

## **Pre-Treatment Techniques for Enhancing Seed Germination Behavior and Early Growth of *Bitaog* (*Calophyllum inophyllum* L.)**

**Karen F. Rabena**

University of Northern Philippines, Vigan City

### **Abstract**

*The study determined the effects of different seed soaking techniques on the germination and growth of bitaog. The treatments used were the following: C-control, T1-use of tap water, T2- use of molluscan shell, and T3-use of NaCl. Statistical design of the experiment was Randomized Complete Block Design (RCBD) with three replicates. The seed coat of bitaog was removed before soaking the seed for sixteen hours.*

*It was observed that germination was improved when seeds were subjected to the different treatments. The percentage germination increased when treated with NaCl and molluscan shell. Early growth of bitaog plant was improved when treated with NaCl. Root length significantly improved with the technique used.*

*With the results of the study, it is recommended that when hastening germination of bitaog seeds, the shell must be removed and soaked solution of sodium chloride and molluscan shell can be used as an alternative to tap water. To help sustain the environment, use of waste materials such as pulverized molluscan shell can be an effective soaking solution. The technique is recommended for study in other tree species or agricultural crops.*

**Keyword Index:** *pre-treatment, enhance, seed, germination growth, bitaog, Calophyllum inophyllum L*

### **INTRODUCTION**

Seed germination is one factor that determines the success of plant establishment. Early and uniform emergence of plant is an essential requirement to reach yield potential, quality and ultimately profit in crops (Parera and Cantliffe, 1994) as cited by Subedi and Ma (2005).

Nowadays, farmers are considering soaking their seeds to hydrate it using tap water. This traditional practice is known to improve the synchronization and percentage of germination as well as its growth. Seeds like any other living thing need moisture, heat and oxygen. When treating seeds by soaking in water, the moisture is absorbed through the seed coat. This results to swelling of the seed parts and making fast vital activities which means that growth has started. This enhances the life process of the dormant embryo ([www.rain.org](http://www.rain.org)).

There is a need to expand the use of pre-treatment of seed not only in agricultural crops but also in timber trees in order to obtain good germination and high quality of harvest. This technique can be applied to sustain quality seeds of trees which can be of potential use. *Bitao* is one plant that can be of good purpose because of its many uses such as in medicine, agroforestry and in cosmetic industry. It is native in the Ilocos region especially near coastal areas, rivers, and swamp.

It is important to promote the tree particularly on its sustainable use and biodiversity conservation to the modern world. Friday and Ogoshi (2011) suggested on their study about *hitaog* that further research on trials at different locations as to best growing conditions must be done.

*Bitao*, scientifically known as *Calophyllum inophyllum* L is a perennial plant widely used as ornamental plant because of its aromatic flowers, attractive leaves and spreading crown. Historically, the trees were considered sacred relics by carving it prior to the conversion of Polynesian to Christianity (Dweck and Meadows, 2002). According to old folks in Vigan and nearby towns, the oil is used in lighting lamps and also cooking as early as 1940s. The hard and strong wood of the tree is best suited for construction or boat building. It was known to be a good source of dark green oil with high-valued medicinal use. Cosmetic manufacturers make use of this plant as ingredient in skin creams because of the presence of an active ingredient in the oil which helps regenerate tissue. ([en.wikipedia.org](http://en.wikipedia.org))

Dweck and Meadows (2002) identified some constituent of the plant. Tannin and the oleoresin is found in bark of the plant. In the leaf are friedeline and triterpene from friedeline group namely canophyllal, canophyllol and canophyllic acid. The oil has two major components namely calophyllic acid and lactone which is believed to have antibiotic properties. The seed has calophyllolide which has non-steroidal anti-inflammatory action. These active ingredients are components of the commercially known *Tamanu oil* which is effective for rashes, sores, swelling and abrasions. ([www.naturalstyles.com](http://www.naturalstyles.com))

## Pre-Treatment Techniques for Enhancing Seed Germination Behavior of *Bitao*

The oil is also used as an analgesic and cures ulcers and bad wounds. The dark yellow oil contains poisonous resin which is alcohol soluble. The seed contains fatty acid methyl ester which is a major biodiesel requirement in the US. (en.wikipedia.org)

Friday and Ogoshi (2011) provided a comprehensive botanical description of the tree which is from Guttiferae now Clusiaceae family. It is large, reaching a height of 12-20 meters, low-branching and slow-growing with a broad, irregular and spreading crowns and horizontal branches. The bark has a gray color with flat ridges. It has a sticky and white sap with fragrant flowers and yellow stamens which appear in racemose or panicle inflorescence in clusters of 4-15. Leaves are heavy, glossy, light green to green with oval, rounded tips and base and parallel veins. Fruits are round balls with green to yellow to brown colors and became wrinkled when ripe. The embryo is encased in a hard shell with corky inner layer.

