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Development of Low-cost and Rapid Multiplication Techniques of Tissue-cultured *Musa acuminate* (AA Group) cv. 'Lacatan' Banana Seedlings

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Abstract

In-vitro multiplication and nursery management techniques were developed for the production of *Musa acuminata* (AA Group) cv. 'Lacatan' banana seedlings at the Mariano Marcos State University from 2005 to 2007.

In-vitro is focused on formulating culture media using low-cost materials as substitute to chemical components, such as Murashige and Skoog (MS) with the addition of 5ppm Benzyl Amino Purine (BAP) 2% sucrose and 4.5g Biolife agar. Substituting the chemical formulation led to comparable results in terms of the growth of plantlets as well as the quality and quantity of plantlets.

The developed culture media was used for shoot proliferation and root induction of *in-vitro* banana. The cost of one shoot/meriplant was P0.02 using the developed media, compared to the P0.20 using the chemical formulation. Likewise, the costs of rooting one plantlet were P0.64 and P0.26 for the existing and for the developed media, respectively. Hence, the total cost of producing one plantlet (from shoot proliferation to root induction) was P0.28 via the developed media, while that of using the existing media was P0.84.

Some nursery management techniques that were established include the following; pot out tissue-cultured banana plantlets in a soil mixture of 2:1 ratio of carbonized rice hull and ordinary garden soil to obtain more vigorous plantlets; pot out 3 to 10cm tall plantlets with bottle covering for seven days; expose the plantlets under the sun with partial shade for three days to obtain a higher plantlet survival.

Another technique for better seedling performance was the application of urea. One tablespoonful was drenched daily to one-month old seedlings. This technique increased their height at four weeks after application (56.73cm). Smaller plants were obtained when urea solution of 2-3tbsp/16 liter of water was drenched every other day (51.49cm) and once a week (43.20cm). The establishment *of in-vitro* techniques and nursery management for the production of cv. *Lacatan* banana seedlings provided some benefits both to the researchers and the farmer-clientele.

Keywords: *banana, in vitro, shoot proliferation, root induction, nursery management techniques*

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Introduction

In Ilocos Region, *Musa acuminata* (AA Group) cv. *Lacatan* banana is being transported and traded from Cagayan Valley provinces. Farmers in the Ilocos plant banana varieties like Cavendish, Bungulan, Matabia (native variety), and *Cardava* but not *Lacatan*. However, the most preferred and used as desert at home or in parties is *Lacatan.*

Banana is still the leading fruit crop in terms of area, volume, and value of production. The national average yield is 9.4t/ha while corporate plantations produce 40t/ha. Banana industry can be a source of livelihood of farmers and it can flourish like any other agricultural industry. However, it can be devastated by the outbreak of the Banana Bunchy Top Virus (BBTV) disease, and consequently, farmers income can be greatly affected. The virus causes stunting and significantly reduces yield, as such bananas infected at the early growth stages do not bear fruit (Aquino *et al*, 1999). In addition, securing planting materials from infected mother plants intensifies the spread of the disease.

Tissue culture production of plantlets is recommended to solve the problem. Tissue culture is now a standard practice in banana propagation (Shiragi *et al*, 2008). Recently, molecular genetic engineering techniques have become available and new approaches have been suggested to control banana virus diseases. Among which is meristem culture that offers an efficient method in producing virus-free materials and germplasm in plants (Cronauer and Krikorian, 1984; Hwang, *et al*, 1994, and Helloit *et al*, 2002). At MMSU and in other institutions, *in-vitro* propagation techniques for banana have already been established using the Murashige and Skoog's (MS) media formulation with the addition of 5 ppm Benzyl Amino Purine (BAP), 2% sucrose and 4.5g distilled water to volume one liter of culture media. The effectiveness of BAP over other cytokinins in inducing multiplication has been reported in different banana cultivars (Buah *et al*, 2010). Substitution of chemical formulation was done to obtain the same quality and quantity of plantlets that incur lower production cost. In addition, nursery management techniques for *Lacatan* banana, specifically under Ilocos conditions, were undertaken; hence, this study was conducted.

Methodology

Experiments were undertaken both under *in-vitro* and nursery conditions to produce banana seedlings. Cheaper sources of the different components of the culture media formulation were used for *in-vitro* shoot proliferation and root induction. Moreover, different nursery techniques such as using potting media, applying fertilizer, and using bottle covering were evaluated.

