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COMPARITIVE EVALUATION OF EMBRYO CULTURE PROTOCOLS IN COCONUT

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ABSTRACT

Four coconut embryo culture protocols, *developed from* PCA, Philippines, UPLB, Philippines, IRHO, France and CPCRI, India were tried with two tall and two dwarf cultivars. There was no significant difference among the protocols for per cent embryos germinated. However, difference among the cultivars and cultivar-by-protocol interaction was significant. The *in vitro* growth was slow in CPCRI protocol but the survival of the plants and *ex vitro* establishment were good. Vitrification was noticed only in liquid medium. Least number of plants was retrieved through IRHO protocol.

Key words: Coconut (*Cocos nucifera* L) Embryo culture, *In vitro* culture, *Ex vitro* establishment.

INTRODUCTION

Embryo culture technique is an effective way of collection and exchange of coconut gemplasm. Rillo and Paloma (1990) and Karun *et al.,* (1999) compared media formulation for *in vitro* culture of coconuts. International Coconut Embryo Culture and Acclimatization Workshop at PCA, Philippines (27-31 October 1997) identified four protocols viz., PCA, Philippines, UPLB, Philippines, IRHO, France and CPCRI, India (Appendix–I). To improve/upgrade these protocols for mature (11 months after fertilisation) embryos and their adaptability among cultivars, COGENT has promoted through collaborative DFID funded project. Experiment was conducted at 14 international laboratories viz., IRHO France, CPCRI, India, CPATC Brazil, CIIF Cuba, SPC Fiji, RICP Indonesia, CNRA, Cote d'Ivoire, CICY Mexico, CCRI Papua New Guinea, PCA-ARC Philippines, PCRDF Philippines, UPLB Philippines, CRI, Sri Lanka, MARI Tanzania with the following objectives: a) to study the effect of different protocols on germination and growth of the embryos, b) to study the interaction between these protocols on two each of local and exotic cultivars. The salient findings of the experiment conducted at CPCRI are discussed in this paper.

MATERIAL AND METHODS

Two dwarf and two tall cultivars *viz.,* COD (Chowghat Orange Dwarf), MYD (Malayalan Yellow Dwarf) and WCT (West Coast Tall), Laccadive Ordinary Tall (LCT) were used in the experment. A total of 960 embryos inoculated in nutrient media and experimental design followed was RBD with three replications each consisting of 20 embryos/ treatment. The embryos of dwarf cultivars were made available from ICGB-SA, Kidu and the tall cultivars from CPCRI Kasaragod.

Culture medium: Out of 4 media protocols, for germination, 3 were liquid state and one (CPCRI) was solid state. Agar 5.5 g/l was added to the CPCRI protocol for gelling. Whatman no. 1 filter paper bridges were immersed in liquid medium to give support to the embryos. Sub-culturing was done once in every 30-45 days intervals.

Culture conditions: Inoculated embryos of different cultivars were incubated in dark conditions with 27± 2°C temperature, 85 % RH till germination took place. Then the cultures were transferred to light room (2500 –3000 lux) with 16 hrs photo-period.

Observations like weight of the embryo (g), germination (%), contamination (%), abnormalities (%) were recorded during every subculture and no. of leaves, length of shoot (cm), length of root (cm), root volume (ml), collar girth (cm) of the seedling at the time of transferring to pots. Standard procedures of statistical analysis

as in SPSS software were followed. Replication wise data was used for comparing the germination, whereas, for other characters, the data from germinated embryos was used and hence treated as one-way classified data.

RESULTS & DISCUSSION

Swelling of the embryos and protuberance of plumule was observed after 15-20 days after inoculation in dwarf (COD and MYD) and 35-45 days in case of tall cultivars. Browning of the embryos was noticed in IRHO medium cultures (Plate: 1). It might be due to the high salt medium (MS) compared to Y3 medium (Appendix: 1). The germination percentage after 45 days of inoculation is presented in Table 1.

60 days after inoculation: The per cent germination of embryos of tall varieties improved during the period of 45-60 days of inoculation (Table 1). There was no significant difference observed between treatments with regards to germination after 60 days. All the protocols gave average germination between 85.4 to 89 %. The cultivar differences and cultivar 'x' protocol interaction were significant at 5% level. Maximum germination was observed in MYD (96.7%) and lowest in WCT (76.7%). COD gave 90.8% germination and LCT 85.8%. The response of MYD in all the protocols were on par.

