Scientific Research Publishing

Open Journal of Ecology, 2023, 13, 106-118
https://www.scirp.org/journal/oje
ISSN Online: 2162-1993
ISSN Print: 2162-1985

Effect of Pre-Sowing Treatments on Mamalis (*Pittosporum pentandrum* Blanco Merr.) Seeds Germination under Nursery Condition

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How to cite this paper: Tano, R.L. (2023) Effect of Pre-Sowing Treatments on Mamalis (*Pittosporum pentandrum* Blanco Merr.) Seeds Germination under Nursery Condition. *Open Journal of Ecology*, **13**, 106-118.
https://doi.org/10.4236/oje.2023.132009

Received: January 5, 2023
Accepted: February 12, 2023
Published: February 15, 2023

Abstract

Seeds were subjected to three different pre-sowing seed treatments: immersion in lukewarm water for 2 hours, immersion in concentrated sulfuric acid (H_2SO_4) for 5 minutes, hilar removal, and a control in which the seeds were sown without being treated. The experiment was laid out in the Completely Randomized Design (CRD) with four replicates and 60 seeds per treatment. Seeds were sown in an improvised Seedbox in October 2019. Germination was monitored daily for one month. The results showed that Mamalis seeds treated with lukewarm water have the earliest germination of twelve days, with a germination percentage of 66.67%. The germination rate of another treatment ranges from 0 - 44 percent, compared to 45 percent for the control treatment. It seems prudent to conclude that to enhance the vegetative propagation methods is to soaking in warm water at 37.5˚C for 2 hours could provide the best growth.

Keywords

Pittosporum pentandrum, Seed Germination, Pre-Treatments, Sulfuric Acid, Seed Dormancy

1. Introduction

Pittosporum is a genus of about 200 species in the family Pittosporaceae in the division of Angiosperms (Flowering plants) found all over the world [1]. When planted in well-draining soil and exposed to full sun or slight shade, they thrive. In shady areas, they grow taller but keep their naturally spherical appearance. Pittosporum plants can range in height from two to thirty meters, depending on the species [2].

Pittosporum pentandrum (Bl.) Merr. locally known as Mamalis is endemic in the Philippines, it can be found in secondary forests at low medium altitudes (1400 to 2300 m) from Northern Luzon to Palawan and Mindanao [3]. Also, it is a lesser-used species that reaches 20 meters high; the flowers are small, white, and fragrant and cluster on small flowering branches. The leaves are alternate and pointed at both ends and the fruit is small and globular with an average size of 1cm and has a light green color when it is young and becomes yellow or orange when mature. In Laguna province, the seed collection is from September to October. But in the other provinces, like Misamis the seed collection is from November to January; September to November in Bohol; October to December in Leyte, and April to July in Bukidnon. Collection of seeds can be done when the fruit is not yet open and the seeds on the ground are not recommended since the seeds on the ground are easily attacked by fungus and other microorganisms. In addition, extraction and cleaning of the seeds are done by rubbing the fruit pulp with very fine soil to remove the oil and storing the seeds in bottles and plastic bags and storing them inside the refrigerator. The viability is up to 6 months. Further, in folklore, the leaves extracts are processed into herbal medications for the treatment of colds, fever, and cough, and also the aromatic decoction of leaves is used by women for postpartum baths and prolonged illness. The bark is used for antipyretic in large doses as a general antidote. The fruit is the main source of Mamalis oil which is used for medicine as it consists of dihydroterpene. And the wood of this tree has a beautiful surface and is used for jewelry beads and firewood [4].

The Philippines continues to experience an alarming rate of destruction of these important resources which is brought about by overexploitation, deforestation, land degradation, climate change, and pollution (including biological pollution), among others. As of now, the Philippine government put up a policy and program that alleviate the impact of massive forest degradation. One of the purposes of the Comprehensive Agrarian Reform Program (CARP) on Hunger Mitigation Program and National Greening Program (EO 26 and 193) is to return the once-denuded forest into a luscious green forest [5] [6]. There is a need to consider the selection of appropriate species for reforestation especially in the lowland because most of these areas are open and dominated by Cogon (*Imperata cylindrical*) and Talahib (*Saccharum spontaneum*) where planting of seedlings is hard and survival is difficult. On the other hand, some native tree species can survive in this kind of area like *P. pentandrum*. *P. pentandrum* can suppress and inhibit the growth of Cogon (*Imperata cylindrical*) and Talahib (*Saccharum spontaneum*); and it produces good soil cover in denuded areas which is useful for vegetative rehabilitation of degraded areas [4].

