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Original Article

Seed storage and pre-sowing treatment affect germination of the timber tree *Prunus arborea*

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Abstract

In many countries, the demand for timber from native trees is increasing due to its beauty and durability but timber logging in natural forests has been prohibited for various reasons. Consequently, plantations of native tree species are increasing in area. The aim of this study was to examine the effects of storage and pre-sowing treatment on seed germination in *Prunus arborea* (Blume) Kalkman, the timber of which is used for furniture and construction. Four storage treatments (S1, clean seeds stored in a cloth bag under room conditions; S2, clean seeds stored in a cloth bag in a refrigerator at 5°C; S3, clean seeds stored in a cloth bag in a refrigerator at -30°C; and S4, clean seeds stored in wet sand at a nursery) and five pre-sowing treatments (PT1, soaked in normal water for 12 hours; PT2, soaked in normal water for 6 hours; PT3, soaked in 40°C–50°C water for 6 hours; PT4, soaked in 70°C–80°C water for 6 hours; and PT5, no pre-sowing treatment as a control) were tested. The results indicated that seeds in the S2 group retained a germination rate of 54% after 9 months of storage, while seeds in the other groups failed to germinate after 1 month of storage for S3, 3 months of storage for S4, and 6 months of storage for S1. Among the pre-sowing treatment groups, the lowest germination rate was found in PT4 (33.1%), which was half of that in PT1 (60.4%), PT2 (63.9%), PT3 (64.6%), and PT5 (62.8%). These findings indicate that seeds of *P. arborea* should be stored in a refrigerator at 5°C for less than 9 months and should be soaked in 40°C–50°C water for 6 hours before being sown in a wet sand bed.

Keywords: Hot water, Moisture, *Prunus arborea*, Seed dormancy, Storing duration

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Introduction

Plantations of exotic species are increasing in area and play a significant role in landscape management and economic development in many countries (FAO, 1993), particularly those in which timber logging in natural forests has been prohibited. In Vietnam, timber logging has been prohibited in most natural forests as

part of the Reduce Emissions from Deforestation and Forest Degradation program for protecting natural forests and the environment (Tran et al., 2018), resulting in more than 90% of the total plantation area being covered with exotic species, mainly acacias and eucalyptus by 2013 (Kien et al., 2014). However, an increasing demand for timber has seen a growing interest in the planting of native timber species by both the Government and local people of Vietnam (Hoang et al., 2018).

P. arborea (Blume) Kalkman (*Pygeum arboreum* Endl.) is a deciduous timber species in the family Rosaceae that has a straight and cylindrical bole and can reach a height of 30 m and a diameter at breast height of 50 cm at maturity (Lim and Gan, 2009) (Fig. 1). This tree is naturally distributed in East Asia, including Pakistan, India, China, Myanmar, Thailand, Cambodia, Laos, and Vietnam, where it has been over-logged for local use and trade of its timber (Hoang et al., 2020) , which is used for furniture and construction (Lim and Gan, 2009). In addition, this plant is used for its medicinal properties; for example, a decoction of the leaves is drunk during labor to precipitate childbirth (Wiart, 2006).

Planting *P. arborea* for the production of timber has recently attracted attention in Vietnam (Hoang et al., 2020), and growing healthy seedlings is the first step toward obtaining good quality timber and high-production plantations. Previous studies (Alamgir and Hossain, 2005; Olatunji et al., 2013) on the effects of seed storage and pre-sowing treatment on germination in a range of species have shown that longer periods of storage and no pre-sowing treatment generally lead to lower germination rates, although the effect of pre-sowing treatment varies among species (El-Juhany et al., 2009; Martine et al., 2009; Amoakoh et al., 2017; Nguyen et al., 2020). However, internal factors, including the physiology, anatomy, and morphology of the plant, also affect seed germination (Vidyasagaran et al., 2016). Therefore, the objective of this study was to examine the effects of storage and pre-sowing treatment on seed germination in *Prunus arborea* (Blume) Kalkman.