Germination and initial growth was more precausious in liquid media (Plate 1). Dwarf variety germinated after and better germination was notified in CPCRI. Comparisons of various embryo culture protocols revealed that best performance was obtained with CPCRI protocol (Damasco, 2000; Oropera, 1999; Angolo, 1999; Weerakoon, *et al.* 1999)? opined that in the liquid medium, the absorption of nutrients and gas interchange is more efficient than the solid medium and it also contributes to the radicle and foliar growth.

However, Cueto and Rillo (1999) noticed that embryos in UPLB protocol gave highest percentage of transplanted seedlings followed by CPCRI protocol.

Cultivar variation was seen in *in vitro* culture of coconut zygotic embryos (Kullaya, 1999). Weerakoon *et al.*, 1999 and Kullaya (1999) reported that CPCRI protocol responded well to germination of tall variety (EAT) than dwarf types viz., MYD and PRD. Angelo (1999) observed that MYD was more sensitive than RIT with change in culture conditions.

Characterization of *in vitro* growth:

The data on weight of embryos at 60, 120 and 200 days after inoculation, length of shoot at 120 and 200 days after inoculation and length of root at 200 days after inoculation were subjected to the Multivariate tests for repeated observations as well as the univariate split block. The anova Weight at three different time points (Table 2) are not uniform among the protocols, but only the former test showed significant Cultivar 'X' time interaction. The Protocol XTime interaction is highly significant for the linear trend. No three-way interaction was observed. The lowest weight gain was found in CPCRI, India protocol. It might be due to the solid state of medium. Maximum weight gain was noticed UPLB Philippines, IRHO, France, and followed by PCA, Philippines. All the varieties recorded

Table 1. Per cent germination at 45 and 60 days after inoculation

Cultivars	Germination at 45 days of inoculation				Germination at 60 days after inoculation			
	CPCRI	UPLB	PCA	IRHO	CPCRI	UPLB	PCA	IRHO
LCT	16.66	36.20	57.62	42.27	71.66	90.00	91.87	90.00
WCT	15.00	42.59	43.33	37.28	78.33	70.00	81.67	76.66
COD	86.66	36.66	63.33	63.33	93.33	91.67	86.67	91.67
MYD	96.33	88.33	98.33	80.00	98.33	96,66	95.0	96.67

CD (5%) for comparison germination at 60 days after inoculation = 9.81; CV = 6.3%

Table 2. Mean weight of embryos (g.) at different points of time

	Wt. after	LCT	COD	WCT	MYD
CPCRI	60 days	.49	.25	.53	.33
	120 days	.85	.65	.95	.93
	200 days	1.21	.72	1.72	1.24
UPLB	60 days	1.02	.79	.98	.86
	120 days	1.96	1.70	1.84	1.79
	200 days	3.39	3.25	2.87	3.33
PCA	60 days	1.19	.78	.94	.62
	120 days	2.48	1.48	1.92	1.41
	200 days	4.25	3.79	2.78	2.40
IRHO	60 days	1.27	0.77	1.07	.66
	120 days	2.56	1.63	1.85	1.17
	200 days	4.22	3.10	3.08	2.33

low weight in CPCRI protocol (Table 2; Fig.1). Weight of the embryo was more in liquid media than in solid medium. There was no Cultivar*Time interaction for gain in weight (Fig. 2).

The percentage of plants having shoot and root after 4th month of inoculation is shown in Table 3. The root formation in CPCRI protocol was the minimum; maximum was in IRHO protocol (Plate.3). At CPCRI we recommend IBA (5 mg/l) and NAA (1 mg/l) supplementation in rhizogenesis medium. The CPCRI plants were sub-cultured to this medium.

Rillo and cue to (2000), reported that in terms of average leaf production, seedlings in CPCRI protocol formed the highest number of leaves followed by PCA , UPLB and IRHO protocols. The lowest root formation was observed in CPCRI medium. However, higher percent of complete seedlings development was obtained from UPLB protocol followed by CPCRI.

Vitrification: The vitrification was noticed only in liquid medium (Plate.5). The percentage of vitrification was recorded in Table: 4. vitrification was more in dwarf varieties compared to tall ones. This type of abnormalities was completely absent in solid medium. Carandang (2000) was also reported this type of abnormality in liquid medium.

***Ex vitro* studies:** A total of 90 plants of various treatments were pot planted. As in the CPCRI protocol, the plants were treated with Bavistin /Carbendazim 1 gm/l and subsequent

Fig. 1. Graphical representation of protocol 'X' time interaction. The CPCRI protocol showed slow gain of weight; other protocols are on par.