According to [3], that *P. pentandrum* is a potential source of renewable energy, but the seeds are difficult to germinate. Germination may be delayed for days, weeks, months, or even years. Seed pre-treatment can ensure both success in seed germination and germination speed guarantee that germination is quick

and homogenous [7]. On the other hand, pre-germination is applied to *P. pentandrum* seeds to break dormancy and hasten germination. This pre-germination treatment includes soaking, stratification, or even keeping them in the dark [8]. Some literature defined pre-germination as a different method on how to break the dormancy of different seeds which includes acid scarification, nicking, and soaking with hot or cold or both [9] [10] [11]. *P. pentandrum* is a native species found all over the Philippines with a unique characteristic that is needed for extensive production for reforestation activities. This species is hard to propagate by seed because of the prolonged process of germination usually a two to three months germination period of the seeds, so pre-germination is needed to hasten the breaking of seed dormancy. Though macro and micropropagation protocols have been developed, mass propagation requires further refinement of poor seed germination [12]. Seed germination is one of the most viable tools for ex-situ conservation of threatened germplasm [13].

Given the high demand for indigenous planting material, limited distribution, slow regeneration, and other factors, the natural propagation of *P. pentandrum* is hampered by prolonged dormancy, low germinability, and poor seed viability. It is critical to have research and understanding about its germination stage to address the enormous knowledge gap on this species towards better production and utilization. Therefore, the objective of the study was to determine the percent germination and percent germinative energy of *P. pentandrum*.

2. Review of Related Literature

2.1. Botanical Description

Mamalis (*Pittosporum pentandrum*) belongs to the genus *Pittosporum* of the family Pittosporaceae. A small tree or shrub, the bark greenish-white, with conspicuous lenticels; branchlets brownish pubescent. Leaves obovate-lanceolate or elliptic-lanceolate, 4 - 11 cm long, 1.3 - 4 cm broad, acute at both ends, entire to crenate; petioles 0.4 - 1.5 cm long. Inflorescence in the terminal, small, crowded, brown-pubescent panicles, 2 - 8.6 cm long, the flowers small, crowded about 5 mm across; sepals 5, ovate, 1 - 2 mm long, distinct; petals 5, oblong-linear, 5 mm long truncate [14]. The seeds in each fruit are about eight, flattened seeds covered with glossy red oily, and sticky mucus and with an odor reminiscent of petroleum. The seeds can be collected from the healthy mother trees in the natural stand. The mature fruits have a golden yellow color [4].

2.2. Seed Germination and Seed Dormancy

The ultimate function of seeds is to produce offspring and preserve species. As a result, plants have developed a variety of strategies to ensure the successful germination of this genetic delivery system. Proper seed germination distribution, both temporally and spatially, is critical for seed plant survival and proliferation. The spatial distribution of germination is generally controlled by seed and fruit morphology, which improves offspring dispersal from the maternal habitat [15].

Seed germination is the critical stage for species survival [16]. Seed germination is a period in the life cycle of a plant when growth is temporarily suspended or very minimal to the point of undetectable visibility. Further, germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis [17].

Dormancy is a survival adaptation that ensures seeds germinate only when environmental conditions are favorable. The conditions required for seeds to "break" dormancy and germinate can vary greatly between species, within species, and among seed sources of the same species [18]. Seed dormancy is a natural seed property that defines the environmental conditions under which the seed can germinate. It is determined by genetics, with significant environmental influence mediated, at least in part, by the plant hormones abscisic acid and gibberellins [19]. Dormancy should not be confused with the absence of germination; rather, it is a seed characteristic that determines the conditions required for germination [20] [21] [22]. Seed dormancy is a limiting factor for efficient seed production in sunflowers because it delays crop sowing. Various dormancy-breaking techniques have been discovered to reduce the period of seed dormancy, allowing for maximum seed production in the shortest amount of time [23] can be imposed by constructing a simple physical barrier around the seed that prevents gas exchange and water passage [24]. Other types of dormancy, such as physical dormancy due to the impermeable seed coat or even physiological dormancy, cannot be ruled out depending on the species. Although a combination of physical and physiological dormancy has been highlighted for some Passiflora species, studies on P. incarnata are very limited and inconclusive. Mechanical scarification was the most commonly used method for removing seed dormancy in P. incarnata; however, it never produced satisfactory results and posed the risk of embryo damage [25]. Although chemical scarification appeared to be more effective in breaking dormancy in this species, prolonged soaking could reduce seed viability and germination rate [26].

