Research paper

Seed Germination and Storage of *Cycas taitungensis* (Cycadaceae)

Ching-Te Chien,[1,2] Shun-Ying Chen,[1] Shu-Hwa Chang,[1] Jeng-Der Chung[1]

【Summary】

Cycas taitungensis is endemic to an area of about 290 ha in Taitung County, eastern Taiwan. However, this species has been attacked by a scale insect (*Aulacaspis yasumatsui*) and larvae of the butterfly, cycad blue (*Chilades pandara peripatria*), and many plants have died in the last 20 yr. To establish *ex situ* conservation of *C. taitungensis*, seeds were collected from a managed plantation and the *Cycas taitungensis* Nature Reserve for germination. Some of the seeds from the plantation were stored at 5℃ for 8 and 16 mo and germinated at 30/20℃ to test germinability. The embryo length was measured during seed incubation at 25/15 and 30/20℃. Germination of seeds from 2007 from the plantation began in weeks 17~18, and final germination was about 58~60% at 30/20, 25/15, and 25℃ incubation. Germination of seeds from 2008 from the same plantation began in week 18, and final germination percentages were 13, 64, 75, and 84% at 20/10, 25/15, 30/20, and 25℃ incubation temperatures, respectively. Thus, favorable germination temperatures were 30/20 and 25℃. Initial germination of seeds from 7 individual plants from the Nature Reserve extended from weeks 3 to 18, and final germination ranged 30~88%. These differences may have been due to the wide range in time of pollination in the Nature Reserve, thus resulting in different degrees of seed maturity. Fresh seeds air-dried for 24 h and stored at 5℃ for 16 mo retained their original germination, whereas seeds stored with moist moss at 5℃ for 16 mo showed decreased germination from 73% (fresh seeds) to 50%. Cold storage at 5℃ shortened the time to begin seed germination regardless of the storage conditions with or without moist moss. The underdeveloped embryo of *C. taitungensis* seeds must increase in length by about 117% before seed germination can occur. We concluded that seeds of *C. taitungensis* exhibit morphophysiological dormancy, a high temperature at 30/20 or 25℃ increases seed germination, and air-dried seeds can be stored at 5℃ for at least 16 mo.

Key words: *Cycas taitungensis*, morphophysiological dormancy, seed germination, seed storage, underdeveloped embryo.

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研究報告

台東蘇鐵種子發芽與儲藏

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摘 要

台東蘇鐵是台灣特有種植物，僅分布在台東縣轄區，面積約290公頃。過去20年間許多的台東蘇鐵因遭受白輪盾介殼蟲和東陞蘇鐵小灰蝶幼蟲為害死亡。為建立台東蘇鐵區外保育，採自林務局設置的台東蘇鐵自然保留區和台東大武鄉一處私人經營的台東蘇鐵栽種區的種子，分別進行不同溫度的種子發芽試驗。另外，採自私人栽種區的種子部份於5℃儲藏8個月和16個月，取出後在30/20℃下發芽，以瞭解台東蘇鐵種子儲藏後之發芽能力。種子胚長度量測亦在種子播種後同時進行。2007年採自私人栽種區種子開始發芽時間是在第17~18週，發芽率在30/20、25/15和25℃下達58~60%；採自同地區2008年種子開始發芽時間是在第18週，發芽率在25℃下最高達84%，在30/20、25/15和20/10℃下分別為75、64和13%。因此，適合台東蘇鐵種子發芽溫度為30/20和25℃。採自台東蘇鐵自然保留區7株台東蘇鐵種子，開始發芽時間從第3週至第18週不等，且發芽率從30%至88%不等，這些差異可能是保留區母株間受粉時間不一，導致種子成熟度不同所引起的。台東蘇鐵種子經室內空氣乾燥24小時，於5℃儲藏16個月後仍能保持原有新鮮種子的發芽率，但種子與濕水苔混合於5℃儲藏16個月，發芽率從新鮮種子73%降至50%，顯示種子乾藏比濕藏佳。乾、濕種子儲藏於低溫5℃皆能縮短開始發芽的時間。台東蘇鐵種子有發育不完全胚，胚長度必須增加約1.17倍，胚根才會突破種皮發芽。結論台東蘇鐵種子有形態生理的休眠，高溫30/20℃和25℃增加種子發芽率，種子陰乾24小時後可儲藏5℃至少16個月。

