Research paper

Germination and Storage Behavior of Seeds of *Scaevola taccada* (Gaertner) Roxb.

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

【Summary】

The purpose of this study was to examine the germination characteristics of seeds of *Scaevola taccada*. Effects of the seed moisture content (MC) and storage temperature on germination were investigated to determine its seed storage behavior. Results showed that seeds of *S. taccada* were non-dormant. The majority of fresh mature seeds germinated during 2~5 wk under fluctuating temperatures of 30/20℃ with 8 h of light, and few seeds irregularly germinated in the subsequent 5~20 wk. It was speculated that the difference in germination speed was related to the degree of seed maturity. Moreover, stratification at 4℃ neither improved germination nor decreased the mean germination times (MGTs) of seeds of *S. taccada*. In other words, stratification at 4℃ is not practical for nursery operations because it cannot promote more-uniform germination. Besides, the seeds faced a great risk of dying during moist storage at 4℃ for more than 3 mo; thus, moist storage at 4℃ should not be applied to short-term storage of seeds of *S. taccada*. However, seeds of *S. taccada* dried to 1.9~5.9% MCs (on a fresh-weight basis) still maintained viability after 2 yr of storage at -20, 4, and 15℃. Seeds of *S. taccada* can tolerate desiccation and sub-zero temperatures, which qualifies them as orthodox. In short, seeds of *S. taccada* can be stored over a long-term period to assure future seed supplies. If seed storage of *S. taccada* is used for plant germplasm collection and long-term conservation, we recommend that seeds of *S. taccada* should be dehydrated to 3~7% MCs and then hermetically sealed before storage at -20℃.

Key words: *Scaevola taccada* (Gaertner) Roxb., germination, orthodox, seed storage behavior, stratification.

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研究報告

草海桐種子的發芽與儲藏性質

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摘　要

本研究探討草海桐種子的發芽特性，並以種子含水率與儲藏溫度對發芽率之影響來判定其種子儲藏性質。結果顯示草海桐種子不具休眠性，當新鮮的成熟種子以30/20℃變溫發芽時，大多數會在第2~5週中發芽，但可能有少數種子會在第5~20週零散發芽，推斷此與種子成熟度有關。4℃層積無法有效提升草海桐種子的發芽率，亦無法降低其平均發芽日數使其在短期內整齊發芽，即4℃層積處理在本種的育苗作業上不具實用價值，而且當4℃濕藏時間超過3個月後就有大量死亡的危險，故4℃濕藏並非短期儲藏本種種子的可行方法。當草海桐種子被乾燥至含水率1.9~5.9%，在-20、4與15℃經二年儲藏後活力仍未有下降趨勢，顯示其能耐乾燥且耐零下低溫環境，故判定其屬長壽命的正儲型，亦即可將種子進行長期儲藏以供日後利用，建議未來以儲存種子來進行本種種源的收集與長期保存時，應先將種子含水率降低到3~7%，然後密封儲藏在-20℃環境中。

關鍵詞：草海桐、發芽、正儲型、種子儲藏性質、層積。

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INTRODUCTION

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

Unlike orthodox seeds, recalcitrant seeds have a low degree of desiccation tolerance. When recalcitrant seeds are dried to < 12~31% MCs, seed viability decreases with concurrent prolongation of the drying duration (Roberts 1973). Many recalcitrant seeds are extremely sensitive to low temperatures, and at temperatures of < 10~15℃, their viability often deteriorates, especially seeds from tropical areas (Bonner 1990). Hong and Ellis (1996), therefore, differentiated recalcitrant seeds into temperate and tropical subtypes according to the level of temperature tolerance of recalcitrant seeds. Temperate-recalcitrant seeds cannot tolerate desiccation but can survive a temperature close to 0℃ and MCs of 35~50%. Air circulation plays an important role in the respiration of temperate-recalcitrant seeds during storage; i.e., seed longevity is more likely to reach 1~3 yr after storage at high MCs with appropriate air circulation (Wang et al. 1995). Different from temperate-recalcitrant seeds, tropical-recalcitrant seeds have a weak tolerance of desiccation and low temperatures. They are almost unable to survive a temperature close to 0℃ and are even sensitive to 15~20℃ (Hong and Ellis 1996). As tropical-recalcitrant seeds are very prone to losing viability at low temperatures, the period for which they can be stored is short even under optimal storage conditions (with the highest possible MCs and good air circulation). In other words, viable seeds begin to quickly germinate after storage at high temperatures. To prolong the storage duration, we employ a low-temperature approach to inhibit germination. However, optimal storage conditions are difficult to determine, and seeds often lose their viability. Thus, it is not easy to preserve tropical-recalcitrant seeds for long-term periods. Moreover, relative to the longevity of temperate-recalcitrant seeds, that of tropical-recalcitrant seeds is shorter. Take the Dipterocarpaceae for example. Among 79 species of tropical-recalcitrant seeds, the maximum seed longevity is 1 yr, and the minimum is only 14 d (Tompsett 1987, 1989).