This study will provide information on enhancing seed germination with technique such as removing of shell or husk then soaking it with different pre-treatment techniques. The use of molluscan shell for seed treatment will also address recycling of waste. Pre-treatment techniques can help farmers and researchers determine the factors that would affect the growth performance of this plant particularly on germination and early growth of *bitao*. With the market demands and practical uses of *bitao*, it is therefore important to improve the production practices and conservation measures in order to produce high quality *bitao* for medicinal, energy production and other uses.

*Bitao* has promising potential for biodiesel production. This will be helpful in addressing the dependence of Philippines on fossil fuels, protect the environment and create jobs by investing in clean energy solutions for the future. Therefore, it is essential that farmers be helped in their production practices and conservation techniques.

### Objectives of the Study

The study determined the seed germination behavior and early growth of *bitao* as affected by seed priming. Specifically, this study determined the following:

- I. Percentage germination of *bitao* seeds after subjecting to different seed pre-treatment techniques for sixteen (16) hours namely:
  - a. control,
  - b. tap water,
  - c. sodium chloride (NaCl), and

- d. molluscan shells;
2. Effect of seed pre-treatment techniques on germination and early growth parameters such as:
    - a. weight of plant,
    - b. height of plant,
    - c. root length, and
    - d. stem diameter;
  3. Differences in the germination and early growth of *bitaog* subjected to four pre-treatment techniques in terms of the above mentioned parameters.

## Review of Literature

Seed soaking is an old strategy used by farmers to lessen the time of seed sowing and germination. The seed requires water, heat and oxygen to live. The water is provided through the seed coat making the seed to swell and quickens its vital activities. Pre-soaking of seeds aims to attain faster germination rate and greater number of seed germinated (Miller, 1996).

Rhoades provided some essential reasons for this seed soaking strategy. One reason is the inherent capacity of the seed to defend itself from harsh condition. The seeds have developed a system to survive in the wild. When seeds are soaked, it is conditioned to break its natural defense which makes it faster to germinate at a shorter period of time. Second is the moisture content of seeds which play a big role in the seeds' optimal growth. The moisture level is improved when seeds are soaked alerting the dormant embryo to germinate. Lastly, for some seeds, they contain germination inhibitors which prevent seed from germination inside the fruit. These inhibitors must be leached before a seed can germinate. The soaking speeds up this process. ([www.gardeningknowhow.com](http://www.gardeningknowhow.com))

There are many possible seed soaking methods that could substitute water for slightly acidic solutions. Rhoades identified examples such as weak tea or coffee or even acidic chemicals. The acidic chemicals imitate the stomach acid of an animal. For most seeds, they only need tap water. Hot tap water is the safest for seed soaking. Nevertheless, seed with hard seed coat can hasten its germination by techniques other than soaking. Scarification techniques such as rubbing the seed on fine grain sand paper, nicking the seed coat with knife, tapping the seed with a hammer to help crack the seed coat or removal of the entire seed coat to expose the embryo can be done before soaking. These damage the seed coat allowing water to enter into the seed's internal system. All this can help the seed to attain a faster germination rate. The seed

## Pre-Treatment Techniques for Enhancing Seed Germination Behavior of *Bitaoog*

must be soaked only within 12-24 hours and not beyond 48 hours. Seed should not be soaked until they sprout in water. There are seeds which can tolerate longer soaking but careful study must be done to avoid seed drowning.

