



TISSUE CULTURE RESEARCH LABORATORY

**SOUTHERN LUZON STATE
UNIVERSITY,
BRGY. AYUTI, LUCBAN QUEZON,
PHILIPPINES**

TCL PERSONNEL

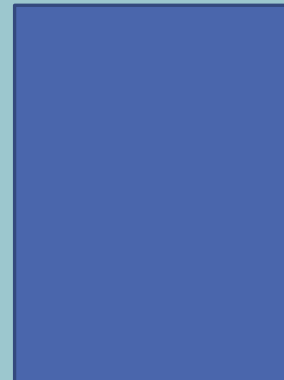
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Head



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Laboratory Technician II



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Laboratory Aide II



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SLSU — TISSUE CULTURE LABORATORY:

- provides students an exposure to a laboratory setting, which will benefit them as future professionals and help them completely comprehend the value of tissue culture.
- This will be possible through educational tour with the approval of the Head of Tissue Culture Laboratory and assistance of laboratory personnel.



SLSU — TISSUE CULTURE LABORATORY:

- Open for students and faculty with **research relating to micropropagation** using tissue culture techniques for the multiplication of plants *in-vitro* aseptically.
- Laboratory personnel are willing to provide their expertise in executing techniques and utilize the facilities and equipment of laboratory in clean and safe manner. The laboratory technician has experience in micropropagation of stem, buds, anther, and ovules of a certain commodity.



SLSU — TISSUE CULTURE LABORATORY

- The existing project in TCL is the **Embryo Culture of Makapuno (ECM).**
- Embryo-cultured makapuno seedlings are available. Depending on the size and age of the seedlings, prices might range from to Php 600.00 to Php 650.00 per seedling.



SIGNIFICANCE/IMPACT TO KNOWLEDGE ADVANCEMENT AND TO THE SOCIETY (SOCIAL IMPACT)

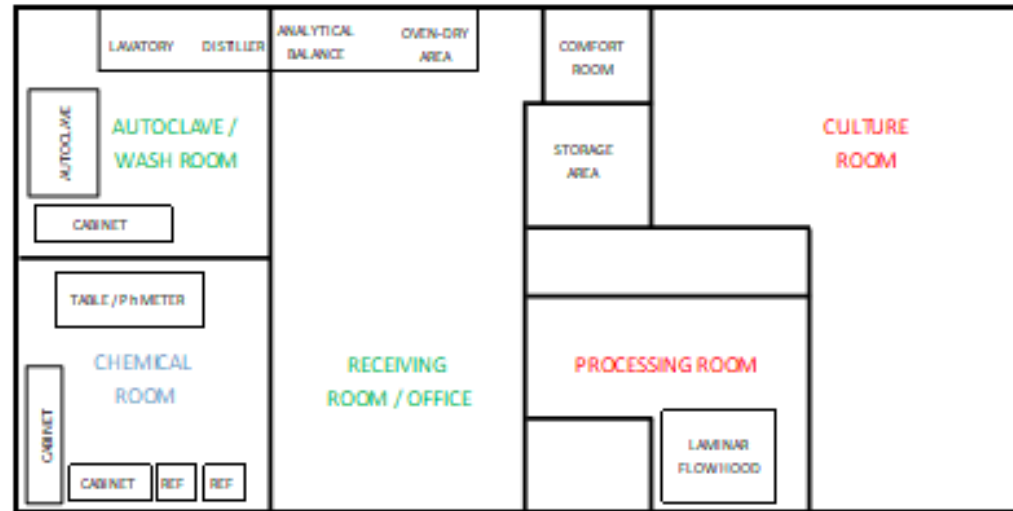
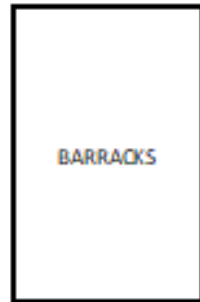
- Through this project we can help in catering the needs for viable makapuno planting materials in our Region. There is a very high demand for Makapuno both from the local primary and secondary processor in the Philippines, but the number of field planted Makapuno-bearing trees are not enough to supply the growing demand. Thus, SLSU plant tissue culture laboratory aims to help in filling the gap in terms of production of Makapuno planting materials through this project. Future collaboration with PCA, some Agencies and other universities concerned in Makapuno industry is anticipated.



