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Influence of media composition on gas exchange parameters, biochemical composition and growth of zygotic embryo-cultured coconut (*Cocos nucifera* L.) plantlets

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ABSTRACT

Coconut zygotic embryo culture has practical value in collection and exchange of germplasm overseas and is done on different media protocols. Differences in plantlet survival and media composition led to a study with an objective to compare growth, leaf gas exchange characteristics and biochemical constituents of plantlets of dwarf and tall cultivars of coconut grown on four media *viz.*, PCA-ARC, UPLB, IRD and CPCRI. A total of 960 embryos from two dwarf (COD and MYD) and two tall (WCT and LCT) cultivars of coconut were inoculated on four different media. The dwarfs germinated earlier than the talls. Maximum vitrification was observed in liquid media, *viz.* PCA-ARC followed by UPLB and IRD. At the time of transplantation to pots for acclimatization, plantlet morphological growth did not vary significantly. However, biochemical composition (reducing sugars, starch, total carbohydrates, free amino acids and proteins; the chl. a, b, total chlorophyll and chl. 'a/b ratio') of seedling leaves varied with cultivar and influenced by medium composition. Development of photosynthetic system was influenced by the composition of medium and readily usable carbon source in media seems to inhibit *Pn.* In general, plantlets raised on PCA-ARC and CPCRI media had higher *in vitro* and *ex vitro* survival rates followed by those raised on UPLB medium. Plantlets from IRD medium had lowest survival capacity. LCT and MYD plantlets survived better when grown on PCA-ARC medium, and WCT and COD plantlets survived better when raised on CPCRI medium. It is suggested that providing solid germinating medium to reduce vitrification loss; addition of auxins and reduction of salt concentrations in growth medium, reduction of sucrose concentration in rooting medium and gradual increase in PAR during rooting may help in early establishment of photosynthetic mechanism and better growth to increase the *in vitro* and *ex vitro* survival rates of coconut seedlings.

Key words: Coconut, medium composition, photosynthetic rate, chlorophyll, stomatal conductance, zygotic embryo culture.

INTRODUCTION

The technique of zygotic embryo culture has gained importance due to its practical use in collection and exchange of coconut germplasm overseas. Collection of coconut germplasm in the form of embryos is not only economical but also ensures the quarantine conditions. Currently, the survival of plantlets in *ex vitro* conditions is around 50%. However, it is important to increase the survival rate further to economize the use of embryo culture technique in germplasm collection. The physiological status of plantlets at the time of transfer from *in vitro* to *ex vitro* conditions largely determines the survival success of plantlets *ex vitro*. Availability of different protocols for embryo culture of coconut and differences in plantlet survival led to a decision to compare the influence of different protocols on growth and development of coconut plantlets at International Coconut Embryo culture and Acclimatization Workshop held at PCA-ARC, Philippines during October 1997 in order to optimize them. The identified protocols for comparison were PCA-ARC, Philippines; UPLB, Philippines; IRD, France and CPCRI, India. Studies were conducted in 14 international laboratories and results on the influence of media on germination and growth of embryos and plantlets are presented elsewhere (Anitha Karun *et al.*, 1).

Since all *in vitro* grown plantlets have to undergo physiological acclimatization for survival in field (Debergh and Zimmerman 3; van Huylenbroeck and Debergh, 23), it is important to study the physiological and biochemical status of the plantlets and to find the influence of media on plantlet growth and survival. Coconut zygotic embryo and *in vitro* grown plantlets can be used for physiological studies (Rival *et al.*, 17). Triques *et al.* (21, 22) reported early establishment of photosynthetic mechanism in *in vitro* development of zygotic embryo-cultured coconut plantlets. Naresh Kumar *et al.* (14), showed that the embryo-cultured coconut plantlets undergo photosynthetic acclimatization with increased PSII efficiency and water use efficiency during acclimatization. Plantlets also undergo chlorophyll and leaf morphological acclimatization (Ranasinghe *et al.*, 15). In the present experiment, objectives were to compare morphological growth, leaf gas exchange characteristics and

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biochemical constituents of the plantlets of different cultivars grown on four selected media during the transfer of *in vitro* plants to *ex vitro* conditions. Since the physiological status of the plantlets during the transfer to *ex vitro* conditions is an important factor determining the plantlet survival rate, this study was expected to give leads towards modification of medium composition to optimize growth and development in order to get high *ex vitro* survival rates.