The experiment was laid out using Completely Randomized Design (CRD) with three replications. Culture media were incubated in a room with 20°C to 25°C for a 16-hour photoperiod. Healthy *Lacatan* plants were selected and grown to produce maiden suckers, which served as initial explants. Data were analyzed using SPSS 14.0 version and the mean differences were contrasted using Duncan's multiple range tests at 0.05 level of significance.

Shoot Proliferation and Root Induction of cv. *Lacatan* Banana Under Aseptic Conditions

Levels of cytokinin for shoot proliferation. Using the MS media formulation, various levels of Benzylamino purine as cytokinin were added:

T1 – MS + 1.0 mg/L BAP
T2 – MS + 2.0 mg/L BAP
T3 – MS + 3.0 mg/L BAP
T4 – MS + 4.0 mg/L BAP
T5 – MS + 5.0 mg/L BAP (check)

The different culture media were monitored regularly and data were gathered at 28 days after initiation. Data gathered include the following: number of days to shoot formation, number of shoots formed, length of shoots, number of leaves, number of roots, and percent of shoot/root formed.

Use of water sources. Three water sources -- distilled, purified, and tap water -- were used in preparing the media for shoot proliferation and root induction. Data gathered on the former were number of shoots produced and percent contamination. On the other hand, those on the latter were percent contamination, plant height, number of leaves, laminar width and length, and number of roots.

Use of carbon sources. Two carbon sources were used -- refined white sugar and analytical sucrose. For each treatment, ten cultures were tested. Data gathered were on the number and length of shoots produced.

Agar sources. Four agar sources were used, namely: biolife agar, commercial agar powder, white gulaman bar, and local seaweeds agar. Two trials were conducted but both followed the same methodology. Cultures were monitored regularly and data on the number of shoots produced and length of shoots were gathered one month after inoculation.

Nursery Management Techniques for Tissue-cultured *Lacatan* Banana Seedlings

Potting media requirement. Tissue-cultured *Lacatan* banana plantlets were potted out in different proportions of carbonized rice hull and ordinary garden soil (1:1, 2:1, 3:1 and 4:1 ratios). Plant height was gathered at 1, 2, 3, 4 and 8 weeks after potting.

Frequency of applying foliar fertilizer. One-month-old seedlings of *Lacatan* banana were applied with foliar fertilizer (Green Bee). Weekly recommended rate of application (5 tbsp/16 li) was drenched once or twice a week. However, no foliar fertilizer was applied to the control plants. Plant height was gathered 1, 2, 3, and 4 week/s after applying the treatments.

Effect of urea concentrations and frequency of application. Different 16-0-0 (NPK) concentrations of 1, 2, and 3tbsp per 16-liter water were applied to one-month-old *Lacatan* banana seedlings in different frequencies - daily, every other day, and once a week. Plant height was gathered 1, 2, 3 and 4 week/s after applying the treatments.

Use of bottle covering in newly-potted out banana plantlets. Wide-mouthed bottles were used to cover the newly-potted plantlets. Five sample plants were used in each treatment. Plantlets that were tall (7-10cm) and small plantlets (3-6 cm) were potted out and treated with bottle covering and without covering. The duration of the covering was also observed (7, 10, and 15 days) on tall and small plantlets. Newly-potted banana plantlets were placed in a totally shaded area. Prior to exposing the plantlets under the sun (25% shade), the number of days to cover removal was also observed such as 3, 6, 9, and 12 days.

Results and Discussion

With all the efforts done to produce low-cost tissue-cultured *Lacatan* banana seedlings, i*n-vitro* multiplication and nursery management techniques were developed for its production.

Generally, the performance of *in-vitro Lacatan* banana inoculated in existing media formulation (containing expensive component sources) had no significant effect compared to those inoculated in cheaper sources (MMSU media formulation).

Cheaper Media Components for *In-vitro* Banana cv. Lacatan

Levels of cytokinin for shoot proliferation. The agronomic characteristics produced by *Lacatan* banana grown *in vitro* (Table 1) indicate that there were significantly more shoots (2.41) produced in meriplants inoculated in culture media

Table 1. Number of shoots, shoot length, number of leaves and number of roots produced by *Lacatan* banana grown *in vitro* as affected by different levels of cytokinin (BAP).