2.3. Pre-germination Treatments

Great efforts are now being made in the search for clean production technologies that are environmentally friendly in order to support the germination processes of agricultural and forestry seeds and encourage plants to develop in a better and higher quality manner [27]. Chemical treatments, which are commonly used to increase the percentage of germinated seeds, are generally unfriendly to the environment, as well as time-consuming and expensive [28] [29]. However, physical methods have been promoted by the scientific community as an alternative to conventional treatments [30] [31] [32], with the use of magnetic and electromagnetic fields gaining importance [33].

Pre-germination can affect the physiological age of seed tubers. Cold-stored seeds (4˚C - 5˚C) should not be ground immediately. Seed potatoes age over time and this process is accelerated when the seed is exposed to higher temperatures [34]. The few germination assays performed on various Opuntia species and

methods used mechanical and chemical scarification with acid or growth regulators to help break the seed coat and facilitate seedling emergence [35]. Both are used as pregerminated seed treatments, but it should be noted that their mechanisms are completely different. Permanent magnets (constant field) and magnetic coils (constant or variable field depending on the following electric current) can produce magnetic fields, whereas electrically charged objects produce electromagnetic fields [36]. The specific differences in the effects presented by each as pregerminated seed treatments, however, have not been described.

3. Material and Methods

3.1. Materials

The materials used in this study were 960 seeds for germination testing (**Plate 1**), a beaker for soaking the seeds, sulfuric acid, one gallon of distilled water for rising the seeds to remove the sulfuric acid, a scalpel for hilar removal, a basin for soaking the seeds in lukewarm water, a wooden box as a container for seed germination, and a loam substrate for germination. In addition, a notebook, pencil, or ballpen for data recording, a camera for photo documentation, and a water sprinkler for watering seedlings are recommended.

3.2. Methods

3.2.1. Site Description

This research was employing field data collection in a vacant lot Barangay Tabuc Suba, Lapaz Iloilo City, Iloilo, Philippines. Tabuc Suba is situated at approximately 10.7310, 122.5602, on the island of Panay. Elevation at these coordinates is estimated at 12.0 meters or 39.4 feet above mean sea level. The area was flat, and the soil is slightly Alkaline at 7.63 pH.

3.2.2. Plot Establishment

The experimental plot was established on seed germination consisting of control and three treatments that were replicated four times and were randomly assigned into each block following the Complete Randomized Design (CRD) (**Figure 1**).

Plate 1. Mamalis seed used for pre-sowing treatments.

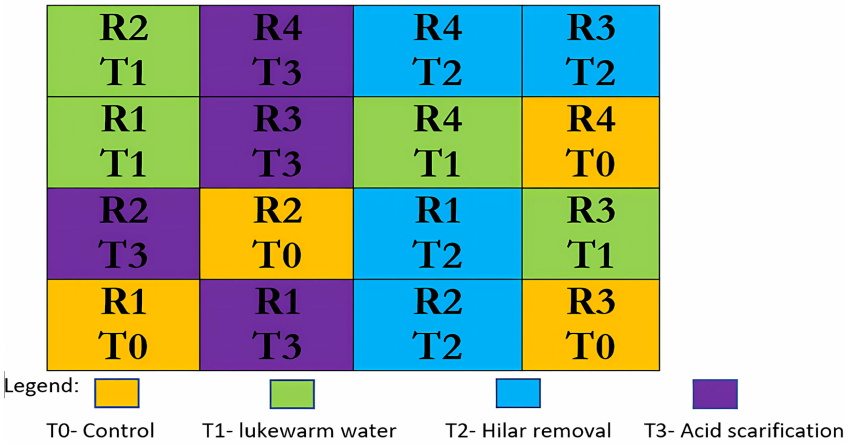

Figure 1. The design of the experimental plot (pre-treatment).

Pre-sowing treatments were applied to the seeds for early germination. The pre-germination treatments were (T1) soaking in lukewarm water, (T2) hilar removal, (T3) acid scarification (Aliero, 2004), and (T0) control. Soaking of seeds was done by placing the seeds in a basin filled with lukewarm water (37.5˚C) for 2 hours. Acid scarification was done by placing the seeds in a beaker and slowly pouring the sulfuric acid (50%) until all seeds were covered for 2 minutes. Afterward, the seeds were removed and rinsed with distilled water to wash off the acid. Hilar removal was done by using a scalpel. The seeds were placed in a wooden seedbox with a dimension of 12 × 12 inches and a 1-foot depth. The seedbox for germination was lined with gravel at the bottom, followed by a sand layer and then garden soil at a 1:1:1 ratio. Rills of 3 rows per treatment replication were lined with 20 seeds each. A divider was placed to avoid mixing of seeds applied with treatments. Germinated seeds were marked for easier monitoring.

