Chemical Fungicide Pretreatment and Cool-Wet Storage Prolonging Seed Longevity in Pachira macrocarpa (Cham. & Schl.) Schl.

Shen, Rong-Show
Department of Horticultural Science, National Chiayi University

Lu, Shao-Wei
Department of Horticultural Science, National Chiayi University

Hsu, Shan-Te
Department of Horticultural Science, National Chiayi University

Huang, Kuang-Liang
Department of Horticultural Science, National Chiayi University

他

https://doi.org/10.5109/1467625

出版情報：九州大学大学院農学研究院紀要. 59 (2), pp.249-257, 2014-08-29. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **59** (2), 249–257 (2014)

Chemical Fungicide Pretreatment and Cool–Wet Storage Prolonging Seed Longevity in *Pachira macrocarpa* (Cham. & Schl.) Schl.

Rong–Show SHEN[1]*, Shao–Wei LU[1], Shan–Te HSU[1], Kuang–Liang HUANG[1] and Ikuo MIYAJIMA[2]

Institute of Tropical Agriculture, Kyushu University,
Fukuoka 812–8581, Japan
(*Received April 25, 2014 and accepted May 12, 2014*)

Production of *Pachira macrocarpa* seeds in Taiwan is concentrated in the summer and winter months. Furthermore, the seeds harvested are difficult to store or recalcitrant. To resolve the problem of supplying seeds throughout the year, this study explores the effects that fungicide pretreatment and storage temperature have on the storage longevity of *P. macrocarpa* seeds, and develops effective and practical seed storage technology. The seeds were pretreated with the fungicides benomyl and carbendazim, and then stored at 13˚C, 18˚C, and 23˚C. The results showed that the seeds infected rate during storage increased in correlation with the storage temperature and duration. The seeds pretreated with carbendazim and stored at 13˚C and 18 ºC had a seeds infected rate ranging between 0% and 30% after 4 to 16 weeks of storage. The seeds had not developed infected and exhibited 100% germination rate after sowing. The treatments as control group that were not pretreated with fungicide before storage at 23˚C for 8 weeks all developed infected. For the control groups stored at 18˚C and 13˚C, infection development was delayed until week 12. However, the seeds infected rate achieved 100%, and all seeds died. Finally, all seeds pretreated with benomyl and stored at 23˚C for 8 weeks also developed infected, and those stored at 13˚C for 16 weeks showed a seeds infected rate of 73%; After sowing, the germination rate was only 66.7%. This indicates that pretreatment with carbendazim and maintaining a constant humidity or moisture content and cool temperature of 13˚C can significantly reduce infection development during seed storage. Furthermore, the seeds that developed uninfected kept vigor, achieving 100% germination; thus, the storage longevity of *P. macrocarpa* seeds was effectively extended.

Key words: Benomyl, Carbendazim, Malabar chestnut, Recalcitrant seed

INTRODUCTION

In Taiwan, flowering and fruiting of malabar chestnut [*Pachira macrocarpa* (Cham. and Schl.) Schl.] are all year round. The seed production period can be divided into four segments. However, peak seed production occurs during the end of July and the beginning of August when first–season fruits are produced, and accounts for 60% of the total yield. The winter harvest period (second–season fruits), during the end of January in the subsequent year, is also the second largest amount of seeds yield, accounting for 30% of the total. According to the production and marketing survey of malabar chestnut in Taiwan, the fruit production area measures approximately 188.17 ha, and 1,629 t are produced annually. Nantou, Changhua, and Pingtung County cultivate the majority area of *P. macrocarpa* seedlings (Chung, 2008). However, *P. macrocarpa* seeds are poly–embryonic, recalcitrant, and a mature seed loses viability after one week. The seeds are also sensitive to dry conditions. When the seed water content declines to 20%, at least 50% of seeds can become unviable (Li *et al*., 2009). For water–selected *P. macrocarpa* seeds stored at low temperatures, one week of storage at 4˚C provides the better germination rate, and excessively high or low storage temperatures reduce the storage longevity of the seeds; Furthermore, after 2 months of storage, all seeds perish (Sun *et al*., 2011). Currently, no effective methods exist for extending the longevity of *P. macrocarpa* seeds over 1 month. While the seedlings production of braided and green braided *P. macrocarpa*, which are grown based on seeds as propagules, insufficient seed supply caused the industry cannot be cultivated all year round. This phenomenon has seriously affected the international competitiveness of *P. macrocarpa* seedlings industry in Taiwan.

Storage temperature affects the longevity of recalcitrant seeds, excessively high or low storage temperatures would be greatly reduced seed longevity. Ajayi *et al*. (2006) indicates that wet stored *Telfairia occidentalis* Hook. f. seeds at 6˚C, 16˚C, and 25˚C, and found that the seeds stored at 25˚C (a relatively high temperature) exhibited pathogen growth during the storage period. Seeds stored at 25˚C for 2 weeks showed a 57.5% visible infected rate. Storage at 6˚C produced lower pathogen growth during the storage period; however, the storage process reduced the longevity of the seeds. Ajayi *et al*. (2006) also determined that the optimal storage temperature is 16˚C and can extend the longevity of fluted *T. occidentalis* seeds to 4 weeks or more.