BAP LEVEL (mg/L)	NUMBER OF SHOOTS	SHOOT LENGTH (cm)	NUMBER OF LEAVES	NUMBER OF ROOTS
1.0	1.45b	1.92	2.94	2.06a
2.0	1.93b	1.86	2.76	0.57b
3.0	1.74b	1.63	2.98	0.49b
4.0	2.41a	1.40	2.73	0.24bc
5.0	1.84b	1.50	2.47	0.10c
Level of Significance	**	ns	ns	**
CV (%)	11.96	13.47	11.93	25.10

ns - not significant
** - significant at 1% level
cv - coefficient of variation
Means marked with the same letter in a column are not significantly different using Duncan's Multiple Range Test (DMRT).

added with 4ppm Benzylaminopurine (BAP) compared to those inoculated in culture media with 1, 2, 3, and 5ppm BAP. An increasing trend in the number of shoots was observed with increasing level of BAP in the culture medium from 1-4ppm. However, at 5ppm BAP, there was a decrease in the number of shoots produced. This implies that an optimum BAP level is reached at 4ppm and any further increase is not beneficial.

In terms of shoot length and number of leaves, no significant differences among the treatments were observed (ranging from 1.40 to 1.92cm and 2.47 to 2.98 leaves, respectively). This finding suggests that the different BAP levels used had similar effects on the growth of shoots and number of leaves of banana *in vitro*. However, results on the number of roots showed significant differences among the treatments used. A decreasing trend was observed wherein the roots became smaller as the BAP level was increased. Root formation was inhibited when higher BAP level was used, showing its basic use - shoot proliferation. Generally, cytokinins and auxins are used for the multiplication of banana shoots and rooting (Rahman *et al*, 2002). The results of the study concur with the findings of Ali (1996), who reported that higher shoot proliferation in banana on MS media was reinforced by BAP.

Water sources

Shoot proliferation. Table 2 shows that banana culture inoculated in a media for shoot proliferation using distilled, purified and tap water did not have significant differences in terms of percent contamination at 2WAI (0.00, 1.33, and 3.58%

Table 2. Percent contamination and number of shoots produced from *Lacatan* banana as affected by different water sources used in media preparation.

WATER SOURCES	PERCENT CONTAMINATION		NUMBER OF SHOOTS	
	2 WAI	6 WAI	3 WAI	4 WAI
Distilled water	0.00	4.44b	1.90	6.17a
Purified water	1.33	2.22b	1.77	4.43b
Tap water	3.58	30.56a	1.87	4.53b
Level of significance	ns	*	ns	*
CV (%)	28.0	39.0	20.7	13.7

ns - not significant
** - significant at 1% level
CV- coefficient of variation
Means marked with the same letter in a column are not significantly different using DMRT.

respectively) and number of shoots at 3WAI (1.90, 1.77, and 1.87%, respectively). However, at 6 WAI, significantly higher contamination was observed in culture media with tap water. According to Gitonga *et al (*2010), the use of alternative sources of water such as rain or tap water can help reduce the propagation cost of local banana.

On the other hand, the number of shoots produced was significantly higher in banana cultures inoculated in media using distilled water (6.17) compared with those that used purified water and tap water (4.43 and 4.53, respectively).

Root *induction.* As shown in Table 3, tissue-cultured banana inoculated for root induction using distilled, purified, and tap water added to MS media formulation did not have significant differences in percent contamination at 2WAI (2.22, 4.45, and 6.67%, respectively) but significant differences at 6WAI (4.47, 6.67, and 33.33%, respectively). All the other parameters gathered such as the number of shoots produced, plant height, number of leaves, laminar width, and length and number of roots did not differ significantly. The results derived by Gitonga *et al* (2010) manifest that different sources of water used such as tap, rain, and distilled did not affect growth of meriplants. The same authors noted that water is one of the major components used in preparing the culture media; however, distilled water is expensive in developing countries. Thus, the use of alternative sources of water such as rain or tap water can help reduce the propagation cost of local banana.

Carbon sources. Table 4 shows the comparison between sucrose with refined white sugar on the number and length of shoots of banana plantlets. It can be observed that substituting sucrose with refined white sugar did not have a negative effect on the

Table 3. Percent contamination, number of shoots produced, plant height, number of leaves, laminar width, length and number of roots of *Lacatan* banana as affected by different water sources used in media preparation.