Material and Methods

Experiments

Naturally fallen ripe fruits of *P. arborea* were manually collected from the floors of natural forests over a 5-day period in January (Fig. 1b). Fruits were collected from beneath 15 mother trees, all of which had diameters at breast height >20 cm, were healthy, and had straight and cylindrical boles. Immediately after collection, the fruits were cleaned with water to remove their covers and collect their seeds, which were mixed for the experiments. The fruit had an average diameter of 10–11 mm, with approximately 1,370–1,430 fruits weighing 1 kg, while the seeds had an average diameter of 8.1–9.3 mm, with approximately 2,430–2,930 seeds weighing 1 kg (Fig. 1c & 1d).

Seed storage

Four seed storage treatments were tested: S1, clean seeds stored in a cloth bag under room conditions; S2, clean seeds stored in a cloth bag in a refrigerator at 5°C; S3, clean seeds stored in a cloth bag in a refrigerator at -30°C; and S4, clean seeds stored in wet sand at a nursery.

The germination rate of each treatment group was examined after 1 month, 3 months, 6 months, and 9 months of storage by washing the seeds with water and sowing them in wet sand (Fig. 1). There were four replicates per treatment group, each of which contained 200 seeds.

Pre-sowing treatment

Five pre-sowing treatments were tested: PT1, soaked in normal (room temperature) water for 12 hours; PT2, soaked in normal water for 6 hours; PT3, soaked in 40°C–50°C water for 6 hours; PT4, soaked in 70°C–80°C water for 6 hours; and PT5, no pre-treatment as a control. The seeds in each treatment group were then sown in wet sand to measure their germination rate (Fig. 1).

Clean seeds were used in this experiment immediately after their collection from the field, and there were four replicates per treatment group, each of which contained 200 seeds.

Germination control

The sand bed in which the seeds were sown was watered once per day in the morning to maintain moisture. The bed was covered by a shading layer that blocked 40%–50% of sunlight and a nylon layer to avoid rainfall and water evaporation. Any rotten seeds were removed from the bed immediately and recorded as non-germinated seeds.

Seed moisture content

The seed moisture content was determined by measuring the initial mass of 50 randomly selected

seeds and then oven-drying them at 90°C until they reached a constant mass. The seed moisture content (%) was then estimated as [(initial mass−final mass)/initial mass] × 100.

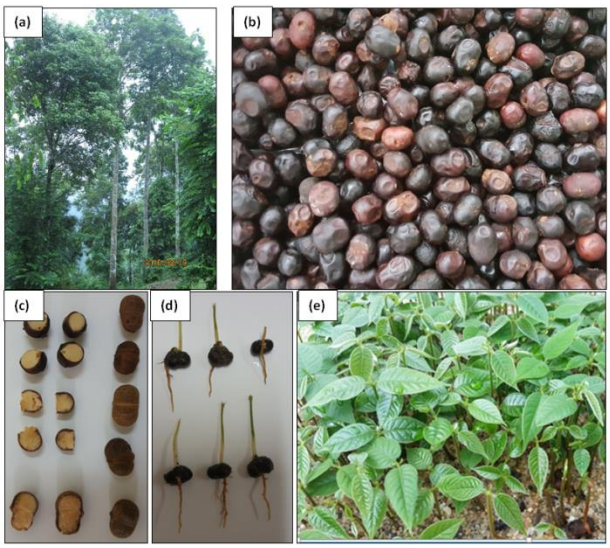

Figure-1. Adult individuals of *Prunus arborea* (a), ripen fruits (b), seeds and kernels (c), germinated seeds (d), and seedlings (e)

Data collection

The number of germinated seeds was checked and recorded every day and all germinated seeds were removed from the sand bed. The number of days to first germination and the timing of the end of germination (defined as the date when no germination had been observed for five consecutive days) were then recorded for each replicate of each treatment.