Benomyl and carbendazim are systemic fungicides that offer fungal infection prevention (Lee, 2008). Both fungicides can inhibit pathogen growth for *in vitro* experiments involving petri dishes (Iqbal *et al*., 2010). Saleem

[1] Department of Horticultural Science, National Chiayi University, Chiayi 60004, Taiwan, ROC.
[2] Institute of Tropical Agriculture, Kyushu University, Fukuoka 812–8581, Japan
* Corresponding author: (E–mail: garden@mail.ncyu.edu.tw, telephone: +88652717431, fax: +88652775472.)

et al. (2012) reported that pretreating corn seeds with 3–3.5 g·kg^{-1} of benomyl prior to storage can effectively reduce disease on seed surfaces without affecting seed germination. This technique has also been widely applied to control and prevent diseases that occur during corn seed storage.

Therefore, to prevent dehydration during storage considering seed sensitivity to dry condition, and identification of the specific storage temperatures that can mitigate infection and rots during wet storage have become important topics for extending the storage longevity of *P. macrocarpa* seeds. This study explored the effects that fungicides and storage temperatures had on the storage longevity of *P. macrocarpa* seeds, to investigate how fungicides inhibit infection and rots during storage. The study also identified the ideal temperature for storing *P. macrocarpa* seeds to extend the seed storage longevity, and provides a valuable application for all–year round production of *P. macrocarpa* seedlings. Thereby, these contributions can increase the international competitiveness of *P. macrocarpa* seedlings production in Taiwan.

MATERIALS AND METHODS

Plant materials

Mature *P. macrocarpa* fruits were purchased in mid–February 2012 from a farm in Nantou managed by Mr. Lin. The fruits were harvested in the Nantou mountain region, Taiwan, and delivered to the laboratory within 1 day after harvesting for testing.

Test methods

P. macrocarpa fruits were washed with tap water, soaked in 2% neutral cleaning solution (SODOSIL® RM 02; Sigma–Aldrich Co., LLC., Germany) for 2 min, and then placed in a cool and clean shaded area to allow the fruit to split naturally. After the fruit had split, the seeds were separated, the fresh seeds underwent screening, which involved a visual inspection of their external plumpness. Mature seeds with a fresh weight of over 2.5 g were selected for testing. The selected seeds were again soaked in a 2% neutral cleaning solution for 2 min. After the cleaning solution drip dried naturally, the seeds were immediately soaked in either 330 mg·L^{-1} benomyl (EC. 50% Methyl–1–(butylcarbamoyl)–2–benzimidazole carbamate; Fulon Chemical Industrial Co., Ltd., Taiwan) or 400 mg·L^{-1} carbendazim (EC. 60% Methyl benzimidazol–2–ylcarbamate; Fulon Chemical Industrial Co., Ltd., Taiwan) for 5 min. The seeds in the control groups were soaked in pure water. Subsequently, the seeds were left in a cool shaded location to allow the surface to dry. A Plantma® container (A–Tech Bioscientific Co., Ltd., Taiwan) was used to store the seeds. The seeds were stored in the upper level of the container, and the lower level was filled with 100 mL of pure water. After the container setup was complete, the containers were separately subjected to storage in reefers at 13°C, 18°C, and 23°C for periods of 4, 8, 12, and 16 weeks. Each treatment was repeated 3 times using 15 seeds each. The test period was from February 7, 2012, to June 30, 2012.

Investigated items

Seeds infected during storage (%): After storage, the rate of infection developed on the exterior of the seeds during the storage period was assessed through visual inspection.

Seeds germination during storage (%): After storage, the seed germination rate during storage was assessed through visual inspection. Seed germination was defined as the radicle having emerged from the seed coat.

Index of infection: After storage, the index of infection rate of the seed exterior during the storage period was visually inspected. The indices were divided into 6 levels: 0 indicated no infection on the seed surface; 1 indicated the presence of infected symptoms, and infection on less than 20% of the seed surface area; 2 indicated a infection area of less than 40%; 3 indicated a infection area of less than 60%; 4 indicated a infection area of less than 80%, and that infection symptoms had begun to appear on the seed interior; and 5 indicated that infection was present on the entire seed exterior, with the seed interior completely infected.

Germination (%): When assessing the germination rate, from each batch of seeds prepared using the 3 processes, 10 seeds with no apparent infection were selected and sown after removing the seed coat. The purpose of this procedure was to investigate the seed germination percentage.

Mean germination time: In the formula $\Sigma(f \times v)/N$ (used for calculation), f represents the number of seedlings germinated from one seed per day, v represents the number of days after the seeds were sown in the plug tray, and N represents the total number of germinated seedlings.

Number of seedlings/seeds: The number of seedlings grown from each germinated seed sown.

Statistical analysis

The completely randomized design (CRD) was adopted for all tests, and the results analyzed using CoStat 6.4 statistical software (CoHort Software, Monterey, CA, U.S.A.). An analysis of variance (ANOVA) was conducted to assess the significance of the results, and a least significant difference test (LSD) was performed to identify significant differences between each pretreatment process ($p \leqq 0.05$, $p \leqq 0.01$, and $p \leqq 0.001$). The experimental data in percentage form were converted using a Bliss conversion table before conducting statistical analysis. SigmaPlot® 10.0 statistical plotting software (Systat software Inc., Richmond, CA, U.S.A.) was used to plot the figures and compare and analyze the test results.

