S & T interventions on quality planting material production of Batikuling (*Litsea leytensis* Merr.) for the wood-based industry of Paete, Laguna, Philippines

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Abstract. The production of high quality planting stocks should not be dependent on the volume and seasonal availability of the seeds of the target forest tree species to be planted. Adopting a scientific way of producing and conserving indigenous forest trees for agroforestry could prevent the use of inferior planting materials. Quality planting material production of a threatened indigenous forest tree species like Batikuling (*Litsea leytensis* Merr.) is necessary to increase its declining population in forestlands. Wood of Batikuling is used in Paete, Laguna for carving statues, architectural additions, and souvenirs that are known in the country and the world. With the existing clonal forestry facility of Southern Luzon State University, the tree species was produced through vegetative reproduction and raised in a nursery adopting the tested protocol. The process will assure the production of the needed volumes of quality planting stocks at any time of the year without waiting for the flowering or fruiting of forest trees in the wild. The project encouraged greater participation from the local government unit, tree farmers and youth as they showcased their contribution in conserving this important indigenous tree species to increase economic productivity while securing sustainability of ecological services.

1. Introduction
Science and technology (S & T) interventions to improve system and practices of agroforestry and “to protect, restore and promote sustainable use of terrestrial ecosystems...” (Sustainable Development Goal 15 of the 2030 Agenda for Sustainable Development) could pave the way towards the conservation of threatened indigenous forest tree species as well as preservation of traditional knowledge for the wood-based industry of the country in general.

Paete in the province of Laguna was declared as the “Carving Capital of the Philippines” by virtue of Presidential Proclamation No. 809 on March 15, 2005. For over the century, Paete and carving wood of Batikuling (*Litsea leytensis* Merr.) have co-existed and its artisans have produced countless statuaries, architectural embellishments and souvenirs that are known in the country and other parts of the world. Unfortunately, its wood supply is not abundant and over-exploitation may have decreased supply to a critical level [1] that established it as an endangered species in DAO 2007-01 [2] and
vulnerable in the IUCN Red List of Threatened Species [3]. According to [4], the people of Paete must learn how to replace Batikuling (Litsea leytensis Merr.) tree felled for wood carving as the only hope of preventing the ecological, social, and human tragedy imperilled by the industry. Also, it is found in the list of endangered trees with zero number of ex situ collections based from reports of Fernando et al. in 2008 as cited by [5]. Thus, with the expanded National Greening Program (eNGP) through Executive Order No. 193, s. 2015 [6], Southern Luzon State University through its clonal forestry nursery facility aimed to be involved in quality planting material production of Batikuling (Litsea leytensis Merr.) and reforestation of unproductive, denuded and degraded forestlands that will encourage development of tree farms integrated with agricultural crops in Paete, Laguna with greater participation from local government unit, nursery managers and tree farmers.

The general objective of the project is to generate and promote S&T interventions as a platform in the production of quality planting materials of Batikuling (Litsea leytensis Merr.) to support the quality planting materials needs of Paete, Laguna in their agrisilvicultural system. It specifically identified plus trees of Batikuling; amplified the rbcL gene of Batikuling using PCR; assessed the effect of available mycorrhiza and rooting hormone products used in nursery production of quality planting stocks through clonal propagation; determined the cost of production applying the package of technology; capacitated stakeholders on planting materials production and nursery management; and disseminated S&T technologies on nursery and tree farm establishment and management.

2. Materials and Methods
S & T interventions in quality planting material production of Batikuling (Litsea leytensis Merr.) included 1) tree improvement, 2) molecular characterization, 3) use of mycorrhiza technology and rooting hormones, 4) analysis of production cost, 5) production of information bulletin, and 6) capacity building of major stakeholders. The plus or mother trees of Batikuling (Litsea leytensis Merr.) were identified following the recommended criteria by DENR-EMB through DAO 2010-11 [7]. Phenotypic characteristics were the basis for selection. Traits observed were: stem straightness, stem forking/multi-stem, branch angle, branch circularity/twisting, tree health, branch thickness, and branch pruning/persistence recorded in a data sheet indicating the location, species name, date, and description of the area, among other relevant information. Selected plus trees were sources of planting materials in the establishment of the clonal facility’s hedge garden. Management of Batikuling (Litsea leytensis Merr.) stock plants include watering, weeding, fertilizer application, pest and disease monitoring and control, and pruning.