In addition to orthodox and recalcitrant seeds, Ellis et al. (1990a) found that *Coffea arabica* L. seeds survived desiccation to 5~10% MCs but failed to survive at a storage temperature of < 10℃. Such seeds are considered intermediate; that is, intermediate seeds are not like orthodox seeds in that they show strong desiccation tolerance and are vulnerable to low-temperature damage. According to Hong and Ellis (1996), most intermediate seeds can survive desiccation to 10~12% MCs, but their viability declines with a concurrent reduction in the MC.

Scaevola taccada (Gaertner) Roxb., also known as sea lettuce or beach naupaka, is an evergreen standing shrub or small tree in the family Goodeniaceae. This species is usually < 5 m in height and distributed in areas of Madagascar, Southeast Asia, tropical Australia, Micronesia (which consists of islands extending across the north of the equator and to the east of the Philippines), islands of northeastern Australia, and Hawaii. In Taiwan, *S. taccada* is widely found from coastal sandy soils to coral rocks (Huang 1998). This species is a typical seaside plant with a preference for a sunny, wet, high-temperature environment. Still, it is also the best native plant used to establish windbreak forests on the coast because of its advantages of salt tolerance, desiccation tolerance, cold tolerance, strong wind resistance, rapid growth, and high survival rates of plantations in the wild (Deng et al. 2006). The ball-like drupes of this species are about 10~15 mm in diameter. When mature, the outer skins of fleshy fruits change from green to white, and the middle fleshy layer contains suberin, which helps disperse seeds by floating on the sea. There are 2 locules of an ovary and an ovule in each locule. The 2 maturation periods are from February to March and August to September every year in southern Taiwan. The appearance of *S. taccada* seeds is round with a sharp thorn, and an irregular and protruding edge, and the diameter of seeds is about 4 mm (Fig. 1). However, a single ball-like seed is actually composed of 2 hemispheric seeds which have only about 10% chance of fully developing at 1 time. Usually, only 1 seed or even neither of them totally grows.

There were very few studies on seeds of *S. taccada* in the past, e.g., Liao et al. (1995). They conducted a series of experiments on germination of wild seeds of *S. taccada* collected from Neishih Village of Shihzih Township in Pingtung County. From the results, seeds collected in May had the highest germination percentage, while those collected in July had the lowest. In addition, they also found that the optimal temperature for germination was under fluctuating temperatures of 35/20℃ with 12 h of light, and seeds under a constant temperature of 25℃ with 12 h of light almost did not germinate. Furthermore, such pretreatments as high-concentration sulfuric acid (H_2SO_4), hot water, mechanical damage, and red light irradiation all failed to increase the germination percentage. Consequently, this study used a more-careful research design to investigate germination and storage behaviors of seeds of *S. taccada*. The results can offer useful information about nursery operations for plantation programs of native coastal forest species in the future.

MATERIALS AND METHODS

Seed collection and processing

Table 1 gives detailed collection information of the 2 seedlots used in this study. Only mature white fruits were collected at the time of seed collection of the 2 seedlots. Because of outer skins and suberin 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, 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. Seeds of seedlot 1 were washed and extracted on August 30, and seeds of seedlot 2 were processed on September 11, 2007. Of the pure seeds with no debris left, small-sized and damaged seeds were removed manually. Seed characteristics of fresh mature seeds of the 2 seedlots are also given in Table 1. There were no significant differences in the MC, seed length, seed diameter, number of seeds, or thousand-seed-weight between seedlots 1 and 2.

Determination of the MC

Seed MCs were determined gravimetrically with 4 replicates. For each replicate, 10 filled seeds of each seedlot were randomly selected and cut into pieces of < 4 mm in length. Then, the selected seeds were dehydrated. MCs of the seeds were determined using a low-constant-temperature oven method (103±2℃ for 17±1 h) (International Seed

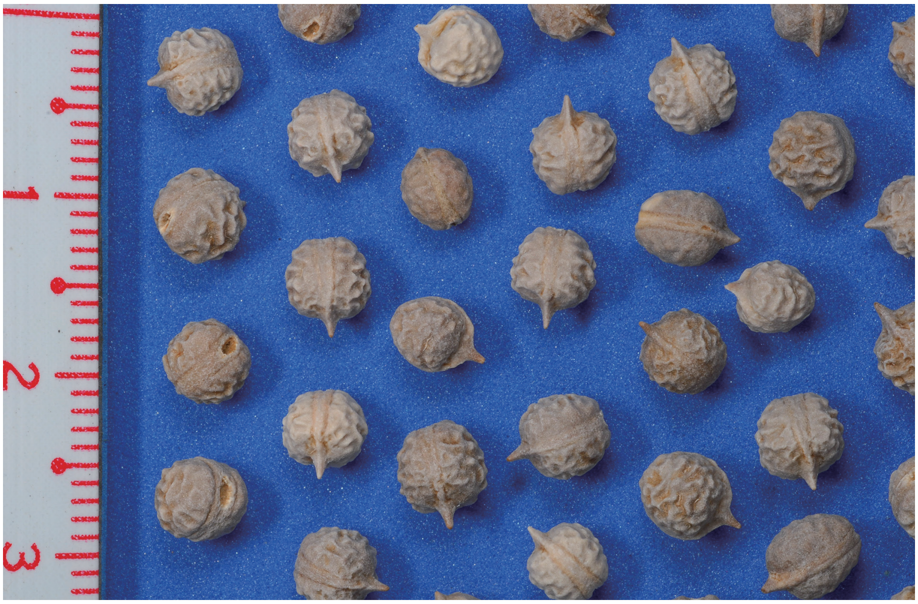

Fig. 1. Seeds of *Scaevola taccada*.