RESULTS

Mature *P. macrocarpa* seeds were pretreated with the fungicides benomyl and carbendazim, and then subjected to storage in reefers at 13°C, 18°C, and 23°C. The results showed that for the seeds that underwent this

processing and a storage time of 4 weeks, the seeds infected rate during storage increased in correlation to temperature increases. Seeds pretreated with benomyl and carbendazim fungicide exhibited a lower infection rate compared to the control group (Table 1 and Fig. 1A). The interaction analysis results indicated that storage temperature had an extremely significant relationship with seeds infected rate, and that the use of fungicide also demonstrated a significant relationship with the seeds infected rate. The results also showed that after 4 weeks of storage, the storage temperature had a more substantial influence on the extent of seed infection, although no interaction was observed. After 4 weeks of storage at 13˚C and 18˚C, the seeds achieved a 100% germination rate, while the germination rate of the seeds stored at 23˚C was 90%. Furthermore, all seeds processed maintained better viability. Each seed produced 1.3 or more seedlings number, and the mean germination time (days) was less than 2.5 d (Table 1).

After wet storage for 8 weeks, the seeds pretreated

Table 1. Effect of chemical fungicide pretreatment and storage temperature on 4–week wet storage of *Pachira macrocarpa* seeds

Fungicide	Storage temp. (°C)	Seeds infected during storage (%)	No. of seedlings / seed	Germination (%)	Mean germination time (days)
CK	13	7.8 cde[z]	1.7 abc	100.0 a	1.5 c
	18	25.6 bcd	1.5 e	100.0 a	1.5 c
	23	77.8 a	1.3 f	90.0 b	1.5 c
330 mg·L^{-1} Benomyl	13	6.7 de	1.4 bcde	100.0 a	1.8 bc
	18	16.7 cde	1.3 cde	100.0 a	2.0 b
	23	65.6 ab	1.3 de	96.7 ab	2.3 ab
400 mg·L^{-1} Carbendazim	13	0.0 e	1.7 abc	100.0 a	1.9 b
	18	3.3 e	1.9 ab	100.0 a	2.1 ab
	23	24.4 bc	1.8 a	96.7 ab	2.5 a
Significance					
Fungicide		*[y]	***	ns	***
Storage temp.		***	***	ns	**
Fungicide × Storage temp.		ns	***	ns	***

[z] Means within a column followed by the same letter(s) are not significantly different at 5% level by LSD test. Percentage data were arcsine–square–root transformed prior to analysis.
[y] F–test of ANOVA. ns, non–significant; *, ** and *** significant at 5%, 1% and 0.1% level, respectively.

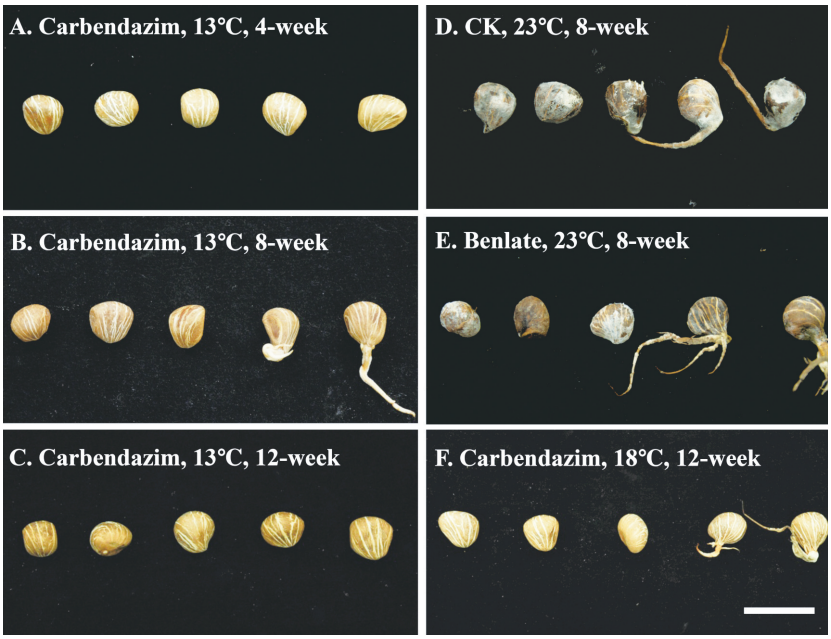

Fig. 1. The condition of chemical fungicide pretreatment and wet storage of *Pachira macrocarpa* seeds. (Bar=3 cm).

with carbendazim and stored at 13℃ exhibited 0% infected rate during seeds storage (Table 2 and Fig. 1B). However, as the storage temperature increased, the infection rate also increased. Nevertheless, the seeds stored at 23℃ exhibited a infection rate of only 18.9% and maintained a 100% germination rate. The average time required for germination was less than 2.3 d, and each seed could grow at least 1.6 seedlings. The seeds pretreated with benomyl and those in the control group exhibited similar seed infection results after 8 weeks of

Table 2. Effect of chemical fungicide pretreatment and storage temperature on 8–week wet storage of *Pachira macrocarpa seeds*