關鍵詞：台東蘇鐵、形態生理的休眠、種子發芽、種子儲藏、發育不完全胚。

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INTRODUCTION

Cycas taitungensis Shen, Hill, Tsou & Chen, previously misidentified as *C. taiwaniana*, is endemic to a specific area of about 290 ha in Taitung County, eastern Taiwan (22°51’30”~22°52’30”N, 120°57’30”~121°01’00”E) (Shen et al. 1994). The *Cycas taitungensis* Nature Reserve was established by the Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan in 1970 and announced in accordance with the *Cultural Heritage Preservation Act* of Taiwan in 1986. The flora in the *Cycas taitungensis* Nature Reserve was studied, and 293 species were found with the 5 largest families being the Fabaceae, Asteraceae, Poaceae, Lauraceae, and Euphorbiaceae (Tzeng et al. 2005). The plant communities in the Nature Reserve were classified into 9 types by a matrix cluster analysis and the dominant tree species were *Cyclobalanopsis glauca*, *Zelkova serrata*, *Liquidambar formosana*, *Fraxinus formosana*, *Cinnamomum philippinense*, *Lagerstroemia subcostata*, etc. (Tzeng et al. 2005). *Cycas taitungensis* has been attacked by cycad scale insects (*Aulacaspis yasumatsui* Takagi) and larvae of the butterfly, cycad blue (*Chilades pandara peripatria*), and many *Cycad* plants have died in the last 20 yr (Hsu 1989, Lan 1998, Bailey et al. 2010). Investigations in the Nature Reserve showed that many scale insects were found year-round on leaves, twigs, and rachides, but larvae of cycad blue were found in spring when new leaves occurred.

Depending on the species, germination of *Cycas* seeds can extend for 4~12 mo after sowing, and there is no need to scarify the hard seed coat (Broome 2011). Field seed collection of several Australian *Cycas* species germinated with no treatment, and they can germinate only after the embryo is fully developed (Dehgan and Schutzman 1989). Thus, one objective of our study was to determine the best temperature to germinate seeds of *C. taitungensis*.

Cycas seeds generally are large, the weight per seed is 6.3~13.7 g (Zheng 2000), and seeds cannot be dried when they are stored, otherwise germination decreases (Dehgan 1999, Broome 2011). The optimum storage conditions to extend *Cycas* seed longevity and reduce storage costs must be determined. Thus, our second objective was to find the best way to store the seeds for more than 1 yr without loss of germinability.

A seed that does not have the capacity to germinate in 30 d under suitable environmental conditions such as temperature, light, and water is termed dormant (Baskin and Baskin 1998, 2004). Five classes of seed dormancy are recognized, i.e., morphological dormancy, physiological dormancy, morphophysiological dormancy, physical dormancy, and combined dormancy (physical + physiological dormancy) (Baskin and Baskin 2004). Seeds exhibiting both morphological and morphophysiological dormancy have underdeveloped embryos that must grow inside the seeds before the radicle emerges. Thus, our third objective was to determine if the seeds of *C. taitungensis* exhibit morphological or morphophysiological dormancy.

Approximately 82% of *Cycad* species in the world are threatened, and some of them are in danger of extinction (Donaldson 2003). This paper provides information on the storage of *Cycad* seeds and propagation of the taxon from seeds for plantation use to increase populations in the future.

MATERIALS AND METHODS

Seed collection and handling

Mature reddish-orange to vermilion seeds of *C. taitungensis* were collected both from the *Cycas taitungensis* Nature Reserve, Taitung County on 19 December 2007 and from a managed plantation in Tawu (22°22'N, 120°54'E), Taitung County, Taiwan on 8~10 December 2007 and 2008 (Fig. 1). Seeds from the Nature Reserve were collected and individually numbered for the germination test. Seeds from > 20 plants of the the managed plantation each year were mixed as a seed lot for the germination test. The seed consists of an embryo surrounded by a large megagametophyte, a stony sclerotesta, and a fleshy outer sarcotesta. Seeds without the fleshy sarcotesta collected from managed plantation in 2008 were 49.79 ± 2.55 mm long, 32.32 ± 1.69 mm wide, and 24.08 ± 1.17 mm thick (n = 20) (Fig. 2). There are 35 seeds L^{-1} and 52 seeds kg^{-1}.

Due to scale insects and fungi present on the seed surfaces, all seeds were treated with 2000 ppm Benomyl (a fungicide and pesticide) for 1 h and air-dried in the laboratory for 24 h before using them in subsequent experiments.