WATER SOURCE	%		PLANT HEIGHT (cm)	NO. OF LEAVES	LAMINAR WIDTH (cm)	NO. OF ROOTS (cm)	LENGTH OF ROOTS (cm)
	2WAI	6WAI					
Distilled water	2.22	4.47b	8.80	3.52	1.23	6.49	5.09
Purified water	4.45	6.67b	8.76	3.27	1.27	6.84	4.98
Tap water	6.67	33.33a	9.61	3.09	1.10	6.83	7.36
CV (%)	19.0	16.0	8.7	16.3	6.1	18.6	13.7
Level of Significance	ns	*	ns	ns	ns	ns	ns

ns - not significant
** - significant at 1% level
CV - coefficient of variation
Means marked with the same letter in a column are not significantly different using DMRT.

Table 4. The number and length of shoots (mm) produced one month after inoculation of *Lakatan* banana *in vitro* as affected by carbon source.

TREATMENT	NO. OF SHOOTS	LENGTH OF SHOOTS (mm)
Sucrose	3.1	14.4
Refined white sugar	3.5	17.7
Level of significance	ns	ns
CV (%)	0.20	0.08

ns – not significant

number of shoots produced (3.1 and 3.5), as well as shoot length produced by the explants one month after inoculation. The use of market sugar instead of sucrose has been reported to reduce the cost of *in-vitro* conservation of banana, with no significant effect on regeneration compared to sucrose (Agrawal *et al*, 2010). Sugar was used to reduce the overall cost of micropropagating and testing the response of local banana. This finding greatly lowers the cost of producing tissue-cultured banana seedlings since one kilo of sucrose costs P700.00 while one kilo of refined white sugar costs only P38.00.

Agar sources. Agar sources used for shoot proliferation (Table 5) of banana *in-vitro* such as biolife agar, commercial agar powder, and white gulaman bar had no significant differences in terms of the number of shoots produced from both the first

Table 5. Growth performance of *in vitro Lacatan* banana inoculated in MS media formulation for shoot proliferation with different agar sources .

AGAR SOURCE	TRIAL I		TRIAL II	
	No. of Shoots	Length of Shoots (cm)	No. of Shoots	Length of Shoots (cm)
Biolife agar	3.27a	3.13ab	2.55	1.80a
Commercial agar powder	2.23a	1.57b	3.60	2.00a
White gulaman bar	2.40a	4.07a	2.80	1.48ab
Seaweeds agar (local)	0.50b	0.43c	2.43	0.88b
Level of significance	*	**	ns	*
CV (%)	23.41	17.34	45.6	32.2

ns - not significant
** - significant at 1% level
cv - coefficient of variation
Means marked with the same letter in a column are not significantly different using DMRT.

(ranging from 2.23 to 3.27 shoots) and the second trial (ranging from 2.55 to 3.60 shoots). However, the meriplants inoculated in media with local seaweeds agar had the least number of shoots (0.50) in the first trial.

In the first trial, longer shoots were observed in meriplants inoculated in media with white gulaman bar (4.07cm) but they were not significant when compared with those in media with biolife agar (3.13cm). The shortest shoots were observed in those inoculated in media with seaweeds local agar. In the second trial, however, the longest shoots were observed in meriplants inoculated with commercial agar powder (2.00cm) but were comparable with those inoculated with biolife agar and white gulaman bar. Based on the results, the expensive agar source (Biolife agar) can be substituted with more affordable source of either commercial agar powder or white gulaman bar. These are also available in local market outlets. The above results coincide with the data derived by Gitonga *et al*, 2010. As added by (Bhattacharya *et al*, 1994), water potential is decreased in gelling agents such as gulaman bar compared to support matrices, which limit nutrient uptake.

Comparison between the existing and the developed media formulations. Table 6 shows the comparison between the existing media formulation (expensive media) and the MMSU formulation for shoot proliferation. No significant differences were observed in all the data gathered. This implies that other media components can be substituted by a cheaper source without any negative effect on the growth of *in-vitro Lacatan* plantlets. The over-all results of the study was almost the same as those derived by Gitonga *et al* (2010). This suggests that it is possible to develop a low-cost tissue culture protocol that produces banana plantlets within short periods.

