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Rapid and Cost-effective Embryo Culture Technique for Commercial Production of Makapuno Seedlings

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Abstract

An embryo cultured Makapuno (ECM) production scheme which is simpler, faster and more low-cost than the current protocol has been developed. With improvements in the *ex vitro* establishment environment, the cut-off age for *in vitro* stage of seedlings before they can be transferred to soil has been lowered from 7 to 4 months resulting in a shorter ECM production cycle from 13 to 10 months. The improvements simplified the current ECM production technique by doing away with several complicated *in vitro* cultural practices and *ex vitro* establishment steps. Overall, the new ECM production scheme lowered the cost of production resulting in more affordable ECM seedlings for the farmers.

Financial analysis of a 10 year ECM seedling production venture projected much higher Internal Rate of Return, Benefit Cost Ratio and Net Present Value as well as shorter Payback Period with the use of the new ECM production scheme. With the new scheme, the break-even prices using marginal and full-cost operations were approximately 4 to 8 times lower than the current selling price for ECM seedlings. With these results, it is expected that more entrepreneurs will take up the technology for commercial production with a consequent massive planting of ECM and greater production of Makapuno in the country.

Keywords: Coconut, Makapuno, Embryo Culture, MYD, MAKT, IRR, NPV, BCR, Break-even Price, Payback Period

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Introduction

Makapuno nut production is a lucrative business in the Philippines offering an annual income per hectare (160 palms/ha) starting from PhP130,000 during the initial year to PhP350,000 during the later years of production. This is the reason why demand for true-to-type Makapuno seedlings remains high. However, even with currently high and increasing demand for ECM seedlings, entrepreneurs seem reluctant to venture into this agribusiness undertaking due to its long payback period (PSMSI, 1999) and high production cost (Areza *et al.*, 2003) even with the use of the current coconut embryo culture protocol which takes about 13 months to produce seedlings ready for field planting. This production cycle needs to be shortened in order to attract capitalists to venture into the embryo-cultured Makapuno (ECM) production business.

Moreover, many complicated cultural practices and steps involved in the current ECM production may be eliminated or simplified to reduce production costs in order to make ECM seedlings more affordable for the coconut farmers.

The lower age limit of *in vitro* seedlings that could undergo autotrophic growth for an earlier shift to *ex vitro* conditions needed to be established. Studies on photosynthetic parameters such as chloroplast ultrastructure, PEPC and RubisCO have shown that plantlets at the end of the *in vitro* culture process possessed several photosynthetic characteristics similar to those of acclimatized plants (Verdeil, 1996). However, these studies used completely hardened *in vitro* plants (ca. at least 7 month old) and did not investigate younger *in vitro* seedlings.

At the same time, the optimal ambient environment for *ex vitro* establishment of *in vitro* grown seedlings should also be identified before results of *in vitro* and *ex vitro* treatments on *ex vitro* survival can be analyzed more accurately. The soil support medium and cover to conserve the relative humidity during soil establishment also needed to be improved and biological factors such as quality of starting embryos and role of haustorium on growth of seedlings *in vitro* and subsequent survival *ex vitro* needed investigation. Likewise, the relationship of root and leaf attributes with survival of seedlings when transferred to soil needed to be established.

This study set out to improve the current art and technique of Makapuno embryo culture. It aimed to shorten the incubation period *in vitro* and simplify the various steps involved in the current protocol for ECM seedling production by: (1) determining the earliest stage of *in vitro* seedlings that can reliably undergo photoautotrophic growth when transplanted to soil; (2) improving ambient environment for *ex vitro* establishment of *in vitro* grown seedlings; and (3) looking at relevant factors that may cause slow development of the seedlings *in vitro* and low survival rate *ex vitro*.

Finally, it is necessary to analyze the financial aspect of ECM production using the current Makapuno embryo culture protocol and the new ECM production scheme developed in this study. The resulting estimates for the Net Present Value (NPV), Benefit Cost Ratio (BCR) Internal Rate of Return (IRR), and Payback Period (PP) can then serve to stimulate entrepreneurs who may be interested in ECM production.

Methodology

Preliminary trials were conducted to identify the range of treatments to be included in the actual experiments. Actual experiments were conducted using embryos collected from nuts of Malayan Yellow Dwarf (MYD) populations and non-Makapuno nuts from previously embryo cultured Makapuno palms (MAKT) growing at PCA-Albay Research Center. Final verification experiments were conducted using Makapuno embryos. *Ex vitro* survival was assessed six (6) weeks after soil transplanting. Whenever appropriate, data were analysed statistically using ANOVA and DMRT, together with data from previous ECM production studies to look at trends relevant to *in vitro* growth and *ex vitro* survival of different ages of *in vitro* seedlings. These were used to establish the statistical significance of the results of experiments conducted in this study.

The following experiments were conducted:

1). Photosynthetic capacity of different ages of *in vitro* seedlings

Two (2) and three (3) month-old seedlings were cultured in Hoagland's nutrient medium for a single passage before they were transplanted to the soil support medium. *Ex vitro* survival of the treated seedlings were compared with the control treatment (current protocol).

Ex-vitro establishment of the treated seedlings (3 and 4 month-old) was also tried on soil-less Hoagland's medium. A pump controlled by a time switch provided a periodic flow of circulating Hoagland's nutrient solution for the plants every 15 minutes. The seedlings were kept in place in holes in Uratex® pads on an ordinary 100 mm diameter PVC pipe through which the Hoagland's solution flowed (Figure 1a). The whole set up was contained inside a humidity tent (Figure 1b) which was manually ventilated to prevent any high temperature build-up (*i.e.* more than 40°C). *Ex vitro* survival rates of treated seedlings were compared to the control.

2). Improvement of ambient environment for *ex vitro* establishment of *in vitro* grown seedlings

A 1:1 mixture of garden soil and cocopeat was compared with the routinely used river sand as support medium in the establishment of *in vitro* grown seedlings. Sterilization of the soil support medium was also evaluated by comparing sterilized and non-sterilized soil support medium in terms of survival of seedlings. Sterilization of the medium was done for 30 minutes in a pressure cooker. Three (3) and four (4) month-old seedlings grown from embryos of MYD and normal nuts of MAKT were used in the experiments. Ordinary clear plastic bags were used as cover to maintain high relative humidity for the transplanted seedlings.

In a separate experiment, utilization of a communal humidity tent (Figure 1b) to preserve high relative humidity during the initial phase of *ex vitro* establishment of seedlings was compared with the use of individual ordinary clear plastic bag covers (Figure 2) as used in the *ex vitro* establishment procedure of the current protocol. Three (3) month-old *in vitro* grown MYD seedlings were used in the experiment.

Figure 1. a) Ex vitro establishment set-up using circulating Hoagland's solution. b) Humidity tent covering the whole set-up

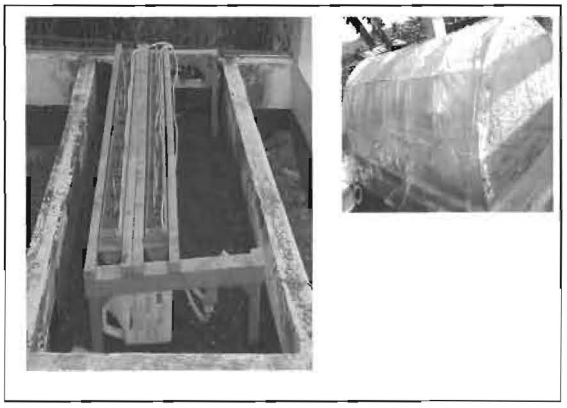
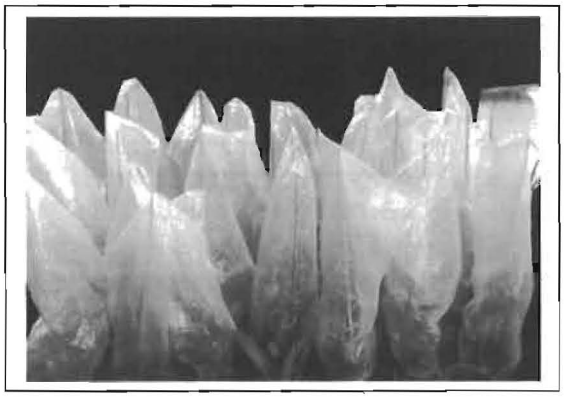