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

	Seedlot 1	Seedlot 2
Collection date	2007/08/21	2007/09/5
Collection location	Hengchun	Taimalee
Latitude, longitude	21°67′N, 120°43′E	22°37′N, 121°00′E
Elevation (m)	30	15
Moisture content (%, FW[1] basis)	11.6±0.1	12.7±0.2
Seed length (mm)	5.34±0.25	4.77±0.46
Seed diameter (mm)	3.94±0.22	4.05±0.37
Number of seeds/L	15,113±332	16,488±160
Thousand-seed weigt[2] (g)	38.9±1.4	36.8±1.4
Germination percentage (%)	71.0±11.1	75.7±10.8
Mean germination time (d)	40.4±3.9	26.0±1.8

[1] FW, Fresh-weight.

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

Testing Association 1999). All MCs are presented on a percentage fresh-weight basis.

Germination assay

To avoid imbibition damage by rapid rehydration, seeds with different treatments were placed above water in a sealed container for 1 d before the germination test so that these 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 (14×10 cm and 0.04 mm thick) with adequate air inside. Excess water of the moss was squeezed out until a water content of about 450% by mass (on a dry-weight basis) was reached (Lin and Chen 1993). To estimate the germination capacity of fresh mature seeds and seeds for dry storage, 25 seeds from seedlot 1 and 35 seeds from seedlot 2 were randomly sampled in 4 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 20-wk test period, the number of protruding seeds was counted once a week. Seeds with a radicle reaching 5 mm were also counted as having germinated. Meanwhile, about 5 ml of water was added to the moss each week. Furthermore, because ball-like seeds of *S. taccada* are composed of 2 hemispheric seeds, 2 radicles might turn into 2 seedlings one after the other. Therefore, to avoid repeated counting, the entile ball-like seed was counted as having germinated when one of the 2 hemispheric seeds had a radicle that had reached 5 mm.

Germination speed

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

4℃ stratification treatment

Table 2 shows durations of stratification at 4℃ for which seeds of the 2 seedlots were stored. The results were used to understand the effects of low-temperature stratification on seed germination of *S. taccada* and the impact of low-temperature moist storage on this species. The stratification method was to thoroughly mix seeds with wet sphagnum moss in a PE bag (14×10 cm, and 0.04 mm thick) and then place it at 4℃ for storage. In each bag, about 2/3 of the volume of air was left for seed respiration, and during storage, water was added and air was refreshed every 2 weeks.

Dry storage and determination of MCs of dehydrated seeds

Sub-seedlots exhibited different MCs, and the range was about 2~15%. When a sub-seedlot reached the desired MC during the dehydration process, seeds were placed in an aluminum foil bag and stored at 15℃ for about 3~5 d before the average equilibrium MC was determined. As soon as the desired MCs of each sub-seedlot were achieved, seeds were sealed in double-layered aluminum foil bags to stabilize the MCs. Table 2 shows storage temperatures and durations of dehydrated seeds of the 2 seedlots. 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 and pure water were used to dry and moisten the seeds, respectively. In seedlot 1, the MC of fresh mature seeds was 11.6%. After dehydration and humidification treatment, the 5 moisture contents were controlled to 1.9% (76 h of desiccation), 5.9% (5 h of desiccation), 8.7% (2 h of desiccation), 11.6% (untreated), and 14.5% (30.5 h of humidification) (Table 2). In seedlot 2, the MC of fresh mature seeds was 12.7%. After treatment, the 5 moisture contents were 2.4% (44 h of desiccation), 5.9% (5 h of desiccation), 8.6% (2.8 h of desiccation), 11.2% (0.6 h of desiccation), and 14.8% (27 h of humidification) (Table 2).

Data analysis

An analysis of variance (ANOVA) was used to analyze the seed germination percentage and MGT to evaluate the effects of the stratification period on germination. Additionally, germination results of the combination of different storage temperatures, seed MCs, and storage periods were statistically analyzed by variables implemented in Tukey's test of the PROC GLM procedure of the SAS statistical package (SAS, Cary, NC, USA).