Cost *of producing one shoot/meriplant in-vitro.* As shown in Table 7, cost of producing one plantlet using the existing media is P0.20, while that of using the developed media is P0.02. The lower cost of the proposed media for shoot proliferation of *in vitro Lakatan* banana concurs with the findings of Gitonga *et al* (2010), which consequently lessened the production cost of the plantlets.

Table 6. Growth performance of *in-vitro* cv. *Lacatan* banana meriplants inoculated in different media formulation for shoot proliferation.

GROWTH CHARACTERISTIC	MEDIA FORMULATION	
	Existing Media	Developed Media
No. of shoots 1MAI	2.73	3.60
Length of shoots 1MAI	1.40	1.63
Length of shoots 1.5MAI	1.50	1.73
No. of roots 1.5MAI	0.03	0.07
Length of roots 1.5MAI	0.03	0.07
Level of Significance	ns	ns
CV (%)	0.35	0.36

MAI- month after inoculation
ns - not significant
CV - coefficient of variation

Table 7. Partial cost of existing and developed low-cost media for shoot proliferation of *in-vitro* cv. *Lacatan* banana.

ITEM	COST (₱) (Per liter media preparation)	
	Existing Media	Developed Media
Benzyl amino purine, 5 ppm	1.90	-
Benzyl amino purine, 4 ppm	-	1.52
Distilled water	7.37	-
Purified water	-	1.56
Sucrose (2%)	16.87	
Refined white sugar(2%)	-	0.76
Biolife agar	18.00	-
Commercial agar powder	-	0.85
Total Cost	44.14	4.69
No. of shoots/meriplant per liter (80 culture bottles)	80x2.73 shoots/ bottle=218	80x3.60 shoots/ bottle=288
Cost per plantlet	P0.20	P0.02

Cost of producing *one rooted plantlet in-vitro*. Table 8 shows the partial cost of the existing and the developed low-cost media for shoot proliferation of *in-vitro Lacatan* banana. The cost of producing one plantlet using the existing media was P0.84 while that of using the developed media was P0.28. Conventional plant tissue culture has been applied for decades, however, the high cost of tissue production is a drawback for laboratories with limited resources, especially in the developing countries. In fact, the cost of the micropropagules production precludes the adoption of the technology for large - scale micropropagation (Gitonga *et al*, 2010).

Nursery management technology for tissue-cultured banana seedlings. Management techniques in producing low-cost banana seedlings under nursery conditions were developed by using different soil potting mixtures, applying foliar fertilizer, and applying NPK fertilizer based on the recommended rate and frequency.

Table 8. Partial cost of existing and developed low-cost media for root induction of *in-vitro* cv. *Lacatan* banana.

ITEM	COST (P) (Per liter media preparation)	
	Existing Media	**Developed Media**
Distilled water	7.37	-
Purified water	-	1.56
Sucrose (3%)	25.30	
Refined white sugar(3%)	-	1.14
Agar	18.00	18.00
Total Cost	50.67	20.70
No. of plantlets per liter (80 culture bottles)	80 plantlets	80 plantlets
Cost per rooted plantlet	P0.64	P0.26
Total cost of producing one plantlet	P0.20 + P0.64 = P0.84	P0.02 + P0.26 = P0.28

Prices:

a. Benzyl amino purine = P9490.50 per 25 grams
   g = P37.96 needed to prepare 100ppm stock solution
   (use 40 and 50ml li $^{-1}$)
b. Distilled water (16 li) = P118.00
c. Purified (16 li) = P25.00
d. Sucrose (2 kg) = P1687.00
e. Refined white sugar (1 kg) = P38.00
f. Biolife agar (500g) = P2000.00
g. Commercial agar powder (500g) = P85.00

Potting media requirement. The growth performance of cv. *Lacatan* seedlings potted out in different proportions of carbonized rice hull and ordinary garden soil had no significant differences (Table 9). This was noted primarily on the rate of growth at one to three weeks after potting (WAP). However, at 4 WAP, faster growth was observed in seedlings planted in 2:1 ratio of CRH and OGS (13.26cm) but comparable with 1:1 ratio (12.18cm). That was followed by the 3:1 ratio (9.44cm), which is comparable with 4:1 ratio (8.93cm). At 8 WAP, seedlings planted in 2:1 ratio were significantly taller (23.92cm) than those in the other proportions (20.97, 17.62, and 17.47cm, respectively). It was noted that in the 1:1 ratio, the growth of roots was inhibited, which could have increased seedling height. On the other hand, the formulation of higher amount of CRH in the media had negative effects on growth. Due to the low water retention and the very light media, the seedlings had the tendency to bend when they were about 6 to 8 WAP. It was found by Rai *et al* (2012) that the mixture contained subsoil land, carbonized rice hull, residue of tea decomposed in proportion had greater plantlet survival.

