhoifolia* need short-term storage within 2 years, decreasing the MC to 5-11% and storing seeds at 4℃ are suggested, which will not decrease the germination percentage (Figures 6, 7).

CONCLUSIONS

Seeds of *M. rhoifolia* exhibited deep dormancy. No seeds could germinate during 12 weeks under fluctuating temperatures of 30/20℃ with 8 hours of light per day. Stratification at 4℃ can efficiently improve the germination percentage and significantly decrease MGT. When the stratification period is extended to 3 months, seed dormancy may become entirely broken, enabling viable seeds to fully germinate within 4 weeks. Furthermore, stratification at 4℃ is not appropriate for storing seeds of *M. rhoifolia* because they began to germinate under moist dark conditions after 4 months, and all viable seeds completely germinated within 4-8 months. Thus, pretreatment of *M. rhoifolia* seeds by stratification at 4℃ for 4 months is suggested for nursery operations. Seeds of *M. rhoifolia* dehydrated to MC levels of 2.7-11.7% still maintained viability after storage at -196 and -20℃ for 2 years. Thus, seeds of *M. rhoifolia* are defined as having orthodox storage behavior due to their tolerance to dehydration and high viability at sub-zero temperatures. We recommend that seeds of *M. rhoifolia* be dehydrated to an MC of 5-7% and then hermetically sealed before long-term sub-zero storage. When seeds of *M. rhoifolia* need short-term storage within 2 years, the MC of seeds should be decreased to 5-11% and then stored at 4℃ after being hermetically sealed.

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研究報告

山豬肉種子的發芽與儲藏性質

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摘 要

背景

山豬肉是臺灣原生的清風藤科落葉大喬木，因本種具有每年開花、花序佔樹冠的覆蓋率大、豐富的蜜粉為昆蟲及蜜蜂所喜食等特點，故山豬肉是臺灣最具蜜源造林潛力的樹種之一。本研究探討山豬肉種子的發芽特性，並以種子含水率與儲藏溫度對發芽率的影響來判定其種子儲藏性質，以評估將種子儲藏作為本種種原長期保存之可行性。

材料與方法

供試的2批山豬肉種子分別為2018與2019年採於臺北市陽明山及新竹縣尖石鄉的單一野生母樹，將洗淨種子以水選及人工篩選剔除空輕粒及劣粒後，以獲得試驗用精選種子，此時2批新鮮種子的含水率分別為15.1及14.2%。在種子發芽特性上，以4℃層積經0, 1, 2, 3, 4, 6, 9, 12, 18, 24個月等10個時間點，來探討不同低溫層積時間對其種子休眠解除之效應。在種子儲藏性質的研究上，將第1批種子控制成2.7、5.2、8.6、11.7與15.1%；第2批種子控制成3.0、5.4、9.0、11.0與14.4%等各5級含水率，立即以雙層鋁箔袋密封儲藏於-196、-20、4及15℃等3或4種溫度，於0、3、6、12、18及24個月等6個儲藏時間點，檢驗各不同處理的種子發芽率，以探討不同含水率與儲藏溫度在儲藏期間對種子活力的影響。本研究各不同處理的發芽試驗均是作3重複，第1批每重複30粒，第2批每重複100粒，以30/20℃變溫發芽(每日8小時的30℃；約5,000 Lux光照，及16小時的20℃；黑暗)，胚根正常突出5 mm視為發芽，發芽期為20週，每週記錄1次發芽數。

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結果與討論

結果顯示山豬肉種子具深度休眠性，當新鮮的成熟種子以30/20℃變溫發芽(光照8小時)，在12週內完全不會發芽。4℃層積可以有效提升山豬肉種子的發芽率及顯著降低其平均發芽日數，以4℃層積3個月能完全解除其種子休眠，使種子在4週內完全發芽。再者，4℃層積並非儲存山豬肉種子的良好方法，因當層積時間超過4個月後，種子會開始在黑暗濕潤的低溫環境中發芽，且具有活力的種子會在層積4~8月內全部自行發芽。因此，建議在育苗播種前應先進行的發芽前處理為「4℃層積3~4個月」。此外，當山豬肉種子被乾燥至含水率2.7~11.7%，儲藏在-196與-20℃經2年後，其活力均未顯著下降，以其能耐乾燥及零下低溫的特性，故將本種種子歸類為正儲型。因山豬肉種子屬長壽命，故可採用儲藏種子方式來進行其種原的長期保存工作，建議應先將種子含水率乾燥到5~7%，並立即密封儲藏於零下低溫環境，若為2年內的短期儲存，則可將種子含水率降到5~11%後封存於4℃。

結論

山豬肉種子具深度休眠性，以4℃層積3個月能完全解除其種子休眠，可使種子在4週內完全發芽。山豬肉種子具有耐乾燥及零下低溫的特性，故將其歸屬為長壽命正儲型，長期保存時的最佳儲藏條件為先將種子含水率乾燥到5~7%，並立即密封儲藏於零下低溫環境中。

關鍵詞：山豬肉、發芽、正儲型、種子儲藏性質、層積

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