

## EFFECT OF DIFFERENT NUTRIENT MEDIA AND 'ECOHUME' ON DIFFERENTIATION, PROLIFERATION AND GROWTH OF DENDROBIUM CV 'Jo MUTANT' PROTOCORM IN VITRO

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### Abstract

*In-vitro* micropropagation of protocorm of *Dendrobium* cv 'Jo Mutant' was investigated to standardize the media and to standardize the concentration of 'Ecohome' a commercial growth enhancer. The full strength Murashige and Skoog medium (MS) was best among the half strength MS medium, full strength Vacin and Went medium (VW) and half strength VW medium, in terms of number of shoots (3.40) and leaves (3.45) per culture. Among all the concentration used, the MS medium supplement with 0.25 per cent of 'Ecohome' was more effective in terms of mean number of shoots (2.26), leaves (3.46) and roots (2.22).

**Keywords:** *Dendrobium*; Humic acid; Ecohome; Protocorm like bodies; In-vitro; Orchids

### INTRODUCTION

Orchids consist of a number of ornamental species having global importance as cut flowers and pot plants. These are the most beautiful plants belonging to the family Orchidaceae. The numerical strength of orchids is estimated to be 25,000 to 35,000 species and 600 to 800 genera (Singh, 1997). The demand for their cut flowers increased rapidly over the year, because of higher number of flower per inflorescence and recurrent flowering. *Dendrobium* are known to be 'splendid' among the orchids and form the second largest and diverse genera in the orchid family, with about 1600 species and innumerable hybrids which have the ability to flower all through the year. *Dendrobium* remains as a favourite orchid of the cut flower industry.

Orchid is the first horticulture crop cloned by tissue culture method on a commercial Scale (Griesbach, 1986). Micropropagation of orchids has proven to be a constant means of rapid clonal propagation (Dhawan, 2002). Though *in vitro* propagation technique has

long been in vogue in *Dendrobium*, several factors are known to influence the per cent success, with special reference to shoot or root formation, as well as the performance of plantlet *in vitro*. The rate of growth of plantlets is extremely slow and the explants available per plant are also low.

In many orchid genera including, *Dendrobium* the *in vitro* development of protocorm like bodies is generally slow (Arditti and Ernst, 1993). Studies on rapid proliferation and differentiation from *in vitro* produced PLBs into plantlet have been very scanty when compared to the large size of the orchid family (Lakshmanan et al., 1995).

Hence the present investigation was taken up to standardize the media and to fine out the effect of 'Ecohome' on proliferation and growth of *Dendrobium* cv 'Jo Mutant' protocorm *in vitro*.

### MATERIALS AND METHODS

In the present investigation light greenish protocorms of *Dendrobium* cv 'Jo Mutant' was surface sterilized by dipping explants in 0.05 per cent of mercuric chloride (HgCl<sub>2</sub>) for the

period of 4-6 minutes, followed by 4-5 times washing in sterile distilled water under aseptic conditions inside laminar air flow cabinet. Though *in vitro* material was taken as explants, they were surface sterilized with Hgcl<sub>2</sub> as some bacterial growth was encountered in culture. The protocorms were inoculated into the medium with the aid of

sterile forceps. The full strength MS, half strength MS, full strength VW and half strength VW were supplemented with 30 gl<sup>-1</sup> sucrose, 8 gl<sup>-1</sup> agar and 1 mg l<sup>-1</sup> Benzyl amino purine (BAP). MS is supplemented with different concentration of 'Ecohme' (Table 2).

Table 1: Effect of different nutrient media on production of protocorms like bodies (PLBs) and shoots Dendrobium Cv 'Jo Mutant'

Media	120 days after inoculation		
	Mean number of PLBs per culture	Mean number of shoots per culture	Mean number of leaves per culture
MS	10.20	3.40	3.45
VW	20.40	3.30	2.62
½ MS	9.40	2.26	1.80
½ VW	9.80	2.12	1.90
F-test	**	**	**
SEm	1.67	0.20	0.21
CD @ 5%	5.01	0.59	0.61

[MS: Murashige and Skoog medium, VW= Vacin and Went medium, 1/2: Half strength]

Table 2: Effect of different concentration of 'Ecohme' on shoot in Dendrobium cv 'Jo Mutant'

Media (MS+ Ecohme)	120 days after inoculation		
	Mean number of shoots per culture	Mean number of leaves per culture	Mean number of roots per culture
Plain MS	1.12	1.94	0.96
MS+ Ecohme (0.25%)	2.26	3.46	2.22
MS+ Ecohme (0.25%)	1.82	2.68	1.36
MS+ Ecohme (0.25%)	1.22	2.04	1.20
MS+ Ecohme (0.25%)	1.20	2.01	0.90
MS+ Ecohme (0.25%)	1.00	1.62	0.42
F-test	**	**	**
SEm	0.12	0.10	0.11
CD @ 5%	0.36	0.29	0.34

[MS: Murashige and Skoog medium, \*\* Indicates significant at 1 per cent]

The inoculated culture bottles were transferred to a culture room having temperature of 24±2 °C. Light intensity of 2000 lux was supplied using white fluorescence tubes for 16 hours photoperiod and 8 hours dark period. The mean number of PLBs, shoots, leaves and roots were recorded

120 days following inoculation. The experiment was laid out using completely randomized block design for each treatment five replicates were used. The data was recorded and analyzed using ANOVA.

## RESULTS AND DISCUSSION

The protocorms as explants when cultured in full strength MS medium resulted in maximum mean number of shoots (3.40) per culture as compared with other media. Full strength MS medium was found the best medium for multiple shoot production in *Dendrobium* cultures Gandawidjaja (1980), Shimasaki and Uemoto (1987), Sharma and Tandon (1990). The full strength VW medium was found to have a better influence on the mean number of PLBs (20.40) production per culture. The full strength VW medium was suggested to be good for culturing of different species of *Dendrobium* by many investigators (Kim et al., 1970; Fernando, 1979). The full strength MS medium recorded the maximum mean number of leaves (3.54) per culture. This could be due to the higher concentration of salts and nutrients in the full strength MS medium (Fu, 1978; Ganga et al., 1999).

Ecohume is a commercial product manufactured by Ajinkya Chemtech Private Limited, Bangalore, India. Ecohume is a product extracted from biologically processed vegetable refuse. It contains humic acid 6 per cent (w/w), Soluble energies 4 per cent (w/w) and aqua base 90 per cent (w/w). The presence of humic substances accelerates plant metabolic processes and enhance the nutrients uptake, helps in overcoming moisture stress, increases yields parameters thus increasing crop yields (Sanderson and Jameson, 1986, Liu et al., 1998).

In the present study among the different concentration of 'Ecohume used 0.25 per cent was found best in terms of means of number of shoots (2.26), leaves (3.64) and roots (2.220) per culture obtained. Humic acid content small but identifiable amount of auxins, cytokinins and antioxidant, they have an influence on accelerating plant metabolism. The positive influence of humic acid in *Dendrobium* tissue

culture could be owed to these properties of humic acid. (Sanderson and Jameson, 1986, Crouch et al., 1992, Zhang and Ervan, 2004).

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