When soaking seed, it is ideal to determine the species growth requirements. *Bitaoog* is a perennial tree with a hard seed coat which delays its germination. Scarification can be done or complete removal of seed coat can enhance germination behavior. Although, natural regeneration occurs near the mother tree, the time for its germination is observed to be long. But when seed soaking is involved before sowing the seed, there is fair germination capacity. Studies cited in World Agroforestry Center conducted revealed that *bitaoog* germination is reduced from 57 to 22 days and enhanced percentage germination from 63 percent to 93 percent.

### METHODOLOGY

The Randomized Complete Block Design (RCBD) was used in the study. There were four (4) treatments with 3 blocks. The 4 treatments were T<sub>0</sub> – control, T<sub>1</sub>- tap water, T<sub>2</sub>- molluscan shell, and T<sub>3</sub>-NaCl. The concentration of sodium chloride and molluscan shell was 2.5 percent each. The seeds were planted in plastic pots and placed in a nursery and were observed daily. The pots were filled with soil composed of 1 part sand and 2 parts loam taken from the upper (30 cm) layer of the soil. The bottom of each plastic pot was provided with an outlet to discharge excess water. The pots were watered with tap water at field capacity. Five (5) grams of complete fertilizer was applied for each pot upon sowing and 15-20 days after emergence.

A total of 120 seeds of *bitaoog* were used during the conduct of the study. Thirty (30) seeds were used for each treatment. The seed was dried and seed shell was removed then soaked in the solutions. These seeds were soaked in 200 mL of water, solution of sodium chloride and molluscan shell for sixteen (16) hours. Ten (10) seeds were sown in each plastic pot to observe germination behavior and later thinned into one (1) to observe early growth parameters. Except for the seeds for the control treatment, they were not soaked in any solution rather only their seed coats were peeled.

The number of seeds germinated was counted on a daily basis. Germination percentage was obtained by dividing the number of seeds which germinated by the total number of seeds sown. Average number of germinated seeds was derived and the percentage was computed for each treatment. A seed is said to have germinated if the coleoptiles exceeds 2 mm above the soil surface. Thinning operation was done after seedling emergence and only one seedling grown in pot per treatment was left to

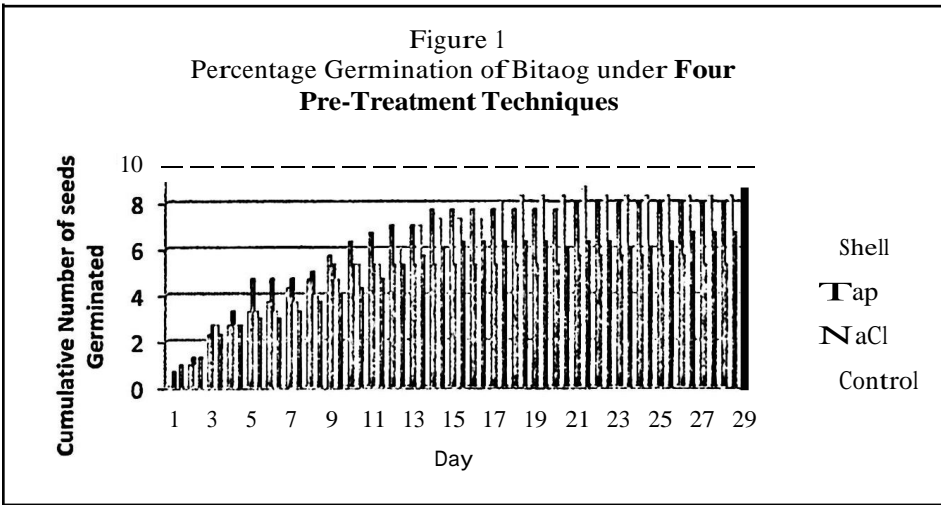
measure early growth parameters. Measurement of height and diameter was imposed 60 days after planting.

The frequency count, mean, and percentages were used to measure germination behavior on a daily basis. Analysis of Variance was used to determine difference between and among priming techniques on early growth parameters of *bitaog* in terms of weight, height, root length, and stem diameter.

## RESULTS AND DISCUSSION

### Percentage Germination of Bitaog

The effect of different soaking techniques on the germination of *bitaog* is shown in Figure 1. Germination commenced seventeen (17) days after the seeds were sown. This corroborates with the findings made by Parras cited by Friday and Ogoshi (2001) that germination averaged 3 weeks for fully shelled seeds but 5 weeks for seeds with cracked shells and 8 weeks for unshelled fruit.



