

EFFECTS OF THE HOT WATER PRE-TREATMENTS AND STORAGE DURATIONS ON THE SEED GERMINATION OF FALCATA (*FALCATARIA FALCATA* (L.) GREUTER & R. RANKIN)

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Keywords: Falcata, Hot water, Storage, Germination

Abstract

Seed dormancy in *Falcataria falcata* (L.) Greuter & R. Rankin is well documented. This study determined the effect of hot water pre-treatment and storage duration to attain a higher seed germination rate. This study found a highly significant difference ($p < 0.05$) between the mean Cumulative Germination Percentage (CGP) between treatments. Hot water pre-treatment significantly increased the CGP of seeds. However, storage duration and its interaction with different hot water pre-treatment did not significantly affect the CGP of seeds. Overnight (12 hours) soaking seeds in water with an initial temperature of 80°C yields the highest CGP regardless of the storage duration. On development, it was found that radicle emergence of imbibed seeds generally starts within 1-2 days.

Introduction

In recent years, *Falcataria falcata* (L.) Greuter & R. Rankin had been one of the most in-demand tree species (DENR-FMB 2021). It is a popular tree plantation species in Mindanao and is widely cultivated across the CARAGA region (Paquit and Rojo 2018). Falcata logs are mainly utilized for veneer production and are highly in demand (Alipon *et al.* 2021). The vast demand prompted researchers to explore ways to propagate the species through macrosomatic means. The absence of published reports and protocols, however, indicates that attempts to asexually propagate the species have been generally unsuccessful. This being the case, regeneration from seed remains the most common method of propagation of *F. falcata* (Sajeevukumar *et al.* 1995). Depending on geographic location, Falcata trees flower as early as three years after planting (Krisnawati *et al.* 2011). Approximately 38,000-44,000 cleaned seeds per kg (Parrotta 1990). The availability of viable seeds has yet to be reported as a major concern.

Despite the availability of seeds, dormancy due to hard seed coat has been reported by various sources (Dell 1980, Sajeevukumar *et al.* 1995). Aside from an impermeable seed coat and micropylar plug, water-soluble inhibitors also cause dormancy in *F. falcata*. These are serious constraints as they can cause delayed and irregular germination (Sajeevukumar *et al.* 1995). Dried *F. falcata* seeds can be stored for at least 1.5 years at 4-8 °C without losing viability (Parrotta 1990). However, proper seed storage is not usually followed, which can result in poor germination rates. Duration of seed storage can be one of the factors that cause seed deterioration (Garoma *et al.* 2017). Generally, a gradual decrease in the seed quality parameters was observed as the storage period increased, more so if the proper environment was not followed (Gebeheyu 2020). If the aim is the large-scale production of *F. falcata* to meet the demand, one must overcome the constraint of seed dormancy and storage limitations.

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Various pre-treatment approaches have been tested, but soaking in hot water is considered the most common pre-germination treatment for *F. falcata* seeds (Rupinta *et al.*, 2020). Sajeevukumar *et al.* (1995) reported the highest germination rates out of all published reports in this regard. However, the storage condition of the seeds was not included in their investigation. This study attempted to replicate the work of Sajeevukumar *et al.* (1995) but will include duration of storage as a potential factor. The study hypothesizes that hot water pre-treatment and short-term stored seeds can attain better germination rates than the control (no hot-water treatment) and long-term stored seeds. This study aimed to gain more insights on this topic and provide a simple and cost-effective protocol to maximize the germination rate of *Falcata* that would benefit local *F. falcata* farmers.

Materials and Methods

The experiment was done in a forest nursery at Central Mindanao University (CMU) in Mindanao, Philippines. Seeds were obtained from two sources. Long-term storage seeds were acquired from the Mindanao Tree Seed Center (MTSC) of the Forest and Wetland Research Development and Extension Center (FWRDEC) in Bislig, Surigao del Sur. In contrast, short-term storage seeds were obtained from CMU College of Forestry Clonal Forest Nursery. The experiment was laid out in a 2 x 4 factorial arranged in a Completely Randomized Design (CRD) with three replications (Table 1). Factor A was the two storage durations (< 1.5 years and > 1.5 years), while Factor B was the four hot water pre-treatments (tap water, 28, 40, 60, and 80°C). A total of eight treatments were compared. Before treatment, immature, empty, or broken seeds were removed by water flotation (Krisnawati 2011). Two sets of seeds based on storage duration were prepared. Each set was then divided into four subsets to form eight sets corresponding to treatments 1 through 8. Each was then wrapped in white cloth, ready for soaking. Treatments T1 and T5, T2, and T6, T3 and T7, and T4 and T8 were soaked in water (1 liter in a beaker) with an initial temperature of 28, 40, 60, and 80°C, respectively. Using a laboratory thermometer, the initial temperature of the water was set accordingly, and the decline was monitored as it cooled down. The seeds were soaked overnight, from 8 PM to 8 AM. After the pre-treatment, the seeds were placed in a petri dish lined with tissue paper. The exact amount of water was applied daily to keep the seeds moist and avoid desiccation. A total of 24 experimental plots were observed in the study. With ten seeds per replication, 240 seeds have been used. The experiment commenced in April 2023, and the actual observation period covered two weeks. The randomization was performed using draw-by-lots. Labels per plot were written on paper, cut out, and then drawn randomly from 1 through 24 in a 4 x 6 layout. A total of 24 experimental plots arranged in a CRD were laid out (Table 1)