Figure 2. Clear plastic bag covers used to maintain high relative humidity during ex vitro establishment of in vitro coconut plants as recommended in the current protocol



3). Internal factors that may influence development of seedlings *in vitro* and survival *ex vitro*

3a). Partial removal of haustorium

During *in vitro* stage of the culture, the treatments were applied to germinated embryos as follows:

T1 Haustorium partially removed after germination

T2 Haustorium partially removed 4 weeks after germination

T3 Haustorium intact (control)

Only embryos that germinated after 4 weeks were used in the experiment. Shoot length and fresh weight of cultures just before they were established *ex vitro* were compared among the three (3) treatments used. The cultures were transplanted after 5 months *in vitro* following the current *ex vitro* establishment procedure with modifications according to the results of Experiment 2.

3b). Density of embryo

Coconut embryos either float or submerge when they are placed into liquid nutrient medium. Germination and further development as well as survival during *ex vitro* establishment of initially floated and submerged embryos were compared.

4). *In vitro* hardening by proper positioning of seedlings *in vitro*

Two (2) month-old rooted seedlings were planted in such a way that only the roots and tips of the haustoria were in contact with the liquid medium. The plants were fixed at this position inside the test tube using sterilized pieces of polyurethane foam. The usual planting procedure wherein the plants were planted with parts of the stem submerged in the medium served as control.

Initial and final fresh weights of the plants were recorded in order to determine the weight gained for each treatment. The final weights were recorded two (2) months after treatment. *Ex vitro* survival rates for the two (2) treatments were compared.

5). Analysis of data from previous ECM production studies

Relevant raw data gathered from previously conducted research were re-consolidated and analyzed. Analysis was focused on possible *in vitro* growth and *ex vitro* survival trends that may relate to the results of the experiments

6). Integration of results to the Makapuno embryo culture protocol: verification and economic analysis of the modified protocol

Experiments using Makapuno embryos were conducted to confirm results of the experiments that were conducted using embryos from normal nuts. Based on results of the confirmatory experiments, necessary adjustments were made on the current embryo culture protocol to come up with a new scheme for ECM production.

An economic analysis of a 10-year ECM production project involved the use of the current and the new protocols. Annual cash flows were estimated based on 2006 prices. NPV, BCR, IRR and PP were computed and compared between the two protocols. Factors of 7, 10 and 15% were considered in the discounted analysis. Break-even analyses for 4-10 month incubation periods *in vitro* were also made using marginal costing (*i.e.*, only variable costs were involved) and full costing (*i.e.*, fixed and variable costs were involved).

Results and Discussion

1). Photosynthetic capacity of different ages of *in vitro* seedlings

The intervening one (1) month passage in Hoagland's medium prior to *ex vitro* establishment did not affect *ex vitro* survival of MAKT seedlings. On the other hand, the same treatment resulted in significantly lower percentage of *ex vitro* survival for MYD seedlings (Table 1). The apparent sensitivity of MYD seedlings to the quality of nutrient medium *in vitro* may be attributable to the higher metabolic rate of MYD, which are earlier maturing than MAKT.

Nevertheless, the observed ability of both cultivars to survive during the 1 month *in vitro* incubation in Hoagland's medium suggested that *in vitro* seedlings are capable of autotrophic growth even at an early age of two (2) months. The plants relied on CO_2 from the air as source of carbon for autotrophic growth because Hoagland's solution lacks sugar. The observed

photosynthetic readiness of 2 month-old *in vitro* seedlings suggests that they maybe already fit for *ex vitro* establishment.

Ex vitro survival of three (3) month-old seedlings was generally comparable with 4 month-old seedlings. This result indicated that under PCA-ARC laboratory condition, 3 month-old coconut embryo cultured seedlings were ready for soil establishment. However, *in vitro* growth of seedlings in Hoagland's was obviously slower than in Y3 medium. Likewise, secondary roots did not develop or were poorly developed in Hoagland's nutrient medium (Figure 3). Nevertheless, the hardiness of the 3 month-old seedlings was comparable to well-rooted 4 month-old seedlings grown in Y3 medium as indicated by their survival during *ex vitro* establishment.

Results of another experiment showed that survival rates of three (3) and four (4) month-old normal MAKT and MYD seedlings grown *in vitro* were not significantly different with respect to the two (2) *ex vitro* establishment methods tried (Table 2). The result indicated that *in vitro* seedlings can also be established *ex vitro* in soil-less medium. Established seedlings continued to grow when transplanted to soil inside the screenhouse or nursery. This *ex vitro* establishment method has great potential for large scale commercial production of elite coconuts, such as Makapuno, using embryo culture techniques. Since the nutrient composition of Hoagland's solution is more exactly defined than the soil-cocopeat mixture, which varies unpredictably from one batch of mixture to another, using this solution will make a consistent *ex vitro* establishment medium. Furthermore, this will pave the way for a mechanized *ex vitro* establishment procedure for *in vitro* grown coconut or other crops whenever applicable.

2). Improvement of ambient environment for *ex vitro* establishment of *in vitro* grown seedlings

Ex vitro survival of three (3) month-old MYD in the routinely used sterilized pure sand medium was significantly higher when a communal humidity tent was used (Table 3). The higher survival of seedlings inside the humidity tent could be due to the good headspace provided for the seedlings. Due to the limited size of the clear plastic bag covers, build up of high temperature within the head space of the seedling may occur and may adversely affect the survival of seedlings.

Illuminance inside the humidity tent was around 45,000 Lux at noon on a partly cloudy day when illuminance outside was around 75,000 Lux. With a clear sky, when illuminance outside reached 130,000 Lux it was 72,000 Lux inside the humidity tent. The highest temperature recorded inside the humidity tent was 38°C. Temperature inside the plastic bag cover reached as high as 49°C.

In a separate experiment, survival of 3 month-old seedlings, noted 6 weeks after soil planting, was significantly higher in soil-cocopeat mixture than in pure sand. For four (4) month-old seedlings, the two (2) soil support media were not significantly different in terms of *ex vitro* survival of four (4) month-old seedlings. Soil-cocopeat mixture provides better aeration and has higher water holding capacity than pure sand. These facts could well explain the higher survival of three (3) month-old seedlings in the soil-cocopeat medium. The beneficial effect of this soil support medium was probably insignificant for older or more hardened four (4) month-old seedlings hence, their survival rates in the two soil support medium were comparable.

Sterilization of the oil support medium had no significant effect on *ex vitro* survival of either three (3) or four (4) month-old seedlings (Table 4). Sterilized and non-sterilized soil support media did not differ significantly in terms of survival of seedlings during *ex vitro* establishment. This suggests that *in vitro* seedlings as young as three (3) month *in vitro* can tolerate any harmful effects of microorganisms that may be present in non-sterilized soil medium. It may also be inferred that the fungicide solution used to dip the seedlings prior to soil planting is enough to avoid fungal infection during soil establishment of the seedlings.