It is shown in Figure I that *bitaog* treated with tap water and control had the early germination. At the start, control obtained the highest rate with three (3) seeds germinated on the first block. When germination was terminated, *bitaog* treated with the different techniques such as sodium chloride, molluscan shell, control and tap water obtained high percentage germination with nine, eight, seven and six seeds

## Pre-Treatment Techniques for Enhancing Seed Germination Behavior of *Bitaog*

germinated respectively. Greatest percentage germination was observed in seeds soaked in sodium chloride (90%) followed by ground molluscan shell (80%). This finding implies that *bitaog* seeds soaked can possibly increase the rate of germination and promote uniform emergence. It also implies that sodium chloride (NaCl) is the best soaking agent. Further, the table shows that *bitaog* seed treated with tap water gave the lowest percentage germination. This implies that among the four (4) priming techniques, tap water is the poorest. Therefore, when treating the seed embryo of *bitaog*, salt is the best technique to be employed (Figure 2).



Figure 2  
Germination of *Bitaog* Seeds using Different Soaking Agents

The effects of different pre-treatment techniques on the growth of *bitaog* were determined using parameters such as weight, height, root length and stem diameter.

Table I. Early Growth of *Bitaog* as affected by Different Pre-Treatment Techniques

Treatments	Mean Early Growth			
	Weight (g)	Height (cm)	Root Length (cm)	Stem Diameter (mm)
Control	8.40	22.00	9.83a	3.70
Tap water	11.30	24.67	10.00b	3.90
Molluscan shell	13.63	26.00	20.67	4.40
NaCl	16.10	27.00	27.00ab	4.50

Legend: Significant difference

Same letter means significantly different from each other

Seeds treated with NaCl obtained the highest value for weight while control had the lowest. However, statistical analysis showed that there are no significant differences between and among the treatments at 5 percent level of significance on weight.

The tallest plant was observed in plants treated with NaCl, followed by those soaked with pulverized molluscan shell, while smallest was obtained in control. Analysis of variance revealed that there is no significant difference between and among the height of the plants treated with different soaking techniques.

Length of roots had the highest value on plants treated with NaCl. Next to it were those plants treated with molluscan shells and lowest value was obtained on control. Analysis of the variance showed that root length differed significantly from each other. This means that one or two of the treatments differed significantly from each other. In comparing which of the treatments differed significantly from each other using Scheffé' test, it was found out that root length of plants treated with NaCl differed significantly from the root lengths of plants under control and tap water. The rest of the different pairs of treatments compared were just the same.

This means that root length of *bitaog* is significantly improved when seeds were soaked with salt and shell. Rapid development of the seedlings root-systems can be observed while the soil conditions around the seed are still good which eventually results to more vigorous crops. ([www.gaia-movement.org](http://www.gaia-movement.org))

Stem diameter was high on NaCl (0.45 mm). This was followed closely by plants treated with molluscan shell and lowest was observed on control. The mean stem diameters of the different treatments however did not differ significantly from each other.

The data above resulted to NaCl and molluscan shell as the best agent for rootings. The seeds of *bitaog* should be soaked in order to obtain a good stand establishment and yield. Figure 3 shows the growth of *bitaog* after the conduct of study.





**Figure 3**  
**Growth of *Bitaog* using different Pre-Treatment Agents**

A study made by Banez and Lazo (1998) on the effect of molluscan shells on the growth of eggplant revealed that growth of eggplant is better when the molluscan shells were incorporated into the soil in pulverized form. Therefore, molluscan shells can be used to improve growth and yield of plants.

### CONCLUSION

Higher root germination was observed in seeds soaked in sodium chloride. Seeds soaked with tap water gave the lowest percentage germination. *Bitaog* seeds subjected to different soaking techniques resulted to high percentage germination as compared to the control.

In terms of growth parameters, the seeds soaked with NaCl obtained the highest value for weight, height, root length, and stem diameter. This means that treating seeds with NaCl is of great potential in improving early growth of plant. The seeds of *bitaog* devoid of shell should be soaked in order to obtain a good stand establishment and yield. ANOVA showed that there is a significant difference between and among the different seed soaking techniques on root length. Plants treated with NaCl significantly differed to plants under control and tap water. There is no significant difference between NaCl and molluscan shell. This means that root length of *bitaog* is significantly improved when soaked in salt solution.

