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Effect of antioxidants on oil palm leaf *in vitro*

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.), an allogamous species belonging to coccoid group of palms, is an important source of edible oil, contributing about 16.5% of total world production of oil and fats. Large number of seedlings are required for expansion of area under oil palm cultivation, and large-scale propagation through tissue culture offers an alternative method for producing high-quality planting material. Efforts are on for several years to regenerate oil palm *in vitro* and several methods are available for its propagation through somatic embryogenesis (Jones, 8; Corley *et al.*, 3; Nwankwo and Krikorian, 14; Raju *et al.*, 15; Karun and Sajini, 9). However, much remains to be understood about the metabolic changes occurring in the tissue in response to media constituents. In the present experiment attempts were made to study the effect of different antioxidants, viz. activated charcoal, polyvinyl pyrollidone (PVP) and diethyl dithiocarbamic acid (DIECA); incubation time and media state (liquid vs solid) on metabolic changes occurring in the explant.

MATERIALS AND METHODS

Young leaves from *in vitro* plantlets, newly emerged leaves from 2-year-old net-house seedling and fully expanded leaf from field-grown adult oil palm were cut into approximately 1 cm long pieces and surface sterilized for 5 min. with 0.1% (w/v) mercuric chloride followed by several washes with sterile distilled water. The explant was cultured on liquid and solid half strength MS medium supplemented with activated charcoal (1 g/litre), PVP (100, 200 or 300 mg/litre) or DIECA (1 or 2 g/litre).Half strength MS medium without any antioxidant was used as the control medium. The media contained sucrose (30 g/litre) as the sole carbon source and the pH was adjusted to 5.8 prior to autoclaving. The tubes were incubated in dark at 27±2°C with 70-80% relative humidity. Then 1 g leaf per treatment was sampled at 24, 48, 72, 120 and 168 hr, ground in 5 ml of hot alcohol in pestle and mortar and centrifuged at 15,000 g for 10 min. The supernatant was used for colorimetric estimation of total phenol (Bray and Thorpe, 2) expressed as chlorogenic acid, orthodihydroxy phenol (OD phenol) (Mahadevan, 11) expressed as catechol, total sugar (Mahadevan and Sridhar, 12) with glucose as standard, and free amino acids (Moore and Stein, 13) with glycine as standard. The residue was oven dried, treated with trichloroacetic acid (TCA) and ether : ethanol (3:1) and used for protein estimation (Lowry *et al.*, 10) using crystalline bovine serum albumen as standard. Simultaneously 1 g leaf per treatment was oven dried and used for tannin estimation (Sadasivam and Manickam, 16). Liquid medium was used for estimation of leaching of phenol and OD phenol.

RESULTS AND DISCUSSION

The antioxidants did not significantly affect the endogenous levels of inhibitory metabolites, viz. phenol and OD phenol in the explant. How-

ever, OD phenol content was higher in DIECA-supplemented medium (Table 1). Similarly, PVP and DIECA reduced the phenol content more than AC and the control. These two metabolites especially phenols are believed to be responsible for browning and subsequent necrosis of the explant during tissue culture of perennial crops, and antioxidants such as activated charcoal (Areza *et al.*, 1) and PVP (Siuqeira and Inoue, 18; Sarathchandra *et al.*, 17) are widely used to overcome this problem. Activated charcoal is shown to adsorb inhibitory phenolic metabolites affecting morphogenesis (Fridborg *et al.*, 7), enhancing somatic embryogenesis and regeneration (Ziv and Godasi, 21), and *in vitro* rooting (Dumas and Monteuuis, 6). Similarly, PVP is also known to improve the response of the explant in repeated subcultures (Dalsaso and Guevara, 4; Dass *et al.*, 5) and *in vitro* rooting (Standardi and Romani, 19), probably by altering the phenolic and other inhibitory compounds, whereas DIECA when added in root induction media had adverse effect

Table 1. Effect of antioxidants on metabolite content of oilpalm leaf tissue *in vitro*.

Antioxidant	Content (mg/g FW)					
	Phenol	OD phenol	Total sugars	Free amino acid	Protein	Tannins
Activated charcoal (1 g/litre)	23.49	35.82	23.74	2.83	3.05	4.26
PVP (100 mg/litre)	19.99	37.62	21.59	2.44	2.98	4.67
PVP (200 mg/litre)	21.57	37.11	28.82	2.66	3.14	4.60
PVP (300 mg/litre)	20.42	34.08	26.29	3.44	3.26	4.95
DIECA (1 g/litre)	20.96	40.46	29.48	1.73	2.66	4.58
DIECA (2 g/litre)	20.05	47.35	25.23	1.71	2.72	3.87
Control	24.51	37.15	32.41	4.01	2.76	4.61
C.D. at 5%	NS	NS	NS	NS	NS	NS

Table 2. Effect of incubation time on metabolite content of oilpalm leaf tissue *in vitro*.