Table 1. Percentage *ex vitro* survival of 3 and 4 month-old MAKT and MYD seedlings as affected by intervening 1 month passage in Hoagland's medium prior to *ex vitro* establishment

Treatment (Final subculture medium *in vitro*)	Survival of Seedlings (%)			
	MAKT ns		MYD *	
	3 month-old	4 month-old	3 month-old	4 month-old
Hoagland's	98.22	97.32	96.43 b	93.75 c
Y3 (control)	98.22	98.22	99.11 a	97.32 b

ns = *Not significantly different at 5% level*

* = *Significantly different at 5% level; values followed by the same letter in the same block (cultivar) are not significantly different.*

Table 2. Percentage *ex vitro* survival of 3 and 4 month-old MAKT and MYD seedlings in two (2) support medium for *ex vitro* establishment

Treatment (Support medium for *ex vitro* establishment)	Survival of Seedlings (%) ns			
	MAKT		MYD	
	3 month-old	4 month-old	3 month-old	4 month-old
Circulating Hoagland's solution	99.11	99.11	96.43	93.75
1:1 soil-cocopeat mixture	97.32	98.22	99.11	97.32

ns = *Not significantly different at 5% level*

Table 3. *Ex vitro* survival of 3 month-old MYD seedlings in communal humidity tent and ordinary clear plastic bag covers

Treatment	*Ex vitro* Survival of Seedlings (%)
T1 Humidity tent (communal)	100 *a*
T2 Clear plastic bag (individual)	96 *b*

Table 4. *Ex vitro* survival of 3 and 4 month-old MAKT and MYD seedlings as affected by types and sterilization of soil support medium

Treatment	Ex vitro Survival of Seedlings (%)			
	3 month-old		4 month-old	
	MAKT	MYD	MAKT	MYD
Soil support	*	*	*ns*	*ns*
Soil - cocopeat mixture	99.11 a	98.22 a	99.11	97.32
Pure sand	97.32 b	96.43 b	98.22	98.22
Sterilization	*ns*	*ns*	*ns*	*ns*
Sterilized	98.22	99.11	98.22	95.54
Non-sterilized	98.22	96.43	97.32	95.54

*Significantly different at 5% level. Means followed by the same letter in the same block are not significantly different at 5% level of DMRT

Ns Not significantly different at 5% level

The experiment clearly pointed out the beneficial effect of using soil-cocopeat mix over pure sand. Elimination of sterilization procedure for the soil support medium saved time, effort and logistics during soil establishment of *in vitro* grown coconut seedlings. This will contribute to lowering the cost of producing true-to-type Makapuno seedlings using embryo culture technique.

3). Internal factors that may influence development of seedlings *in vitro* and survival *ex vitro*

3a). Partial removal of haustorium

Partial removal of the haustoria immediately after or 1 month after germination of embryos had no significant effect on *in vitro* growth performance of seedlings in terms of shoot length, fresh weight and *ex vitro* survival of MYD seedlings (Table 5). While the haustorium plays a significant role in endosperm hydrolysis and mobilization of nutrients during germination and initial shoot growth *in vivo*, this may not be true for *in vitro* condition considering the insignificant effect of partial removal of the haustorium in terms of growth of seedlings. The possibility that it can store nutrients that can be consumed by the seedlings during *ex vitro* establishment is unlikely since survival rates of seedlings with intact and partially removed haustoria were not significantly different. It was also observed that the haustoria of developing seedlings were often under-developed and sometimes inconspicuous *in vitro*. In contrast, when growth of the haustoria sometimes filled the circumference of the test-tubes it was difficult to take out the seedlings during transferring. Based on these findings, haustoria may be partially removed after germination in order to avoid this identified difficulty during subculturing. This will contribute to higher efficiency especially when commercial application of the embryo culture technique is involved.

3b). Density of embryo

Of all the embryos planted, 96% floated on the medium while only 4% submerged (Table 6). Except when contaminated, submerged embryos floated after 3 - 7 days in culture. The germination rates were comparable between embryos that initially floated and those that submerged upon planting. With respect to growth of seedlings, embryos that floated at planting displayed significantly lower frequency of stunting. Subsequently, significantly higher survival rate during *ex vitro* establishment was exhibited by seedlings which developed from embryos that initially floated during planting. With this result, efficiency of the coconut embryo culture technique may be improved by initially culling the submerged embryos. Although submerged embryos may be discarded when the number of embryos is not limiting, this may not be wise for Makapuno embryos due to the rarity of this type of coconut.

4). *In vitro* hardening by proper positioning of seedlings *in vitro*

In vitro growth of MYD seedlings in terms of shoot length and fresh weight was generally slower when seedlings were planted in such a way that only the roots and tip of the haustorium were in contact with the nutrient medium (T1) compared when the roots, haustorium and part of the stem were submerged in the medium (T2) (Figure 4, Table 7) showing that absorption of nutrient by the growing seedlings was greatly reduced when only the roots and tip of the haustorium were in contact with the nutrient medium. Hence, growth of the seedlings relied greatly on nutrient absorption through the stem and petioles. However, survival of seedlings during ex-vitro establishment was not significantly different between the two treatments. This indicated that orienting the seedlings *in vitro* in such a way that the only the roots and tip of the haustorium are submerged in the nutrient medium is not necessary to increase the *ex vitro* survival of seedlings. Furthermore, readiness of the seedlings for *ex vitro* establishment should not be measured by the size or weight of the seedlings. For better growth *in vitro*, it is suggested that the basal part of the stem and petioles be kept in contact with the nutrient medium. Trimming of the roots may be necessary to keep the seedlings in proper contact with the medium.

Figure 3. Three (3) month-old seedlings from Malayan Yellow Dwarf embryos that have been planted in Hoagland's (seedlings 1 & 2) for 1 month and Y3 medium (seedlings 3 & 4) continuously

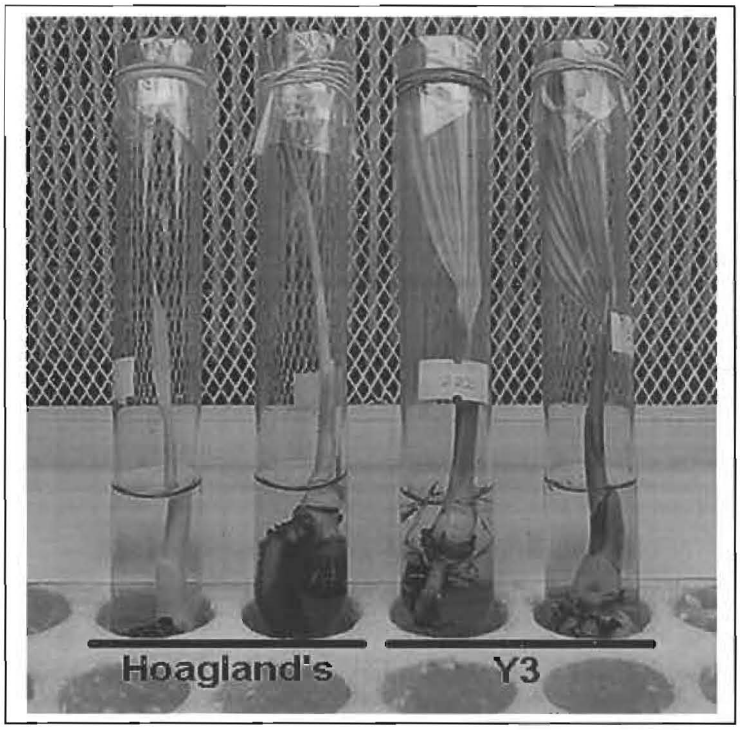


Figure 4. Four (4) month-old MYD seedlings planted normally (T2) and oriented in such a way that only the roots and tips of haustoria are in contact with the liquid medium (T1)

