EFFECTS OF WATER QUALITY, pH AND STATE OF THE MEDIUM ON GROWTH AND DEVELOPMENT OF COCONUT EMBRYOS *IN VITRO*

By

M.B.B. Areza, E.P. Rillo, C.A. Cueto,
A.W. Ebert & O.D. Orense [1]

ABSTRACT

The effects of the quality qfwater, pH and state qf the medium on growth and development of coconut embryos in vitro were evaluated. Germination of embryos in Y3 liquid medium was not affected by neither wau ,r quality nor pH adjustment. These suggest that tap water could replace glass distilled water and gelling agent could be eliminated in embryo culture qf coconut. However, pH qf the medium has to he adjusted.

INTRODUCTION

One of the types of coconut (Cocos nucifera L.) is the Makapuno coconut which is characterized by a soft enclosperm that fills the nut. Embryos of Makapuno coconuts do not germinate in situ clue to this abnormality of the enclosperm. The team of de Guzman of UPLB clemonstrated the possibility of extracting the normal Makapuno embryo and growing it in vitro to produce true-to-type, high Makapuno yielding coconut palms (de Guzman & del Rosario, 1964; Balaga & de Guzman, 1971a; Rosario and de Guzman, 1976).

The PCA-ARC tissue culture team further improved the technology by looking into the other media formulations to support growth and development of coconut embryos in vitro (Rillo & Paloma, 1990). Using Murashige and Skoog (MS) and Eeuwens (Y3) mineral formulations supplemented. with activated. charcoal, germination was hastened. Altogether the incubation was shortened from 6-8 months to 4-6 months.

REVIEW OF LITERATURE

Water in tissue culture researches should be given great attention since it constitutes 95% of the nutrient medium. Tap water contains high levels of unwanted ions. Therefore, it is passed through a series of purifying filters to free the water of contaminating ions, especially cations (positively charged ions), organic contaminants and even micro-organisms. For research purposes, water that has been glass distilled is recommended, while for research the photoplasts, cells and meristems, water which has been double distilled (Pierik, 1987).

The pH of the nutrient medium should also be given. an important consideration for a successful in vitro growth performance of any crop. It is predicted that a pH in the range of 5.0-6.5 is suitable for growth. pH lower than 4.5 or higher than 7.0 generally stops growth and development of cultures in vitro (Pierik, 1987). Butenko (1968) reported that if the pH is too low the agar becomes too, sloppy; phosphate and iron salts may precipitate; Vitamin 131 (thiamine and aneurine) and pantothenic acid become less stable; and uptake of ammonium ions is retarted.

The state of the medium is also important. Based on preliminary studies on the growth and development of zygotic coconut embryos in vitro, there was longer and greatbr primary root formation in Y3 solid than in Y3 liquid, MS solid or MS liquid media. Secondary roots were already

[1] Philippine Coconut Authority, Albay Research Center Banao, Guinobatan Albay 4503, Philippines

evident in seedlings cultured in Y3 solid medium. The average number of scale leaf formation, however, was the same in Y3 solid, Y3 liquid and MS solid. After another four weeks in culture, it was observed that there were more formation of expanded leaf and greater ramification of secondary roots in Y3 solid medium than in the other media (PCA-Annual Report, 1990). However, since sampling was not enough to really come up with conclusive statements, a follow-up study on the effect of the water source, pH, and state of the medium was conducted.

OBJECTIVES

Generally, this study aims to determine if mass propagation cost of Makapuno using embryo culture could be reduced.

Specifically, it aims to investigate the effects of water quality, pH and state of the medium on growth and development of coconut embryos in vitro.

MATERIALS AND METHODS

Embryos were excised from 10-11 month-old nuts collected from Laguna Tall palms. They were cultured following the protocol established by Rillo and Paloma (1992). The treatments were assigned completely at random in a three-factor experiment using single-factor experimental design. The following factors were considered:

Water quality (W)
Wl glass distilled water
W2 tap water

pH (P)
PI adjusted to 5.8
P2 not adjusted

State of the medium (S)
Sl solid
S2 liquid

Treatments were replicated 4 times with 20 samples per replicate.

Germination of embryo, shoot and root lengths were recorded after one month in culture. Plant height and further root elongation were regularly measured, 2, 3, and 4 months after initial culture.