Incubation time (hr)	Content (mg/g FW)					
	Phenol	OD phenol	Total sugars	Free amino acid	Protein	Tannins
24	25.45	44.94	28.79	2.06	2.42	5.38
48	21.70	42.89	23.07	2.77	2.01	4.93
72	19.98	30.25	25.40	3.64	2.38	3.91
120	20.45	28.06	31.26	2.94	4.19	4.01
168	20.27	46.42	25.44	2.03	3.68	4.30
C.D. at 5%	NS	9.389***	NS	NS	0.757***	0.712**

**Significant at 1%, ***significant at 0.1%

Table 3. Effect of media, tissue type and interaction on metabolite content of oil palm leaf tissue *in vitro*.

Treatments	Content (mg/g FW)					
	Phenol	OD phenol	Total sugar	Free amino acid	Protein	Tannins
Type of medium						
Solid	22.51	36.73	19.14	3.63	2.78	4.58
Liquid	20.63	40.29	34.45	1.75	3.10	4.43
C.D. at 5%	NS	NS	4.885***	12.706**	NS	NS
Type of tissue						
In vitro	22.15	36.32	25.05	1.94	2.98	NE
Net house	20.67	39.19	41.69	5.31	2.01	3.38
Adult	21.89	40.03	13.64	0.82	3.80	5.62
C.D. at 5%	NS	NS	5.983	4.303***	0.586***	0.45***
Interactions						
Tissue × time						
S.Em.	3.015	5.803	4.774	1.174	0.467	0.357
C.D. at 5%	0.45**	16.26	13.38	NS	1.31***	1.01***
Antioxidant × tissue						
S.Em.	3.56	6.866	5.648	1.39	0.553	0.423
C.D. at 5%	NS	NS	15.83	NS	NS	1.19
Antioxidant × time						
S.Em	4.61	8.864	7.292	1.794	0.714	0.668
C.D. at 5%	NS	NS	NS	NS	NS	NS

**Significant at 1%, ***significant at 0.1%; NE = not estimated

on rooting in apple (Standardi and Romani, 19). Results from this experiment suggest that compounds other than phenols may also be involved in browning of tissue during initial stages of the culture. Other metabolities, viz. total sugar, amino acids and proteins, are also not affected by type and concentration of antioxidant (Table 1).

OD phenol, protein and tannin contents were significantly different with incubation time, whereas sugars and amino acids remained unaffected by the time interval (Table 2). OD phenol and tannin content decreased gradually up to 120 hr and then increased at 168 hr. Protein showed an increase at 120 hr after remaining constant up to 72 hr. Protein is an important metabolite and change in its content reflects change in metabolism. These results indicate that response of tissue to the media constituents may start at about 120 hr after inoculation resulting in metabolic changes.

Only total sugar and free amino acid contents differed significantly between solid and liquid media (Table 3). Total sugar was higher in the

explant inoculated in liquid meidum, whereas free amino acid content of the explant was more in solid medium. Since tissue was inoculated in high sucrose containing medium, liquid medium may facilitate faster absorption of sugar, resulting in higher content in liquid medium. As the availability of sugar and other nutrients is more in liquid medium, tissue seems to be metabolically more active, as indicated by low amino acid and higher protein content in this medium.

Tissue type had significant effect on total sugar, amino acid, protein and tannins (Table 3). However, phenol and OD phenol levels were not affected by the tissue type. Total sugar content in adult tissue was minimum, indicating that endogenous sugars are being utilized by the tissue during initial phase, resulting in reduction in its content, because adults have much higher levels of endogenous sugars, both reducing as well as non-reducing, compared with *in vitro* plantlets (Subronto *et al.*, 20). Absorption of sugar from the medium by net house and *in vitro* tissues for their utilisation may be responsible for the high content of total sugars in these tissues. Amino acid levels were maximum in net house and minimum in adult tissue, whereas the reverse was true for protein content. This may be due to high metabolic activity in field-grown adult tissue. Tannin, a secondary metabolite, was significantly higher in adult tissue. Interaction between tissue and incubation time was significant for all the metabolites except free amino acids (Table 3).