Fungicide	Storage temp. (℃)	Seeds infected during storage (%)	No. of seedlings / seed	Germination (%)	Mean germination time (days)
CK	13	36.7 c[z]	1.2 bcd	90.0 bc	2.0 b
	18	53.3 bc	1.0 cd	86.7 c	2.2 ab
	23	100.0 a	–[y]	–	–
330 mg·L^{-1} Benomyl	13	17.8 cd	1.4 bcd	100.0 a	2.0 b
	18	54.4 bc	0.8 e	90.0 bc	2.1 ab
	23	100.0 a	–	–	–
400 mg·L^{-1} Carbendazim	13	0.0 d	1.8 a	100.0 a	2.0 b
	18	5.6 d	1.7 ab	100.0 a	1.9 b
	23	18.9 cd	1.6 ab	100.0 a	2.3 a
Significance					
Fungicide		***[x]	***	***	***
Storage temp.		***	***	***	***
Fungicide × Storage temp.		ns	ns	***	**

[z] Means within a column followed by the same letter(s) are not significantly different at 5% level by LSD test. Percentage data were arcsine–square–root transformed prior to analysis.
[y] Lack of germination test attributed to over 80% fungal infection during storage.
[x] F–test of ANOVA. ns, non–significant; *, ** and *** significant at 5%, 1% and 0.1% level, respectively

Table 3. Effect of chemical fungicide pretreatment and storage temperature on 12–week wet storage of *Pachira macrocarpa* seeds

Fungicide	Storage temp. (℃)	Seeds infected during storage (%)	No. of seedlings / seed	Germination (%)	Mean germination time (days)
CK	13	100.0 a[z]	–[y]	–	–
	18	100.0 a	–	–	–
	23	100.0 a	–	–	–
330 mg·L^{-1} Benomyl	13	67.8 b	1.3 b	90.0 b	2.3 a
	18	77.8 ab	–	–	–
	23	100.0 a	–	–	–
400 mg·L^{-1} Carbendazim	13	34.4 c	1.8 a	100.0 a	2.5 a
	18	33.3 c	1.3 b	100.0 a	2.8 a
	23	68.9 b	–	–	–
Significance					
Fungicide		***[x]	***	***	***
Storage temp.		***	***	***	***
Fungicide × Storage temp.		ns	***	***	***

[z] Means within a column followed by the same letter(s) are not significantly different at 5% level by LSD test. Percentage data were arcsine–square–root transformed prior to analysis.
[y] Lack of germination test attributed to over 80% fungal infection during storage.
[x] F–test of ANOVA. ns, non–significant; *, ** and *** significant at 5%, 1% and 0.1% level, respectively

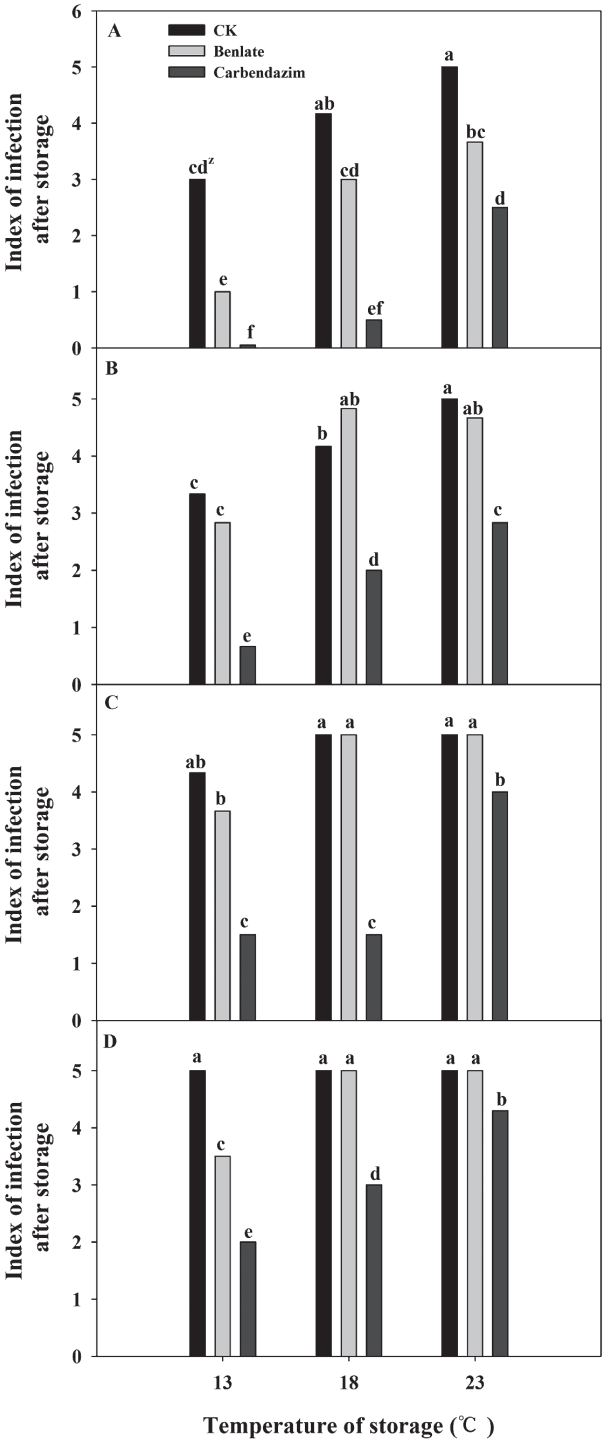

Fig. 2. Effect of chemical fungicide pretreatment and storage temperature on index of infection after 4(A), 8(B), 12(C) and 16(D) weeks wet storage of *Pachira macrocarpa* seeds. [z] The index of infection during storage is the level of infection: Level–0 indicates no infection on the surface; Level–1 indicates that the infection symptom was appeared, but infected area was lower than 20% of seed superficial area; Level–2 indicates infected area was lower than 40%; Level–3 indicates infected area was lower than 60%; Level–4 indicates infected area was lower than 80%, and the infected symptom was appeared inside the seed; Level–5 indicates all seed superficial area and internal were appeared 100% infection. [z] Means within a storage period followed by the same letter(s) are not significantly different at 5% level by LSD test.