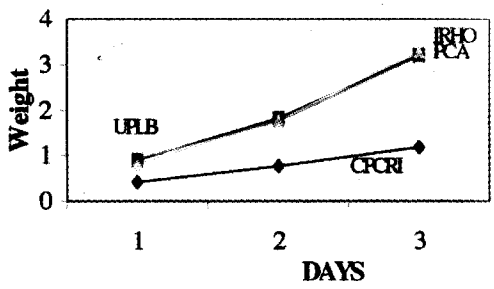

Fig.2. There is no Cultivar 'x' Time interaction for gain in weight

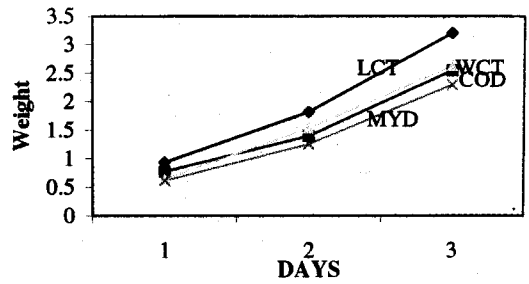

Table 3. Per cent plants with shoot and root formed after 120 days of culture initiation

Protocol	CPCRI		UPLB		PCA		IRHO	
Cultivars	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
LCT	76.66	20.00	68.33	68.33	88.3	80.00	81.16	73.33
WCT	58.33	31.66	63.33	51.66	66.66	40.00	71.66	60.00
COD	70.00	23.33	73.33	53.38	81.66	51.66	81.66	68.33
MYD	95.00	28.33	91.66	65.00	85.00	46.66	90.00	81.66
Mean	**74.99**	**25.83**	**74.16**	**59.59**	**80.41**	**54.58**	**81.12**	**70.80**

dip the root zone in IBA 1000 ppm for 1 hour each. At the time of transferring to pots, the observations like shoot length, Number of leaves, root length, Number of roots collar girth, and root

Table 4. Per cent vitrification observed after 60 days of inoculation

Cultivars	CPCRI	UPLB	PCA	IRHO
LCT	0.00	1.66	8.33	10.00
WCT	0.00	3.33	13.33	11.66
COD	0.00	30.00	46.66	21.66
MYD	0.00	18.33	20.00	10.00

Table 5. Average measurements of plants at the time of transfer to pots

Protocols	Plant height (cm)	Root-volume (ml)	Leaf-length (cm)	Leaf-width (cm)
CPCRI	20.54	4.18	14.27	1.68
IRHO	18.50	2.78	12.54	1.47
PCA	23.73	5.11	16.50	1.93
UPLB	21.15	4.37	13.91	1.72

volumes were measured (Table 5). Significant protocol differences were notice with respect to plant height, volume of root, and length and width of leaves. No significant differences were noticed among the cultivars and also there was no porotocol cultivar interaction.

Sucrose is playing an important role in pot establishment. It was noticed that higher percentage of success at lower concentration of sucrose at rooting medium. Triques *et al.*, (1997) emphasized that the lower sugar concentration

Plate 1. embryos of Laccadive Ordinary (Tall variety) in different medium after 30 days of culture initiation.
1. PCA, Philippines,
2. CPCRI, India,
3. UPLB, Philippines,
4. IRHO, France. (Note the browning in IRHO Protocol)

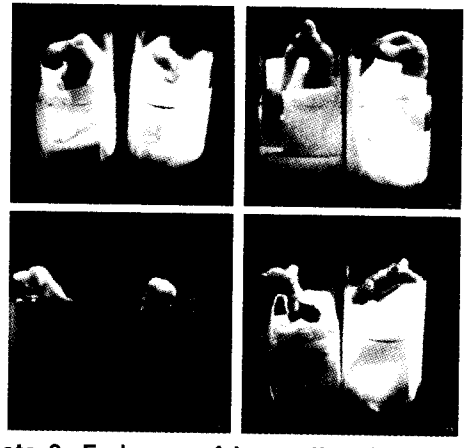

Plate 2: Embryos of Laccadive Ordinary in different protocol after 60 days of inoculation.
1. PCA, Philippines,
2. UPLB, Philippines,
3. CPCRI, India,
4. IRHO, France (Note the browning of embryos in IRHO medium)