Table 4. Effect of antioxidants and tissue type on leaching of inhibitory metabolites from oil palm leaf tissue *in vitro*.

Treatments	Leaching (mg/g FW)	
	Phenol	OD phenol
Antioxidants		
Activated charcoal (1 g/litre)	5.53	8.32
PVP (100 mg/litre)	8.18	6.98
PVP (200 mg/litre)	10.25	6.30
PVP (300 mg/litre)	7.57	5.96
DIECA (1 g/litre)	8.25	5.83
DIECA (2 g/litre)	9.47	6.82
Control	8.44	6.52
S.Em.	1.348	0.857
C.D. at 5%	NS	NS
Type of tissue		
In vitro	10.55	9.0
Net house	5.88	7.21
Adult	8.29	3.80
S.Em.	0.883	0.561
C.D. at 5%	2.48*	1.579*

*Significant at 0.1%

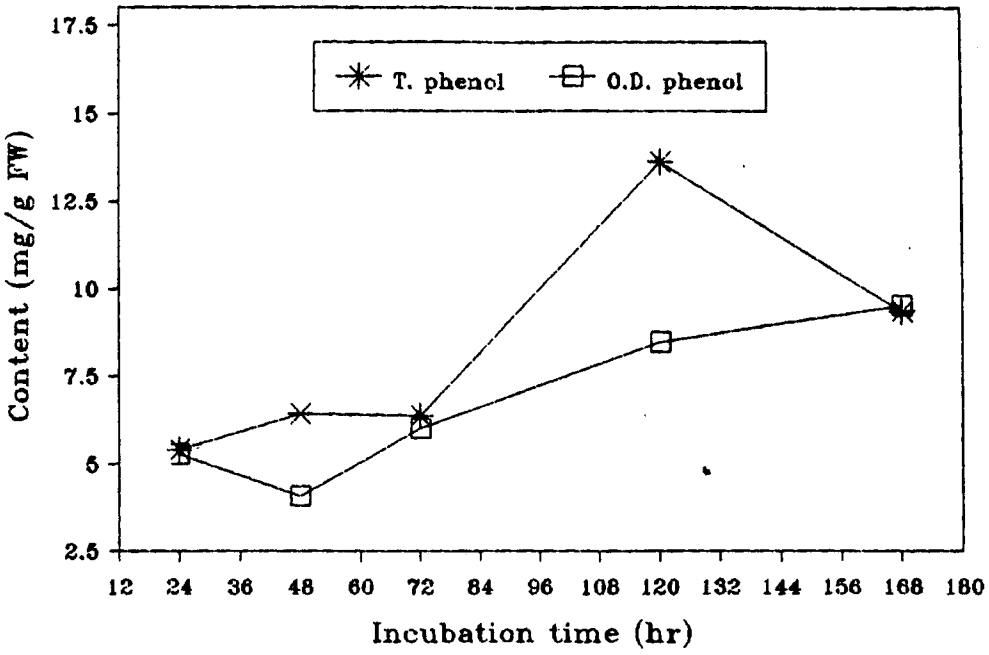

Fig. 1. Effect of incubation time on leaching of inhibitory metabolites from oil palm leaf *in vitro.*

Similarly, interaction of tissue and antioxidants had significant effect on total sugar and tannin content.

Although leaching of phenol and OD phenol into the liquid medium was not significantly influenced by the type and concentration of antioxidants, it was minimum in the medium supplemented with activated charcoal (Table 4). It may be due to better adsorption capability of activated charcoal (Fridborg *et al.,* 7) than of PVP and DIECA. Leaching was significantly different between tissue type (Table 4) and incubation time (Fig. 1). Maximum leaching of phenol and OD phenol was observed from *in vitro* and net house tissues respectively. Significant change in leaching of phenol and OD phenol was observed at 120 hr, once again indicating that response of tissue starts at that time.

SUMMARY

Biochemical changes in oil palm-leaf explant of three different stages, viz. *in vitro,* net house and adult at different periods and in different antioxidants were studied *in vitro.* Antioxidants didnot significantly influence the level of metabolites in the explant as well as their leaching in the medium. The content of phenols and OD phenols starts increasing 120 hr after inoculation. Total sugars were higher in the tissues cultured in liquid medium, whereas amino acid was high in solid medium. Antivated charcoal adsorbed phenolic leachates better in the liquid medium.