storage. The seeds stored at 23˚C showed a 100% infection rate (Figs. 2D and 2E), and the seeds stored at 18˚C showed a infection rate of 50% or more. The seed germination rate for those seeds stored at 23˚C and 18˚C was 90% and 86.7%, respectively (Table 2). The control and benomyl–treated seeds that were stored at 13˚C for 8 weeks exhibited a successful germination rate of 90% to 100%. Besides, the seed index of infection can be adopted to observe the effects of carbendazim pretreatment. The index of infection for the untreated control group increased in correlation to the temperature. After 8 weeks of storage at18˚C and 23˚C, the index of infection for all control group specimens exceeded 4.5, and the seeds stored at 13˚C exhibited an index of infection over 3.5. The seeds that were pretreated with carbendazim and stored at 13˚C for 8 weeks showed an index of infection below 1. Although the index of infection rose with increases in storage temperature, the seeds stored at 23˚C showed a lower infection rate than that of the control group stored at 13˚C (Fig. 2).

The control groups all developed infections during wet storage for 12 weeks (Table 3); therefore, a germination test was not conducted. The seeds pretreated with benomyl showed a 100% infected rate when stored at 23˚C, and a 67.8% and 77.8% infected rate at 13˚C and 18˚C. Although the seeds pretreated with carbendazim exhibited a higher infected rate at 12 weeks than 8 weeks,

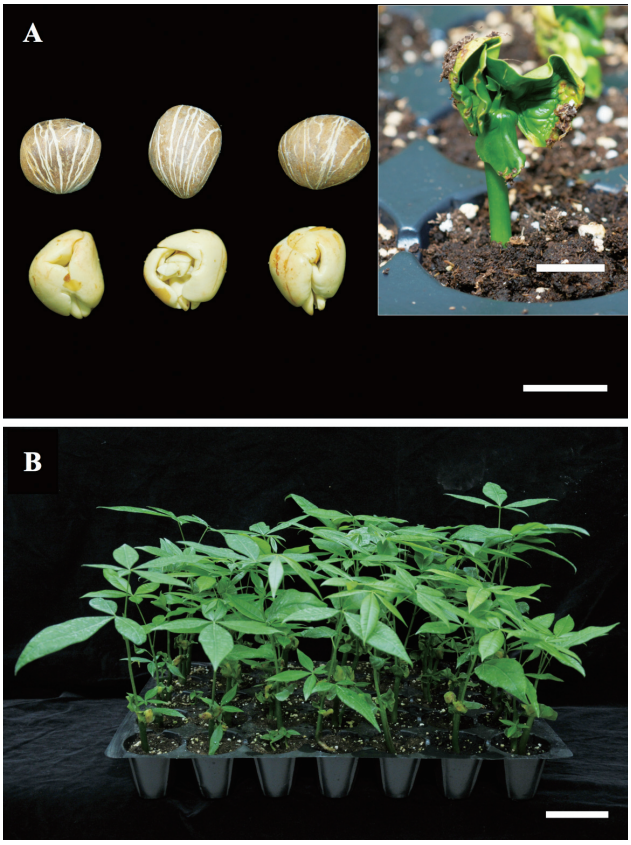

Fig. 3. The condition of carbendazim pretreatment after 16–week cool–wet storage at 13˚C of *Pachira macrocarpa* seeds.
A, The aspect of whole seed, seed coat removed and 3–day after sowing (insert) (bar=2 cm)
B, The seedling growth for 30–day after sowing (bar= 10 cm)

Table 4. Effect of chemical fungicide pretreatment and storage temperature on 16–week wet storage of *Pachira macrocarpa* seeds

Fungicide	Storage temp. (°C)	Seeds infected during storage (%)	No. of seedlings / seed	Germination (%)	Mean germination time (days)
CK	13	100.0 a[z]	–[y]	–	–
	18	100.0 a	–	–	–
	23	100.0 a	–	–	–
330 mg·L^{-1} Benomyl	13	73.6 a	1.3 c	66.7 b	2.8 a
	18	97.6 a	–	–	–
	23	100.0 a	–	–	–
400 mg·L^{-1} Carbendazim	13	24.4 b	1.7 a	100.0 a	2.8 a
	18	27.7 b	1.6 b	100.0 a	2.8 a
	23	100.0 a	–	–	–
Significance					
Fungicide		***[x]	***	***	***
Storage temp.		***	***	**	***
Fungicide × Storage temp.		***	***	**	***

[z] Means within a column followed by the same letter(s) are not significantly different at 5% level by LSD test. Percentage data were arcsine–square–root transformed prior to analysis.
[y] Lack of germination test attributed to over 80% fungal infection during storage.
[x] F–test of ANOVA. ns, non–significant; *, ** and *** significant at 5%, 1% and 0.1% level, respectively

only the seed infected rate when stored at 23°C was 68.9%. The seeds stored at 13°C and 18°C had less than a 35% infected rate, and achieved a 100% germination rate after sowing. However, the mean germination time increased slightly to 2.5–2.8 d (Table 3 and Fig. 1F).