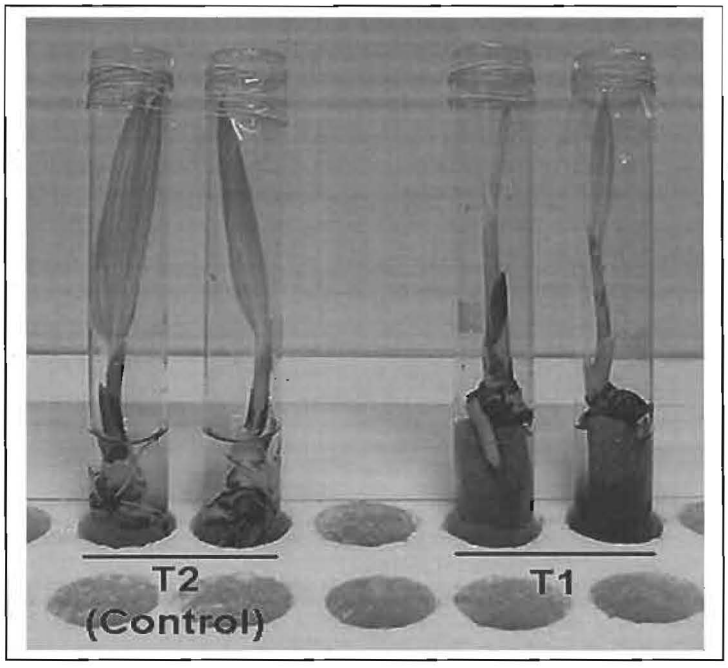


Table 5. Effect of removing the haustorium on *in vitro* growth performance of embryos from open pollinated MYD and survival of seedlings during *ex vitro* establishment

Treatment		Growth Parameter		Ex vitro Survival ^{ns} (%)
		Mean Shoot Length ^{ns} *(cm)*	Mean Fresh Weight ^{ns} *(g)*	
T1	Haustorium partially removed after germination	12.23	5.86	100
T2	Haustorium partially removed 1 month after germination	12.54	6.20	100
T3	Haustorium Intact	12.14	5.88	100

Table 6. Frequency of occurrence and germination rate of floating and sinking MYD embryos and subsequent growth of seedlings

Parameter	Proportion *(%)*	Growth performance		*Ex vitro* survival *(%)*
		Germination *(%)* ns	Stunted Growth *(%)*	
Floating	96 *a*	73.18	11.54 *b*	82.28 *a*
Submerged	4 *b*	72.73	18.18 a	63.64 *b*

Table 7. *In vitro* growth performance (Data gathered after 5 month *in vitro*) and ex-vitro survival of seedlings grown from embryos of MYD as affected by two planting orientations

Treatment (Part of the seedlings submerged in the nutrient medium)		Growth Parameter		Ex vitro Survival ^{ns} (%)
		Mean Shoot Length *(cm)*	Mean Fresh Weight *(g)*	
T1	Only the roots and tip of the haustorium	7.68 *b*	4.83 *b*	80.12
T2	Roots, haustorium and base of stem (Control)	10.14 *a*	6.23 *a*	78.03

5). Analysis of data from previous ECM production studies

Analysis of ECM production data from embryos initiated from January to May 2005 revealed that the ages of ECM taken out of the laboratory for soil establishment ranged from 3 - 11 months (Figure 5). Most of the seedlings transplanted to the soil were 4-8 months old. A decreasing trend for survival was seen as the seedlings aged *in vitro*. This finding suggests that younger *in vitro* seedlings have a better chance of survival during soil establishment than older seedlings. It was obvious that most of the seedlings were established in the soil too late because of the requirement for profuse root as recommended in the current protocol.

Moreover, analysis of other data revealed that the periodic increment in shoot or leaf length noted from the second to the fifth passage periods had a declining trend curve indicating a declining periodic growth rate as the seedlings aged *in vitro* (Figure 6). The higher growth rate of younger seedlings *in vitro* may be attributable to higher adaptability of 3 month-old seedlings to *ex vitro* conditions. On the other hand, the lower percentage of survival of older seedlings may be due to culture shock. The long incubation *in vitro* could have caused excessive dependency of older seedlings to *in vitro* conditions, negatively affecting their adaptability when transferred to the *ex vitro* environment.

Further analysis of the production data put values to some important points of concern in the production of Makapuno using embryo culture technique (Table 8). Germination of Makapuno embryos was realized on 72% of all embryos planted. Discarded cultures due to contamination reached 4% contributing to the total loss *in vitro* of about 30%. With soil establishment survival of about 97%, the overall percentage recovery from initial embryos to nursery hardened seedlings was estimated to be about 68%.

6). Integration of results to the Makapuno embryo culture protocol: verification of the modified protocol and comparative economic analysis

Experiments using Makapuno embryos were carried out based on analyses of results of previous experiments.

The efficiency of a communal humidity tent over plastic bag covers, in conserving relative humidity during *ex vitro* establishment of MYD and normal MAKT seedlings, was verified using ECM seedlings. Survival of selected three (3) month-old rooted Makapuno seedlings which possessed at least one true leaf was significantly higher when communal humidity tent was used than when individual clear plastic bag cover was utilized during *ex vitro* establishment (Table 9). The use of communal humidity tent proved more efficient in ensuring higher *ex vitro* survival of *in vitro* grown Makapuno seedlings.

Culture of Makapuno embryos revealed that at three (3) months *in vitro*, ECM seedlings had varying extent of root and leaf development. During soil establishment, survival of seedlings with at least one (1) true leaf differed according to the extent of root development (Table 10). Surprisingly, three (3) month-old seedlings with only primary root(s) exhibited a higher percentage survival during soil establishment than seedlings possessing both primary and secondary roots. This indicated that secondary roots of three (3) month-old seedlings are better initiated *ex vitro*. The presence of more secondary roots during soil establishment may not be advantageous. The tender secondary roots are subject to breakage or injury during soil transplanting and may cause death of some seedlings.

According to the current Makapuno embryo culture protocol, seedlings should be ready for soil establishment when profuse secondary and tertiary roots have developed. Due to variation in their development *in vitro*, seedlings were taken out of the culture vessels for soil establishment at different times as they become ready (*i.e.,* the seedlings that have developed profuse secondary and tertiary roots)

The findings that younger seedlings had a higher survival rate than older seedlings will revolutionize the Makapuno embryo culture technique. This will clearly shorten the *in vitro*

Figure 5. Proportion of different ages of Makapuno seedlings (%) taken out of the culture vessel for ex vitro establishment and their corresponding percentage survival ex vitro. (From January–May 2005 ECM Production Data, PCA-Albay Research Center)

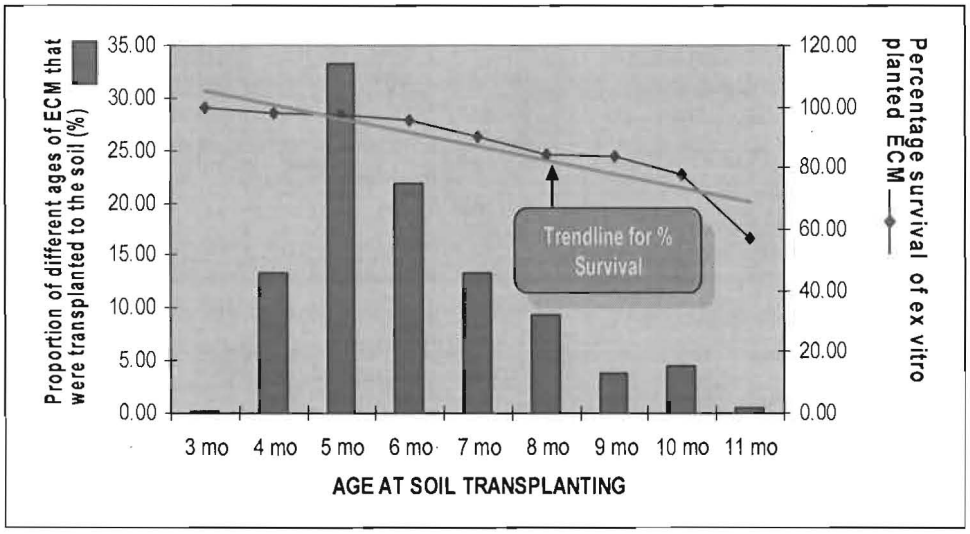


Figure 6. In vitro growth pattern of Makapuno seedlings in terms of periodic (monthly) increment in shoot/leaf length after embryo germination until the 5th passage period