SIGNIFICANCE/IMPACT TO KNOWLEDGE ADVANCEMENT AND TO THE SOCIETY (SOCIAL IMPACT)

- Creation of livelihood for the community (in the near future) - Makapuno meat can be processed into sweets/delicacies or another product/s that can be sold.





LAYOUT

PROCESSING AREA (RED AREA)



- Also known as **Transfer Room** or **Sterile Room**
- Consist of **Laminar Air Flow Hood** with **High Efficiency Particular Air (HEPA) filter** that provides a sterile atmosphere within the work area by forcing air through microfilters and into the hood.
- The room is air-conditioned to keep the room relatively free from dust.

CULTURE ROOM (RED AREA)



- Consist of **6 lighted shelves** with a capacity of **600 – 1000 cultures**
- Temperature is kept at **25 - 27°C** for optimum growth.

CHEMICAL ROOM (BLUE AREA)



- Where media preparation happens.
- Consists of pH meter, refrigerator, and cabinets for storing chemicals, glassware and labware.

RECEIVING ROOM AND OVEN-DRY AREA (GREEN AREA)



- Analytical balance and hot air oven are placed in a concrete platform.

WASH ROOM (GREEN AREA)



- Consists of autoclave, distilling apparatus, sink, water dispenser and storage space for glassware and labware.

SHADE HOUSE



- Where hardening stage happens.



