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BRIEF COMMUNICATION

Photoautotrophic *in vitro* multiplication of the orchid *Dendrobium* under CO_2 enrichment

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Abstract

An attempt to reduce the production cost on tissue cultured plants, photoautotrophic culture of a high value orchid *Dendrobium* was established under CO_2-enriched conditions. The shoot length and the number of leaves were almost equal in plantlets grown on medium with 2 % sucrose or without sucrose and under normal or enhanced (40 g m^{-3}) CO_2 concentration, whereas the fresh and dry masses were higher in cultures grown in sucrose containing media or under CO_2 enrichment. Development of roots was observed only on media without sucrose, but CO_2 enrichment did not have significant effects on *in vitro* rootings.

Additional key words: growth parameters, micropropagation, sugar-free medium.

Micropropagation is a challenging and exciting area in commercial horticulture, that has the advantage of rapid production of genetically identical and physiologically uniform plantlets over conventional vegetative propagation. However, its widespread commercial use is still restricted because of its relatively high production cost. Media cost (mainly sucrose), and losses due to biological contamination and low survival rates during acclimatization are some of the major reasons for high cost of tissue cultured plants (Mitra 1994).

Plantlets grown *in vitro* have often been considered to have a low photosynthetic ability to provide a positive carbon balance, and therefore require sugars and grow in

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a mixotrophic mode using both CO_2 from air and organic carbon source from the medium (Kozai 1988). These cultures not only involve an additional expenditure on the carbon source (usually sucrose), but also invite bacterial and fungal contaminations (Jeong *et al.* 1995). In addition, these mixotrophic plantlets need to be acclimatized to photoautotrophy after transplanting into natural conditions. On the other hand, sugar-free medium eliminates the risk of biological contamination and offers additional advantage in generating photoautotrophic cultures which are amenable to large scale culture techniques (Sharma 1992). Photoautotrophic cultures use CO_2 as their sole carbon source like higher plants. Environmental factors such as irradiance, CO_2 concentration, temperature, humidity play the major role towards the development of photoautotrophism in plantlets grown without sugar in the media (for review see *e.g.* Kozai 1991, Buddendorf-Joosten and Woltering 1994, Pospíšilová *et al.* 1997). The present report concerns the comparison of *in vitro* growth and multiplication of a highly valued orchid, *Dendrobium* under photomixotrophic and photoautotrophic conditions.

In vitro grown shoots of *Dendrobium* (kindly provided by the A.V. Thomas & Co. Ltd., Cochin for this study) were cultured on Vacin and Went (1949) medium without or with 2 % sucrose at ambient air or CO_2-enriched (40 g m^{-3}) atmosphere as essentially described by Mitra *et al.* (1997). All cultures were incubated for 30 d at the growth chamber (temperature 25 ± 3 °C, relative humidity 60 - 70 %, irradiance 42 - 45 μmol m^{-2} s^{-1}, 16-h photoperiod). Each treatment had five culture vessels and each glass jar (250 cm^3) with a plastic screw cap contained five shoots. To increase the number of air exchanges per hour, the vessel cap was punctured (hole 0.8 cm^2) and a gas permeable cotton wool filter was attached.

Table 1. Growth parameters of *Dendrobium* after 4 weeks on medium with 0 or 2 % sucrose and under ambient or enriched CO_2 concentration [0.6 or 40 g (CO_2) m^{-3}]. Means of 5 replicates ± SD.

Growth parameter	Initial explant	0.6 g(CO_2) m^{-3} 2 %	0 %	40 g(CO_2) m^{-3} 2 %	0 %
Shoot length [cm]	1.2 ± 0.01	1.4 ± 0.15	1.5 ± 0.19	1.4 ± 0.35	1.7 ± 0.25
Number of leaves	5 ± 0.8	6 ± 0.7	5 ± 0.85	5 ± 0.54	6 ± 0.7
Leaf length [cm]	1.1 ± 0.1	1.1 ± 0.15	1.1 ± 0.22	1.4 ± 0.15	1.1 ± 0.15
Leaf width [cm]	0.3 ± 0.01	0.5 ± 0.02	0.3 ± 0.02	0.3 ± 0.03	0.4 ± 0.01
Number of branches	1 ± 0.15	1 ± 0.15	1 ± 0.15	1 ± 0.15	1 ± 0.15
Number of roots	-	-	2 ± 0.7	-	2 ± 0.7
Root length [cm]	-	-	0.3 ± 0.02	-	0.2 ± 0.01
Fresh mass [mg]	71 ± 2.9	264 ± 5.17	99 ± 2.91	284 ± 4.47	190 ± 3.16
Dry mass [mg]	4.9 ± 0.1	13 ± 1.58	4 ± 0.7	12 ± 1.58	8 ± 0.7

The shoot length and number of leaves were almost equal under all culture conditions. Plantlets grown on medium with sucrose had higher total fresh mass and total dry mass than those grown on sugar-free medium. Also Kirdmanee *et al.* (1992) found higher total fresh mass in plantlets grown on sucrose-containing media. The

dry mass of the plantlets using both CO_2 and sucrose as carbon and energy sources produced more leaf area and accumulated more dry matter than plantlets grown strictly photoautotrophically as was observed earlier on tobacco plantlets (Tichá 1996).

Also CO_2-enriched air almost always had positive influence on the growth and morphogenesis (Table 1). The results agree with the observation on other plants like carnation (Kozai 1991), *Cymbidium* (Kirdmanee *et al.* 1992), and strawberry (Deng and Donnelly 1993). Under CO_2-enriched atmosphere, the CO_2 in the vessel during the light period was never depleted to concentrations lower than those saturating plantlet photosynthesis (Solárová *et al.* 1989). Therefore increase in biomass accumulation in photoautotrophic cultivation was mostly a consequence of increased photosynthetic rate of plantlet *in situ* due to control of environmental conditions (Solárová and Pospíšilová 1997).

However, also under elevated CO_2 concentration, growth parameters are higher on the medium with 2 % sucrose than on the medium without sucrose. Similar findings were reported earlier for other plantlets like *Dianthus*, *Nicotiana* and *Solanum* (Pospíšilová *et al.* 1997). Nevertheless the photoautotrophic cultivation minimise the risk of losses due to contamination, reduce the cultivation cost, enable better acclimatisation under *ex vitro* conditions (Kozai 1991).

Response in growth and biomass accumulation of *in vitro* grown shoots of *Dendrobium* under photoautotrophic condition were comparable to those grown under photomixotrophic condition. Development of roots was observed only on media without sucrose, but CO_2 enrichment did not have significant effects on *in vitro* rootings. Since for commercial micropropagation the length and number of shoots are more important than dry mass, the photoautotrophic culture under sugar-free medium in combination with CO_2 enrichment holds promise.

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