3.2.3. Field Measurements

Monitoring of seed germination was done daily for one (1) month.

The number of seeds germinated in each treatment was recorded every alternate day (every 2 days). The starting and finishing dates of germination were also recorded. At the end of the germination period, the germination Percentage [37] and [38] were calculated using the following equation:

$$\text{Germination Percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100 \quad (1)$$

$$\text{Germinative Energy}(\text{GE}) = \frac{\text{Number of seeds that germinated up to the peak period}}{\text{Total number of seeds sown}} \times 100 \quad (2)$$

3.2.4. Data Analysis

Analysis of Variance (ANOVA) was conducted using an open-source calculator SPSS. One-way ANOVA was performed from the Experiments. The treatment mean values were compared with the least significant difference (LSD) at the 1% level.

4. Results and Discussion

4.1. Number of Days to Emergence of *P. pentandrum* Seeds

Figure 2 shows that T1 (lukewarm water) germinated after 16.75 days, followed by T0 (Control) in 17 days. T3 (acid scarification) germinated in 19.25 days, while T2 (hilar removal) germinated in 20 days. In this pre-germination study, there was a lack of uniformity in the germination period. The early germinated seeds were not prone to fungi and other agents because the more extended germination period made the seedlings weak and susceptible to environmental stresses. In a report by Yu [39] [40], Similarly, the study by Asiedu *et al.* [41] highlights that the germination of seeds with hard seed coats is enhanced if soaked in tap water for 1 - 2 days at room temperature.

The results could be explained by the fact that the Mamalis seeds do not respond well to hilar removal and acid scarification as pre-germination treatments. The hilar treatment damages the embryo as the seeds are tiny in size. The acid treatment removes the mucilage and oil that protects the seeds from fungi.

P. pentandrum seed contains oily seed coats that resulted in prolonged germination; seeds are also susceptible to deterioration and infection by fungi [42]. Besides, the researcher found out that some *Pittosporum* spp. seeds appeared to die due to fungal and bacterial infection and the removal of mucilage from the seeds. In contrast, soaking in lukewarm water likely did not altogether remove the mucilage, resulting in its higher percent germination. The more prolonged germination may be due to other factors like after repining dormancy, mucilage, and hard seedcoat.

Sharma [43] found that the untreated seeds of *Albizza lebbek*, *Albizzaprocera*, *Peltophorumpterocarpum*, *Acacia auriculiformis*, *and Leucaena leucocephala* showed maximum germination by 12, 9, 12, 11, and 11% at 20, 17, 19 and 20 days, respectively. Also, the treatments reduced the duration of maximum germination from 17 - 20 days in Control to 4 - 6 days in treated seeds of all the species. Thus, it shortened the period of germination and increases seed germination. Sharman [43] cited that hot water treatment enhances germination by affecting seed coat permeability since water promotes seed hydration. This result supports the studies conducted by Connor [44] that germinated seeds with hard seed coats are enhanced if soaked in tap water for 1 - 2 days at room temperature. Delayed and lower percent germination was obtained with the use of sulfuric acid as a pre-germination treatment. This treatment is similar to the study of Imani *et al.* [45], the researchers treated seeds with sulfuric acid. Similarly, in the study of Farajollahi *et al.* [16], sulphuric acid treatment did not improve the seed germination of *Calotropis persia*. The result demonstrated that seeds with sulfuric acid treatment had a destructive effect on the embryo, supporting this study's outcome. It appears that the constant moist condition of the seedboxes positively improved germination of the Control and lessened its difference with those seeds soaked in lukewarm water and those given sulfuric and hilar removal treatments.