ZYGOTIC EMBRYO CULTURE OF COCONUT

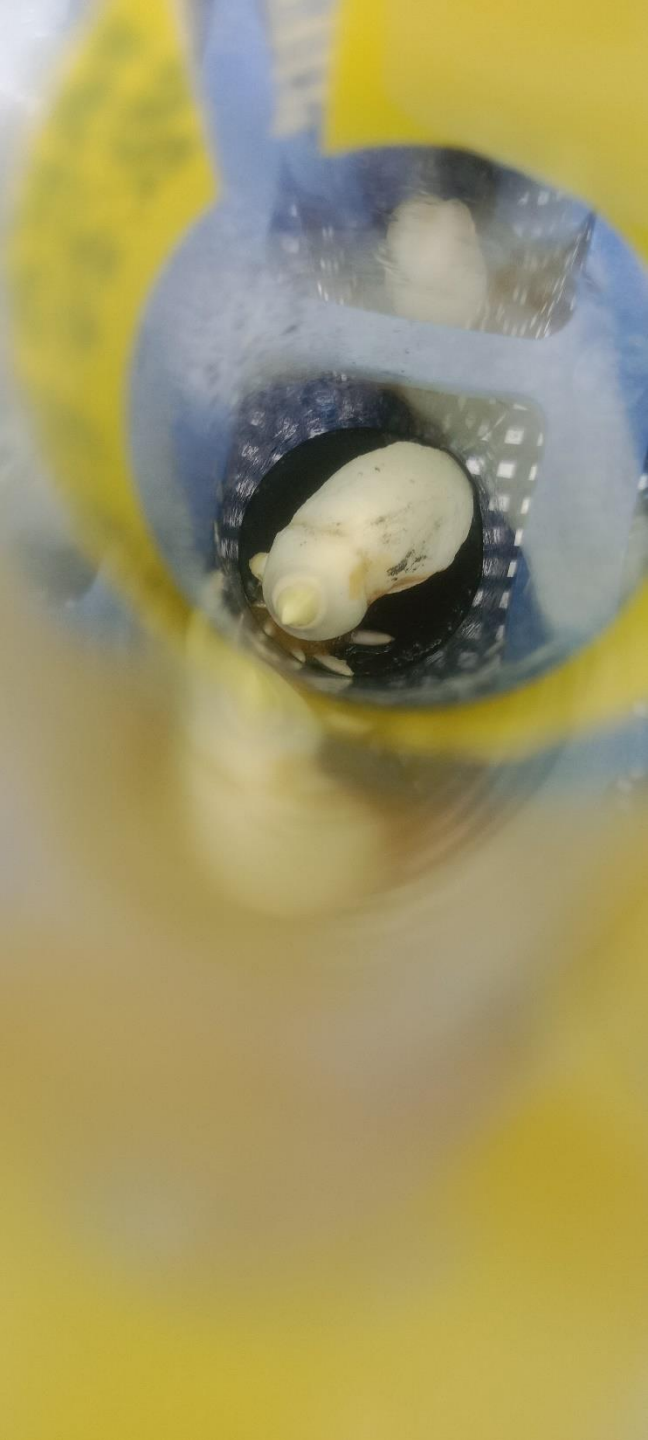


INTRODUCTION

There is an urgent need in the country to mass propagate high yielding varieties of coconuts for massive replanting programs of the Philippine Coconut Authority. There is a projected shortage of 7 million coconut seedlings (PCA) which are needed to be planted to replace those coconut palms that are already unproductive and senile, or those affected by calamities of pest, diseases and damages due to typhoons and other natural causes. This means millions of seed-nuts will be diverted for seed gardens and thus will be unavailable for oil production, which is the main activity of the industry.

INTRODUCTION

At SLSU, this is an on-going research project in which we intend to use the zygotic embryo culture techniques on elite coconut varieties developed by PCA to produce viable plantlets. Here, only the embryo is rescued and used in culture and the rest of the meat is made available for production of copra and oil. Embryo rescue techniques are currently being used for Makapuno plantlet production, which will be extended to the mass production of elite variety plantlets to supplement the seedlings, for planting purposes.



OBJECTIVE

To develop working protocol for zygotic embryo-culture of elite, high-yielding Philippine coconut varieties, to produce viable plantlets that can be field-planted and grown to maturity.

2 to 20%
Makapuno coconuts
from normal nuts of
makapuno-bearing
palms

VS.

75-100%
Makapuno coconuts
from embryo-
cultured seedlings*

***if planted together and/or isolated from other coconut palms by a pollen barrier.**

ELITE HIGH YIELDING VARIETIES

- Synvar
- Aromatic
- Tacunan
- Catigan Dwarf

MAJOR SUPPLIERS OF MAKAPUNO EMBRYOS



Makapuno Processors

- Nadel Food Corp. in Nagcarlan
- Escaba Sweets in San Pablo, Laguna



SLSU MAKAPUNO PLANTATION

Consists of 52 Makapuno
Dwarf Trees

- Monitored and maintained by Mr. Rolando S. Oblenida.



FIELD COLLECTION

Harvesting

- mature nuts (11 to 14 months-old). Nut is color break of the husk from green to brown
- **Dehusking and splitting of nuts**
- **Extraction or excision of endosperm cylinders**
 - The embryo embedded in the solid endosperm is located under one of the three “eyes” of the coconut.



SURFACE STERILIZATION

(INSIDE THE LABORATORY)

- Sterilized with KCl, Bleach and other disinfecting agents rinse with sterilized distilled water. Decant.



IN-VITRO TECHNIQUE

(INSIDE THE LAMINAR FLOW HOOD)

- Excision
- Sterilization
- Inoculation in modified Y3 artificial nutrient medium aseptically.



CULTURE CONDITIONS

- Incubate cultures at 28-30°C in dark for 1 to 2 months. The practice is usually 1 month.
- Subculture to fresh medium at monthly interval.
- Check periodically for contamination.



After 1 month incubation in dark



A week after subculture from liquid
to solid medium

CULTURE CONDITIONS

- Embryos growth at different rates. Generally, **6-8 weeks** after roots and shoots are formed. The earliest record time to transfer *ex vitro* is **4 months**.
- Altogether the culture period could be **a year or more**.