LITERATURE CITED

1. Areza, M.B.B., E.P. Rillo, M.B.F. Paloma, A.W. Ebert, C.A. Cueto and O.D. Orense (1993). Prevention of browning of coconut (*Cocos nucifera* L.) inflorescence and leaf tissues in *in vitro* cultures. *Phil. J. Coconut Stud.* **18**(2) : 20-3.

2. Bray, H.G. and W.V. Thorpe (1954). Analysis of phenolic compounds of interest in metabolism. *Methods Biochem. Analysis* **1**: 27-57.

3. Corley, R.H.V., J.N. Barrett and L.H. Jones (1977). Vegetative propagation of oil palm via tissue culture. *Oil Palm News* **22** : 2-7.

4. Dalsaso, L. and E. Guevara (1989). Tissue culture of avocado cv. Fuerte. *Agronomia Costariceanse* **13**(1): 61-71.

5. Dass, H.C., R.K. Kaul, S.P. Joshi and R.R. Bhansali (1989). *In vitro* regeneration of date palm plantlets. *Curr. Sci.* **58**(1): 22-4.

6. Dumas, E. and O. Monteuuis (1995). *In vitro* rooting of micropropagated shoots from juvenile and mature *Pinus pinaster* explants: influence of activated charcoal. *Plant Cell Tissue Organ Cult.* **40**: 231-5.

7. Fridborg, G., M. Pedersen, L.E. Landstrom and T. Eriksson (1978). The effect of activated charcoal on tissue culture: absorption of metabolites inhibiting morphogenesis. *Physiol. Pl.* **43**: 104-6.

8. Jones, L.H. (1974). Propagation of clonal oil palms by tissue culture. *Oil Palm News* **17**: 1-8.

9. Karun, A. and K.K. Sajini (1996). Plantlet regeneration from explants of oilpalm. *Curr. Sci.* **71**(11): 922-6.

10. Lowry, O.H., N.J. Rosebrough, A.L. Fan and R.J. Randall (1951). Protein measurement with the Folin- phenol reagent. *J. Biol. Chem.* **193**: 265-75.

11. Mahadevan, A. (1966). Biochemistry of infection and resistance. *Phytopath. Z.* **57**: 96-9.

12. Mahadevan, A. and R. Sridhar (1986). Estimation of total soluble sugars. (In) *Methods in Physiological Plant Pathology,* pp. 147-8.

13. Moore, S. and W.H. Stein (1948). Photometric method for use in the chromatography of amino acids. *J. Biol. Chem.* **176**: 367-88.

14. Nwankwo, B.A. and A.D. Krikorian (1983). Morphogenetic potential of embryo and seedling-derived callus of *Elaeis guineensis* Jacq. var. *pisifera* Becc. *Ann. Bot.* **51**: 65-76.

15. Raju, C.R., K.K. Sajini, S.M. Balachandran, K.V. Saji, K. Geetha Maheshan, P.E. Rajasekharan, L. Geetha and K.V.A. Bavappa (1988). Clonal multiplication of oilpalm (*Elaeis guineensis* Jacq.). *J. Plantation Crops* **16** (suppl.): 17-20.

16. Sadasivam, S. and A. Manickam (1992). (In) *Biochemical Methods for Agricultural Sciences,* 246 pp. Wiley Eastern Ltd. New Delhi.

17. Sarathchandra, T.M., P.D. Upali and P.V. Arulpragasam (1990). Progress towards the commercial propagation of tea by tissue culture techniques. *Sri Lanka J. Tea Sci.* **59** (2): 62-4.

18. Siqueira, E.R. de and M.T. Inoue (1991). Control of browning in coconut tissue culture. *Pesquisa Agropecuaria Brasileira* **26** (7): 949-53.

19. Standardi, A. and F. Romani (1990). Effect of some antioxidants on in vitro rooting of apple shoots. *Hort. Sci.* **25** (11): 1435-6.

20. Subronto, H., T., G. Ginting and Fatmawati (1995). Metabolite compsoition and isozyme variation between plantlets and their ortets in the oilpalm. (in) *Recent Development in Oilpalm Tissue Culture and Biotechnology,* pp. 195-204 (Rao, V., I.E. Henson and N. Rajanaidu, Eds.).

21. Ziv, M. and G. Godasi (1986). Enhanced embryogenesis and plant regeneration from cucumber (*Cucumis sativus* L.) callus by activated charcoal in solid/liquid double layer culture. *Pl. Sci.* **47**: 115-22.