RESULTS

Germination of fresh mature seeds

Seeds of *S. taccada* exhibited no dormancy. In seedlot 1, when fresh seeds were incubated at fluctuating temperatures of 30/20℃ for 20 wk, their germination percentage was 71.0% and MGT was 40.4 d (Table 1). Seeds mainly emerged during 2~4 wk, but few seeds irregularly geminated in the subsequent 4~20 wk (Fig. 2). In seedlot 2, the ger-

Table 2. Durations of stratification at 4℃ and dry storage durations of seeds at 5 moisture contents (MCs) stored at 3 temperatures of the 2 seedlots of *Scaevola taccada*

	seedlot 1	seedlot 2
4℃ moisture storage duration (mo)	0, 2, 4, 6, 8,10,12, 15, 18, 24	0, 1, 2, 3, 4, 5, 6, 8, 10,12, 15, 18, 24
MCs of dry storage (FW[1] basis, %)	1.9±0.2, 5.9±0.1, 8.7±0.1, 11.6±0.1, 14.8±0.1	2.4±0.1, 5.9±0.1, 8.6±0.1, 11.2±0.2, 14.8±0.3
Dry storage temperature (℃)	-20, 4, 15	-20, 4, 15
Dry storage duration (mo)	0, 3, 6, 9, 12, 18, 24	0, 3, 6, 9, 12, 18, 24

[1] FW, Fresh-weight.

mination percentage and MGT of fresh seeds under the same germination conditions as seedlot 1 were 75.7% and 26.0 d, respectively (Table 1). Seeds mainly emerged during 2~5 wk, and there was no germination after 13 wk (Fig. 2). The above results showed that seeds of *S. taccada* exhibited no dormancy, but few seeds took a longer time to germinate, which might be related to seed maturity. The date on which the seeds of seedlot 2 were collected was later by about 15 d than that of seedlot 1, so that seeds of seedlot 2 might have exhibited better maturity and enabled them to uniformly germinate in a shorter time (Fig. 2). In addtion, at the time of collection, the white fruits of these 2 seedlots were mature, but the seeds inside might not have fully developed maturity.

Effects of stratification at 4℃ on germination

Stratification at 4℃ was believed to neither increase the germination percentage of seeds of *S. taccada* nor decrease the MGTs for concentrating germination. In seedlot 1, the germination percentage of seeds with 4-mo stratification at 4℃ was still 57.0%, and there was no significant difference in germination ($p > 0.1$) compared to fresh seeds (71.0%). When the stratification period was extended to 6 mo, the germination percentage decreased to 2.0%, and the seeds had totally lost viability after 8 mo (Fig. 3). Seeds of seedlot 2 with 3-mo stratification at 4℃ still maintained higher viability. Germination percentages were 59.3~67.1%, and there was no significant difference ($p > 0.05$) in germination

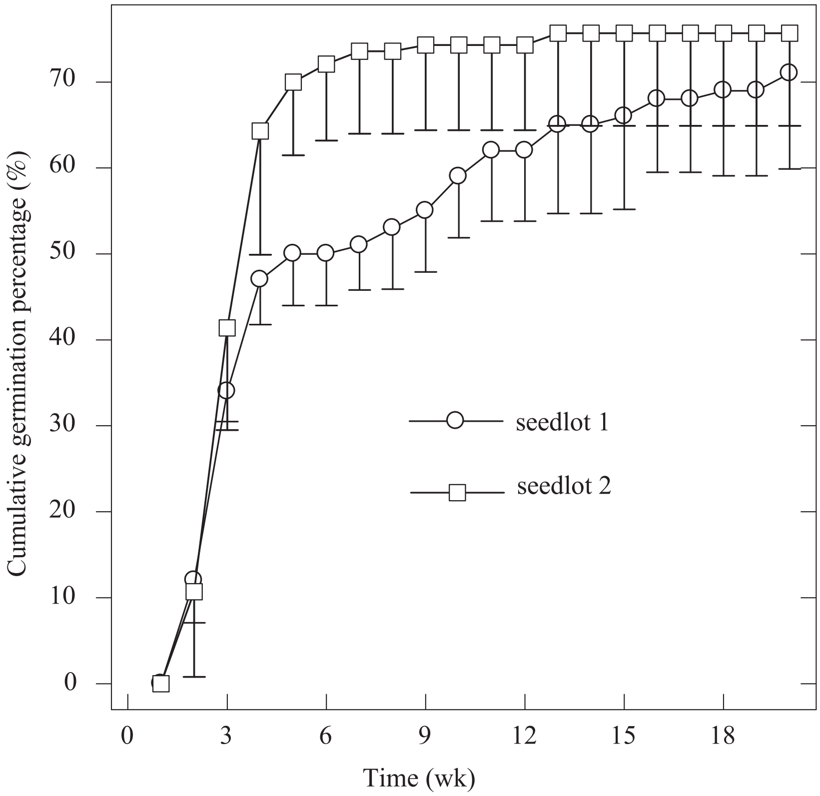

Fig. 2. Cumulative germination percentage of fresh mature seeds of the 2 seedlots.

compared to fresh seeds (75.7%). When the stratification duration was extended to 4 mo, the germination percentage sharply decreased to 46.4% ($p < 0.01$), and the germination percentage continued to decrease during stratification for 4~8 mo ($p < 0.005$). Seeds with 8-mo stratification had almost completely lost viability (only 0.7% germination left) (Fig. 3).