Use of bottle covering in newly-potted out banana plantlets. High survival of tissue-cultured cv. *Lacatan* banana plantlets potted out and covered with wide-mouthed bottles was observed (Table 10). Results revealed that placing bottles as covering to newly-potted out banana plantlets, tall or medium-sized, had higher survival rate. The taller plantlets (7-10cm) had 100% survival and those smaller (3-6cm) had 86.67%. On the other hand, plantlets, which were potted out without cover had lower survival on tall

Table 9. Plant height of tissue-cultured cv. *Lacatan* banana potted-out seedlings.

POTTING MEDIA RATIO BRH : OGH	PLANT HEIGHT (cm)				
	1WAP	2WAP	3WAP	4WAP	8WAP
1:1	5.92	6.14	7.87	12.18a	20.97b
2:1	5.96	6.37	8.52	13.26a	23.92a
3:1	5.93	5.97	6.98	9.44b	17.62c
4:1	6.05	6.37	7.04	8.93b	17.47c
Level of Significance	ns	ns	ns	**	**
CV (%)	9.9	10.7	11.2	8.0	7.1

ns - not significant
** - significant at 1% level
CV - coefficient of variation
WAP - weeks after potting out
Means marked with the same letter in a column are not significantly different using DMRT.

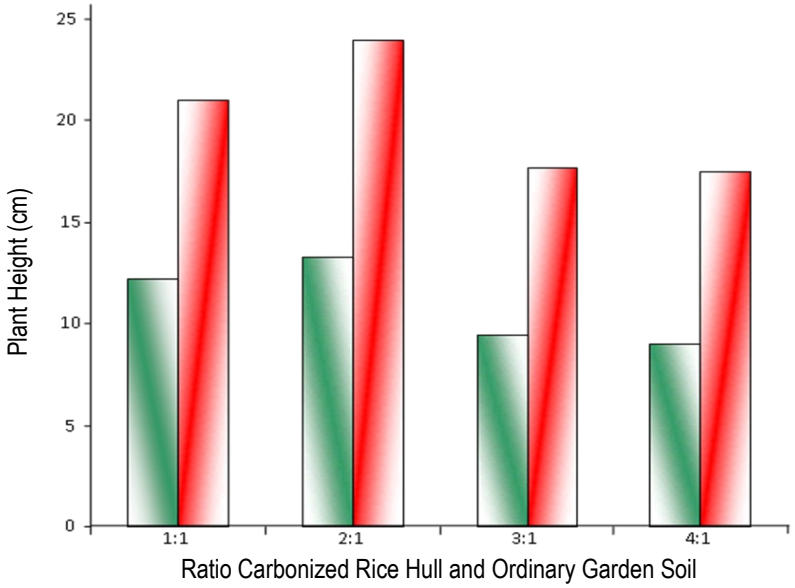

Fig. 2. Plant height of cv. *Lacatan* banana seedlings potted out in different proportions of carbonized rice hull and ordinary garden soil at 4 and 8 weeks after potting out.

Table 10. Survival of tissue-cultured cv. *Lacatan* banana plantlets potted out with covers.