Data were analyzed using ANOVA. Means were compared using the Duncan's Multiple Range Test (DMKr).

DISCUSSION OF RESULTS

Statistical analysis showed that quality of water and pH of the media affected root length but not percent germination and shoot length of l0 to 11 month-old Laguna embryos (Fig. l a & b) after one month in culture. Longer roots and shoot lengths were observed in embryos germinated in Y3 liquid media which used glass distilled water or pH adjusted to 5.8 (Table 1). However, their interaction effect was not significant.

After a 2-month incubation, the increase in plant height was significantly affected by state and pH of the medium but not by the quality of water. Although not significantly different, the increment in plant height was higher in tap water. Higher increment in plant height was observed in Y3 solid than in liquid medium. Likewise, adjustment to ph to 5.8 resulted to taller seedlings. The increment in root length of the seedlings was not significantly affected by any of the variables tested (Table 2).

Incubation of the seedlings in their respective medium for three to four months showed that only pH significantly affected plant height (Table 2, Fig. 2). The effect of pH in terms of increment in root length was observed only after 3 months. This effect, however, became negligible after the fourth month in culture. No interaction effect among the three factors was detected.

Table 1. Mean pereent germination, shoot and root length after one month incubation in Y3 liquid germination medium

VARIABLES	% Germination	Shoot length	Root length (mm)
Glass distilled water (W 1)	85.3 a	1.5 a	7.4 a
Tap water (W2)	86.9 a	1.4 a	6.2 b
+ pH (P 1)	88.1 a	1.8 a	7.6 a
- pH (P2)	84.1 a	1.2 a	5.9 b

* Means with the same letter in the same block are not significantly different at 5 % level of DMRT

Table 2. Mean increment in plant height and root length after two to four months incubation *

Variables	Increment in Plant Height (mm)			Increment in root Length (mm)		
	2 mo	3 mo	4 mo	2mo	3mo	4mo
Glass distilled water (W1)	9.7a	14.0a	34.3a	12.6a	8.0a	5.8a
Tapwater(W2)	11.4a	16.2a	38.0a	13.2a	7.2a	6.0a
solid medium(S1)	11.4a	15.2a	37.1a	13.7a	7.6a	5.0a
liquid medium (S2)	8.7b	15.0a	35.2a	11.3a	7.5a	6.8a
+pH (P1)	12.1a	16.9a	40.3a	14.5a	8.7a	6.4a
-pH (P2)	8.9b	13.4b	32.0b	11.3a	6.4b	

* Means with the same letter in the same block are not significantly different at 5 % level of DMRT

Chemical analysis of the tap water collected from Albay Research Centre showed that it contained 4.76 ppm potassium, 26.0 ppm calcium, 10.8 ppm magnesium, 14.4 ppm sodium, 2.0 ppm chloride, 2.4 ppm sulfur and nil amount of nitrogen, phosphorus and boron. From the results, it is safe to assume that the ions present in the tap water did not significantly affect growth and development of coconut embryos. The pH of the Y3 media which used glass distilled water and tap water ranged from 4.8-5.0 and 6.2-6.4, respectively (Table 3). Skirvin et al (1986) reported that pH changes after autoclaving. If the starting pH is in the range of 5.0-7.0, it generally lowers by 0.3-0.5 unit. The pH range of 5.0-6.5 is predicted suitable for growth with an optimum at about 6.0 (Pierik, 1987). Hence, assuming a reduction of 0.3-0.5 units in pH due to autoclaving, the pH of the autoclaved culture media which used tap water with or without pH adjustment was within the predicted suitable range for growth (Table 3). The pH after autoclaving of culture media which used distilled water could be below the pH range for growth if pH was not adjusted prior to autoclaving.

Table 3. pH range of the culture medium before and after autoclaving

	pH OF THE CULTURE MEDIUM		
	Before Autoclaving		After Autoclaving[a]
	Before Adjustment	After Adjustment	
W1P1S1	4.9-5.0	5.8	5.3
W1P2S1	4.9-5.0	-	4.4-4.5
W1IP1S2	4.8-5.0	5.8	5.3
W1P2S2	4.8-5.0	-	4.3-4.5
W2P1S1	6.2-6.4	5.8	5.3
W2P2S1	6.2-6.4	-	5.7-5.9
W2P1S2	6.2-6.4	5.8	5.3
W2P2S2	6.2-6.4	-	5.7-5.9

[a] - Ph range assuming a reduction in pH by 0.5 units.