Effect of temperature regimes on seed germination

Fresh seeds with dried sarcotesta collected from the managed plantation in 2007 and 2008 and mixed with moist sphagnum moss (cut into small pieces) were placed in sealable polyethylene (PE) bags and incubated at 12

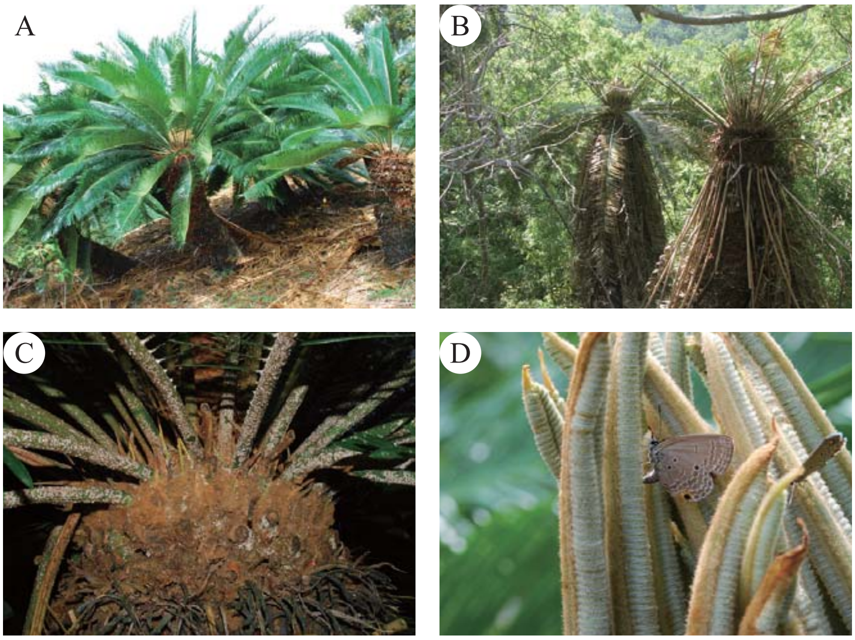

Fig. 1. ***Cycas taitungensis*** **in a managed plantation from which old leaves are pruned in winter (A), in the** ***Cycas taitungensis*** **Nature Reserve (B) with many cycad scale insects on the leaves, twigs, and rachides (C), and a cycad blue butterfly laying eggs on growing leaves (D).**

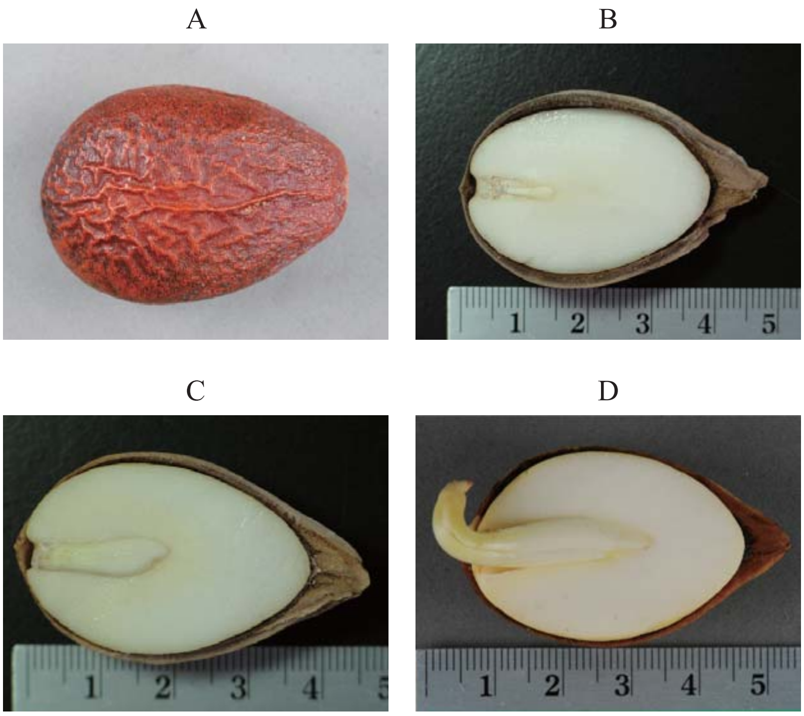

Fig. 2. Intact seed of ***Cycas taitungensis*** **with a reddish-orange sarcotesta (A), longitudinal section of a fresh seed with an underdeveloped embryo (B), seed with a fully grown embryo before root emergence (C), and a seed after radicle emergence (D). The scale marks are millimeters.**