Regarding the seeds that were wet stored for 16 weeks, the benomyl–treated seeds stored at 13°C exhibited infected rate of 73.6%, and the carbendazim–treated seeds stored at 13°C and 18°C exhibited a infected rate between 24.4% and 27.7% during storage. The remaining treatments had seeds infected rate of 100% (Table 4). However, benomyl–treated seeds stored at 13°C achieved a germination rate of only 66.7% after sowing. In addition, the carbendazim–treated seeds stored at 13°C and 18°C maintained a germination rate of 100% (Fig. 3B), and grew at least 1.6 seedlings per seed. However, the mean germination increased to 2.8 d (Table 4). In addition, the carbendazim–treated seeds stored at 13°C for 16 weeks showed no interior infection following seed coat removal (Fig. 3A). After sowing, the seeds germinated within 2.8 d (insert, Fig. 3A), achieving a 100% germination rate. Regarding the index of infection, the carbendazim–treated seeds stored at 13°C for 16 weeks had an index of infection of approximately 2. However, the index increased with increases in the storage temperature. These results were superior compared to those of the control group and the group pretreated with benomyl. Moreover, the carbendazim–treated seeds stored for 16 weeks had a lower index of infection compared to the control group that was stored for 4 weeks (Fig. 2).

DISCUSSION

In an effort to extend the storage longevity of *P. macrocarpa* seeds, this study explored the effects that fungicide pretreatments, different temperatures, and varying storage durations have on *P. macrocarpa* seed storage longevity. The physiological characteristics of *P. macrocarpa* seeds conform to the dehydration and storage intolerance characteristics of recalcitrant seeds proposed by Win (2008). Therefore, wet storage is the primary method employed to extend the longevity of *P. macrocarpa* seeds (Lu, 2013). In a previous study, *T. occidentalis* Hook. f. seeds were soaked in a 3.5% sodium hypochlorite solution, and semi–translucent plastic buckets were used for wet storage at 6°C, 16°C and 25°C. The results showed that during the seed storage period, the infection rate increased in correlation to the storage temperature and duration. Seeds stored at 25°C for 6 weeks showed severe infection, and although seed storage at 6°C inhibited mold growth during storage, it also reduced seed viability. The more suitable seed storage temperature was 16°C, under which seeds could maintain infection rate of less than 30% and achieve a germination rate of 90% or above (Ajayi *et al.*, 2006). In the present study, the control group of untreated *P. macrocarpa* seeds stored at 23°C (a relatively high temperature) for 4 weeks exhibited seeds infected rate during storage of 77.8% (Table 1), and the seeds stored at 13°C exhibited seeds infected rate of approximately 7.8% or below. These results indicate that cool–wet storage can inhibit pathogen growth, and that seeds that had not developed infection could still achieve 100% seed germi-

nation viability. The test results corresponded to those of the *Telfairia occidentalis* seed storage study conducted by Ajayi *et al*. (2006); however, the optimal storage temperature for *P. macrocarpa* seeds was determined to be 13˚C, slightly lower than the 16˚C for *T. occidentalis*. Because the *T. occidentalis* seeds were not pretreated with fungicides before storage, the seeds infected rate became excessively high after 6 weeks of storage, reducing seed survival rates. This study applied fungicides to accommodate storage temperature processing for *P. macrocarpa* seeds. The results indicate that in addition to the importance of a 13˚C storage temperature, fungicide pretreatment has a significant influence on seed storage longevity.

The method proposed by Mata and Moreno–Casasola (2005) for storing *Pachira aquatica* Aubl. seeds involved floating the seeds on water. The resulting seeds achieved an 80% germination rate after 90 d. For *Laurus nobilis* L. seeds stored at 5˚C, although wet storage provided superior results, long–term storage remained unachievable (Konstantinidou *et al*., 2008). These results indicate that recalcitrant seeds are intolerant to dry conditions; thus, they require different storage strategies compared to orthodox seeds. Decreases in seed moisture content disrupt the cell membranes and organelles, resulting in seed death. Based on previous research, this study infers that recalcitrant seeds are intolerant to limited moisture or humidity; hence, low–moisture or humidity storage processing was not conducted. In past research, *Coffea canephora* Pierre seeds were stored with varying seed moisture content to demonstrate the effects that differing moisture content has on the storage longevity of recalcitrant seeds. As the moisture content of seeds declined, the radicle protrusion (%) and germination rate decreased (Rosa *et al*., 2005). Similarly, *Carica papaya* L. seeds exhibited superior germination rate when stored with moisture content of 8% to 11% (which was reltaively high). After storage for 9 months, the seeds still achieved a germination rate of over 80% (Dias *et al*., 2010). When the water content of fresh *P. macrocarpa* seeds declines below 1.4 $gH_2O \cdot g^{-1}$ DW, all seed viability is lost (Lu, 2013). Based on this outcome and the rapid decline in germination rates for *C. canephora* and *C. papaya* after being exposed to dry conditions and dehydration, it is speculated that a low seed moisture content is not beneficial for storing *P. macrocarpa* seeds. The wet storage device employed in this study greatly enhanced the maintenance of *P. macrocarpa* seed longevity. The Plantma® container comprises upper and lower layers that are connected by a plastic tube. The seeds were stored in the upper layer, and pure water was placed in the lower layer. This device can provide an interior relative humidity of 100% (results not shown) without the seeds directly contacting the water or other aqueous medium. In addition to delaying seed germination during storage, this device can drastically reduce infection.