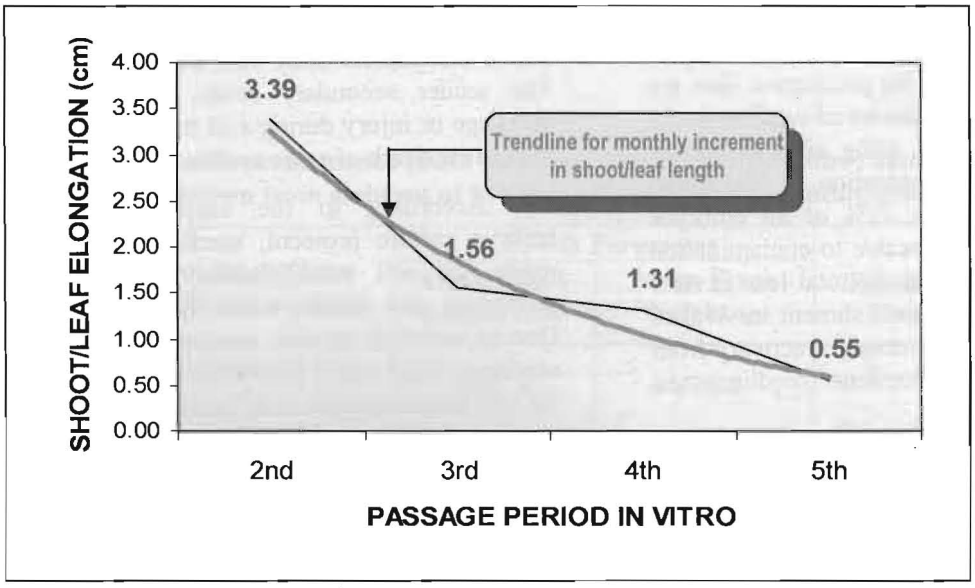


Table 8. Assessment of important points of concern in the production of Makapuno seedlings using the current Embryo Culture Technique. (*Data from Jan-May 2005 ECM Production Data, PCA-Albay Research Center*)

Factor / Concern	Value (%)
A. Germination	72.57
B. Contamination *in vitro*	3.53
C. Survival *in vitro* [C = A x [(100–B)/100]	70.00
D. Survival *ex vitro*	97.00
E. Recovery from embryo to hardened seedlings (E = C x D)	67.90

Table 9. *Ex vitro* survival of three (3) month-old Makapuno seedlings (with at least 1 expanded leaf and 1 primary root) in communal humidity tent and ordinary clear plastic bag cover

Treatment	*Ex vitro* Survival (%)*
T1 Humidity tent (communal)	100 *a*
T2 Clear plastic bag (individual)	92 *b*

* *Assessed 6 wk after soil planting*

Table 10. Percentage survival of 3 month old Makapuno seedlings with different stages of root development regardless of leaf development

Type of Root Present	*Ex vitro* Survival (%)
Primary Roots Only	100.00
Primary + Secondary Roots	85.00

Table 11. Frequencies of selected leaf and root attributes of 3 and 4 month-old *in vitro* Makapuno seedlings

Attribute	3 month-old (%)	4 month-old (%)
With at least 1 true leaf (partially or fully expanded)	78.38	99.07
With at least 1 primary root	100	100

incubation of the seedlings leading to a shorter production cycle. Although 3 month-old seedlings could be taken out of the culture vessels for soil establishment, it was observed that significant numbers had under-developed shoots (Figure 7a). Comparing the leaf and root attributes between 3 and 4 month-old *in vitro* Makapuno seedlings, all have developed at least one (1) primary root. Only about 78% of 3 month-old seedlings have a partially or fully expanded leaf (Table 11). Almost 100% of 4 month-old *in vitro* seedlings have this leaf attribute (Figure 7b) which is required in order to qualify the seedlings for soil establishment.

With all these findings, it was recommended for commercial production of Makapuno using embryo culture technique, that four (4) *in vitro* months be set as the cut-off age prior to *ex vitro* establishment of seedlings. Moreover, as the seedlings are incubated in long test tubes (25 x 200 mm) during this stage of development they are still easy to handle (Figure 7c) With the current protocol, the seedlings have to be transferred to bigger bottles with clear plastic bag extension after this stage to accommodate the growing seedlings (Figure 7d). Bigger bottles occupy larger space inside the autoclaves during sterilization; hence, less bottles are autoclaved at one time. Besides, fixing the plastic bag extension is laborious and requires care and skill to avoid contamination of the cultures. Based on these considerations, the current Makapuno embryo culture protocol was revised. The *in vitro* stage was shortened by 3 months (*i.e.,* from 7 to 4 months). With a period of six (6) months for *ex vitro* establishment and hardening of ECM seedlings in the screenhouse and nursery before they can be sold and planted out, the whole cycle from embryo to nursery-hardened seedling was reduced from 13 to 10 months (Table 12).

Due to the shorter duration of incubation *in vitro*, ECM seedlings that were produced using the new scheme were smaller than when they were produced using the current protocol (Figure 8). Nevertheless, seedling size was not expected to affect their survival in the field. The smaller size, though, may initially affect the trading of the produced ECM due to the acknowledged (but unfounded) preference of farmers for bigger seedlings. On-farm trials and roadside demonstration plots will solve this problem. On the other hand, experiments related to nutritional requirement for optimal growth of ECM seedlings in the nursery is on-going at the PCA-Albay Research Center. Faster growth of ECM in the nursery will improve turn-over and satisfy the preference of farmers for bigger plants.

With a grant from DOST-PCARRD, the results prompted the construction of a strong and large capacity humidity chamber (Figure 9) for full adoption of the new ECM production scheme.

The profitability of a 10-year Makapuno seedling production project using the shortened protocol which is being proposed in this paper was compared with that using the current protocol. Both projects involved the construction of a laboratory building screenhouse and nursery in a leased 500 sqm lot which is accessible to water and electric supply. Rental for the lot was PhP2,500 per month with the agreement that all lot developments automatically go to the landowner after the 10-year project. An advanced payment of PhP30,000 corresponding to one (1) year rental was effected during the construction of infrastructures. All the necessary equipment, labware and initially required chemicals were purchased prior to the commencement of operation. Makapuno nuts were bought at PhP25/kg or approximately PhP16.65/pc assuming that three (3) nuts weigh two (2) kg. For the 1,200 embryos needed each month, the projects spend PhP20,000/month for the Makapuno nuts. Establishment and maintenance of seedlings *in vitro* involved the use of coir dust (PhP25.00/sack) black polypropylene bags for potting out, pesticides and other minor things. These were estimated at PhP2,520/mo. *Ex vitro* establishment and hardening of seedlings in the screenhouse and nursery before they were dispatched took a period of 6 months.

For the project involving cut off age of 4 month for *in vitro* incubation, 2 laboratory and 2 nursery workers were hired at PhP6,000 and

Figure 7. a) 3 month-old Makapuno seedlings with unopened leaves; b) same age of Makapuno seedling with opened leaves; c) 4 month-old Makapuno seedlings in long test tubes ready for soil establishment; and d) 5 month-old Makapuno seedlings in milk bottles with extended plastic cover

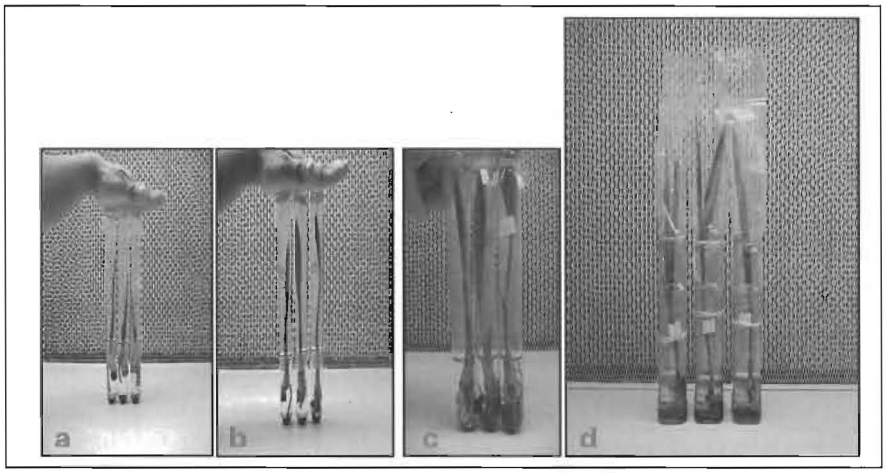


Figure 8. Comparative growth of completely hardened ECM seedlings produced using the new and current production scheme