h/12 h of alternating temperature regimes of 30/20, 25/15, and 20/10℃ and at a constant temperature of 25℃. Likewise, fresh seeds collected from the Nature Reserve in 2007 and mixed with moist sphagnum moss in PE bags were tested at 30/20℃ for germination. The water content of the sphagnum moss inside the PE bags was about 400% of the dry mass. The daily photoperiod of 12 h in the incubators (60~80 μmole m^{-2} s^{-1}, 400~700 nm) was at the high temperature. Each treatment consisted of 3 replications of 25 seeds each, except seeds from the Nature Reserve which consisted of 2 replications of 10~28 seeds each depending on the collection number. The outer sarcotesta was removed after several weeks of incubation. Germination, i.e., a radicle at least 2 mm long, was recorded weekly for 32~60 wk. Results are expressed as a germination percentage (%). Due to the coarse nature of the sphagnum, most seeds received some light, but at any given point in time a few may have been in darkness. However, at weekly intervals the contents of each bag were poured out on a table to facilitate examination of seeds for germination. After germination was monitored, seeds and the sphagnum moss were returned to the bag, resulting in a re-shuffling of seeds with regard to their position in the sphagnum and thus the light they received. Consequently, all seeds were in light part (or all) of the time the lights were on in the incubator.

Embryo growth measurement

To monitor embryo growth, seeds of *C. taitungensis* collected from the managed plantation in 2007 with the sarcotesta removed were mixed with moist sphagnum moss, placed in sealable PE bags, and incubated at 30/20 and 25/15℃ for 8 mo. Incubation conditions were the same as these described above for the germination test. Growth of embryos was measured at 4-wk intervals. Seeds were sectioned longitudinally using a mini electric saw, and lengths of 5 embryos from the above 2 incubation temperatures were measured.

Effect of 5℃ storage on seed germination

Fresh seeds (with the sarcotesta intact) collected from the managed plantation in 2008 were treated with Benomyl solution and air-dried in the laboratory for 24 h (as described above) and then separated into 2 portions: 1 portion of seeds mixed with moist sphagnum moss was placed in sealable PE bags and stored at 5℃ in darkness for 8 and 16 mo. The moist moss-stored seeds at 5℃ were opened each month to allow the seeds to breathe. The other portion of seeds in sealed PE bags was directly stored at 5℃ for 8 and 16 mo without moist sphagnum moss. These stored seeds were incubated at 30/20℃ for a germination test. Water contents in the sclerotesta and megagametophyte + embryo of the fresh seeds were 12.9 and 43.5% on a fresh-weight (FW) basis, respectively, as determined by oven drying for 17 h at 103℃ (ISTA 1999).

Statistical analysis

Means (3 replicates) and standard errors of the germination percentage were calculated based on treated seeds. Means of the percent germination were compared by an analysis of variance (ANOVA) and the least significance difference (LSD) test at the 0.05 level of significance (SAS Institute, Cary, NC). Percentage data were arcsine square-root-transformed before analysis, but only non-transformed data are shown in the figures. Embryo length data based on 5 seeds of an incubation time at 30/20 and 25/15℃ were measured, and means and standard errors were calculated.

RESULTS

Effect of temperature regimes on seed germination

Germination of seeds collected from the managed plantation in 2007 began in weeks 17~18, and final germination was 58~60% after incubation at 30/20, 25/15, and 25℃ for 56 wk (Fig. 3). However, the germination rate was lower at 25/15℃ than at 30/20 and 25℃. Seeds from the same plantation in 2008 incubated at 30/20, 25/15, 20/10, and 25℃ for 59 wk germinated to 75, 64, 13, and 84%, respectively (Fig. 4). The optimum temperatures for seed germination were 30/20 and 25℃.

Seeds collected from 7 plants of the *Cycas taitungensis* Nature Reserve in 2007 germinated to 30~88%, and the time to beginning of germination was in weeks 3~18 (Fig. 5). For example, seeds from a plant began to germinate in week 3, and seeds of other plants began to germinate in weeks 7, 10, and 18.

Embryo growth measurement

The mean (±S.E.) length of embryos of fresh seeds collected from the managed plantation in 2008 was 8.50±0.45 mm, and the critical embryo length just before the seed germinated was 18.42±1.06 mm (Figs. 2, 6). Thus, embryo length increased 117% before seeds germinated. After 16 wk, embryo growth in seeds was faster at 30/20℃ than at 25/15℃ (Fig. 6).

Effect of 5℃ storage on seed germination

Germination of air-dried seeds stored at 5℃ for 8 and 16 mo retained the same percentages compared to seeds without storage, but those mixed with moist sphagnum at 5℃