MATERIALS AND METHODS

A total of 960 mature embryos excised from the nuts of uniform age from dwarf (COD-Chowghat Orange Dwarf; MYD- Malayan Yellow Dwarf) and tall (WCT- West Coast Tall; LCT-Laccadive Ordinary Tall) cultivars of coconut were inoculated in four different media in RBD with three replications (each treatment 20 embryos/replication). The embryos of dwarf cultivars were collected from International Coconut Gene Bank for South-Asia, Kidu, Karnataka.

Two types of basal media *viz.,* Eeuwens Y3 (Eeuwens, 6) and MS (Murashige and Skoog, 13) were tried for culturing the embryos. PCA-ARC, CPCRI and UPLB media are Y3 based and that of IRD is MS based. Compositions of all media are given in Table 1. Three media are of liquid state and one (CPCRI's) is solidified, which contains agar (5.5 g/l). In liquid media, all sub-culture conditions in PCA-ARC and IRD protocols are on liquid media, while in UPLB protocol, germinating medium was liquid followed by sub-culturing to solid medium and then on liquid rooting medium. In CPCRI protocol, initial cultures and sub-cultures were on solid medium followed by sub-culturing on liquid rooting medium (Table 1). Whatman No.1 filter paper bridges were immersed to provide support to the embryos in liquid media. Sub-culturing was done at 30-45 days interval. Inoculated embryos were incubated in dark at 27 ± 2 °C temperature and 85% relative humidity till germination. The germinated cultures were transferred to light (~60 mol/m^2 on sec.) with a photoperiod of 16 hours.

The plantlets were acclimatized under controlled conditions and then transferred to net house. The survival percentage of plantlets was registered three months after transfer to *ex vitro* conditions. After passing this phase of acclimatization, all plantlets generally be ready for field planting. Observation on germination percentage, number of leaves, leaf width and length, and root length were taken at every subculture while root volume (by water displacement method), collar girth were taken at the time of transfer to pots. Gas exchange characteristics were measured immediately after taking out plantlets from the culture tubes and just before transferring to pots for acclimatization in room conditions at high humidity levels using potable photosynthesis system (LCA-4 and PLC 4, ADC, UK). The leaf chamber was given a flow rate of 200 ml/min. At the time of measurement, the PAR levels on leaflet were maintained at ~60 mol/m^2/sec and the leaf chamber RH was close to 75% at 22°C. Boundary layer resistance to H_2O was 0.2 m^2/s/mol. During the period of photosynthetic observations, roots of plantlets were supplied with adequate amount of distilled water (in a sterile polymer) to ensure stable conditions. Readings were allowed to stabilize (*Ci* and *Pn*) before logging. At least ten observations were recorded in each treatment and replication. The data thus obtained were corrected using the measured leaflet width to obtain the values for uniform leaf area.

For the biochemical analysis, sampling of leaflets was done at the time of plantlet transfer to pots. They were oven dried at 100 °C for 2 h and at 70 °C for next 24 h, till the constant dry weights achieved. The spectrophotometric estimations (UV-Vis 160 A, Shimadzu, Japan) of concentrations of proteins (Lowrey *et al.,* 11), free amino acids (Spies, 20), starch (Hodge and Hofreiter, 7), reducing sugars (Somogyi, 19) and total carbohydrates (Dubois *et al.,* 5) were done. Chlorophyll concentrations in fresh leaf tissue were estimated after dark extraction in acetone: water (80% v/v). The optical density was read on spectrophotometer and then used formulae as given by Witham *et al.* (25) for calculating the concentrations of chlorophyll components (a and b) and total chlorophyll. While sampling for chlorophyll estimations, fresh weights of unit leaf area were taken to compute the chlorophyll concentrations on area basis.

The 2-way ANOVA was done to find the significance of difference using SPSS software and critical differences (CD) were used to compare the means. Further, upon finding the significant differences, contrast analysis was done to find the significantly differing treatments using SPSS (ver. 10.0) package.

RESULTS AND DISCUSSION

Embryos of dwarfs (COD and MYD) germinated earlier than those of talls (LCT and WCT) and germination percentage on 60 days after inoculation was from 85.4 to 89%. Germination was maximum in MYD (~97%) followed by COD (92%), LCT (90%) and WCT (~77%). Germination and initial growth was more precocious in liquid media. However, vitrification was also more as compared to that in solid medium. On 120th day after inoculation, the shoot formation was almost similar on all media, even though the root formation was high in IRD medium. Significantly more vitrification was observed in liquid medium, it can be substituted with solid germinating medium to reduce vitrification thereby to increase the germinated embryo out turn for subsequent culturing.