Figure 9. Humidity chamber for ex vitro establishment of in vitro Makapuno seedlings

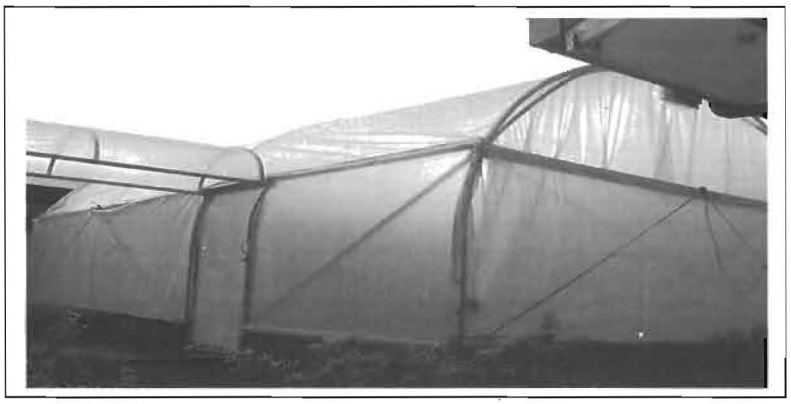


Table 12. Different developmental stages of the original and revised Makapuno Embryo Culture protocol

Protocol	Culture Stages (months)												
	1	2	3	4	5	6	7	8	9	10	11	12	13
ORIGINAL	In vitro stage							Ex vitro stage					
	Germination	Further growth						Establishment	Further growth and hardening				
PROPOSED	In vitro stage				Ex vitro stage								
	Germination	Further growth			Establishment	Further growth and hardening							

Table 13. NPV, BCR, IRR and PP for a 10-year Makapuno production project involving 4 months *in vitro* incubation at different discount factors (DF) and selling prices of seedlings

SELLING PRICE OF ECM (*PhP*)	PARAMETER	7% DF	10% DF	15% DF
500	Present Value	16,052,586.74	13,601,842.17	10,487,381.03
	BCR	3.36	3.22	3.01
	IRR (%)	102.51		
	Payback Period (*yr*)	3.33		
400	Present Value	11,556,355.69	9,723,208.40	7,395,606.75
	BCR	2.70	2.59	2.42
	IRR (%)	81.46		
	Payback Period (*yr*)	3.95		
300	Present Value	7,060,124.64	5,844,574.63	4,303,832.47
	BCR	2.04	1.96	1.82
	IRR (%)	57.92		
	Payback Period (*yr*)	4.97		

PhP5,000 per month with an annual year-end bonus equivalent to one (1) month's salary. An annual 10% increase in salaries of project staff was added as an incentive to keep them until the end of the project; turn over of staff would hamper smooth implementation of the project. Procurement of nuts and initiation of embryos were discontinued 6 months before the end of the project. The final 6 months of the project were purely devoted to *ex vitro* establishment. The cost of laboratory chemicals varied each year depending on the rate of consumption of each chemical being used. The cost of laboratory chemicals amounted to PhP252,346.00 for the whole duration of the project. Distilled water was bought at PhP30.00/liter amounting to PhP2,088.00/ month for an estimated 69.6 liters utilized monthly. Cost of tap water was PhP600.00/ month (PhP300.00 for the lab and PhP300 for the screenhouse and nursery. Cost of electricity, estimated at PhP8.50/KWH, was PhP3,151.68/ mo. Other items used in the laboratory such as LPG, plastics bags, rubber bands and detergents were estimated to cost PhP1,470/mo.

For the project involving a seven (7) month cut-off age for *in vitro* incubation, four (4) laboratory and two (2) screenhouse and nursery workers were hired at the same rate and the same monetary incentives. The longer incubation period of seedlings *in vitro* and the intricate procedure involved in the shift from the use of test tube to wide-mouth bottle (with plastic bag extension) necessitated hiring of an additional 2 workers in the laboratory. Besides, more labor is needed in the preparation and sterilization of nutrient media. The cost of laboratory chemicals amounted to PhP979,824.00 for the whole duration of the project. This amount was almost four (4) times the cost of chemicals for the new protocol (PhP252,346.00). The cost of distilled water amounted to PhP9,648.00/month for an estimated 321.6 liters utilized monthly. This volume of distilled water was 4.62 times higher than the monthly requirement of 69.6 liters for the project involving the modified protocol. Longer *in vitro* incubation and the use of bigger culture vessels required the use of more medium. Consequently, more distilled water was needed. Cost of electricity, estimated at PhP5,346.88/month was also approximately 70% higher than the cost for the project involving the modified protocol. Cost of tap water was PhP750.00/month (PhP450.00 for the lab and PhP300 for the screenhouse and nursery). Increased requirement for water was expected with longer incubation period of seedlings *in vitro*. Other items used in the laboratory such as LPG, plastics bags, rubber bands and detergents were estimated to cost PhP3,360/mo. This is more than double of the cost incurred when the modified protocol is used.

Cash inflows were computed from sale of the ECM seedlings using PhP500, 400 and 300 selling prices. Sale of Makapuno meat at PhP5.00/kg of split nuts (after the collection of the embryos) was also considered.

Annual cash flows of costs and benefits for the 10-year Makapuno production project involving four (4) month and seven (7) month passages period *in vitro* are presented in Annex Tables 1 and 2. Understandably, the investment analysis revealed higher values for the three (3) investment decision tools (NPV, BCR and IRR) when the shortened Makapuno embryo culture cycle was considered (Tables 13 and 14). Moreover, a shorter PP was achieved with use of the shortened ECM production scheme.

At a selling price of PhP500 per ECM seedling, an IRR of 102.51% was realized when the shortened protocol was used (Table 13). This value is about 64% higher than the estimated value for the project involving the currently used protocol. Using the current protocol in 2002, ECM production with a capacity of 6000 embryos resulted to an IRR of only 26.80% and BCR of 2.11 (Areza-Ubaldo *et al.*, 2003). Using the new production scheme, the project gave net benefits with present values of PhP16,052,586.74, PhP13,601,842.17, and PhP10,487,381.03 at 7%, 10% and 15% discount factors, respectively. Considering the BCR, every PhP1.00 that will be invested will return a benefit of 3.36, 3.22 and 3.01 at 7%, 10% and 15% discount factors. All these values were lower than their corresponding values

when the old protocol was used. The PP for the new protocol was estimated at 40 months which was approximately 16 months earlier than the PP of 56 months for the old protocol. This was much shorter than the 1999 estimate of 62 months (PSMSI, 1999).

With all these values of profitability measures, the new scheme clearly outruns the current ECM production protocol. Nevertheless, both schemes remain profitable even at a low ECM selling price of PhP300 per seedling.

Break-even analysis was done using both marginal (*i.e.*, only variable costs were considered) and full (*i.e.*, fixed and variable costs were considered) costing considering the same assumptions used in the investment analysis. Break-even analysis involving marginal costing would be useful for entrepreneurs who are already operating a tissue culture laboratory and would like to expand its operation on ECM production. On the other hand, full costing analysis would benefit those who do not have existing tissue culture laboratory facilities

With marginal costing, ECM production projects running for 10 years gave break even prices of PhP112.07, PhP138.90, PhP150.32, PhP175.49, PhP203.66, PhP228.98, and PhP255.77 for 4, 5 ,6, 7, 8, 9, and 10 month durations of *in vitro* incubations, respectively (Table 15). These measures will give existing plant tissue culture laboratory operators an idea of how much they will sell their ECM seedlings for if they decide to include Makapuno embryo culture in their endeavor. The break even prices increased as the duration of *in vitro* incubation is prolonged. An average of PhP23.94/seedling was lost for every 1 month delay in *ex vitro* establishment of seedlings. This is equivalent to a monthly loss of PhP14,364 considering the 600 seedlings produced per month.