TREATMENT	PERCENTAGE SURVIVAL
***Use of Cover*[1]**	**
With cover	
Tall plantlets (7-10 cm)	100.00a
Small plantlets (3-6 cm)	86.67b
Without cover	
Tall plantlets (7-10 cm)	53.33a
Small plantlets (3-6 cm)	40.00b
***Duration of cover*[2]**	ns
7 days covered, tall	100.00
7 days covered, small	80.00
10 days covered, tall	100.00
10 days covered, small	100.00
15 days covered, tall	100.00
15 days covered, small	80.00
***Days to placing under 25% shade*[3]**	ns
3 DTU *	100.00
6 DTU	100.00
9 DTU	100.00
12 DTU	100.00

* - days to uncovering ns - not significant
** - significant at 1% level cv - coefficient of variation
Means marked with the same letter in a column are not significantly different using DMRT.
[1]CV (%) = 11.7, [2] CV (%)= 2.3, [3] CV (%)= 1.1

or small-sized plantlets (53.33% and 40.00%, respectively). In addition, the survival of plantlets ranged from 80-100% when the plantlets were covered for 7, 10, and 15 days using tall and small-sized plantlets. Covering the plantlets for seven days wass sufficient to grow healthy plantlets and to shorten the growth duration of the plantlets. Placing the plantlets under a 75% sunlight (25% shade) secured a 100% survival. This suggests that banana plantlets can be grown under a 25% shade at 3, 6, 9, and 12 days before removing their covers.

Hence, uncovering the plantlets for three days then placing them under 25% shade would result in high seedling survival.

Frequency of applying foliar fertilizer. Table 11 shows that frequency in applying foliar fertilizer had significant effects on the growth of tissue-cultured cv. *Lacatan* banana seedlings at 1, 3, and 4 weeks after treatment (WAT). At 1 WAT, seedlings applied with Green Bee weekly were the tallest (17.23cm) but they were comparable with those applied biweekly (16.59cm). At 3 WAT, tallest seedlings were those applied with Green Bee biweekly (30.11cm) but comparable with those applied weekly (27.45cm). The shortest seedlings were those under the controlled set up. At 4 WAT, plants applied with foliar fertilizer biweekly were significantly taller (33.87cm) compared with those applied weekly and those under the controlled treatment. Likewise, it was observed that more fertilizer was needed by the plants as they grew bigger and older.

Banana is always considered as a gross feeder and requires large amounts of macronutrients such as NPK and micronutrients like calcium (Ca) and magnesium (Mg) to maintain high yields (Abdullah *et al*, 1999; Robinson, 1996). Likewise, applying foliar

Table 11. Plant height of tissue-cultured cv. *Lacatan* banana seedlings applied with foliar fertilizer (Green Bee ™) at different frequencies.

TREATMENT	PLANT HEIGHT (cm)			
	1WAT	2WAT	3WAT	4WAT
Control	15.25b	23.61	26.08b	27.32c
Once a week	17.23a	24.03	27.45ab	30.23b
Twice a week	16.59ab	25.16	30.11a	33.87a
Level of Significance	*	ns	*	**
CV (%)	4.2	5.8	5.4	3.8

ns - not significant
** - significant at 1% level
CV - coefficient of variation
WAT - weeks after treatment
Means marked with the same letter in a column are not significantly different using DMRT.

fertilizer affects physiological limitation in N-storage capacity. As deficiency symptoms quickly develop, extra N must be frequently applied even on fertile soil (Robinson, 1996).

*Effect of urea concentrations and application frequency***.** The application of urea to one-month old tissue-cultured banana seedlings at different rates and frequencies (Table 12) significantly affected the growth of seedlings at 1 and 2 WAT. Plants applied with 1tbsp urea/16 li water were taller compared with those applied with 2 and 3tbsp urea/16 li water. However, at 3 and 4 WAT, no significant differences among treatment means were observed. This means that 1tbsp/16liter of water is enough to optimize plant growth.

On the other hand, the frequency of applying urea significantly affected the growth of banana seedlings at 2, 3, and 4 WAT. Seedlings applied with urea daily were the tallest at 2 and 3 WAT, however, they were comparable with those applied with urea every other day. Shortest plants were those applied with urea once a week. At 4 WAT, however, significantly taller plants were observed on those applied with urea daily (56.73cm) followed by those applied with the same fertilizer every other day (51.49cm), and those applied once a week (43.20cm). NPK application in the form of urea can be dissolved instantly in which banana plants effectively utilized the accurately -placed fertilizer in the active root zone area resulting in vigorous growth (Mustafa and Kumar, 2012).

Table 12. Plant height of tissue-cultured cv. *Lacatan* banana seedlings applied with different rates of 16-0-0 (NPK) at different frequencies.