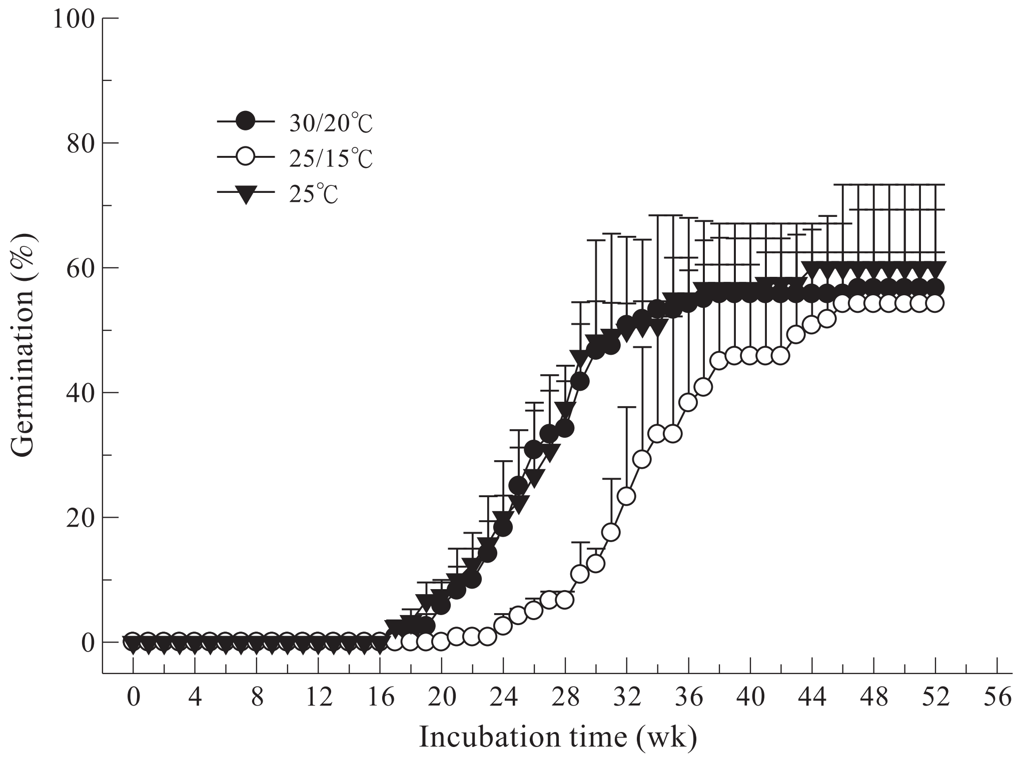

Fig. 3. Effect of temperature on the germination of *Cycas taitungensis* seeds collected from a managed plantation in 2007. Seeds mixed with sphagnum moss were incubated at 30/20, 25/15 and 25℃ for germination. Vertical bars are±the standard error.

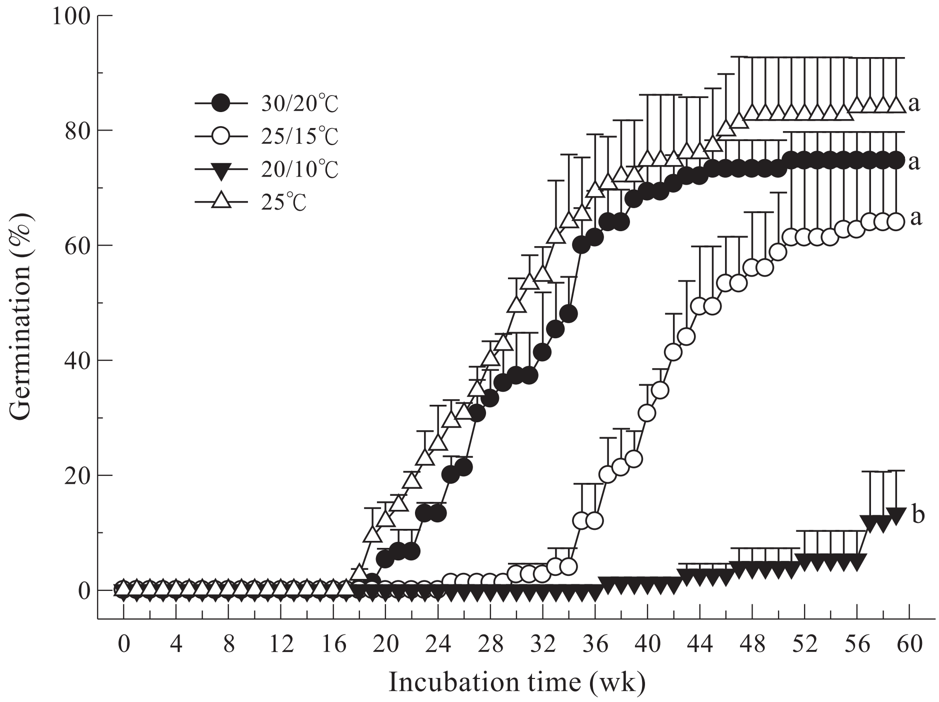

Fig. 4. Effect of temperature on the germination of *Cycas taitungensis* seeds collected from a managed plantation in 2008. Seeds mixed with sphagnum moss were incubated at 30/20, 25/15, 20/10 and 25℃ for germination. Vertical bars are ± the standard error. Points with different letters significantly differ at 59 wk of incubation (LSD, $p = 0.05$).