Table 1. Different media compositions used for coconut embryo culture (mg/l).

Sl.No.	Chemical	Protocol			
		PCA-ARC	UPLB	CPCRI	IRD
		Y3	Y3	Y3	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, organics and growth regulators			
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	0.025
15.	$NaMoO_4.H_2O$	0.24	0.24	0.24	0.025
16.	$NiCl.6H_2O$	0.024	0.024	0.024	-
17.	$Fe_2(SO_4)_3.7H_2O$	13.9	41.7	13.9	24.9
18.	Na_2EDTA	37.3	55.8	37.3	26.1
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-pantpothenate	0.05	-	-	1.0
24.	Biotin	0.05	0.05	0.05	0.01
25.	Folic acid	-	0.05	-	-
26.	Glycine	-	1.0	2	-
27.	Na-ascorbate	-	-	-	100
28.	BAPκ	-	-	0.5	-
29.	NAAκ	-	-	0.5	-
30.	NAAκκ	-	-	1.0	-
31.	IBAκκ	7.0	-	5	-
32.	Activated charcoal	2.5 g/l	2.5 g/l	1 g/l	2 g/l
33.	Sucrose	45 g/l	60 g/l	60 g/l κ 30 g/l κκ	60 g/l
34.	pH	5.8	5.6	5.7	5.5
		State of medium			
	Germination medium (agar)	Liquid (-)	Liquid (-)	Solid (5.5 g/l)#	Liquid (-)
	Growth medium (agar)	Liquid (-)	Solid (7.0 g/l)	Solid (5.5 g/l)	Liquid (-)
	Rooting medium (agar)	Liquid (-)	Liquid (-)	Liquid (-)	Liquid (-)

κ Germinating medium, κκ Rooting medium, # - Values in parenthesis indicate the concentration of agar.

At the time of transfer from *in vitro* to *ex vitro* conditions for acclimatization, the morphological growth of plantlets was not significantly different due to change in medium. Generally, plantlets at this stage had two to three leaves of 12 to 17 cm length. However, plantlets grown on PCA-ARC medium were significantly taller than those grown on other media (Fig. 1). Development of root system also did not significantly vary due to change in medium. In general, 2 or 3 roots were seen at the time of transplantation to pots. Even though root length varied from 4 to 7 cm, the root volume was in the range of 3.1 to 5 cm^3. Collar girth, an important parameter for nursery grown seedlings for assessing the vigour, also did not vary significantly among the plantlets.

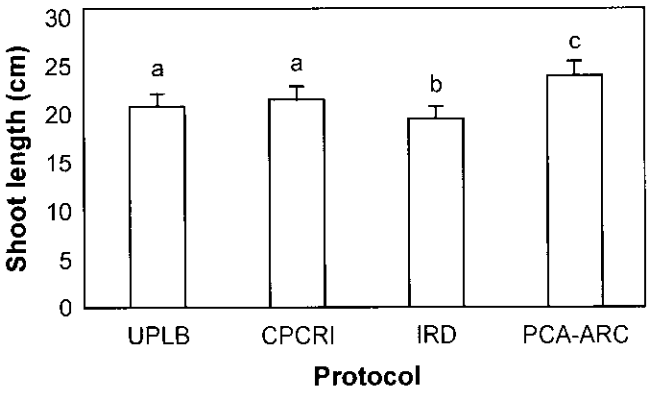

Fig. 1. Influence of culture media on shoot length of embryo-cultured coconut plantlets at the time of transfer from *in vitro* to ex *vitro* conditions. Contrast analysis indicates that bars with different alphabets are significantly differing. Bars with same alphabets indicate non-significant difference between those two.

Addition of sodium in medium seems to benefit the growth of plantlets as evident from better growth on three Y3 based media compared to MS based medium. Enhancement effects of Na^+ and Cl^- on coconut growth were reported (von Uexkul, 24; Magat and Margate, 12; Bopaiah *et al.*, 2). High concentrations of other salts in MS based protocol proved to be detrimental for *in vitro* plantlet growth. Addition of auxins in combination i.e., IBA and NAA, as done in CPCRI medium seems to have synergistic effect on growth and rooting of culture plants. Since survival percentage is positively correlated to shoot length and root volume in this study, inclusion of auxins, NAA and IBA should be advantageous for obtaining higher survival of plantlets. Presence of excess nitrogen in the form of nitrate as in IRD medium protocol seems to affect the growth of plantlets. Excess nitrogen in medium also causes competitive inhibition of uptake of other anions. Further, results indicate providing solid medium in beginning will ensure better growth and development of plantlet.