With full costing, ECM production projects running for 10 years gave break-even prices of PhP134.96, PhP162.00, PhP173.63, PhP199.01, PhP227.41, PhP252.95, and PhP279.98 for 4, 5, 6, 7, 8, 9, and 10 month durations of *in vitro* incubations, respectively (Table 16). The break-even prices increased as the duration of *in vitro* incubation is prolonged. An average of PhP24.17/seedling was lost for every 1 month delay in *ex vitro* establishment of seedlings. This is equivalent to a monthly loss of PhP14,502 for the project considering the 600 seedlings produced per month. Using the current protocol, analysis of a 10 year ECM production project with a capacity of 6,000 embryos cultured per month resulted to a 2002 break-even price of PhP265.45 (Areza-Ubaldo *et al.*, 2003).

Understandably, full costing gave higher break-even prices than marginal costing because the costs of infrastructure and equipment were added in the full-cost analysis. Nevertheless, it is clear that Makapuno seedling production involving either full or marginal costing is highly profitable considering the current price of PhP500-1,000 per seedling.

Conclusion and Recommendations

With the improvement in the ambient environment for *ex vitro* establishment of *in vitro* grown seedlings it was found that seedlings with at least one (1) partially or fully exposed true leaf and one (1) primary root with or without lateral growths are very much ready for soil establishment. The use of 1:1 soil-cocopeat mixture as soil support medium together with a good headspace provided by communal humidity tent has assured higher *ex vitro* survival rate of *in vitro* seedlings even at an earlier age of three (3) month after initiation of embryos.

Supported by experimental results and analyses of data from previous studies, this paper recommends modifications on the existing Makapuno embryo culture protocol by shortening the *in vitro* culture duration to four (4) months from the earlier duration of seven (7) months. The modifications also include simplification of the *in vitro* cultural practice by doing away with the plastic bag extensions attached to the culture bottles during transferring since 4 month-old *in vitro* seedlings can still be accommodated in long test tubes. Likewise, *ex vitro* establishment procedure is modified by using non-sterilized 1:1 soil-cocopeat mixture as soil support medium and by

Table 14. NPV, BCR, IRR and PP of a 10-year Makapuno production project involving 7 months *in vitro* incubation at different DF and selling prices of seedlings

SELLING PRICE OF ECM (*PhP*)	PARAMETER	7% DF	10% DF	15% DF
500	Present Value	12,242,698.83	10,143,693.92	7,494,096.73
	BCR	2.25	2.16	2.00
	IRR (%)	62.67		
	Payback Period (*yr*)	4.69		
400	Present Value	8,577,365.72	6,988,211.65	4,984,366.42
	BCR	1.94	1.85	1.71
	IRR (%)	48.60		
	Payback Period (*yr*)	5.58		
300	Present Value	4,245,690.53	3,268,255.57	2,042,308.59
	BCR	1.46	1.40	1.29
	IRR (%)	30.63		
	Payback Period (*yr*)	7.05		

Table 15. Break-even price analysis for a 10-Year Marginal Cost ECM Production using 4-10 month passage *in vitro*

ITEM	COST AT VARIOUS PASSAGE PERIODS *IN VITRO* (*PhP*)						
	4 month	5 month	6 month	7 month	8 month	9 month	10 month
INPUT							
Labwares	229,628.00	253,085.00	276,543.00	300,000.00	323,457.00	339,096.00	370,372.00
Salaries and wages	3,979,300.00	4,907,500.00	4,921,500.00	5,832,900.00	6,768,900.00	7,638,900.00	8,556,900.00
Lab Chemicals	252,346.00	501,058.00	751,282.00	979,824.00	1,238,492.00	1,476,438.00	1,718,718.00
Makapuno nuts	2,220,000.00	2,200,000.00	2,180,000.00	2,160,000.00	2,140,000.00	2,120,000.00	2,100,000.00
Distilled water	231,768.00	506,880.00	776,952.00	1,041,984.00	1,301,976.00	1,556,928.00	1,806,840.00
Electricity	359,291.52	517,614.72	563,579.52	609,544.32	767,867.52	813,832.32	859,797.12
Tap water	68,400.00	73,400.00	78,300.00	83,100.00	87,800.00	92,400.00	96,900.00
Maintenance of seedlings in the screenhouse and nursery	292,320.00	289,800.00	287,280.00	284,760.00	282,240.00	279,720.00	277,200.00
Other consumables	163,170.00	231,000.00	297,570.00	362,880.00	426,930.00	489,720.00	551,250.00
Total Input	**7,796,223.52**	**9,480,337.72**	**10,133,006.52**	**11,654,992.32**	**13,337,662.52**	**14,807,034.32**	**16,337,977.12**
OUTPUT							
No of ECM produced	66,000	65,400	64,800	64,200	63,600	63,000	62,400
Sale from Makapuno meat	399,600.00	396,000.00	392,400.00	388,800.00	385,200.00	381,600.00	378,000.00
BREAK-EVEN PRICE PER SEEDLING (*PhP*)	**112.07**	**138.90**	**150.32**	**175.49**	**203.66**	**228.98**	**255.77**

Table 16. Break-even price analysis for a 10-Year Full Cost ECM Production using 4-10 month passage *in vitro*

ITEM	COST AT VARIOUS PASSAGE PERIODS *IN VITRO (PhP)*						
	4 month	**5 month**	**6 month**	**7 month**	**8 month**	**9 month**	**10 month**
INPUT							
Laboratory, screenhouse and nursery	720,000.00	720,000.00	720,000.00	720,000.00	720,000.00	720,000.00	720,000.00
Laboratory equipment	490,500.00	490,500.00	490,500.00	490,500.00	490,500.00	490,500.00	490,500.00
Land Rental (500 sqm)	300,000.00	300,000.00	300,000.00	300,000.00	300,000.00	300,000.00	300,000.00
Labwares	229,628.00	253,085.00	276,543.00	300,000.00	323,457.00	339,096.00	370,372.00
Salaries and wages	3,979,300.00	4,907,500.00	4,921,500.00	5,832,900.00	6,768,900.00	7,638,900.00	8,556,900.00
Lab Chemicals	252,346.00	501,058.00	751,282.00	979,824.00	1,238,492.00	1,476,438.00	1,718,718.00
Makapuno nuts	2,220,000.00	2,200,000.00	2,180,000.00	2,160,000.00	2,140,000.00	2,120,000.00	2,100,000.00
Distilled water	231,768.00	506,880.00	776,952.00	1,041,984.00	1,301,976.00	1,556,928.00	1,806,840.00
Electricity	359,291.52	517,614.72	563,579.52	609,544.32	767,867.52	813,832.32	859,797.12
Tap water	68,400.00	73,400.00	78,300.00	83,100.00	87,800.00	92,400.00	96,900.00
Maintenance of seedlings *ex vitro*	292,320.00	289,800.00	287,280.00	284,760.00	282,240.00	279,720.00	277,200.00
Other consumables	163,170.00	231,000.00	297,570.00	362,880.00	426,930.00	489,720.00	551,250.00
Total Input	**9,306,723.52**	**10,990,837.72**	**11,643,506.52**	**13,165,492.32**	**14,848,162.52**	**16,317,534.32**	**17,848,477.12**
OUTPUT							
No of ECM produced	66,000	65,400	64,800	64,200	63,600	63,000	62,400
Sale from Makapuno meat	399,600.00	396,000.00	392,400.00	388,800.00	385,200.00	381,600.00	378,000.00
BREAK-EVEN PRICE PER SEEDLING (*PhP*)	**134.96**	**162.00**	**173.63**	**199.01**	**227.41**	**252.95**	**279.98**

using a communal humidity chamber instead of the individual plastic bag covers to maintain high relative humidity for the establishing seedlings. With the shortening and simplification of the *in vitro* cultural practice as well as modification of the *ex vitro* establishment procedure, ECM production has been made faster and cheaper.