Furthermore, the MGT of seeds of seedlot 1 with 2-mo stratification at 4℃ was 46.5 d, and there was no significant difference ($p > 0.1$) compared to fresh seeds (40.4 d). However, when the stratification duration was extended to 4 mo, the MGT significantly increased to 76.0 d ($p < 0.0005$), and afterward the seeds rapidly died (Fig. 3). In seedlot 2, MGTs of seeds with 2-mo stratification at 4℃ were 27.3~30.3 d, and there was no significant difference ($p > 0.05$) in MGTs compared to fresh seeds (26.0 d). However, when the stratification duration was extended to 3 mo, the MGT significantly increased to 45.5 d ($p < 0.001$). In addition, MGTs continued to increase during stratification for 4~6 mo and significantly increased to 64.6 d after 6-mo stratification ($p < 0.0001$). Subsequently, seeds quickly lost viability (Fig. 3).

From the above results, there was obviously no practical use to apply pretreatment of 4℃ stratification for nursery operations of *S. taccada*. Moreover, under low-temperature moist storage for more than 3 mo, the viability of seeds of *S. taccada* sharply deteriorated. Thus, low-temperature moist storage was not efficient to temporarily store seeds of *S. taccada*.

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

Seeds of *S. taccada* can tolerate desiccation and sub-zero temperatures, so they show orthodox storage behavior. Figure 4 shows the effects of different MC levels and storage

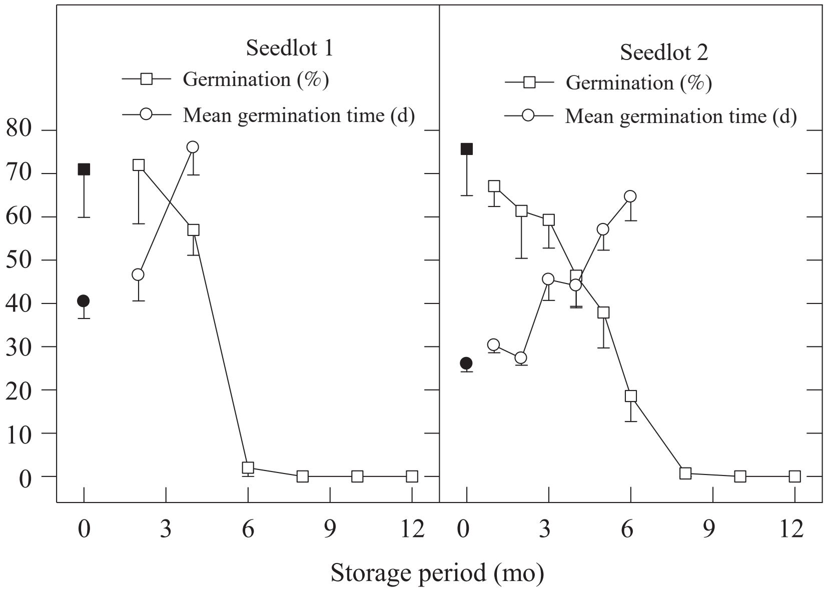

Fig. 3. Effects of stratification at 4℃ for 1~12 mo on the germination percentage (☐) and mean germination time (○) of *Scaevola taccada* seeds. Filled squares (■) and circles (●) respectively represent the germination percentage and mean germination time of fresh mature seeds. Vertical bars represent the standard error of the mean.

temperatures on the germination percentage of seeds of *S. taccada* of seedlot 1 with dry storage for 0~24 mo. Once the 5 desired MC levels were reached, seeds were immediately incubated under fluctuating temperatures of 30/20℃ for 20 wk. Germination percentages of these seeds at MCs of 1.9, 5.9, 8.7, 11.6, and 14.8% were 61.0, 71.0, 63.0, 57.0, and 56.0%, respectively. There was no significant difference ($p > 0.1$) in germination compared to fresh seeds (71.0%). During storage at -20℃, seeds at an MC of 14.8% had totally lost viability after 3 mo. In addition, the germination percentage of seeds at an MC of 11.6% had significantly decreased to 30.0% after 6-mo storage ($p < 0.005$), and the germination percentage was only 19% after 24-mo storage. The other 3 lower-MC seeds had similar germination percentages after 24-mo storage, and germination percentages of these seeds at MCs of 1.9, 5.9, and 8.7% were 58.0, 52.0, and 56.0%, respectively. There was no significant difference in germination compared to fresh seeds ($p > 0.05$) (Fig. 4). During storage at 4℃, viability of seeds at an MC of 14.8% kept dropping, and seeds had totally died after 24 mo. No significant difference in germination was revealed among seeds at the 4 lower MC levels compared to fresh seeds ($p > 0.05$), and germination percentages of these seeds at MCs of 1.9, 5.9, 8.7, and 11.6% after 24-mo storage were 52.0, 62.0, 56.0, and 51.0%, respectively (Fig. 4). During storage at 15℃, viability of the seeds at an MC of 14.8% quickly decreased, and they had completely lost viability after 9-mo storage. The germination percentage of seeds at an MC of 11.6% after 24-mo storage was 43.0%, and there was a significant reduction in germination ($p < 0.05$). Germination percentages of seeds with the other 3 lower MCs did not significantly decrease after 24-mo storage