The cost of producing an in vitro-cultured Makapuno in a solid medium with glass distilled water and pH adjustment, as previously applied for coconut embryo culture, was found expensive (P150.35). Comparing the cost of the previous method (W1P1S1) with the other treatments suitable for embryo culture, the amount saved was P3.61, P15.23, and as much as P18.84 with W1P1S2, W2P lSl and W2PlS2, respectively (Table 4).

Total volume of medium prepared per embryo:

Initial	15 ml
1st subculture	20 ml
2nd subculture	50 ml
3rd subculture	80 ml
4th subculture	80 ml
TOTAL	245 ml - 250 ml

2/ Estimate was based on the salary of a Lab. aide directly involved.

3/ See Table 5 for the computations

4/ Water source:

a. Triple distilled water (BYCON)
P 35.85
x 250 ml = p 8.96 per 250 ml medium
1000 ml

5/ Factor used on finding that 70% of total embryos are lost at different stages of culture.

6/ Computed by subtracting the cost of other treatments from the cost incurred using the previous method (W1P1S1).

Table 4. Estimated cost of production per embryo from initial to rinal subeulture [1/]

ITEM	W1P1S1	W1P1S2	W2P1S1	W2P1S2	W1P2S1	W1P2S2	W2P2S1	W2P2S2
Salary [2/]	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Cost of embryo	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Chemical[3/]	5.31	3.19	0.001	0.001	8.96	8.96	0.001	0.001
Water source[4/]	8.96	8.96	1.17	1.17	-	-	-	-
pH adjustment	1.17	1.17	12.00	12.00	12.00	12.00	12.00	12.00
Light & water	12.00	12.00	15.00	15.00	15.00	15.00	15.00	15.00
Misc Lab supplies	15.00	15.00	5.31	3.19	5.31	3.19	5.31	3.19
TOTAL	88.44	86.32	79.48	77.36	87.27	85.15	78.31	76.19
	x 1.75 5/	x 1.7	x 1.7	x 1.7	x 1.7	x 1.7	x 1.7	x 1.7
Cost/embryo	150.35	146.74	13.12	131.51	148.36	144.76	133.12	129.52
Savings[6/]		3.61	15.23	18.84	1.99	5.59	17.3	20.83

[1/] These estimates did not consider the infrastructure, equipment, glasswares, other supplies and salaries of other people involved in the supervision of the work.

Table 5. Cost of chemical per embryo (in Pesos) depending on the state of the medium

CHEMICAL	G/250 ml[1/]	UNIT COST	Price/gram	Total Cost	
				Solid	Liquid
$Cacl_2$, $2H_2O$	0.007	4899/k	0.899	0.07	0.07
$MgSO_4$, $7H_2$	0.062	631/500g	1.262	0.08	0.08
$NaH_2PO_4H_2$	0.078	892/500g	1.784	0.14	0.14
KNO_3	0.505	625/500g	1.250	0.6	0.63
KCl	0.373	703/k	0.703	0.26	0.26
NH_4Cl_2	0.134	892/k	0.892	0.12	0.12
$MnSO_4$, $7H_2O$	0.002	582/100g	5.820	0.012	0.012
$ZnSO_4$, $7H_2O$	0.002	571/500g	1.142	0.002	0.002
H_3B_3	0.001	495/500g	0.990	0.001	0.001
KI	0.002	1468/250g	5.872	0.012	0.012
$CuSo_4$, $5H_2O$	0.00006	467/250g	1.868	0.0001	0.0001
Na_2MoO_4, $2H_2O$	0.00006	1513/100g	15.13	0.001	0.001
$CoCl_2$, $6H_2O$	0.00006	1532/100g	15.32	0.001	0.001
$NiCl_2$, $6H_2O$	0.000006	1345/250g	5.380	0.0003	0.00003
FeNaEDTA	0.009	2160/250g	8.640	0.08	0.08
Myo-inositol	0.025	1066/100g	10.660	0.27	0.27
Thiamine HCl	0.000125	756/250g	3.024	0.0004	0.0004
Nictonic acid	0.0000125	1003/100g	10.03	0.0001	0.0001
Pyridoxine HCl	0.0000125	1205/10g	120.5	0.002	0.002
Ca D-pantothenate	0.0000125	220.5/5g	44.1	0.001	0.001
d-Biotin	0.0000125	1177/100g	11.77	0.0002	0.0002
Act. Charcoal	0.625	2080/k	2.08	1.3	1.3
Agar-agar Japan[2/]	1.38	700/500g	1.54	2.12	-
NAA[3/]	0.002	200/500g	0.04	0.001	0.001
Sugar	11.25	19/k	0.019	0.21	0.21
TOTAL				**5.31**	**3.19**

