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EFFECT OF ORGANIC ADDITIVES ON IN VITRO PROLIFERATION, GROWTH AND DEVELOPMENT OF DENDROBIUM CV 'JO MUTANT'

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Abstract

Dendrobiums are known to be splendid among the orchids and enjoy a great demand in the international market. *In-vitro* micro-propagation of Dendrobium cv 'Jo Mutant' was investigated to enhance the proliferation, growth and development of protocorms into plantlets by using different types of organic additives in the MS basal medium. The medium supplemented with l5 per cent of coconut water (CW) was found most effective and enhance the mean number of shoots, roots and shoots length, which was followed by 15 per cent green gram and l5 per cent tomato.

Keywords: *Dendrobium*; Protocorm like bodies; In-vitro propagation, Orchids

INTRODUCTION

Orchids are the most beautiful plants belonging to the family Orchidaceae. They are botanically the most advanced types among the flowering plants, highly specialized in many ways. The numerical strength of orchids is estimated to be 25,000 to 35,000 species and 600 to 800 genera (Singh, 1997). Orchid is the first horticulture crop cloned by tissue culture method on a commercial scale (Grieshbach, 1986). *Dendrobium* is known to be 'Splendid' among the orchids and from 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. The delicate and beautiful flower of *Dendrobium* has not been demand in the world orchids market.

In most of the orchids, the propagation by seed has not been successful due to its minute size, heterozygous nature and presence of little endosperm and requirements of association with Fungi (Prasad et al., 2001). Multiplication of the orchids through convention means is very slow, expensive and consumes years to develop an elite clone.

Micropropagation of orchids has proven to be a constant means of rapid clonal Propagation. In tissue culture techniques especially meristem culture and axillary bud culture (Goh, 1989) provide a solution for the problem of sexual propagation in *Dendrobium* and serve as a means of producing a large number protocorm like bodies (PLBs) which subsequently develop into plantlet. 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 are generally slow (Arditti and Ernst, 1993). Studies on rapid proliferation and differentiation from in vitro produced PLBs into plantlets by using organic additives have been very scanty when compared to work done by organic substance (Lakshmanan et al., 1995). Hence, the present investigation was to know the effect of organic additives on in vitro growth and development of protocorms.

MATERIALS AND METHODS

The protocorms of in vitro culture Dendrobium cv 'Jo Mutant' maintained at the plant Tissue Culture Laboratory were used as an explants to study the effect of organic additives. The surface sterilization was carried

out under aseptic conditions inside laminar air flow cabinet by dipping explants in 0.05 per cent of mercuric chloride for the period of 4-6 minutes, followed by 4-5 times washing in sterile double distilled water.

Bigger and healthy protocorms were inoculated into the medium with the aid of sterile forceps.

The different adjuvant used were coconut water, extracts of tomato and carambola fruits, germinating ragi, wheat, green gram and black gram seed (Table 1).

Tender coconut was used for obtaining liquid endosperm. Freshly obtained tomatoes and carambolas were cut into small pieces, weighed, and ground separately in a mixer with required quantity of double distilled water. Bulk volume were collected filtered through cheesecloth, then through filter paper (Watman Filter Paper), distributed in small aliquots, and frozen until needed. The germinated seeds of green gram, black gram, ragi, and wheat were grinded and these materials are filtered through filter paper.

The inoculated culture bottles were transferred to a culture room having temperate 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 shoots, shoots length, leaves and roots were recorded 120 days following inoculation. The data recorded were analyzed with completely randomized block design (CRD) with five replicates for each treatment.

RESULTS AND DISCUSSION

Among the different organic additives viz., coconut water, tomato and carambola fruit extracts, germinated ragi, wheat, green gram and black gram seed extracts, the Murashige and Skoog medium supplemented with 15 per cent of CW was found to be superior over all other Treatment, which was followed, by 15 per cent of green gram and tomato in all the characters except in the case of mean number of leaves per culture which was slightly lower than green gram (Table 1).

Coconut water the mean number of shoots per culture recorded were maximum in both the concentrations, at 5% (3.64) and,l5% (3.78) that was followed by l5% of tomato (3.30) and green gram (3.24) extracts.

In case of mean number of leaves per culture the maximum was recorded at the medium supplemented with l5% green gram (3.32) that was followed by 15 % of CW (3.26) and l5 % tomato (3.21). The maximum mean shoots length was recorded at 15 % CW (2.72), which was followed by 15 % green gram (2.60).

But in the case of mean number of root per culture the maximum was recorded at both the concentration of CW at 5 % (3.46) and l5 % (4.06).

The simulative effect of coconut water might be due to its growth regulator contents (cytokinins). It is commonly added to stimulate the callus or protocorm formation (Goh et al., 1975). Badge and Sharon (1997) observed the best PLBs formation and proliferation when the basal orchid medium is supplemented with l5 per cent coconut water. The influences of CW on shoots and leaves formation was also reported by Kerbary (1984) and Peak et al., (1998). It is probable that the gibberellins-like substances present in CW (George, 1993) may have promoted the elongation of shoots. It is probable that the increased auxin activity in CW as suggested by George (1993) may have promoted the root initiation and elongation. Moral (1964) reported that tomato juice extract was helpful in the subsequent growth especially after the division of protocorms. The beneficial effect of tomato juice could be due to the presence of

specific unidentified substances (Aditti, 1966) Agrawal et al., (1992) obtained rapid proliferation of multiple shoots in vanilla walkkeriae, when MS medium was supplemented with casein (CH). CH is a non-specific organic nitrogen source and act as an amino acid supplement (Skoog and Millar, l957). Peptone is an amino acid that stimulates meristemetic tissue growth and shoot formation.

Table 1: Effect of organic additives on shoot formation in Dendrobium cv 'Jo Mutant'

Media (MS+ Adjuvant, in per cent)	120 days after inoculation			
	Mean number of shoots per	Mean number of leaves per culture	Mean shoots length per culture (cm)	Mean number of roots per culture
Plain MS	1.40	1.51	1.42	2.38
MS+ CW (5%)	3.64	2.86	2.0	3.46
MS+ CW (15%)	3.78	3.26	2.72	4.06
MS+ Tomato (5%)	2.62	2.81	1.74	2.76
MS+ Tomato (15%)	3.30	3.21	2.28	2.84
MS+ Carambola (5%)	1.86	1.65	1.06	1.08
MS+ Carambola (15%)	1.82	1.47	1.22	1.18
MS+ Ragi (5%)	1.28	1.32	1.08	1.00
MS+ Ragi (15%)	1.30	1.47	1.28	1.28
MS+ Wheat (5%)	1.14	1.89	1.16	1.00
MS+ Wheat (15%)	1.26	1.98	1.38	1.26
MS+ Green Gram (5%)	2.88	3.04	2.22	1.54
MS+ Green Gram (15%)	3.24	3.42	2.60	1.88
MS+ Black Gram (5%)	1.90	1.62	1.14	1.42
MS+ Black Gram (15%)	2.18	1.82	1.28	1.68
F.Test	**	**	**	**
SE m	0.19	0.13	0.11	0.15
CD @ 5%	055	037	0.31	0.43

[MS: Murashige and Skoog medium; DAI= Days after inoculation; ** indicates significance at 1 per cent]

In case of orchids, the best regeneration and organogenesis were noted on MS medium supplemented with peptone (Kukulczanka et al., 1989).

In the present study also the green gram was significantly superior over than control (basal MS medium), since this could be the presence of amino acids (64.04 per cent protein), nitrogen (Mesallam and Hamza, 1987) and vitamins specially vitamin C in germinated green gram seed which supplemented the growth and development of protocorms.

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