Table 1. Experimental layout.

T1R1	T1R3	T6R2	T1R2	T3R2	T2R2
T4R1	T3R1	T7R1	T2R1	T5R1	T4R2
T2R3	T5R3	T8R2	T3R3	T6R1	T4R3
T8R1	T7R2	T8R3	T7R3	T6R3	T5R2

T= treatment, R=replication, T1 (short storage, 28°C), T2 (short storage, 40°C), T3 (short storage, 60°C), T4 (short storage, 80°C), T5 (long storage, 28 °C), T6 long storage, 40°C), T7 (long storage, 60°C), T8 (long storage, 80°C).

The number of seed germinations was observed daily for seven days. After the observation period, the Cumulative Percent Germination (CPG) was computed using the following formula. The data was then analyzed using the Analysis of Variance (ANOVA) in Microsoft Excel. Tukey's Honest Significant Difference (HSD) was used for the post hoc test.

$$\text{CPG} = \frac{\text{Number of germinated seeds per treatment}}{\text{Total number of seeds per treatment}} \times 100$$

Results and Discussion

After a seven-day observation period, it was found that the difference in mean Cumulative Germination Percentage (CGP) among treatments was statistically significant at a 5% level (Table 2). Hot water pre-treatment significantly increased the CGP of *F. falcata* seeds. Meanwhile, storage duration and its interaction with different hot water pre-treatment did not significantly affect the CGP of seeds. The CGP of T4 and T8 were significantly higher than the other six treatments (Fig. 1), which were treated with lower water temperature. T4 and T8 were treated with hot water at 80°C for 12 hrs (8 pm - 8 am). The variation between T4 and T8 was not statistically significant. However, T4 obtained a higher germination rate (90%) than T8 (86.67%), which may indicate a slight advantage in CGP for short-storage seeds over long-storage seeds. In a similar trend, T2 and T3 had better CPG than T6 and T7, although their difference was not statistically significant. The lowest germination rates were observed in T1 and T5, wherein seeds from both treatments were only treated with tap water. Valuable information can be drawn from the statistically comparable CGP between *F. falcata* seeds that have undergone short and long storage durations. The present findings supported the idea that proper cold storage can help preserve seed viability. A study by Soerianegara and Lemmens (1993) found that dried seeds of *F. falcata* can be stored for at least 1.5 years at 4-8°C without losing viability. Soerianegara and Lemmens (1993) also reported that the germination rate may still be high (70-90%) after 18 months of storage. Moreover, Parrotta (1990) recommended storing *F. falcata* seeds in sealed containers and placing them in the refrigerator at 3-5°C. Thus, seed viability could still be higher for seeds stored for an extended time as long as they are placed at an ideal temperature. In a field trial using Cashew seeds, Makale *et al.* (2020) found that seeds harvested in 2018 were superior in germination and vigor to those harvested in the previous years. Genes and Nyomora (2018) have noted that long-term storage of seeds under unfavorable conditions leads to loss of viability. Good germination results of Moringa seeds were only up to three months of storage; after that, the germination rate gradually decreased with prolonged storage duration (Mubvuma *et al.* 2013).

Table 2. Analysis of variance of data from a 2 x 4 factorial experiment in a CRD.

Sources of variation	df	SS	MS	Comp F	F crit	P-value
Storage duration	1	150	150	1.20	4.49	0.29 ^{ns}
Hot water pre-treatments	3	19533	6511	52.09	3.24	1.77E-08**
Interaction	3	850	283	2.27	3.24	0.120 ^{ns}
Error	16	2000	125			