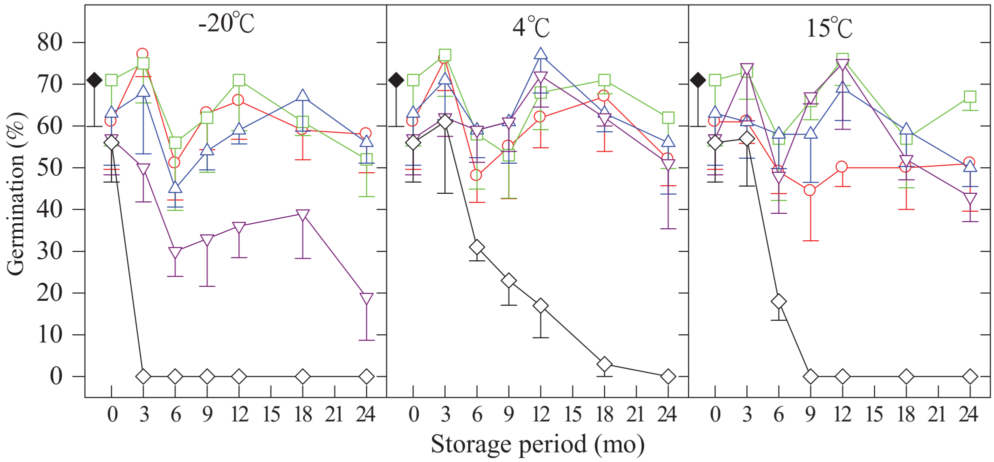

Fig. 4. Effects of storage temperatures (-20, 4, and 15℃) and moisture contents (MCs) (1.9~14.8%, on a fresh-weight basis) on germination percentages of seedlot 1 seeds stored for up to 24 mo. Survival of seeds at the 5 MC levels significantly differed ($p < 0.0001$), but no difference ($p > 0.05$) was observed when seeds of sub-seedlots at MCs of 11.6 and 14.8% were excluded from the analysis for all temperatures. The initial germination percentage of fresh mature seeds was 71.0±11.1% (◆). MCs of seeds: ○, 1.9±0.2%; □, 5.9±0.1%; △, 8.7±0.1%; ▽, 11.6±0.1%; ◇, 14.5±0.1%. Vertical bars represent the standard error of the mean.

($p > 0.05$) (Fig. 4). Survival of seeds at the 5 MCs significantly differed ($p < 0.0001$), but no significant difference ($p > 0.05$) was found if seeds of sub-seedlots at MCs of 11.6 and 14.8% were excluded from the analysis for the 3 storage temperatures (Fig. 4).

Figure 5 shows the effects of different MC levels and storage temperatures on the germination percentages of seeds of *S. taccada* of seedlot 2 after dry storage for 0~24 mo. After the drying process was completed, freshly dried seeds with 5 MC levels were incubated under fluctuating temperatures of 30/20℃ for 20 wk. Germination percentages of these seeds at MCs of 2.4, 5.9, 8.6, 11.2, and 14.8% were 67.9, 73.6, 78.6, 65.7, and 67.1%, respectively. No significant difference ($p > 0.1$) in germination was found compared to fresh seeds (75.7%). When stored at -20℃, seeds at an MC of 14.8% had totally died after 3 mo. Viability of seeds at an MC of 11.2% did not obviously deteriorate during storage for 0~18 mo, until after 24-mo storage, when the germination percentage significantly decreased to 36.4% ($p < 0.005$). Still, germination percentages of the other 3 lower MC seeds at MCs of 2.4, 5.9, and 8.6% with 24-mo storage were 60.7, 62.9, and 55.0%, respectively, and no significant decrease ($p > 0.05$) in germination was found (Fig. 5). After storage at 4℃ for 0~24 mo, viability of seeds at an MC of 14.8% continued to decrease, and seeds had totally died after 24 mo. In addition, there was no significant difference ($p > 0.05$) in germination among seeds at the other 4 lower MC levels and fresh seeds within 24 mo, and their germination percentages at MCs of 2.4, 5.9, 8.6, and 11.2% were 58.6, 74.3, 76.4, and 75.0%, respectively (Fig. 4). The germination result of seeds stored at 15℃ was similar to those at 4℃, but the viability of the seeds at an