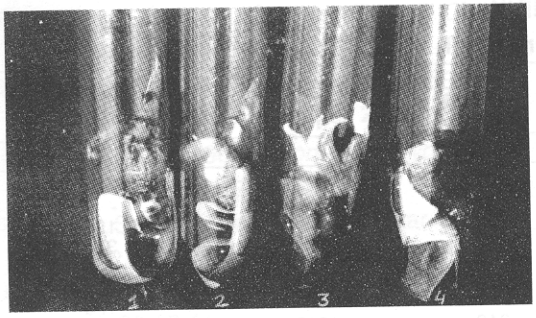

Plate 3. Embryos of Laccadive Ordinay in culture media after 4 months on inoculation.
1. PCA, Philippines 2. UPLB, Philippines
3. CPCRI, India 4. IRHO, France
(Note the longer root length in PCA and UPLB medium)

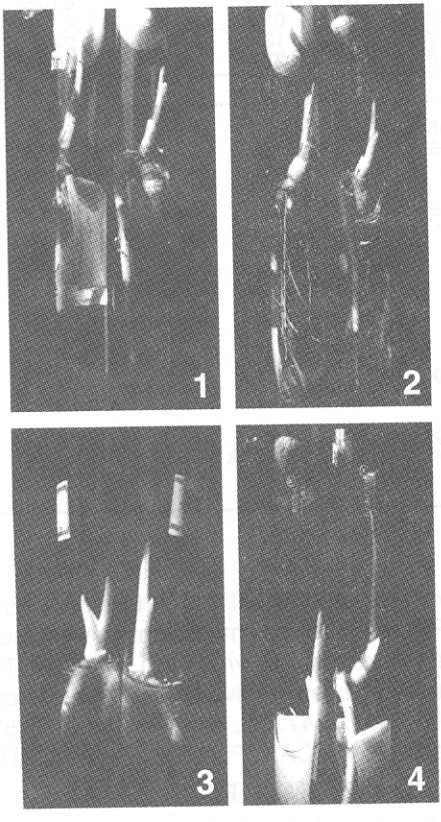

Plate 4. Plants of Laccadive Ordinary in culture media after 7 months of inoculation.
1. PCA, Philippines
2. UPLB, Philippines
3. CPCRI, India (transferred from solid to liquid medium) and
4. IRHO, France

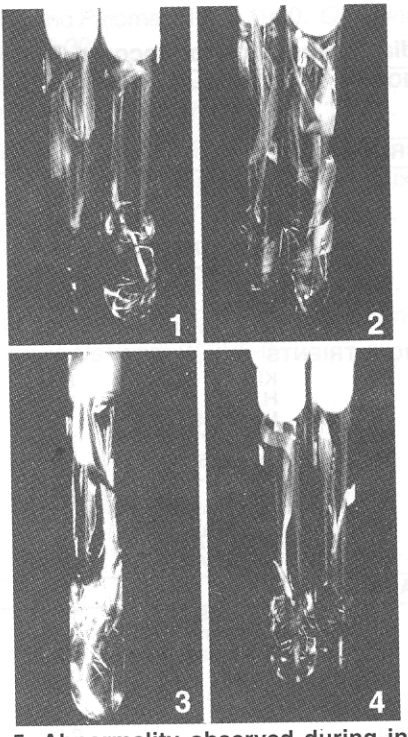

Plate 5. Abnormality observed during in vitro culture (Vitrification noticed only in loquid medium)
1. MYD embryo in IRHO medium
2. MYD embryo in PCA medium
3. COD embryo in UPLB medium
4. LO embryo in IRHO medium

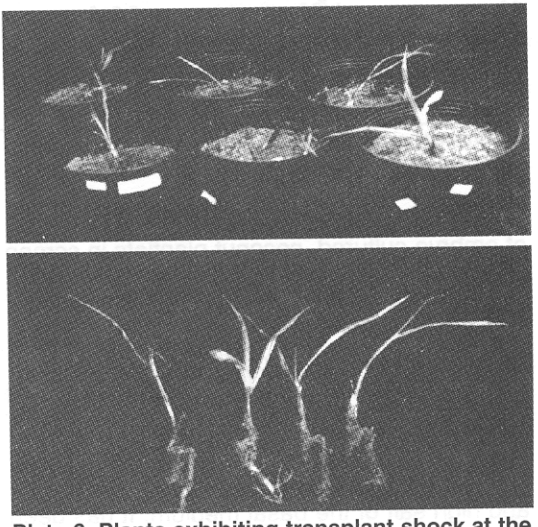

Plate 6. Plants exhibiting transplant shock at the time of pot culture. Plantlets derived from UPLB and IRHO protocol exhibited 66% and 50% mortality respectively in the pots.