Composition of medium has significantly influenced the plantlet biochemical constituents (Table 2). Plantlets grown on CPCRI and IRD media had significantly higher leaf tissue concentrations of carbohydrates, starch and free amino acids, while those grown on UPLB and PCA-ARC media had significantly higher concentrations of reducing sugars and proteins. This may be due to increased protein synthesis with the availability of carbon source due to the presence of adequate sucrose in medium. Results indicate that the survival of plantlets is positively related to the tissue concentrations of reducing sugars, starch and proteins. Presence of high concentrations of starch in plantlets indicate their efficiency for organogenesis thus leading to better survival capacity.

Concentrations of leaf chlorophyll a and b were significantly high in plantlets raised on UPLB and IRD media (Table 3). Consequently, the concentration of chlorophyll, on the basis of leaf fresh weight and area, was high in plantlets grown on these two media. However, the ratio of chlorophyll a to b did not change significantly due to change in growth medium. Higher sugar concentrations in media positively influenced the development of chlorophyll in *in vitro* plants as also was reported earlier in coconut (Santamaria *et al.*, 18). Chlorophyll concentrations were high in tall cultivars and low in dwarf cultivars in all media. Higher concentrations of Mg as in IRD medium seems to favour chlorophyll synthesis since Mg is the main inorganic component of chlorophyll molecule. The data also indicate the positive effect of sucrose in medium on synthesis of chlorophyll.

The photosynthetic mechanism develops during the *in vitro* growth and acclimatization of coconut plantlets. *In vitro* cultured plantlets had low stomatal conductance (-0.005 $\mu mol/m^2/sec.$) and transpiration rates (-0.2 $mmol/m^2/sec.$). However, these did not vary significantly among treatments. Net photosynthetic rates (*Pn*) were negative in all the seedlings indicating that the plants were not autotropic (Fig. 2). Since in this experiment *Pn* rates were estimated at light levels nearer to light compensation point, i.e. - 60 $mol/m^2/sec.$ for C_3 plants, values near zero can be attributed for a developed photosynthetic system. At higher light levels, the *Pn* rates will increase as indicated in earlier experiments (Triques *et al.*, 21,22; Naresh Kumar *et al.*, 14). Apart from this, once plantlets are stopped from getting external carbon source through medium, the photosynthetic rates increase dramatically due to the absence of feedback inhibition by sucrose. Plantlets grown on UPLB and CPCRI media had near zero *Pn* rates indicating the developed photosynthesis system in these plantlets. The PCA-ARC grown plantlets, which had low chlorophyll concentrations also had significantly negative net photosynthesis. This indicates that photosynthesis system in these plantlets is still

Table 2. Leaf biochemical constituents of zygotic embryo-cultured plantlets of different coconut cultivars as influenced by the growth media (Mean values of observation taken on plantlets during transfer of plantlets from *in vitro* to *ex vitro* conditions for acclimatization).