Collection of leaf samples of Batikuling (Litsea leytensis Merr.) was done for DNA extraction, DNA amplification, Agarose gel electrophoresis, PCR purification, DNA sequencing, and DNA sequence analysis. To validate and evaluate the presence of the DNA from leaf samples of the species, the rbcL primer was used as recommended by the CBOL. The PCR program was set to have initial denaturation for 3 minutes at 94 °C, followed by 35 cycles of 94 °C for 45 seconds of secondary denaturation, 30 seconds at 50 °C for the annealing of primers, 1:30 minutes at 72 °C for the primer extension, and finished with 72 °C for 10 minutes for the incubation of DNA and filled up incompletely extended strands. Several trials of PCR using same PCR reactions and same PCR conditions were done after the quantification. Stem cuttings from established hedge garden were gathered for macropropagation following the procedures of ERDB (2010) [8] in which varying concentrations of Alpha Napthalene Acetic Acid (NAA) and Indole Butyric Acid (IBA) rooting hormone (100 ppm and 200 ppm) was applied with two formulations (MykoVAm and Hi-Q VAM 1) and amount of mycorrhiza inoculant (5 g and 10 g) in rooting media. The study followed a split plot design with 3 factorial experiments to determine the response of stem cuttings in a mist propagation system. Survival rate, average length of roots, and average number of roots were gathered and analysed through the Analysis of Variance (ANOVA) and Duncan’s Multiple Range Test (DMRT). To determine positive symbiosis between rooted stem cuttings of Batikuling (Litsea leytensis Merr.) and mycorrhiza, destructive sampling was employed when the stem cuttings have started to have soft and few secondary roots. Using a dissecting microscope, mycorrhiza was determined by washing soil from
roots very gently and by using extraction procedures where observations at this level are normally used to quantify mycorrhiza association. Cost of producing Batikuling (*Litsea leytensis* Merr.) clones was estimated using the actual inputs and labor costs incurred in conducting macropropagation at the clonal nursery. The existing wage rate was used to value labor in which the goal of production is 20,000 clones per species per year and the hedge garden has already been established and is sustainable to serve as planting stocks as sources of stem cuttings.

The publication materials were produced in coordination with the extension unit of the university following standard form, substance and process for technical review and evaluation by experts. The materials produced supported the trainings conducted to farmer co-operators or POs to strengthen the technical know-how of the project. The trainings focused on clonal propagation techniques for the mass production of high quality planting materials of Batikuling (*Litsea leytensis* Merr.) for agrisilvicultural system in the uplands.

### 3. Results and Discussion

The plus trees of Batikuling (*Litsea leytensis* Merr.) with good phenotypic characteristics were found at 300-400 masl within the vicinity of Mt. Banahaw de Lucban. According to ERDB (1999) [9], it was found on its required habitat in low and medium altitude. It is a medium-sized tree with mean height of 13.5 m, 46.79 cm diameter and 9.25 m² crown cover. The optimized condition for the amplification of the *rbcl* gene for DNA barcoding of Batikuling (*Litsea leytensis* Merr.) was: 3 min initial denaturation at 94 °C → (denaturation at 94 °C for 45s → annealing at 50 °C for 30s → extension at 72 °C for 1 min and 30s) 35 cycles → final extension at 72 °C for 10 min. A total of thirteen (13) out of 24 Batikuling (*Litsea leytensis* Merr.) samples were successful in the amplification and was stored at -20 °C which was used to sequence the samples. According to [10], amplified PCR products are essential in DNA barcoding to produce the DNA barcode regardless of the success rate.

![Figure 1](image.png)

**Figure 1.** Electrophoresis gel electropherograms of the *rbcl* fragment of representative samples of Batikuling (Legend Upper Panel: 1 to 7- Paete, Laguna; 8 to 10, and 12 to 15- Lucban, Quezon; 16 to 22 SLSU NGP Clonal Forestry Nursery); 23-25 – 100, 50, 10 ng/uL lambda DNA, 26 – 100 bp ladder. Legend Lower Panel: 9-12 Lucban, Quezon and 17 – 100 bp ladder.
The gene *rbcL* is approximately 1430 base pairs in length and has a fairly conservative rate of evolution. This single copy gene codes for the large subunit of the ribulose-1,5- biphosphate carboxylase/oxygenase (RUBISCO/RuBPCase) [11]. Results from gel visualizations showed that the size of the PCR products were all similar. The product sizes of relatively good sequences using Sanger method approximately range from 600 to 800 bp. No significant sequence variation was detected for all individuals. While this confirmed high genetic similarities within species, variability at populations could not be ascertained due to low variation in nucleotide sequence, and thus, another barcoding gene should be optimized and sequenced. However, it is suggested that a more powerful marker must be optimized or a population genetic analyses using more polymorphic markers such as microsatellites is highly recommended to detect genotypic/allelic variation between individuals or to delineate populations of the species. It will be more useful genetic data for the conservation of the species.