Statistical analysis

Differences in the number of days to first germination, the germination rate in the first 7 days, and the entire duration of germination among treatments were assessed by performing univariate analysis of variance and Duncan's multiple range test. All analyses were conducted using SAS 9.2 at p =0.5.

Results

Storage significantly affected the seed moisture content regardless of the storage duration ($p < 0.05$; Fig. 2). After 1 month of storage, all of the seeds in all treatment groups germinated but the seed moisture content was highest in S4 (53.6%) followed by S3 (41.1%), S2 (39.7%), and S1 (33.8%) (Fig. 2a). After 3 months of storage, the seeds in S4 were dead and therefore were not checked for moisture. Among the remaining groups, the highest seed moisture content was observed in S2 (38.8%), followed by S3 (37.8%) and S1 (32.8%) (Fig. 2b). After 6 months of storage, seeds in S1 and S4 were dead and so were not checked for moisture. Among the remaining groups, the seed moisture content was higher in S2 (37.8%) than in S3 (34.6%) (Fig. 2c). A similar pattern was also observed after 9 months of storage, with seed moisture contents of 36.6% in S2 and 33.0% in S3 (Fig. 2d). Thus, in all treatment groups, the seed moisture content gradually decreased with increased storage duration (Fig. 2). Only seeds in S2 and S3 were alive after 6 months of storage, while those in S4 and S1 were dead after 3 months and 6 months of storage, respectively.

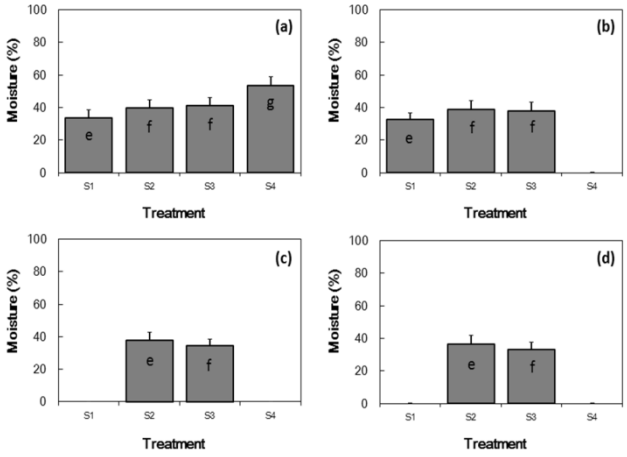

Figure-2. Effects of storage on seed moisture in different treatments.

(a) 1-month storage, (b) 3-month storage, (c) 6-month storage, and (d) 9-month storage. Bars indicate +SE. Different letters $^{e, f, g}$ indicate a significant difference of means at p =0.05. The seeds became dead in S4 (b, c, and d), S1 (c and d), therefore their moisture was not checked

Both storage treatment and storage duration significantly affected seed germination ($p < 0.05$; Fig. 3), with the highest germination rate occurring in S2 for all durations of storage (61.7% after 1 month, 58.7% after 3 months, 56.3% after 6 months, and 54.0% after 9 months of storage). By contrast, seeds in S3 failed to germinate after 1 month of storage (Fig. 3a), while those in S4 and S1 failed to germinate after 3 and 6 months of storage, respectively (Fig. 3b, 3c) and had germination rates of only 35.3% and 14.3%, respectively, after 1 month (Fig. 3a). Thus, the longer

the storage duration is, the lower is the rate of seed germination across all treatments.