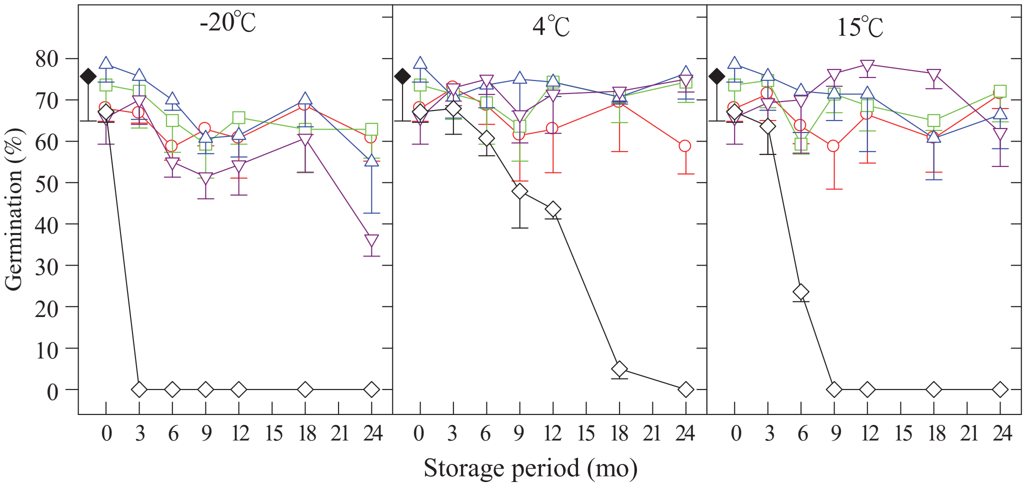

Fig. 5. Effects of storage temperatures (-20, 4, and 15℃) and moisture contents (MCs) (2.4~14.8%, on a fresh-weight basis) on germination percentages of seedlot 2 seeds stored for up to 24 mo. Survival of seeds at the 5 MC levels significantly differed ($p < 0.0001$), but no difference ($p > 0.05$) was observed when seeds of sub-seedlots at MCs of 11.2 and 14.8% were excluded from the analysis for all temperatures. The initial germination percentage of fresh mature seeds was 75.7±10.8% (◆). MCs of seeds: ○, 2.4±0.1%; □, 5.9±0.1%; △, 8.6±0.1%; ▽, 11.2±0.2%; ◇, 14.8±0.3%. Vertical bars represent the standard error of the mean.

MC of 14.8% decreased faster when stored at 15℃, and they had completely died after 9 mo. In addtion, germination percentages of seeds at the other 4 MC levels did not significantly decrease after 24-mo storage ($p > 0.1$) (Fig. 5). Survival of seeds at the 5 MCs significantly differed ($p < 0.0001$), but no significant difference ($p > 0.05$) was found if seeds of sub-seedlots at MCs of 11.2 and 14.8% were excluded from the analysis for all temperatures (Fig. 5). As above, the 2 seedlots showed similar trends in the effects of MCs, storage temperatures, and storage periods on germination percentages (Figs. 4, 5).

DISCUSSION

According to a study by Liao et al. (1995), when collected during the late spring and early summer (in May), seeds of *S. taccada* showed the highest germination percentage, which reached 61%. Seeds collected during the summer season (in July) had the lowest germination percentage, at merely 6.7%. However, in this study, 2 seedlots of *S. taccada* seeds were respectively collected in late August and early September, and they showed better germination under fluctuating temperatures of 30/20℃ for 20 wk (71.0 and 75.7%). Different results were shown between our study and Liao et al.'s, and germination in relation to seed quality collected in different seasons also required investigation with more-careful experiments. Yet, we still found that plants of *S. taccada* growing in southern Taiwan can bloom and fruit throughout the entile year, and 2 obvious maturity periods were found. To obtain a large number of seeds with good quality, we suggest that the optimal time for seed collection is from February to March and from August to September every year when seed crops are abundant. At the time of collection, characteristics of the outer skins can be used to examine the degree of seed maturity. It is the optimal time for seed collection when outer skins have mostly change from green to white and fleshy fruits have begun to swell.

Scaevola taccada is typical of sea-drift plants. The seed-dispersal mechanism is based on the middle fleshy layer of fruits of *S. taccada* with decay-resistant suberin so that they float well in seawater. Seed germinability did not decrease after fruits of *S. taccada* had floated on the sea for 120 d. In non-saline germination medium, seeds in seawater reached a higher germination speed than dried seeds (faster by about 1~2 wk), while in highly saline soils, the germination percentage and germination speed of seeds of *S. taccada* significantly decreased (Lesko and Walker 1969).

Hong and Ellis (1996) used 2 parameters, thousand-seed weight and MC, to categorize seed storage behaviors of species they examined. Their samples were collected from mature seeds of 94 species of 5 families, including the Aceraceae, Araucariaceae, Dipterocarpaceae, Fagaceae, and Myrtaceae. The findings of their study clarified seed storage behaviors as follows. The MC of mature recalcitrant seeds is 36~90%. Mature seeds at an MC of < 35% are not seen as recalcitrant seeds, and those at an MC of < 23% are orthodox seeds. In addition, mature seeds at MCs of 23~55% are intermediate seeds. Furthermore, a thousand-seed weight of < 25 g is classified as orthodox, while a weight above 13,000 g is seen as recalcitrant. If the weight is between 30 and 13,000 g, the seeds could be orthodox, recalcitrant, or intermediate. In this study, seeds of *S. taccada* were considered orthodox seeds, e.g., the MC of fresh mature seeds was about 11~13%, and the thousand-seed weight was about 35~40 g (Table 1). For this species, the relation of the MC and thousand-seed weight with the seed storage behavior was consistent with Hong and Ellis's criteria (1996).