In the macropropagation of Batikuling (*Litsea leytensis* Merr.) stem cuttings, it started to form callus and roots on the tenth week of propagation in all of the treatments used. The results of the study showed significant difference in the means of the treatments in terms of survival rate and average length of roots (Tables 1 and 2). ANOVA table showed significant differences between the means of the treatment concentrations and amount of mycorrhiza inoculant, which is higher than the tabulated value of 5.14 at 5 % level of significance. Average length of roots was 8.0 cm in stem cuttings with NAA rooting hormone and mycorrhiza inoculant (Figure 1). The total average length of roots in the entire treatment with NAA rooting hormone and 10 g of mycorrhiza had a higher average length than treatments with 5 g of mycorrhiza compared to NAA rooting hormone only. In the average number of roots, rooted stem cuttings with 5 g of mycorrhiza inoculant without NAA rooting hormone produced the highest number of roots. DMRT test for survival rate of rooted Batikuling (*Litsea leytensis* Merr.) stem cuttings was highly significant in terms of concentration level of NAA rooting hormone (Table 3).

**Table 1.** ANOVA table of the effect of NAA rooting hormone and mycorrhiza inoculant on the survival rate of rooted Batikuling stem cuttings.

| Source of Variation                              | df  | Fc   | Ftab 0.05 | Ftab 0.01 |
|------------------------------------------------|-----|------|-----------|-----------|
| Replication                                     | 3   | 6.0526 | 4.76     | 9.15      |
| Amount of mycorrhiza (A)                        | 2   | 2.0526 | 5.14     | 10.92     |
| NAA concentration level (B)                     | 2   | 5.5471* | 5.14     | 10.92     |
| Amount of mycorrhiza and Level of Concentrations (A x B) | 4   | 0.8031 | 3.26     | 5.41      |
| Types of Mycorrhiza (C)                         | 1   | 2.0769 | 4.21     | 7.68      |
| Amount of Mycorrhiza and Types of Mycorrhiza (A X C) | 2   | 0.6923 | 3.35     | 5.49      |
| NAA concentration level and Types of mycorrhiza (B x C) | 2   | 0.6923 | 3.35     | 5.49      |

*-Significant
Table 2. ANOVA table of the effect of NAA rooting hormone and mycorrhiza inoculant on the average length of roots of Batikuling stem cuttings.

| Source of Variation | df | Fc      | Ftab (0.05) | Ftab (0.01) |
|---------------------|----|---------|-------------|-------------|
| Replication         | 3  | 7.4564  | 4.76        | 9.15        |
| Amount of mycorrhiza (A) | 2  | 1.5494  | 5.14        | 10.92       |
| NAA concentration level (B) | 2  | 0.2072  | 5.14        | 10.92       |
| Amount of mycorrhiza and NAA concentration level (A x B) | 4  | 6.1482* | 3.26        | 5.41        |
| Type of mycorrhiza (C) | 1  | 0.8539  | 4.21        | 7.68        |
| Amount of mycorrhiza and type of mycorrhiza (A x C) | 2  | 0.6992  | 3.35        | 5.49        |
| NAA concentration level and type of mycorrhiza (B x C) | 2  | 0.926   | 3.35        | 5.49        |

*-Significant

Table 3. DMRT table of the significance of NAA rooting hormone on the survival rate of rooted Batikuling (*Litsea leytenis* Merr.) stem cuttings.

| Source of Variation | Mean Difference | Rp    |
|---------------------|-----------------|-------|
| Control and 100ppm  | 0.7916ns        | 0.7545|
| Control and 200ppm  | 0.0833*         | 0.7545|
| 100ppm and 200ppm   | 0.875*          | 0.7937|

*-Significant
ns-Not significant

Figure 2. Rooted Batikuling stem cuttings (a) with 5 g mycorrhiza only, (b) 100 ppm NAA with 5 g mycorrhiza and (c) 200 ppm NAA with 5 g mycorrhiza at 19th week of observation.
The rooted stem cuttings of Batikuling with mycorrhiza inoculant, after five months have hyphae, arbuscules, and vesicles in root samples under microscopic observation. Based on DMRT, different concentrations of IBA rooting hormone and types of mycorrhiza had a significant effect on the survival rate of rooted Batikuling stem cuttings (Table 4). However, IBA rooting hormone concentration and amount of mycorrhiza inoculant had no significant effect on the average length and average number of roots. However, different concentration of IBA had significant effect (p<0.05) with types of mycorrhiza (Hi-Q VAM and MykoVAM) in length of roots of Batikuling (Table 5). Rooted Batikuling stem cuttings with mycorrhiza inoculant produced longer roots with 6.2 cm - 10.3 cm in 100 ppm with 5g; 5.3 cm - 7.51 cm in 100 ppm with 10 g; 3.48 cm - 7.7 cm in 200 ppm with 5 g; 4.98 cm - 5.1 cm; in 200 ppm with 10 g. The presence of hyphae and vesicles of mycorrhiza in Batikuling root samples were observed in 100 ppm and 200 ppm IBA rooting hormone with 5 g and 10 g of mycorrhiza inoculated in rooting media. The combination of mycorrhiza and IBA rooting hormone has affected root initiation and root growth, however increasing rooting hormone concentration and inoculant does not promote shoot growth. The production cost of 20,000 cloned Batikuling (*Litsea leytensis* Merr.) through vegetative propagation was estimated at PhP16.00 to PhP 19.00 per clone at rooting stage.