** = significant at 5% level and ^{ns} = not significant.

After 12 hours of soaking, a higher mean % of imbibition can already be observed in T4 (70%) and T8 (63.33). On the other hand, seeds soaked in tap water in T1 and T5 had the lowest %

of imbibition, 20 and 6.67%, respectively (Fig. 2A). Hot water causes softening of the seedcoat (Sajeevukumar *et al.* 1995), which allows the entry of water into the seed resulting in a higher % of imbibition. The highest % of imbibition was attained in seeds soaked in water with an initial temperature of 80°C. Meanwhile, germination continues until the 7th day (Fig. 2A). In T4 and T8, 60-70% CGP was already attained in 4-5 days, higher than the other treatments, especially the control. The findings of this study concur with those of Sajeevukumar *et al.* (1995), who observed 93.8% seed germination at 80°C hot water pre-treatment. However, germination rates at 60°C (T3 = 66.67% and T7 = 43.33%) were lower in this study than the findings of Sajeevukumar *et al.* (1995), which was 94.3%. Germination rates observed in this study were higher than the results from recent studies. Rupinta *et al.* (2020) reported a 66% germination rate after 30 seconds of hot water treatment (100°C) followed by overnight soaking. Using the same hot water temperature (100°C), Mazo *et al.* (2020) found that the germination of seeds soaked for 20 seconds was significantly higher than those soaked in tap water. As the results have shown, 80°C hot water pre-treatment appears to be the most effective in promoting seed germination of *F. falcata*. Monitoring of the water temperature revealed that, at an initial temperature of 80°C, seeds were exposed to 50-80°C for at least 30 min (Fig. 3). The exposure of seeds to this water temperature plus the additional 60 min of exposure to a temperature higher than 40°C seemed sufficient in breaking the dormancy of the seeds. This is evidenced by the higher imbibition and CGP of the treated seeds, especially in the 80°C hot water pre-treatment.

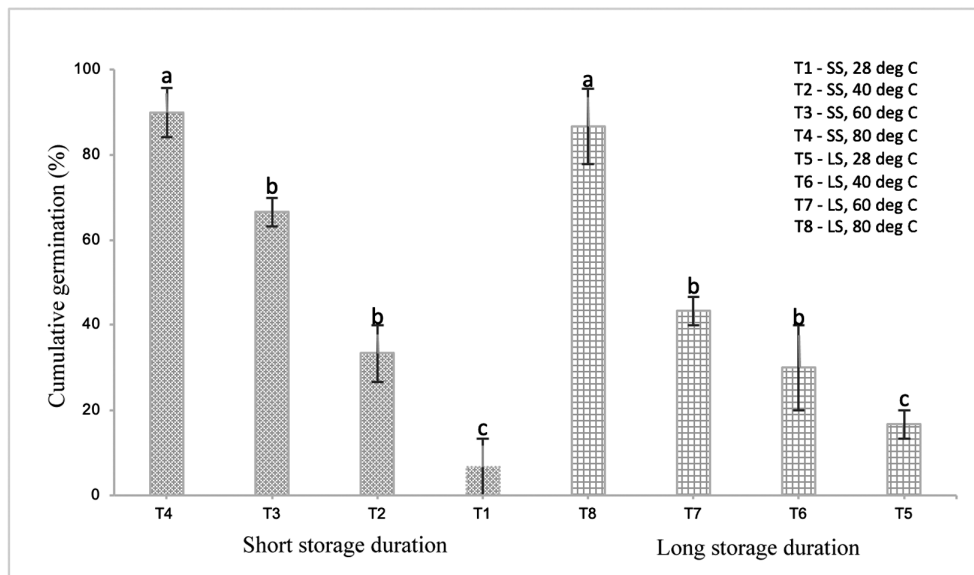


Fig. 1. Variation in CGP among treatments. Error bars represent SEM. Means with the same letter are not significantly different at $p < 0.05$ by Tukey's HSD.

According to Sajeevukumar *et al.* (1995), the initial temperature of 40°C may be insufficient to break the impermeability of the seedcoat as evidenced by poor imbibition. They added that boiling water treatment may be too severe, as evidenced by poor germination. Poor germination response due to boiling water treatment may be due to the sensitivity of the embryo to higher temperatures (Kandya 1990). Rapid uptake of hot water may cause imbibitional damage to seeds (Gilbero *et al.* 2014). Babeley *et al.* (1986) reported that germination of *Albizia* species without pre-treatment was very poor. The poor germination of seeds of *Albizia* spp. has been attributed to

two reasons. One is the slow imbibition caused by the thick, impermeable seed coat (Sniezko and Gwaze 1987). Aside from an impermeable seed coat and micropylar plug, water-soluble inhibitors also cause dormancy in *Falcata* (Sajeevukumar *et al.* 1995). When extracted, the seedcoat extracts of *F. falcata* resulted in the complete inhibition of cowpea seeds (Sajeevukumar *et al.* 1995). Soerianegara and Lemmens (1993) recommended that before sowing, seeds should be soaked in boiling water for 1-3 min or dipped in concentrated sulphuric acid for 10-15 min followed by washing and then 15 min of soaking in cool water to accelerate and ensure uniform germination. Another method is to dip the seeds in boiling water, remove them from the heat source, and allow them to cool at room temperature, leaving them in the water for 24 hours (Parrotta 1990). A higher germination percentage (66%) was also observed in a 30-second soaking in hot water (Rupinta *et al.* 2020). Hot water treatments of 40, 60, 70, and 80°C significantly increased germination only in *Falcata* (Sajeevukumar *et al.* 1995).