## RECOMMENDATIONS

The recommendations are hereby forwarded for improving crop establishment and growth of plant. The seeds of *bitaog* tree should be soaked before sowing. Before soaking, the seed coat must be removed. This could enhance rate and percentage germination and growth of the plant. The commonly used soaking technique by growers is tap water. Sodium chloride of 2.5 percent solution can be used as an alternative to tap water to improve growth of *bitaog* plant. The effects of seed soaking as compared to other techniques seed should be evaluated on other crops with factors such as temperature and salinity conditions.

## LITERATURE CITED

### Books

- Alejar, A.A., Cedo, M.L.O., & del Rosario, A.G. (2001). Laboratory Manual in Plant Growth. 1<sup>st</sup> edition. University of the Philippines-Los Banos, Laguna.
- Banez, S., & Lazo, M. (1998). Moluscan Soil Neutralizer. *University of Northern Philippines-Research Journal Vol VII Nos. 43-51*.

### Online Resources

- Dweck, A.C. & Meadows, T. (2002). Tamanu (*Calophyllum inophyllum*)- the African, Asian, Polynesian and Pacific Panacea. *International Journal of Cosmetic Science*. Blackwell Science Ltd: USA. Retrieved from [http://www.dweckdata.co.uk/Published\\_papers/Tamanu.pdf](http://www.dweckdata.co.uk/Published_papers/Tamanu.pdf)
- European Commission. (2012). Environment: Soil. Retrieved from [http://ec.europa.eu/environment/soil/index\\_en.htm](http://ec.europa.eu/environment/soil/index_en.htm)
- Florido, H.B., & Cortiguerra, F.F.. (2004). Toog and Bitaug. *Research Information Series on Ecosystems*. Volume 16 No I. January-April 2004. Retrieved from [http://erdb.denr.gov.ph/publications/rise/r\\_v16n1.pdf](http://erdb.denr.gov.ph/publications/rise/r_v16n1.pdf)
- Friday, J.B., & R. Ogoshi. (2011). Farm and Forestry Production and Marketing Profile for Tamanu (*Calophyllum inophyllum*). Specialty Crops for Pacific Island Agroforestry. *Permanent Agriculture Resources (PAR)*, Holualoa, Hawaii. retrieved from <http://agroforestry.net/scps>
- Friday, J. B. and Okano, D. (2006). Species Profiles for Pacific Island Agroforestry. Retrieved from <http://www.agroforestry.net/tti/Calophyllum-kamani.pdf>
- Miller, E. and Miller, C. (1996). It's our garden: Starting Seeds & Plants. Retrieved from <http://www.rain.org/~sals/soak.html>

## Pre-Treatment Techniques for Enhancing Seed Germination Behavior of *Bitaog*

Ocampo, A.M. (2004). Integrated Nutrient Management in Corn, *Integrated Crop Management Module and Manual in Corn*. Department of Agriculture-Agricultural Training Institute & Bureau of Agricultural Research, & National Corn ROE Network-Institute of Plant Breeding, College of Agriculture, UPLB

Rhoades, H. Gardening Know How: How to Soak Seeds Before Planting and the Reasons for Soaking Seeds. Retrieved from <http://www.gardeningknowhow.com>

Subedi, K.D. & Ma, B.L. (2005). Seed Priming Does Not Improved Corn Yield in a Humid Temperate Environment. *Agronomy Journal* 97:211-218. American Society of Agronomy, USA. Retrieved from <https://www.soils.org>.

### Other Online Resources

Agro Forestry tree database. A tree species reference and selection guide: *Calophyllum inophyllum*. Retrieved from <http://www.worldagroforestrycentre.org>

*Calophyllum inophyllum*. Retrieved from <http://en.wikipedia.org>

Seed Priming. The **GAIA** Movement Booklet 29. Retrieved from [www.gaia-movement.org](http://www.gaia-movement.org)

Tamanu oil. Retrieved from <http://www.naturalstyles.com/Pure-Tamanu-Oil-p/ns108.htm>