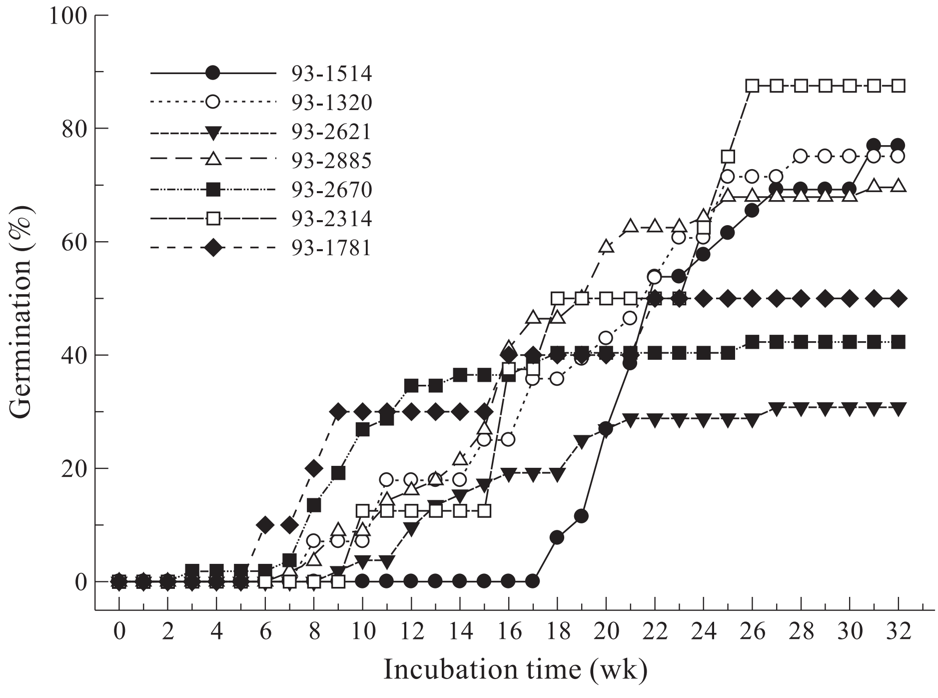

Fig. 5. Cumulative germination percentages of *Cycas taitungensis* seeds collected from the Nature Reserve in 2007. Seeds mixed with sphagnum moss were incubated at 30/20℃ for germination. Each number represents a single female plant.