APPENDIX - I

Media Composition for coconut Embryo Culture (mg/l)

SL.NO	Chemical	PROTOCOLS PCA Y3	U PLB Y3	CPCRI Y3	IRHO MS
MACRONUTRIENTS					
1	NH_4NO_3	-	-	-	1650
2	NH_4Cl	535	535	535	-
3	KNO_3	2020	2020	2020	1900
4	$MgSO_4.7H_2O$	247	247	247	370
5	$CaCl_2.2H_2O$	294	294	294	440
6	KCl	1492	1492	1492	-
7	KH_2PO_4	-	-	-	170
8	$NaH_2PO_4.2H_2O$	312	312	312	-
MICRONUTRIENTS					
9	KI	8.3	8.3	8.3	0.83
10	H_3BO_3	3.1	3.1	3.1	6.2
11	$MnSO_4.4H_2O$	11.2	11.2	11.2	22.3
12	$ZnSO_4.7H_2O$	7.2	7.2	7.2	8.6
13	$CuSO_4.5H_2O$	0.25.	0.25	0.160	0.025
14	$CoCl_2.6H_2O$	0.24	0.24	0.24	.025
15	$NaMoO_4.H_2O$	0.24	0.24	0.24	0.025
16	$NiCl.6H_2O$	0.024	0.024	0.024	-
17	$Fe_2SO_4.7H_2O$	13.9	41.7	13.9	24.9
18	Na_2EDTA	37.3	55.8	37.3	26.1
ORGANICS					
19	Myo-inositol	100	-	100	100
20	Pyridoxine HCl	0.05	0.05	0.05	1.0
21	Thiamine HCl	0.05	0.05	0.5	1.0
22	Nicotinic Acid	0.05	0.5	0.5	1.0
23	Ca-D-panthothenate	0.05	-	-	1.0
24	Biotin	0.05	0.05	0.05	0.0125
Folic acid	-	0.05	-	-	
26	Glycine	-	1.0	2	-
27	Na Ascorbate	-	-	-	100
28	BAPk	-	-	0.5	-
29	NAAk	-	-	0.5	-
30	NAAkk	-	-	1.0	-
31	IBAkk	7.0	-	5	-
32	Agar	-	7.0g/l		
33	L/S/L	5.5g/l			
34	S/S/L	-			
35	Activated charcoal	2.5g/l	2.5g/l	1g/l	2g/l
36	Sucrose	45 g/l	60g/l	60 g/l k	
37	30 g/l kk	60 g/l			
pH	5.8	5.6	5.7	5.5x	

k Germinating medium

kk Rooting medium

of the medium contributed to autotropic conditions of embryo cultured coconut plantlets in screen house.

Transplant shock: The potted plants exhibited transplant shock after two weeks of pot transferring. The plant grown in UPLB and IRHO medium exhibited 66% and 50 % respectively (Plate.6): This might be due to high concentration of sucrose (super optimal levels) and high salt of the basal medium. It has been found that the maximum plantlets survived in PCA Philippines irrespective of the cultivar and were on par with CPCRI and UPLB protocols. In the IRHO protocol, maximum plantlets were found to be dead both *in vitro* and *ex vitro*. The Maximum plantlets survived in different protocols were 58.33 %, 47.9%, 54.18 %, and 37.5%, respectively in PCA, UPLB, CPCRI, and IRHO.

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REFERENCES

Angelo, P., 1999. *Coconut Embryo Culture Network Newsletter,* **22**:9-10.

Carandang, E., 2000. *Coconut Embryo Culture Network Newsletter,* **31**:19

Cueto .C.A. and Rillo. E.P. 1999. *Coconut Embryo Culture Network Newsletter.* **22**:3-4

Damasco, O.P. 2000. *Coconut Embryo Culture Network Newsletter,* **31**:19-20.

Karun, A., Sajini, K.K. and Shivashankar, S. 1999. *Indian Journal of Horticulture,* **564**:348-353.

Kullaya, A. 1999. *Coconut Embryo Culture Net work,* **22**:10-11.

Oropeza, C. 1999. *Coconut Embryo Culture Net work,* **22**: 12-13.

Rillo, E.P. and Paloma, M.B.F. 1990. *Oleagineax,* **45:** 319-323.

Rillo, P.R. and Cueto, A.C. 2000 . *Coconut Embryo Culture Net work,* **3**(1): 17-18.

Triques,K., Rival, A ., Deule,T., Puard, M., Roy, J., Nato, A., Lavergne, D., Havanux, M. and Hamon, S. 1997. *Plant Science,* **127:** 39-51.

Weerakoon, L.K., Vidhanaarachchi, V. and Ranasinghe, S. 1999. *Coconut Embryo Culture Net work* **2**(2):5-6.