Results of this study showed that seeds of *S. taccada* were non-dormant, and stratification at 4℃ neither improved the germination percentage nor enhanced the germination speed. In addition, the viability of seeds began to deteriorate after 3-mo stratification, seed viability significantly decreased after 4 mo, and seeds had almost totally lost viability after 6~8 mo (Fig. 3). Seeds of *S. taccada*, which are intolerant of low-temperature moist storage, are quite different from seeds of temperate zones but are similar to seeds of *Cordia dichotoma* G. Forst. and *Scolopia oldhamii* Hance (Yang et al. 2006a, 2007). Consequently, such pretreatment as stratification at 4℃ is unnecessary in the nursery for seeds of *S. taccada*, and moist storage at 4℃ is not efficient for short-term storage, either.

Results of stratification at 4℃ in this study showed that seeds of the 2 seedlots (with stratification for 4 and 3 mo, respectively) revealed deterioration. A considerable increase in MGTs was found even when there was no significant decrease in germination (Fig. 3). Therefore, when such parameters as the MGT and germination percentage are used to conduct a seed viability test, the former is more sensitive. In other words, seeds might risk deteriorating when values of the MGT significantly increase (Yang et al. 2006b).

In conclusion, effects of storage at 3 temperatures and 5 different MC levels on seed viability within 24 mo presented similar results for the 2 seedlots. Therefore, seeds of *S. taccada* dried to 1.9~5.9% MCs still maintained viability after 24-mo storage at -20, 4, and 15℃; i.e., seeds of this species can tolerate desiccation and sub-zero temperatures. Therefore, we confirm that seeds of *S. taccada* have orthodox storage behavior (Hong and Ellis 1996).

Generally speaking, when seeds at an MC of > 15% are stored at sub-zero temperatures, their free water turns into ice crystals which causes freeze damage to seeds (Tompsett 1985). Before sub-zero storage, MCs should be decreased to < 14% in case the available water in cells freezes and causes membranes to break. That is linked to seed deterioration (Copeland and McDonald 1995). Thus, decreasing the MC keeps seeds from the risk of freeze damage. This study showed that seeds of *S. taccada* 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℃ (Figs. 4, 5). We inferred that the value of MC at which seeds of *S. taccada* suffered freeze damage was about 11%. Apparently, the ability of *S. taccada* seeds to protect against freeze damage was weaker than that of other orthodox seeds. In this study, seeds of seedlot 1 at an MC of 11.6% had sharply deteriorated after 6-mo storage at -20℃ (Fig. 4). Thus, when seeds of *S. taccada* at MCs of > 11% were stored at sub-zero temperatures, freeze damage would occur. As the MC increased, the harmful effect of freeze damage was simultaneously strengthened in a short time (Figs. 4, 5). Many species have similar characteristics, including *Chionanthus retusus* Lindl. & Paxt. (Yang and Lin 2004), *Sapium discolor* Muell.-Arg., *Celtis sinensis* Pers. (Yang et al. 2006b), and *Scolopia oldhamii* Hance (Yang et al. 2007). When the MCs of these 4 species stored at -20℃ were more than 8, 9, 9, and 11%, respectively, they suffered freeze damage and rapidly died. In this study, the seeds of *S. taccada* at MCs of 1.9~8.6% maintained good viability after storage at -20℃ for 2 yr. One reason might be that the period of our test was not long enough so that the effects of freeze damage on the seeds at slightly higher

MCs of 8.7% (seedlot 1) and 8.7% (seedlot 2) did not emerge within 2 yr. In conclusion, when seeds of *S. taccada* need to be stored at sub-zero temperatures for a long time, we recommend following the rules of FAO/IPGRI (1994). The MC of orthodox seeds should be 3~7% to avoid excess moisture causing seed deterioration. If seeds of *S. taccada* need short-term storage within 2 yr, decreasing the MC to < 11% and storing seeds at 4℃ are suggested, which would not decrease the germination percentage (Figs. 4, 5).

CONCLUSIONS

Seeds of *S. taccada* exhibited no dormancy. Under fluctuating temperatures of 30/20℃ with 8 h of light, most seeds germinated in the subsequent 2~5 wk, but few seeds irregularly germinated during 5~20 wk. Stratification at 4℃ neither increased germination of seeds of *S. taccada* nor decreased the MGTs for concentrating germination in a short time. Therefore, low-temperature stratification was not effective for nursery practice. Seeds of *S. taccada* dehydrated to 1.9~5.9% MCs still maintained viability after storage at -20, 4, and 15℃ for 2 yr. Results showed that seeds of *S. taccada* have orthodox storage behavior, and a long-term storage strategy can be adopted for future demands. We recommend that seeds of *S. taccada* should be dehydrated to 1.9~5.9% MCs and then hermetically sealed before long-term sub-zero storage. When seeds of *S. taccada* need short-term storage within 2 yr, the MC of seeds should be decreased to < 11% and then stored at 4℃ after being hermetically sealed.

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