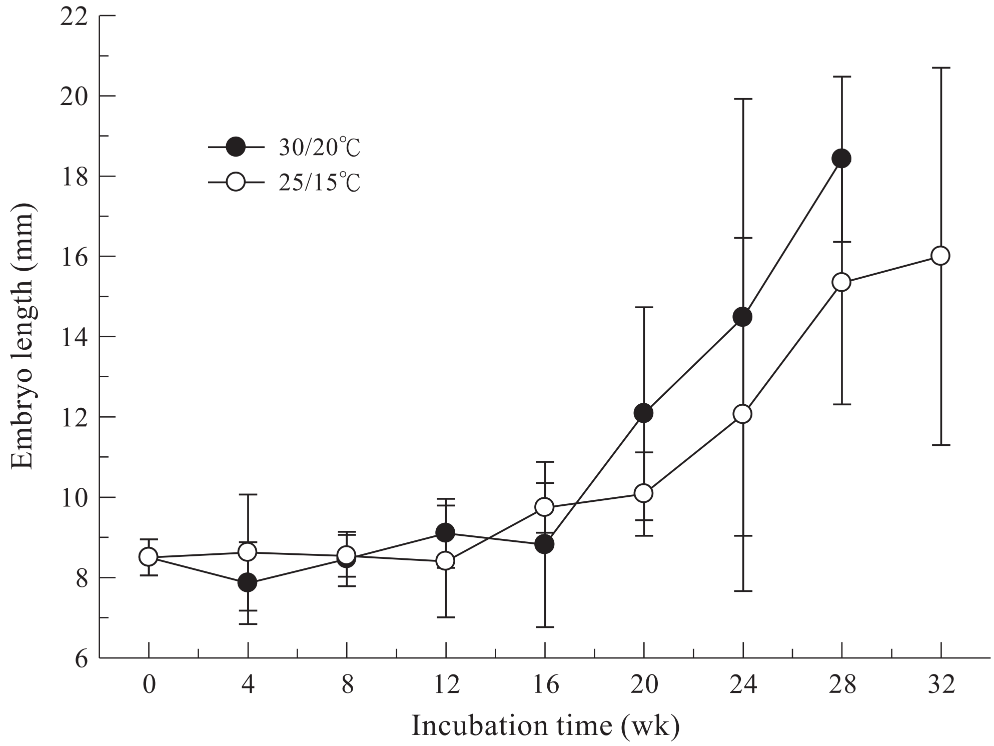

Fig. 6. Effect of temperature on embryo growth of *Cycas taitungensis* seeds collected from a managed plantation in 2007. Seeds mixed with sphagnum moss were incubated at 30/20 and 25/15℃, respectively, and embryo length was measured at 4-wk intervals. Vertical bars are ±the standard error.

decreased by about 18% after 8 and 16 mo of storage (Fig. 7). Final germination percentages between dry-stored seeds and moist-sphagnum-stored seeds significantly differed after 50 wk of incubation, but the final germination percentages of seeds stored for 8 and 16 mo within the same storage treatment did not significantly differ. Storage shortened the time to initiate seed germination from week 20 of fresh seeds to week 14 of 16-mo air-dried stored seeds, or to week 10 of 16-mo moist-sphagnum-stored seeds (Fig. 7). After 16 mo of storage, water contents in the sclerotesta and megagametophyte + embryo of the moist-sphagnum-stored seeds were 28.9 and 45.1%, respectively; water contents in the sclerotesta and megagametophyte + embryo of dry-stored seeds were 14.9 and 44.5%, respectively.

DISCUSSION

The optimal temperatures for maximum germination percentage and rate in seeds of *C. taitungensis* were 30/20 and 25℃. Seeds from the managed plantation began to germinate at these 2 temperature regimes in weeks 17~18, and final germination ranged 58~60% in 2007 and 75~84% in 2008, whereas seeds from 7 individual plants at the Nature Reserve began to germinate at 30/20℃ from weeks 3 to 18, and final germination ranged 30~88%. These differences in seed germination from the Nature Reserve may have been due to the wide range in times of pollination and thus to different degrees of seed maturity and embryo sizes (5~22 mm in length) of seeds. In the managed *Cycas* plantation, old leaves are cut off after seeds are harvested and before new