Leaf biochemical constituent	Protocol	Cultivar				
		LCT	WCT	COD	MYD	Mean
Total carbohydrates (mg/g DW)	UPLB	116.1	163.8	94.6	128.8	125.8
	CPCRI	227.2	116.5	169.2	131.1	161.0
	IRD	125.7	161.9	141.7	206.0	158.8
	PCA-ARC	59.3	118.7	159.7	204.0	135.4
	Cultivar mean	132.1	140.2	141.3	167.5	145.3
CD for comparison the interaction : 21.3.						
CD for comparison factor means : 10.6.						
Free amino acids (mg/g DW)	UPLB	9.4	10.1	9.4	18.4	11.8
	CPCRI	15.2	20.1	16.0	13.5	16.2
	IRD	12.2	15.6	9.3	23.4	15.1
	PCA-ARC	10.1	9.2	17.1	15.9	13.1
	Cultivar mean	11.7	13.7	13.0	17.8	14.1
CD for comparison the interaction : 5.2.						
CD for comparison factor means : 2.6.						
Reducing sugars (mg/g DW)	UPLB	62.1	93.2	129.7	79.0	91.0
	CPCRI	53.7	56.0	59.7	40.5	52.5
	IRD	90.0	86.0	50.2	76.7	75.7
	PCA-ARC	78.3	147.5	53.3	116.3	98.9
	Cultivar mean	71.0	95.7	73.2	78.1	79.5
CD for comparison the interaction : 24.9.						
CD for comparison factor means : 12.5.						
Starch (mg/g DW)	UPLB	14.3	34.5	46.3	11.5	26.7
	CPCRI	13.7	48.0	25.5	39.7	31.7
	IRD	39.5	8.5	3.7	25.0	19.2
	PCA-ARC	5.4	67.5	3.2	19.9	24.0
	Cultivar mean	18.2	39.6	19.7	24.0	25.4
CD for comparison the interaction : 12.0.						
CD for comparison factor means : 6.0.						
Proteins (mg/g DW)	UPLB	138.5	179.0	174.4	159.0	162.7
	CPCRI	144.3	137.7	145.8	140.7	142.1
	IRD	152.0	136.0	119.2	146.3	138.4
	PCA-ARC	141.0	156.0	176.2	158.0	157.8
	Cultivar mean	143.9	152.2	153.9	151.0	150.3
CD for comparison the interaction : 18.1.						
CD for comparison factor means : 9.1.						
Cultivar, protocol and cultivar-by-protocol interaction effects were significant at 1%.						

under developed. Sucrose concentration in medium and the development of chlorophyll in leaf influenced the *Pn* rates. While higher concentrations of sucrose in PCA-ARC medium may provide early impetus for chlorophyll development in plantlets, decrease in sucrose concentrations in rooting medium may help in development of photosynthetic system (Rival *et al.*, 16; Kubota *et al.*, 10). Since higher concentration of sucrose in medium promotes the development of chlorophyll, it inhibits the development of RUBISCo and promotes

Table 3. Leaf chlorophyll components of zygotic embryo-cultured plantlets of different coconut cultivars as influenced by the growth medium (Mean values of observation taken on plantlets during transfer of plantlets from *in vitro* to *ex vitro* conditions for acclimatization).

Leaf chlorophyll component	Protocol	Cultivar				
		LCT	WCT	COD	MYD	Mean
Chlorophyll a (mg/g tissue FW)	UPLB	0.92	1.42	1.05	1.05	1.11
	CPCRI	1.20	1.43	0.93	0.65	1.05
	IRD	1.03	1.60	1.17	0.97	1.19
	PCA-ARC	1.18	1.19	0.81	0.53	0.93
	Mean	1.08	1.41	0.99	0.80	1.07
CD for comparison the interaction : 0.25. CD for comparison factor means : 0.13.						
Chlorophyll b (mg/g tissue FW)	UPLB	0.36	0.55	0.36	0.36	0.41
	CPCRI	0.43	0.54	0.34	0.26	0.39
	IRD	0.38	0.59	0.40	0.33	0.43
	PCA-ARC	0.43	0.46	0.30	0.18	0.34
	Mean	0.40	0.53	0.35	0.28	0.39
CD for comparison the interaction : 0.15. CD for comparison factor means: 0.08.						
Total chlorophyll (mg/g tissue FW)	UPLB	1.28	1.97	1.41	1.41	1.52
	CPCRI	1.63	1.97	1.27	0.91	1.44
	IRD	1.41	2.19	1.57	1.30	1.62
	PCA-ARC	1.61	1.65	1.11	0.71	1.27
	Mean	1.48	1.94	1.34	1.08	1.46
CD for comparison the interaction : 0.30. CD for comparison factor means : 0.15.						
Total chlorophyll on area basis (mg/cm^2 leaf area)	UPLB	0.90	1.39	1.00	0.99	1.07
	CPCRI	1.15	1.39	0.89	0.64	1.02
	IRD	1.00	1.55	1.10	0.92	1.14
	PCA-ARC	1.14	1.16	0.78	0.50	0.89
	Mean	1.05	1.37	0.94	0.76	1.03
CD for comparison the interaction: 0.12. CD for comparison factor means : 0.06.						
Chlorophyll a/b ratio	UPLB	2.56	2.59	2.95	2.94	2.76
	CPCRI	2.80	2.64	2.73	2.50	2.67
	IRD	2.72	2.72	2.96	2.94	2.84
	PCA-ARC	2.76	2.60	2.74	3.00	2.78
	Total	2.71	2.64	2.85	2.84	2.76
CD for comparison the interaction: NS; CD for comparison factor means : NS.						