Investment analysis of a 10-year production project revealed a high IRR of 102.51% for the new production scheme when the produced seedlings are sold at PCA-recommended price of PhP500.00 per seedling. This is about 64% higher than the value for the old production scheme. Using the new production scheme, the project has a BCR of 2.16 corresponding to a net present value of PhP10.14M using 10% discount factor.

Using marginal and full costing, comparative break-even prices of ECM seedling for various production schemes involving 4-10 month culture durations *in vitro* showed the profitability of the ECM seedling production business, considering the PhP500-1,000 current price of ECM seedlings. The computed break-even prices will also be a good decision tool for ECM seedling producers. High selling cost would mean more profit. On the other hand, lower but profitable cost would attract more buyers resulting to increase in demand and therefore, massive planting of ECM in the country. This will eventually pave the way for the development of the Makapuno Industry in the Philippines.

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Annex Table 1. Investment analysis of a full cost 10-Year ECM Production using the proposed 4 month passage *in vitro*

	Yr 0	Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	Yr 6	Yr 7	Yr 8	Yr 9	Yr 10	TOTAL
INPUT												
Laboratory, screenhouse and nursery	720,000.00											720,000.00
Laboratory equipment	490,500.00											490,500.00
Cost of Land rental (500 sqm)	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	0.00	300,000.00
Labwares	229,628.00											229,628.00
Salaries and wages		276,000.00	314,600.00	343,200.00	371,800.00	400,400.00	429,000.00	457,600.00	486,200.00	514,800.00	385,700.00	3,979,300.00
Lab Chemicals	63,170.00	27,158.00	22,248.00	23,192.00	22,152.00	29,318.00	16,122.00	29,318.00	19,668.00	0.00	0.00	252,346.00
Makapuno nuts		240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	60,000.00	2,220,000.00
Distilled water		21,627.00	25,056.00	25,056.00	25,056.00	25,056.00	25,056.00	25,056.00	25,056.00	25,056.00	9,693.00	231,768.00
Electricity		37,820.16	37,820.16	37,820.16	37,820.16	37,820.16	37,820.16	37,820.16	37,820.16	37,820.16	18,910.08	359,291.52
Tap water		5,700.00	7,200.00	7,200.00	7,200.00	7,200.00	7,200.00	7,200.00	7,200.00	7,200.00	5,100.00	68,400.00
Maintenance of seedlings *ex vitro*		20,160.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	292,320.00
Other consumables		17,640.00	17,640.00	17,640.00	17,640.00	17,640.00	17,640.00	17,640.00	17,640.00	17,640.00	4,410.00	163,170.00
Total Input	**1,374,298.00**	**676,105.16**	**724,804.16**	**754,348.16**	**781,908.16**	**817,674.16**	**833,078.16**	**874,874.16**	**893,824.16**	**902,756.16**	**514,053.08**	**9,306,723.52**
OUTPUT												
Sale from ECM (PhP 500)		600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	33,000,000.00
Sale from ECM (PhP 400)		480,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	26,400,000.00
Sale from ECM (PhP 300)		360,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	19,800,000.00
Sale from Makapuno meat		43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	10,800.00	399,600.00
Residual value (equip & labwares, 25%)											180,032.00	180,032.00
Total Output (PhP 500)		643,200.00	3,643,200.00	3,643,200.00	3,643,200.00	3,643,200.00	3,643,200.00	3,643,200.00	3,643,200.00	3,643,200.00	3,790,832.00	33,579,632.00
Total Output (PhP 400)		523,200.00	2,923,200.00	2,923,200.00	2,923,200.00	2,923,200.00	2,923,200.00	2,923,200.00	2,923,200.00	2,923,200.00	3,070,832.00	26,979,632.00
Total Output (PhP 300)		403,200.00	2,203,200.00	2,203,200.00	2,203,200.00	2,203,200.00	2,203,200.00	2,203,200.00	2,203,200.00	2,203,200.00	2,350,832.00	20,379,632.00

Annex Table 2. Investment analysis of a full cost 10-Year ECM Production using *in vitro* incubation period

	Yr 0	Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	Yr 6	Yr 7	Yr 8	Yr 9	Yr 10	TOTAL
INPUT												
Laboratory, screenhouse and nursery	720,000.00											720,000.00
Laboratory equipment	490,500.00											490,500.00
Cost of Land rental (500 sqm)	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	30,000.00	0.00	300,000.00
Labwares	300,000.00											300,000.00
Salaries and wages		358,000.00	486,200.00	530,400.00	574,600.00	618,800.00	663,000.00	707,200.00	751,400.00	795,600.00	347,700.00	5,832,900.00
Lab Chemicals	153,798.00	110,556.00	94,008.00	112,602.00	110,556.00	90,348.00	110,556.00	102,924.00	94,476.00	0.00		979,824.00
Makapuno nuts		240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	240,000.00	0.00	2,160,000.00
Distilled water		74,547.00	115,776.00	115,776.00	115,776.00	115,776.00	115,776.00	115,776.00	115,776.00	115,776.00	41,229.00	1,041,984.00
Electricity		64,162.56	64,162.56	64,162.56	64,162.56	64,162.56	64,162.56	64,162.56	64,162.56	64,162.56	32,081.28	609,544.32
Tap water		5,850.00	9,000.00	9,000.00	9,000.00	9,000.00	9,000.00	9,000.00	9,000.00	9,000.00	5,250.00	83,100.00
Maintenance of seedlings *ex vitro*		12,600.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	30,240.00	284,760.00
Other consumables		40,320.00	40,320.00	40,320.00	40,320.00	40,320.00	40,320.00	40,320.00	40,320.00	40,320.00	0.00	362,880.00
Total Input	**1,730,798.00**	**936,035.56**	**1,109,706.56**	**1,172,500.56**	**1,214,654.56**	**1,238,646.56**	**1,303,054.56**	**1,339,622.56**	**1,375,374.56**	**1,325,098.56**	**456,500.28**	**13,165,492.32**
OUTPUT			6,600	7,200	7,200	7,200	7,200	7,200	7,200	7,200	7,200	64,200
Sale from ECM (PhP 500)		0.00	3,300,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	3,600,000.00	32,100,000.00
Sale from ECM (PhP 400)		0.00	2,640,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	2,880,000.00	25,680,000.00
Sale from ECM (PhP 300)		0.00	1,980,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	2,160,000.00	19,260,000.00
Sale from Makapuno meat		43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	43,200.00	0.00	388,800.00
Residual value (equip & labwares, (25%)											197,625.00	197,625.00
Total Output (PhP 500)		**43,200.00**	**3,343,200.00**	**3,643,200.00**	**3,643,200.00**	**3,643,200.00**	**3,643,200.00**	**3,643,200.00**	**3,643,200.00**	**3,643,200.00**	**3,797,625.00**	**32,686,425.00**
Total Output (PhP 400)		**43,200.00**	**2,683,200.00**	**2,923,200.00**	**2,923,200.00**	**2,923,200.00**	**2,923,200.00**	**2,923,200.00**	**2,923,200.00**	**2,923,200.00**	**3,077,625.00**	**26,266,425.00**
Total Output (PhP 300)		**43,200.00**	**2,023,200.00**	**2,203,200.00**	**2,203,200.00**	**2,203,200.00**	**2,203,200.00**	**2,203,200.00**	**2,203,200.00**	**2,203,200.00**	**2,357,625.00**	**19,846,425.00**