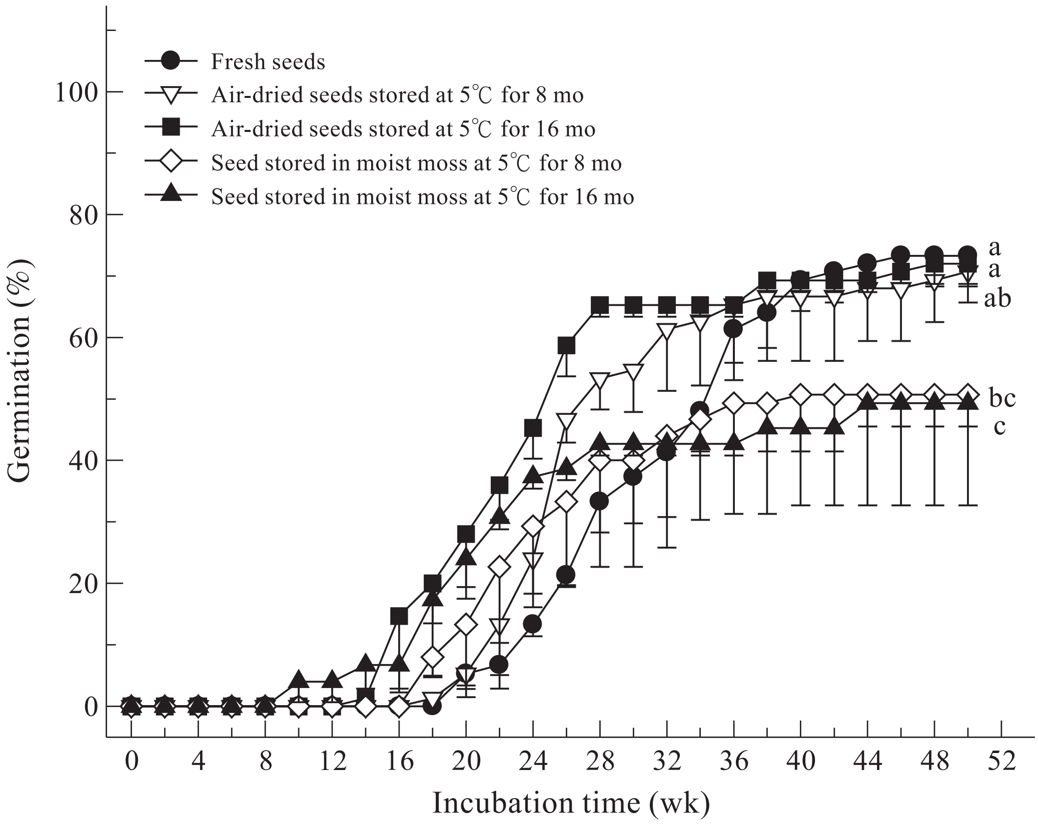

Fig. 7. Effect of storage at 5℃ on germination of *Cycas taitungensis* seeds collected from a managed plantation in 2008. Stored seeds mixed with sphagnum moss were incubated at 30/20℃ for germination. Vertical bars are ± the standard error. Points with different letters significantly differ at 50 wk of incubation (LSD, p = 0.05).

buds emerge in January to reduce the amount of scale insects and avoid cycad blue laying eggs. Thus, *C. taitungensis* plants in the managed plantation flower at the same time, and seeds ripen in October through November and are dispersed in December.

Fresh seeds of *C. taitungensis* have a differentiated (root and shoot can be observed) embryo, and the length of the small underdeveloped embryo had increased about 1.2-fold inside the mature seed before the root and shoot emerge (Figs. 2, 6). Therefore, *Cycas* seeds exhibit morphological dormancy. Since the seeds also required > 30 d for germination, we concluded that seeds exhibit both morphological and physiological dormancy, i.e., morphophysiological dormancy. However, *Cycas* seeds do not exhibit epicotyl morphophysiological dormancy because shoots emerged in 2 wk.

There are 9 levels of morphophysiological dormancy, i.e., non-deep simple, intermediate simple, deep simple, non-deep simple epicotyl, deep simple epicotyl, deep simple double, non-deep complex, intermediate complex, and deep complex (Baskin and Baskin 2004, Baskin et al. 2008, 2009). Which one of the 9 levels of morphophysiological dormancy do seeds of *C. taitungensis* exhibit? Based on the temperature requirements for embryo growth and breaking physiological dormancy, seeds of *C. taitungensis* do not require cold stratification for germination, and embryo growth occurs at warm temperatures, leading to the conclusion that the seeds exhibit non-deep simple morphophysiological dormancy.

Seeds without moist sphagnum stored dry at 5℃ for 16 mo retained their high germinability, and the final germination percentage was nearly the same as those of fresh seeds. Meanwhile, dry-stored seeds at 5℃ germinated rapidly, and the time to initiate germination was shortened. However, seeds mixed with moist sphagnum stored at 5℃ showed a decreased germination percentage, although the time to initiate seed germination was also shortened, indicating that these seeds may have become too wet during cold storage with moist sphagnum. We actually measured the water content of seeds after 16 mo of cold storage and found that the water content in the sclerotesta had increased from 12.9% of fresh seeds to 28.9% of seeds mixed with moist sphagnum, but the water contents of the megagametophyte + embryo were similar. Thus, we recommend that *Cycas* seeds be air-dried for 24 h after harvest and sterilized, placed in sealable PE bags, and stored at 5℃. If water drops in inner PE bag are found during storage, these seeds need to be air-dried again overnight.

Seeds are mainly divided into 2 categories on the basis of storage behavior: orthodox and recalcitrant (Roberts 1973, Hong and Ellis 1996). Orthodox seeds have a low water content following maturation, and can be dried to 5% and stored at subzero temperatures for long periods of time without a loss of viability, whereas recalcitrant seeds shed their high water contents and lose viability rapidly if they are dried to a water content of < 20~30%. *Cycas* seeds were described as exhibiting recalcitrant storage behavior because seeds cannot be dehydrated to a water content below a critical minimum (Dehgan 1999, Broome 2011). Dehgan and Yuen (1983) also indicated that a complete separation between the megagametophyte and sclerotesta in seeds (which produces a rattling sound when shaken) was recognized as indicating that the seeds are inviable. In the present study, we determined that wet storage of seeds of *C. taitungensis* can extend seed longevity, but further study is needed to find the optimal seed storage conditions.

Seed dispersal of *C. taitungensis* occurs in winter. However, newly dispersed seeds require higher temperatures, e.g., 30/20 or 25℃ for germination. We speculated that dispersed seeds may germinate in summer or fall if suitable environmental factors such as water and light are available. To propagate *C. taitungensis* plants, seeds can be air-dried and stored at 5℃ for several months until the next warm season. Under this air-dried storage condition, seeds can be stored for at least 16 mo, which not only extends the storage time but also increases the germination rate (by decreasing the incubation time).

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