PEPCo (Santamaria *et al.*, 18; Kozai, 8; Kozai *et al.*, 9; Desjardins, 4; van Huylenbroeck and Debergh, 23). The RUBISCo activity was low in *in vitro* coconut plants just before acclimatization (Triques *et al.*, 21). While the presence of high concentrations of sugars in growth medium not only inhibits the net photosynthetic rates, the osmotic potential of medium also is increased, thus possibly reducing the nutrient uptake by plantlets. Thus, a step-wise reduction in sugar concentrations in culture medium and gradual increase in PARs during *in vitro* growth may help in early establishment of photosynthetic mechanism in plantlets.

In general, plantlets raised on PCA-ARC and CPCRI media had higher survival rates followed by those raised on UPLB. The plantlets from IRD medium had lowest survival rates (Table 4). The loss of plantlets

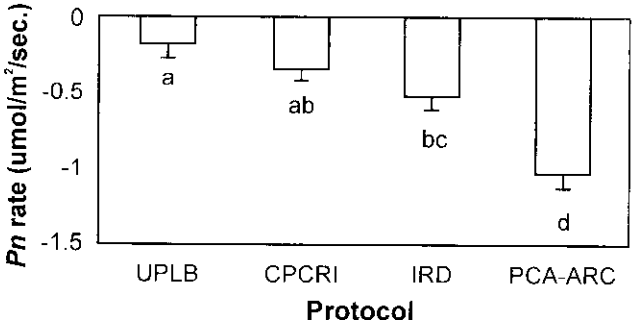

Fig. 2. Influence of culture media on net photosynthetic rates of embryo-cultured coconut plantlets at the time of transfer from *in vitro* to *ex vitro* conditions. Contrast analysis indicates that bars with different alphabets are significantly differing. Bars with same alphabets indicate non-significant difference between those two.

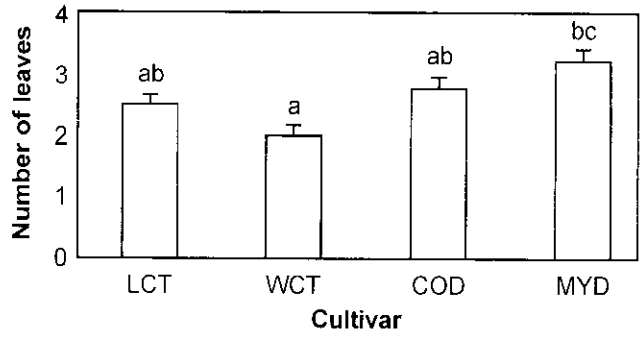

Fig. 3. Differences in number of leaves in embryo-cultured coconut plantlets of different cultivars at the time of transfer from *in vitro* to *ex vitro* conditions. Contrast analysis indicates that bars with different alphabets are significantly differing. Bars with same alphabets indicate non-significant difference between those two.

Table 4. Survival (%) of plantlets of four cultivars grown on different media (after 100 days of acclimatization, loss after this stage was negligible) in pots.

Protocol	Cultivar				
	LCT	WCT	COD	MYD	Mean
UPLB	41.0	57.0	56.0	51.0	51.3
CPCRI	56.0	65.0	64.0	39.0	56.0
IRD	38.0	32.0	41.0	49.0	40.0
PCA-ARC	68.0	62.3	45.0	56.0	57.8
Mean	50.8	54.1	51.5	48.8	51.3

CD for comparison the interaction: 0.52.
CD for comparison factor means: 0.26.
Values are significantly different at P = 0.05.

during *in vitro* was higher on IRD medium. Plantlets raised on this medium had low acclimatization capacity leading to low survival rates.