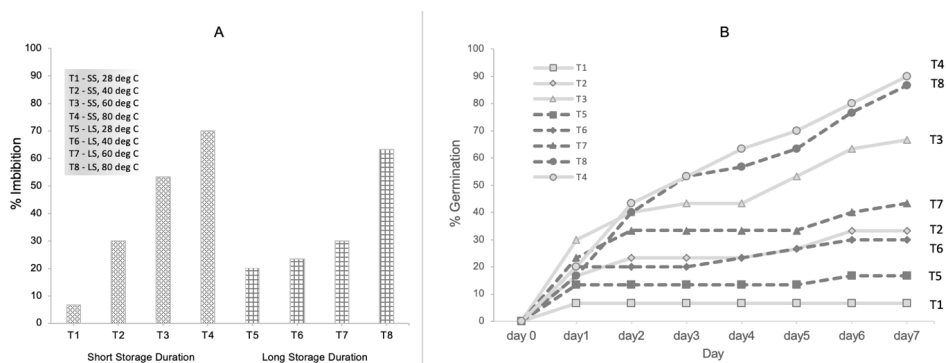


Fig. 2. Variation in % imbibition (A) and trend in daily CGP (B).

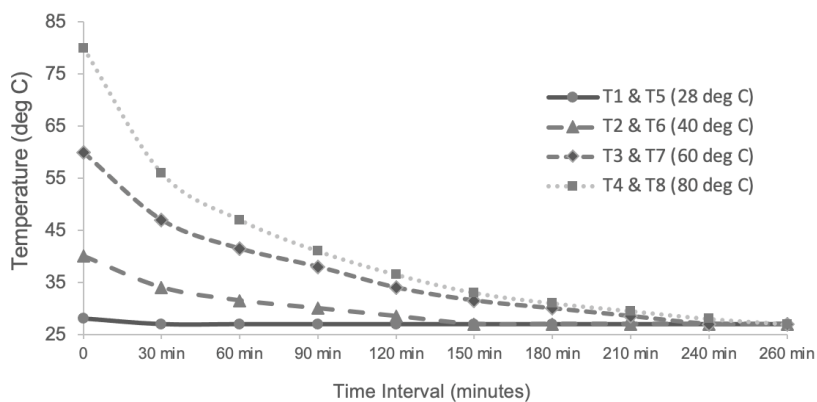


Fig. 3. Trend in water temperature decline.

At day 0, imbibition was already observed after the seeds were removed from soaking in 80°C initial water temperature (Fig. 4A). 60% of the imbibed seeds germinate within 1-2 days, as evidenced by radicle emergence. Complete germination of those seeds can be achieved within 4-5 days. It must be noted that some seeds exhibited delayed imbibition. Shedding of the seed coat and the exposure of the cotyledons marked critical development at days 4-5. This is followed by the emergence of the embryo and exposure of the epicotyl. Seeds planted on soil media exhibited a slightly different timing in developing secondary roots. Development of secondary roots was

achieved in 8 days per observation, but this could have started even earlier. After 14 days, the cotyledons continue to become attached to the developing seedling. In an experiment that was conducted separately, it was observed that cotyledons were still present even after two months.

In summary, this study found that hot water pre-treatment had significantly increased the CGP of *F. falcata* seeds. A higher % of imbibition was observed in T4 and T8, both treated with 80°C water temperature. CGP was also significantly higher in T4 and T8 compared with the rest of the treatments. On development, it was found that radicle emergence starts within 1-2 days (Fig. 4A). Moreover, shoot emergence from the cotyledons occurs within 4-5 days. When grown in soil media, shoot and secondary roots have already been established as early as the 8th day (Fig. 4B).



Fig. 4. Germination and early seedling development of *F. falcata* seed soaked in 80 °C water and then grown on tissue paper media from 0-7 days (A). Early seedling development in soil media from day 8 to day 14 (B).

To improve seed germination, it is recommended that *F. falcata* seeds, regardless of storage duration, are soaked overnight (12 hours) in hot water with an initial temperature of 80°C. Seedling growers can achieve 80°C water temperature by boiling a liter of water, setting it aside, and cooling it for approximately 5 minutes. It is also recommended that seeds germinate in tissue paper for 1-4 days before they are transplanted into soil media.

Acknowledgements

The authors would like to thank the Department of Science and Technology- Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) for funding this study under its GREAT scholarship program.

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(Manuscript received on 22 November, 2023; revised on 02 May 2024)