l/ Total volume of medium prepared for embryo:

Initial	15 ml
1st subc	20 ml
2nd subc	50 ml
3rd subc	80 ml
4th subc	80 ml
	245 ml - 250 ml

2/ Solid medium (6g/l): 1st - 4th subc = subc = 230 ml

3/ NAA 10 mg/l – 0,01 g/l

$$\frac{\text{x} \quad 160 \text{ ml}}{0.002 \text{ g}}$$ (3rd & 4th subc)

CONCLUSION

Percent germination of 10 to 11 month-old Laguna embryos cultured in Y3 liquid medium was, not affected by either water quality or pH adjustment. However, further culture of the coconut embryos showed that pH adjustment is a critical factor. The quality of water and state of the medium are not very important factors. The culture medium for coconut embryos could, therefore, use tap water, without agar but with pH adjustment. The elimination of agar and the use of ordinary tap water will greatly reduce the production cost of *in-vitro* cultured Makapuno coconuts.

RECOMMENDATIONSTOLICY IMPLICATIONS

The results of this study influenced partly the policy of Philippine Coconut Authority to reduce the selling price of in vitro grown Makapuno seedlings from P 500.00 to P300.00 per piece, thereby, allowing more small coconut fariners to afford this high value coconut type. Sociologically and economically, the availment of this type of coconut will benefit the farmers in the form of being able to sell high value coconuts helping them in the short to medium term.

REFERENCES

BALAGA, H. M. & E.V. de GUZMAN. 1971a. The growth and development of coconut 'Makapuno' embryos *in vitro*. II. Increased root incidence and growth and response to media composition and to sequential culture from liquid to solid medium. *Phil. Agric*. 53(10): 551-565.

BUTENKO, R.G. 1968. Plant tissue culture and plant morphogenesis. In: Pierik, R. L. M. 1987. *In vitro* culture of higher plants. *Martinus Nijhoff Publishers*, Dordrecht, Netherlands, 344 pp.

GUZMAN, E.V. de & D.A, del ROSARIO. 1964. The growth and development of Cocos Nucifera L. 'Makapuno' embryos *in vitro. Phil. Agric*. 48(2-3): 82-94.

PCA ANNUAL REPORT. 1990. Performance of Catigan embryos in MS solid, MS liquid, Y3 solid and Y3 liquid media. In: Growth and development of zygotic coconut embryos in vitro-Improvement of root growth. 146 pp.

PIERIK, R.L.M. 1987. In vitro culture of higher plants. *Martinus Nijhoff Publishers*, Dordrecht, Netherlands. 344 pp.

RILLO, E. P. & M. B. F. PALOMA. 1990. Comparison of three media formulations for in vitro culture of coconut embryos. *Oleagineux* 45 (2): 319-323

RILLO, E. P. & M. B. F. PALOMA. 199Z. In vitro culture of Makapuno coconut embryos. *Coconuts Today* 9(l): 90-101

ROSARIO, A.G. del & E.V de GUZMAN. 1976. The growth of coconut 'Makapuno' embryos in vitro as affected by mineral com position and sugar level of the medium during the liquid and solid cultures. *Phil. J. Sci*. 105: 215-222.

SKIRVIN, R. M., M. C. CHU, M. L. MANN, H. YOUNG, J. SULLIVAN & T. FERMANIAN. 1986. Stability of tissue culture medium pH as a function of autoclaving, time and cultured plant material. *Plant Cell Rep*. 5:292-294.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support provided by the Philippine Coconut Authority and the Deutsche Gessellschaft fur Technische Zusammenarbeit (GTZ). This work forms part of the Philippine-German Coconut Tissue Culture Project.