May 27,
2021



June 24,
2021



July 15,
2021



August 10,
2021



September 16,
2021



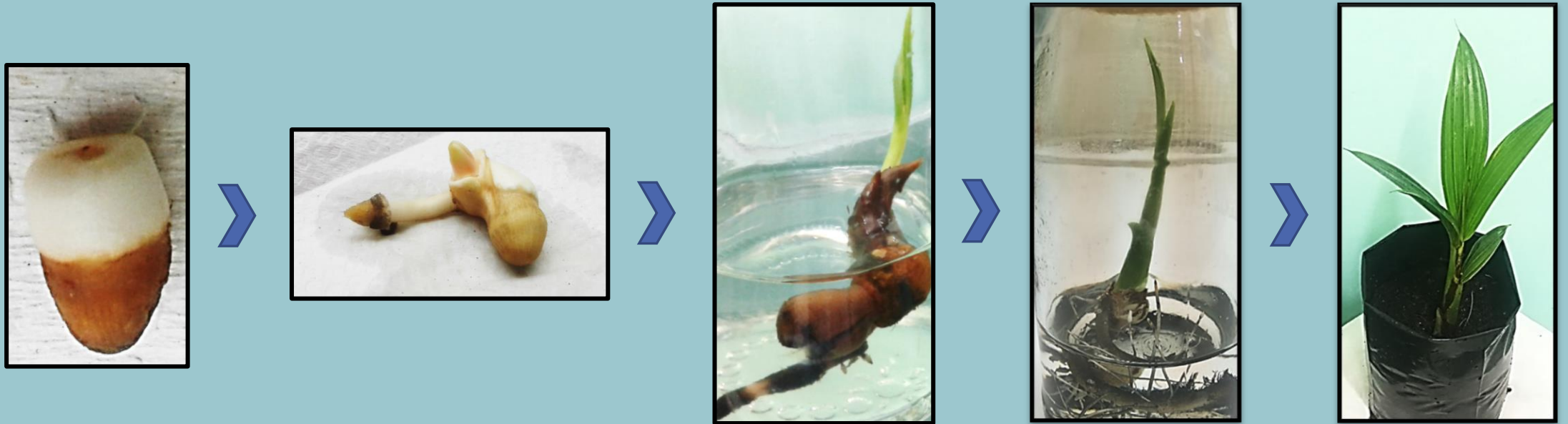
October 19,
2021



November 16,
2021



SUCCESSIVE STAGES OF GROWING COCONUT EMBRYOS OF ELITE VARIETY AROMATIC IN MODIFIED Y3 MEDIUM



Rooting and shooting achieved as early as 4 weeks in Y3 medium.

REGENERATION STAGE

- After about 9-10 months of culture the plants were transferred to poly-bags containing 1:1 sterile river sand and coconut peat and hardened in greenhouse to be finally field planted.
- To maintain high relative humidity, cover the seedlings with plastic bags with a support of bamboo pegs so that will not sag on the leaves of the seedlings. Keep them covered for 4 to 8 weeks or until the seedlings have manifested complete recovery from in vitro conditions.
- After this period, gradually expose the seedlings to screen house conditions by partially lifting the plastic cover.
- Thereafter, the plants can be fully exposed the seedlings to screen house conditions.

STATUS REPORT

Date of Inoculation	Number of New Embryos	Minimum Number of Explants with Visible Growth (60%)	Minimum Number of Plantlets or Explants in Advance Stages (10%)	Minimum Number of Plantlets transferred to Sterile Soil Media (8%)	Minimum Number of Plantlets in Acclimatization Stage (5%)	Minimum Number of Plantlets in Hardening Stage to Established Seedlings (2.5%)
		After 1 month of Incubation in Dark (28 - 30°C)	After 3 months in Solid media	After 1 year <i>in-vitro</i> (may vary to the growth of explants)	After 1 - 3 months (may vary to the growth of plantlets)	After 3 - 9 months (may vary to the growth of embryo-cultured seedlings)
2 nd Quarter (April 16,2021)	150	120 (74.53%)	17(11.33%)	4 (2.67%)	1	-
1 st Quarter (February22, 2022)	200	125 (62.5%)	17 (8.5%) as of April 2022	-	-	-

REFERENCE

Rillo E. (2013). *Technoguide for Makapuno Embryo Culture*, Tissue Culture Division, PCA – Albay Research Center