**Table 4.** DMRT table of the significance of IBA rooting hormone and type of mycorrhiza inoculant on the survival rate of rooted Batikuling (*Litsea leytensis* Merr.) stem cuttings.

| Source of Variation | Mean Difference | Rp          |
|---------------------|----------------|-------------|
| Control and 100ppm  | 0.67*          | 0.631118    |
| Control and 200ppm  | 0.13ns         | 0.631118    |
| 100ppm and 200ppm   | 0.54ns         | 0.663912    |
| Hi-Q VAM and MykoVAM| 0.31ns         | 0.2777      |

*-Significant
ns-Not significant

**Table 5.** ANOVA table of the effect of IBA rooting hormone concentration and Hi-Q VAM and MykoVAM level in average length of roots of Batikuling stem cuttings.

| Source of Variation            | df | F Value | Tabular Value |
|-------------------------------|----|---------|---------------|
|                               |    |         | 0.05          | 0.01          |
| Replication                   | 3  | 1.72ns  | 4.76          | 4.76          |
| Amount of mycorrhiza (A)      | 2  | 0.16ns  | 5.14          | 5.14          |
| IBA concentration level (B)   | 2  | 1.25ns  | 5.14          | 5.14          |
| Amount of mycorrhiza and IBA  | 4  | 2.59ns  | 3.26          | 3.26          |
| concentration level (A x B)   | 1  | 0.11ns  | 4.21          | 4.21          |
| Type of mycorrhiza (C )       |    |         |               |               |
| Amount of mycorrhiza and type of mycorrhiza (A x C) | 2 | 0.68ns | 3.35          | 3.35          |
| IBA concentration level and Type of mycorrhiza (B x C) | 2 | 3.99*  | 3.35          | 3.35          |
| A x B x C                    | 4  | 1.22ns  | 2.73          | 2.73          |

*-Significant
ns-Not significant
Figure 3. Rooted Batikuling stem cuttings (a) control, (b) 100 ppm IBA with 5 g mycorrhiza and (c) 200 ppm IBA with 10 g mycorrhiza at 19th week of observation.

Figure 4. Sample of brochure used during IEC on Batikuling (LitsealeytenisMerr.).

Figure 5. Training of stakeholders of Paete, Laguna on vegetative propagation using stem cuttings of Batikuling (LitsealeytenisMerr.).
Developed IEC materials were in the form of printed matter (Figure 4) and video clip which were utilized as information bulletin to the public through the university’s extension project to capacitate stakeholders of Sitio Gumihan and Sitio Papatahan in Paete, Laguna on quality planting materials production and nursery management of Batikuling (Litsea leytensis Merr.). The IEC campaign is a step towards disseminating information and gets the interests of stakeholders of Paete, Laguna to regrow their forestlands. Also, seminars and trainings were conducted in partnership with the local government unit of the municipality to tree farmers, nursery staff and manager, youth (senior high school students), and women (Figure 5). A demo farm of the cloned tree species was established in their agroforestry learning site at Sitio Gumihan to showcase S & T interventions in quality planting material production and further evaluate field performance of the species.

4. Conclusion

Plus/mother trees of Batikuling (Litsea leytensis Merr.) with phenotypically superior characteristics was found within the vicinity of Mt. Banahaw de Lucban which were sources of stock plants for hedge garden establishment. The PCR conditions for amplifying the rbcL region in Batikuling (Litsea leytensis Merr.) are easily attained by adjusting the number of cycles and annealing temperature, and hence, generation of DNA sequences for DNA barcoding was attained with relative ease. However, it is suggested to have a more powerful marker must be optimized or a population genetic analyses using more polymorphic markers. The use of orthotropic shoots of Batikuling (Litsea leytensis Merr.) with rooting hormone products and mycorrhiza inoculant in rooting media hastened root formation with observed mycorrhiza association.

The partnership of the university and local government showcase their contribution in conserving economically important indigenous tree species such as Batikuling (Litsea leytensis Merr.) for the wood-based industry to increase economic productivity while securing sustainability of ecological services by employing high quality and integrated research and extension projects. However, the success of species conservation does not only rely on quality seedling production but it also considers the involvement of local communities as stewards of a vulnerable ecosystem.

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