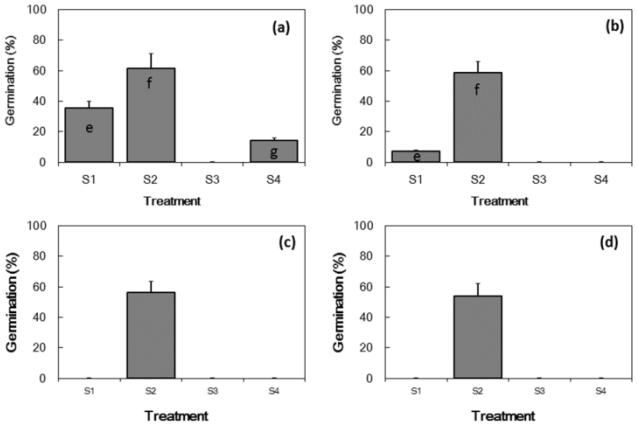

Figure-3. Effects of storage on seed germination in different treatments.

(a) 1-month storage, (b) 3-month storage, (c) 6-month storage, and (d) 9-month storage. Bars indicate +SE. Different letters [e, f, g] indicate a significant difference of means at p =0.05

Pre-sowing treatment significantly affected the number of days to first germination ($p < 0.05$; Fig. 4a), the germination rate in the first 7 days (Fig. 4b), and the total germination rate (Fig. 4c). Seeds germinated earliest in PT1 (soaked in normal water for 12 hours), PT2 (soaked in normal water for 6 hours), and PT5 (direct sowing without treatment), taking around 6 days to first germination in each case, while the latest germination was observed in PT3 (soaked in 50°C–60°C water for 6 hours) and PT4 (soaked in 70°C–80°C water for 6 hours), taking around 8 days to first germination (Fig. 4a).

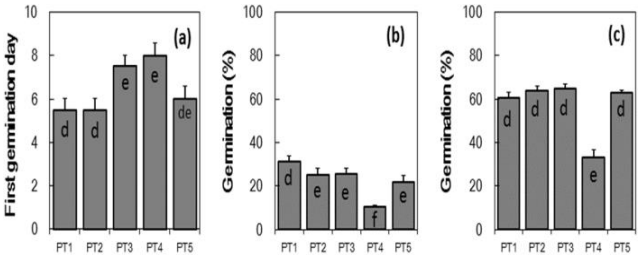

Figure-4. Effects of pre-sowing treatments on seed germination.

(a) A number of days to first germination, (b) germination rate in the first seven days, and (c) total germination. Bars indicate +SE. Different letters [d, e, f] indicate a significant difference of means at p =0.05

The highest germination rate in the first 7 days was observed in PT1 (31.4%), followed by PT2 (25.3%), PT3 (25.5%), and PT5 (21.8%), which were not significantly different from each other ($p > 0.05$), whereas the lowest germination rate was found in PT4 (10.5%) (Fig. 4b). The total germination rate was also lowest in PT4 (33.1%), which was nearly half of that in PT1 (60.4%), PT2 (63.9%), PT3 (64.6%), and PT 5 (62.8%) (Fig. 4c). There was no significant difference in the total germination rate among PT1, PT2, PT3, and PT5 ($p > 0.05$).

Discussion

Seeds can lose moisture through evaporation, which is mainly caused by a low humidity and high temperature; and transpiration, which is caused by respiration to keep them alive. *P. arborea* seeds that were stored under room conditions (S1) had the lowest moisture content, possibly due to a low surrounding humidity and high temperature leading to high levels of evaporation. By contrast, seeds that were stored in wet sand at a nursery (S4) had the highest seed moisture content as a result of the high surrounding humidity/wet sand and low temperature. Meanwhile, seeds that were stored in a refrigerator (S2 and S3) experienced low temperatures and humidities and consequently had moisture contents that were intermediate to those in S1 and S4 (Fig. 2). Seeds in S2 and S3 also exhibited fewer changes in seed moisture content with increased storage duration because the low temperature led to low evaporation and respiration rates in the stored seeds. The fresh seeds that were used in the present study had a relatively high initial moisture content compared with the stored seeds, which likely explains the high germination rates observed, as indicated in a previous study (Martine et al., 2009; Mai et al., 2019; Nguyen et al., 2020). However, a longer storage duration led to a lower germination rate (Fig. 3), as observed in a wide range of other species previously (Martine et al., 2009; Cao and Tran, 2019; Nguyen et al., 2020).