TREATMENT	PLANT HEIGHT (cm)			
	WAT	2WAT	3WAT	4WAT
Rate (R) of urea/16 li water	*	*	ns	ns
1 tbsp	19.65a	30.33a	41.21	51.48
2 tbsp	18.00b	28.65b	40.38	50.62
3 tbsp	17.93b	27.98b	39.80	49.31
Frequency (F) of application	ns	**	**	**
Daily	19.11	30.30a	44.09a	56.73a
Every other day	18.71	29.03ab	41.16ab	51.49b
Once a week	17.76	27.63b	36.14c	43.20c
R x F	ns	ns	ns	*
CV (%)	7.1	5.4	7.0	6.2

ns - not significant
** - significant at 1% level
CV - coefficient of variation
WAT - weeks after treatment
Means marked with the same letter in a column are not significantly different using DMRT.

Figure 3 shows the interaction effects of rate and frequency of urea application on the growth of banana seedlings at 4 WAT. Banana seedlings applied with urea fertilizer daily under the different concentrations showed faster growth.

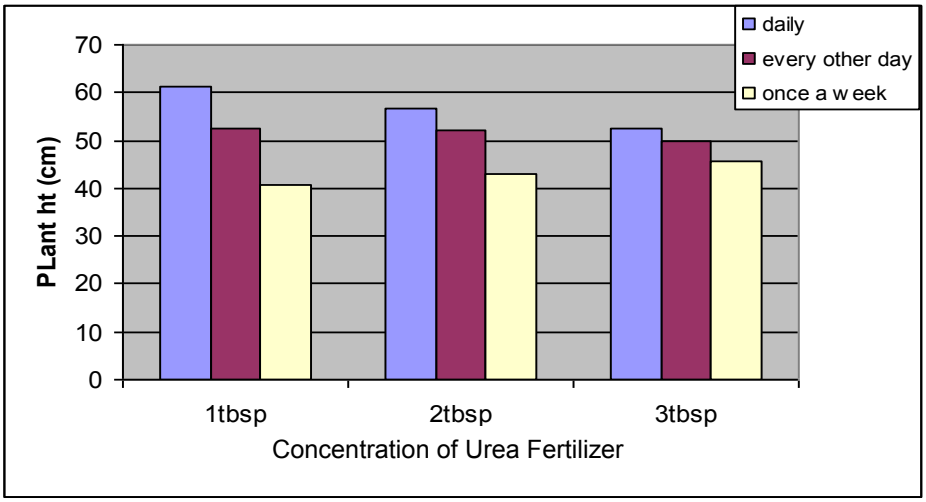

Fig. 3. Plant height of cv. Lacatan banana seedlings applied with urea in different concentrations.

Conclusions and Recommendations

The existing *in-vitro* propagation techniques for banana plantlets, involved the addition of 5ppm Benzyl Amino Purine, sucrose, chemical grade agar, and distilled water to the MS media formulation. It was found that the same quality and quantity of plantlets can be obtained by using affordable and locally-available materials, thereby lowering production cost.

In-vitro multiplication of banana can be done with cheaper components of the MS culture media. Based on the findings, a 4ppm BAP level was enough to produce the most number of shoots and 5ppm was not beneficial. Sucrose can be substituted with refined white sugar; chemical grade agar (Biolife agar) instead of commercial agar powder, and distilled water instead of purified water.

Likewise, nursery management techniques for *Lacatan* banana specifically under Ilocos conditions were developed. A two-part carbonized rice hull and one-part ordinary garden soil is the best medium for establishing/potting tissue-cultured banana seedlings. Moreover, survival of the plantlets is high when potted out with bottles covered for 7, 10, and 15 days. Plantlets had a 100% survival when placed under the sun (with partial shading) for 3, 6, 9, and 12 days after removing the cover.

As such, applying foliar fertilizer twice a week can facilitate the growth of seedlings. Likewise, applying urea at the rate of 1tbsp daily is best for establishing

tissue-cultured banana seedlings.

Additionally, using *in-vitro* techniques combined with proper nursery management in producing cv. *Lacatan* banana seedlings provided benefits to the farmer-clientele. Because of the use of affordable and locally-available materials. Specifically, a mixture of carbonized rice hull and ordinary garden soil (2:1 ratio) is the best medium to pot out banana plantlets from the laboratory. In establishing tissue-cultured banana seedlings, the rate of 1tbsp of urea fertilizer must be applied twice a week for faster growth.

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