In general, WCT and LCT (talls) plantlets had significantly less number of leaves as compared to that in COD and MYD (dwarfs) (Fig. 3). However, all other morphological parameters did not differ significantly among the cultivars at the time of transfer of plantlets from *in vitro* to *ex vitro* conditions. The results indicate that plantlets grown on PCA medium, in general, had better morphological growth than those grown on other media, closely followed by those grown on UPLB, CPCRI and IRD media. The morphological growth of LCT and WCT plantlets was better when grown on either PCA-ARC or UPLB medium, while that of COD and MYD plantlets was better when grown on PCA-ARC and CPCRI media. The leaf biochemical composition significantly varied among the cultivars and was also influenced by the growth medium. The plantlets of MYD had significantly higher concentrations of total carbohydrates and free amino acids. While significantly higher concentrations of reducing sugars and starch were found in WCT plantlets and that of proteins was significantly higher in COD plantlets. The plantlets of LCT, a tall cultivar, had significantly higher concentrations of total carbohydrates, free amino acids and chlorophylls when grown on CPCRI or on PCA medium. The survival percentage of plantlets from these media in pot culture was also significantly more, indicating better suitability of these media for embryo culture of LCT cultivar.

The plantlets of WCT had significantly high concentrations of total carbohydrates and proteins (on UPLB medium), reducing sugars and starch concentrations (on PCA medium) and free amino acids (on CPCRI medium). Survival of WCT plantlets from these media was also significantly higher. As regards the dwarf cultivar COD, the plantlets grown on CPCRI medium had significantly higher concentrations of total carbohydrates and free amino acids in leaf tissue. The COD plantlets grown on UPLB medium had significantly higher concentrations of reducing sugars, starch, proteins and chlorophyll concentrations. The survival of COD plantlets in pots from these media was significantly high. The MYD seedlings had higher concentrations of reducing sugars when grown on PCA medium. The starch concentrations were significantly high in plantlets grown on CPCRI medium while that of proteins and chlorophylls was significantly high in those grown on UPLB medium. The survival of plantlets from UPLB and PCA-ARC media was significantly higher indicating their suitability for MYD embryo culture.

Overall results indicate that the survival percentage was more on CPCRI and PCA media. Plantlets of WCT and COD survived better when grown on CPCRI medium while the plantlets of LCT and MYD survived better when grown on PCA-ARC medium. Variations among cultivars for growth performance *in vitro* and

survival *ex vitro* depending on change in composition of medium indicate variability in response of talls and dwarfs to a given growth conditions. The talls are predominantly cross-pollinated, whereas the dwarfs are predominantly self-pollinated. Since larger variability can be expected predominantly in cross pollinated talls, higher survival rates of plantlets of tall cultivars on CPCRI and PCA-ARC media indicate the suitability of these media for wider range of cultivars. It indicates the possibility of development of media separately suitable for dwarfs and talls. However, a universal protocol is much desirable. The results indicate the possibility that CPCRI and PCA-ARC media composition and protocols can be slightly modified to get higher percentage of plantlet survival.

It can be concluded that the germination and initial growth was more precocious in liquid media. At the time of transplantation to pots for acclimatization, plantlets grown on PCA-ARC and CPCRI media had better morphological growth. Biochemical composition of leaflets and photo-autotrophy at the time of transferring to pots was influenced by the composition of medium. Results indicate that the survival of plantlets is positively related to the tissue concentrations of reducing sugars, starch and proteins. Higher sugar concentrations in media positively influenced the development of chlorophyll in *in vitro* plants, however *Pn* rates were inhibited. Plantlets at this stage are mostly dependent on medium for the supply of nutrients and carbon source. Overall results indicate that the survival percentage was more on CPCRI and PCA media. Plantlets of WCT and COD survived better when grown on CPCRI medium while the plantlets of LCT and MYD survived better when grown on PCA-ARC medium. It is suggested that the liquid geminating medium can be substituted with solid medium to reduce vitrification and to increase the germinated embryo outturn for subsequent culturing. Since survival percentage is positively correlated to shoot length and root volume in this study, inclusion of auxins, NAA and IBA, in growth medium should be advantageous for obtaining higher survival of plantlets. It is suggested that reduction of salt concentrations, addition of sodium and magnesium chloride in growth medium seem to benefit the growth and chlorophyll development in plantlets. A reduction in sugar concentrations in rooting medium and gradual increase in PARs during *in vitro* growth may help in early establishment of photosynthetic mechanism in plantlets. The above mentioned modifications in protocols of CPCRI and PCA-ARC are expected to further improve the survival rates of coconut embryo culture raised plantlets. Mainly solid germinating medium, reduction of sucrose concentrations in PCA-ARC rooting medium and addition of two auxins i.e., BAP and NAA in germinating and rooting media may help to increase the survival percentage of coconut plantlets during *in vitro* culture and also during *ex vitro* acclimatization.

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