Benomyl and carbendazim are systemic mitosis inhibiting agents that offer both prevention and treatment functions. To prevent seed diseases, soaking hastened germinated rice in 400 $mg \cdot L^{-1}$ benomyl can effectively reduce the occurrence of rice blasts (*Pyricularia oryzae* Cav.), Bakanae disease (*Gibberella fujikuroi*), brown spots (*Cochliobolus miyabeanus*), narrow brown leaf spots (*Sphaerulina oryzina* Hara), stem rot (*Magnaporthe salvinii* (Catt.) R. A. Krause & R. K. Webster), and other pathogens that adhere to rice seeds, reducing outbreaks in the seedbed or rice field (Gergon and Prot, 1993). For gourd, cucumber, and bitter melon seeds infected with *Fusarium solani*, pretreatment with 3 $g \cdot kg^{-1}$ of benomyl or carbendazim fungicides significantly increased the seed germination rate, and reduced the seed and seedling death rates (Sultana and Ghaffar, 2010). This study used systemic fungicides benomyl and carbendazim to hinder the proliferation of pathogens during seed storage. The test results showed that although both fungicides are systemic, only carbendazim provided significant effects for *P. macrocarpa* seed storage. This result is similar to that reported for the prevention of disease in stored rice, where carbendazim could only prevent the growth of *Alternaria padwickii* and *Curvularia lunata* (Nghiep and Gaur, 2005).

Because the moisture content of *P. macrocarpa* seeds is high, if the seeds are stored without a fungicide pretreatment, pathogens rapidly proliferate on the seed surface. The coat of *P. Macrocarpa* seeds is approximately 0.42 mm thick, which provides a natural barrier for the seed interior. However, clear germination pores can be observed on the seed coat structure. The results indicate that when pathogens proliferate on the seed coat surface, they can invade the seed interior through germinal pores. The radicle is located under the germinal pores. Once pathogens enter the germination pores, they rapidly infect the radicle, reducing the seed survival rate (results not shown). In addition to the cotyledons accounting for the majority of the internal seed volume, the water content of the endosperm can be as high as 3.0 $gH_2O \cdot g^{-1}$DW (Lu, 2013). Thus, when pathogens invade, they can rapidly infect the endosperm, reducing the seed germination rate. In this study, the seeds were soaked in fungicide and left to dry naturally in a shaded area for a brief period before storage. Hence, the seeds were coated in a thin layer of fungicide, which could prevent pathogens from proliferating on the surface. This process can reduce the likelihood of pathogens invading the germinal pores, which enables the endosperm to maintain normal moisture content without exhibiting signs of infection (Fig. 3A) and greatly enhances the seed survival rate and germination rate (Table 4).

The tests conducted in this study investigating the extension of *P. macrocarpa* seed longevity using fungicide pretreatment and storage at specific temperatures provided the following results: (1) Following 4 weeks of storage, the fungicide pretreatment and storage temperature factors did not exhibit an interaction effect on the seeds infected rate during storage, although the 2 factors had separate effects on the seeds infected rate during storage. Additionally, the fungicides and storage temperature did not significantly affect the seed germination rate after storage (Table 1). (2) After 8 weeks of storage, the fungicide factor's effect on the seeds infected

rate increased significantly, although no interaction was observed. However, the 2 factors had separate statistically significant effects on the seed germination rate (Table 2). (3) After 16 weeks of storage, the fungicide and storage temperature exhibited a significant interaction effect on the seeds infected rate (Table 4). These results indicated that within a 4–week storage period, a storage temperature factor of 13˚C can inhibit seed infection during the storage period. Additionally, most of the indices of infection in the pretreated seeds did not severely affect seed viability, and the germination rate reached 90 to 100%. Thus, the 2 factors of temperature and fungicide had no significant effects on the germination rate. However, when the storage time was increased to 8 weeks, the seeds infected rate during the storage period increased, and the infection inhibiting effects of the fungicide factor during storage were evident. Consequently, the significance of the fungicide factor increased. When the storage period was extended to 16 weeks, all seeds exhibited infected rate of above 80%, except for those pretreated with carbendazim. Most of the seeds exhibited severe infection symptoms. Without a cool storage temperature and carbendazim pretreatment, the infection could not be inhibited. Therefore, the interaction between fungicide and storage temperature showed statistical difference. These results indicated that short–term seed storage at cool temperatures effectively delayed the development of infection on seeds. However, both fungicide pretreatment and cool temperatures must be applied to extend seed longevity for long–term storage.