P. arborea seeds have bulky, hard, and thick covers, which affect water absorption, gaseous exchange, and breaking by the roots and shoots. The germination of such seeds can be enhanced by applying a pre-sowing treatment that can break the seed cover without affecting the kernel inside and which supports water absorption (Azad et al., 2011; Mwase and Mvula, 2011 Cao and Tran, 2019). Examples of pre-sowing treatments include water, growth regulators (e.g.,

chemicals), or mechanical stratification (Airi et al., 2009; Missanjo et al., 2014; Cao and Tran, 2019; Nguyen et al., 2020). However, different methods will be suitable for different species, so it is very important that a suitable method and conditions are determined for each species to maximize seedling production (Bahar, 2015).

Seeds that germinate rapidly after falling from the mother tree need to be sown immediately (Jaganathan et al., 2018). However, the seeds of some species known as long-dormancy species cannot germinate until a long time after falling (Nonogaki, 2019). In the present study, non-stored seeds of *P. arborea* were found to have a significantly higher germination rate than stored seeds (Fig. 3), indicating that this species may not exhibit seed dormancy and so its seeds should be sown as soon as possible after falling from the mother tree. Germination within a short time can help to generate uniform seedlings. However, in the case of *P. arborea*, the maximum germination rate in the first 7 days was 31%, which is considered low. Therefore, further studies on shortening the germination process should be considered to help produce uniform seedlings.

Soaking *P. arborea* seeds in 40°C–50°C water for 6 hours gave the highest germination rate among the pre-treatments tested (Fig. 4c). It is well known that hot water makes the seed coat permeable to water and that the seeds imbibe water and swell as the water cools (Mwase and Mvula, 2011), and it has also been suggested that hot water plays a role in breaking the dormancy of hard-coated seeds (Singh et al., 2019). Therefore, the effects of different water temperatures (e.g., 50°C–70°C) on the germination rate of *P. arborea* should be investigated in the future to determine the optimum pre-sowing treatment for producing seedlings.

The selection of plus trees must be undertaken for *P. arborea* to increase production and improve the quality and form of the stem. This selection could be carried out in plantations if available and/or in natural forests (Hoang et al., 2020). Further studies on the vegetative propagation of *P. arborea* (e.g., through cuttings) should also be conducted to maximize and standardize the quality of seedlings for use in intensive plantations. In addition, progeny tests should be carried out to select the best cultivars for both timber production and disease resistance.

Conclusion

This study investigated the effects of storage and pre-sowing treatment on seed germination in the timber tree *P. arborea*. The results indicated that seeds can maintain a germination rate of 54% after 9 months of storage if they are stored in a cloth bag in a refrigerator at 5°C. Furthermore, pre-treating the seeds by soaking them in 40°C–50°C water for 6 hours led to the highest germination rate (64.6%).

Based on these results, it is recommended that seeds of *P. arborea* are stored in a refrigerator at 5°C for less than 9 months and then soaked in 40°C–50°C water for 6 hours before sowing. A seedbed made of sand is suitable for sowing the treated seeds, but this bed must be covered with shading that blocks 40%–50% of sunlight and a nylon layer, and must be watered once per day to maintain moisture. The seedbed must also be checked regularly to remove any rotten seeds and to immediately transplant any germinated seeds into pots.

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Contribution of Authors

Thang HV: Conceived idea, designed research methodology, data analysis, literature review and manuscript write up

Lang CV: Helped in designing research methodology, data collection and data interpretation

Thanh HV: Helped in designing research methodology, data collection and data interpretation

Dien NT: Helped in designing research methodology, data collection and article write up

Sam PD: Helped in designing research methodology, data collection and article write up

Trung DQ: Helped in designing research methodology, data collection and article write up