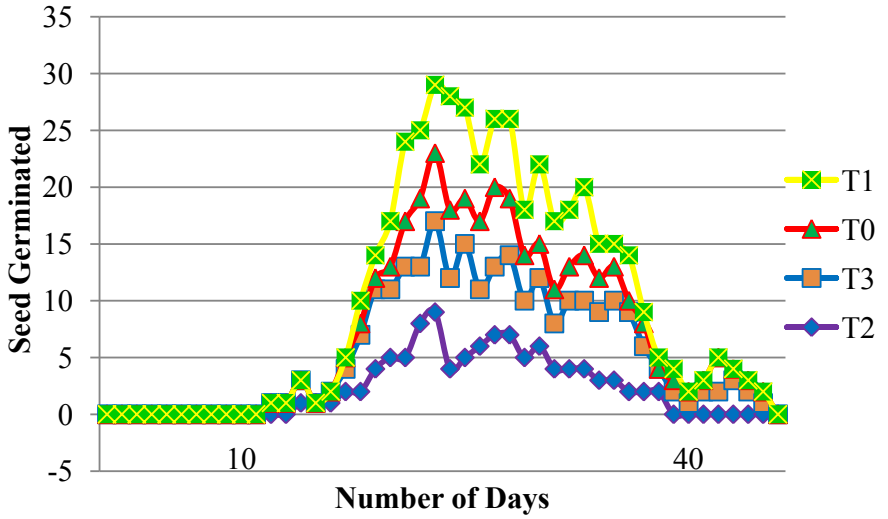

Figure 2. The number of days to the emergence of *P. pentandrum*seeds was affected different pre-sowing treatments.

4.2. Percent Germination

Table 1 shows the results of the pre-germination treatments applied to Mamalis seeds. The highest rate of germination was obtained under T1 (lukewarm water) with 66.67%. The germination rate for T0 (Control) was 45.4%, followed by T3 (acid scarification) at 44.6% and T2 (hilar removal) at 40.8%. The analysis revealed that lukewarm water-treated Mamalis seeds were significantly different from the other pre-germination treatments ($P \leq 0.0001$). Lukewarm water treatment will be advantageous considering the higher labor requirements for hilar removal and the cost of sulfuric acid treatments. After repining seed dormancy, only fully mature fruits should be used for seed-sowing purposes. Argerich and Bradford [46], as cited by Sharma *et al.* [43], reported that a more extended period of germination makes the seed susceptible to deterioration and infection by fungus and other agents.

4.3. Percent Germinative Energy

As presented in Table 2, the highest rate of germinative energy was obtained under treatment 2 (lukewarm water) at 19.17%. The germinative energy rate for treatment 1 (Control) was 17.50%. It was followed by treatment 4 (acid scarification) with a germination energy of 15.42% and treatment 3 (hilar removal) with a germination energy of 15.00%. Based on the results, there were no significant differences in the percent germinative energy of Mamalis seeds among treatments ($P \leq 0.34$). This portrays that the Mamalis seeds under the various treatments had almost the same percent germinative energy. Considering that Mamalis likely had an after-repining seed dormancy, only fully mature fruits should be used for seed sowing purposes. Argerich and Bradford [46], as cited by Sharma *et al.* [43], reported that a more extended period of germination makes the seed susceptible to deterioration and infection by fungus and other agents.

Table 1. Percent germination comparison on the pre-germination of *P. pentandrum* seeds compared to the various treatments.

TREATMENTS	MEAN	F_{VAL}	P_{VAL}	C.V (%)
Pre-germination				
T0 – Control	45.42[b]	16.093**	0.0001	11.81
T1 – Lukewarm Water	66.67[a]			
T2 – Hilar Removal	40.84[b]			
T3 – Acid Scarification	44.58[b]			

Legend: The means of the different letters are significantly different using LSD. * - a significant difference.

Table 2. Percent germinative energy comparison on the pre-germination of *P. pentandrum* seeds compared to the various treatments.

TREATMENTS	MEAN	F_{VAL}	P_{VAL}	C.V (%)
Pre-germination				
Control (T0)	17.50[a]	1.406[ns]	0.304	19.47
Lukewarm Water (T1)	19.17[a]			
Hilar Removal (T2)	15.00[a]			
Acid Scarification (T3)	15.42[a]			

Legend: The means of the different letters are significantly different using LSD. * - a significant difference.

5. Conclusion and Recommendations

This study investigated the effect of pre-sowing treatments on *P. pentandrum* seeds germination. It was revealed that seeds immersed in lukewarm water (at 37.5˚C) could provide better germination than sulfuric acid treatment and hilar removal. The treatments had a significant effect on percent germination, however, not on percent germinative energy. Further study should be conducted on the improvement of hilar removal by applying the technique to *P. pentandrum*-seeds. There is also a need to determine the optimum scarification time to avoid damage to the embryo.

Acknowledgements

The authors were grateful to all the laboratory members for continuous technical advice and helpful discussion. To Benguet State University Graduate School for allowing in conducting this study and to the adviser and panel of this research for full-time support to finish this research. The authors would like to thank the editor and the anonymous reviewers for their helpful comments.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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