The production of *P. macrocarpa* seeds in Taiwan is concentrated at the end of July and January of the next year. The supply of seeds between these 2 periods is nonexistent or sporadic, resulting in a seed shortage for 4 to 5 months between production periods. However, all *P. macrocarpa* products, such as single–trunk or braided seedling with specific ornamental value, require seeds to grow. The annual production of *P. macrocarpa* seedlings depends totally on the seed supply. However, *P. macrocarpa* seeds are recalcitrant, and mature seeds that are not treated can lose nearly all viability 1 week after harvesting (Li *et al*., 2009). Seed longevity when stored at 4˚C is only 1 month (Sun *et al*., 2011). Thus, how to extend the longevity of *P. macrocarpa* seeds to ensure a consistent seed supply for seedling production is crucial for the *P. macrocarpa* industry and enhancing seedling quality. This study examined the effects that fungicide and storage temperature have on *P. macrocarpa* seed longevity. The results show that the storage longevity of *P. macrocarpa* seeds was successfully extended to 4 months. By categorizing and summarizing a cool–wet storage technique in seed of *P. macrocarpa*, the seed shortage for the 4 to 5 months between production periods can be resolved. If this technique can be combined with the existing system for accelerated growth technique of green braided seedling in *P. macrocarpa* (Wang, 2011), it can provide a reference for the seedling industry regarding the all–year production and supply plan of green braided seedling. However, *P. macrocarpa* seeds remain intolerant to dry conditions and dehydration, and the seed storage longevity cannot be compared to that of orthodox seeds. Hence, further tests to determine the physiological changes or responses in the seed interior that occur during dry conditions or storage are required.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Agriculture and Food Agency, Council of Agriculture, Executive Yuan, Taiwan, ROC. 99AS–4.2.2–FD–Z3(20) and 100AS–4.2.2–FD–Z3. We are also grateful to Mr. Wu–Chou Chung and Mr. Chi–Hua Lin for providing the seeds materials and to Miss Ying–Jhen Chen for help investigating.

REFERENCES

Ajayi, S. A., P. Berjak, J. I. Kioko, M. E. Dulloo, R. S. Vodouhe 2006 Responses of fluted pumpkin (*Telfairia occidentalis* Hook. f.; Cucurbitaceae) seeds to desiccation, chilling and hydrated storage. *S. Afr. J. Bot*., **72**: 544–550

Chung, W. C. 2008 The survey report of malabar chestnut production and marketing in Taiwan in 2008. Taiwan Pot Flower Asso., Taipei. Taiwan

Dias, D. C. F. D. S., W. T. Estanislau, F. L. Finger, E. M. Alvarenga, L. A. D. S. Dias 2010 Physiological and enzymatic alterations in papaya seed during storage. *Rev. Bras. Sements*, **32**: 148–157

Gregon, E. B. and J. C. Prot 1993 Effect of benomyl and carbofuran on *Aphelenchoides besseyi* on rice. *Fundam. Appl. Nemalo*., **16**: 563–566

Iqbal, Z., M. A. Pervez, S. Ahmad, Y. Iftikhar, M. Yasin, A. Nawaz, M. U. Ghazanfar, A.A. Dasti, A. Saleen 2010 Determination of minimum inhibitory concentrations of fungicides against fungus *Fusarium mangiferae. Pak. J. Bot*., **42**: 3525–3532

Konstantinidou, E., I. Takos, T. Merou 2008 Desiccation and storage behavior of bay laurel (*Laurus nobilis* L.) seeds. *Eur. J. For. Res*., **127**: 125–131

Lee, L. M. 2008 The induction of plant fungicide use. Handbook of crops diagnosis and safely use of pesticide. Taiwan agricultural chemicals and toxic substances research institute. pp. 61–89

Li, Y. H., Y. M. Ma, L. Han 2009 Relationship between tolerance to dehydration and superoxide dismutases (SODs) in *Pachira marcrocarpa. Scientia Silvae Sinicae*, **45**: 74–79

Lu, S. W. 2013 Seed storage, production and shipment techniques of green braid seedling in malabar chestnut (*Pachira macrocarpa*). MS Thesis, Dept. Hort., Natl. Chiayi Uniy., Chiayi

Mata, D. I. and P. Moreno–Casasola 2005 Effect of in situ storage, light, and moisture on the germination of two wetland tropical trees. *Aquat. Bot*. **83**: 206–218

Nghiep, H. V. and A. Gaur 2005 Efficacy of seed treatment in improving seed quality in rice (*Oryza sativa* L.). *Omonrice* **13**: 42–51

Rosa, S. D. V. F., D. S. B. Júnior, E. V. R. V. Pinho, A. D. Veiga, L. H. C. Silva. 2005 Effect of different drying rates on the physiological quality of *Coffea canephor*a Pierre seeds. *Braz. J. Physiol*. **17**: 199–205

Saleem, M. J., R. Bajwa, A. Hannan, T. A. Qaiser 2012 Maize seed storage mycoflora in pakistan and its chemical control. *Pak. J. Bot*. **44**: 807–812

Sultana, N. and A. Ghaffar 2010 Effect of fungicides, microbial antagonists and oilcakes in the control of *Fusarium solani*, the cause of seed rot, seedling and root infection of bottle gourd, bitter gourd and cucumber. *Pak. J. Bot*. **42**: 2921–2934

Sun, Y. W., S. T. Kuo, M. T. Liu, W. W. Liao, W. C. Chung 2011 The

storage and mutagenic technique in *Pachira macrocarpa*. *Seed Sci. Tech*. **74**: 14–16

Wang, Z. L. 2011 Accelerated growth techniques and simulation shipment in braiding malabar chestnut [*Pachira marcrocarpa* (Cham. & Schl.) Schl.]. MS Thesis, Institute of Agr., Natl. Chiayi Univ., Chiayi

Win, B. 2008 On the compound quantitative characteristic trait of seed recalcitrant. *Acta Bot. Yunnanica* **30**: 76–88