Preservation of the brazilian orchid *Cattleya walkeriana* Gardner using *in vitro* propagation

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ABSTRACT

Cattleya walkeriana is found in different regions of Brazil growing on rocks or trees in areas near lakes, rivers and swamps. This orchid is appreciated by orchid lovers because of its beautiful flowers and is in risk of becoming extinct due to predatory collection and habitat destruction. Murashige and Shoog culture medium modified with half the concentration of macronutrients supplemented with 1, 2, 4 and 6 g/L activated charcoal was used for cultivation, having an overall pH 6.0. Five approximately 1.0 cm high *C. walkeriana* plantlets were inoculated in each flask. A randomized complete block design was used with 45 replications per treatment. Seven months after the start of the experiment the canopy and largest root length, number of roots and shoots and fresh matter weight of the plantlets were assessed. *Cattleya walkeriana* plantlet development increased with elevated activated charcoal concentration and with greater cultivation time. The results obtained in this experiment enabled us to conclude that the addition of 2 g/L activated charcoal to culture medium were beneficial for *in vitro Cattleya walkeriana* propagation.

KEY WORDS: Orchidaceae, tissue culture, culture medium, activated charcoal.

INTRODUCTION

The *Cattleya* genus is found in Mexico and Central America, along the slopes of the Andes and in Colombia and Venezuela (Miller and Warren, 1996). *Cattleya walkeriana* Gardner, an orchid species known throughout the world and appreciated for its hybrids (Houch and Mendes, 1999) is found in Brazil stretching from the Amazon region through Mato Grosso state and the Mata Atlantica (Atlantic Forest) to Rio Grande do Sul state (Miller and Warren, 1996). It is found in various habitats, growing on rocks or trees, in areas close to lakes, river or swamps (Houch and Mendes, 1999).

The culture medium formula is essential for *in vitro* propagation as it supplies the nutrients necessary for plant development and should be mixed according to the requirements of each species. Several authors have reported the use of activated charcoal in culture medium. According to Shi et al. (2000) supplementing culture medium with activated charcoal improved the growth conditions of *Dendrobium officinale* plantlets. George and Ravishanker (1997) stated that activated charcoal induced roots in *Vanilla planifolia* and better rooting was observed in M&S culture medium with half the macronutrients supplemented with 0.2 % activated charcoal.

Vij et al. (1994) also observed that root development of *Cymbidium pendulum* Roxb. was improved with the addition of activated charcoal. The addition of 1.0 mg activated charcoal/liter to modified Murashige and Skoog culture medium promoted *Cymbidium lancifolium* rhizome growth and organogenesis (Kim and Lee, 1992). *In vitro* shoot rooting of *Diuris longifolia* R. Br. was improved with an increase in saccharose concentration to 40g/liter or by the addition of 0.05% activated charcoal to the culture medium (Collins and Dixon, 1992). When transferred to a medium containing activated charcoal, the plantlets grew well and rooted (Choi and Chung, 1989). Activated charcoal proved beneficial in micropropagation of *Dendrobium chrysanthum* Wall., maintaining considerable growth in *in vitro* plantlets (Vij and Promila, 1989).

The objective of the present study was to assess the effect of adding different concentrations of activated charcoal to culture medium for *in vitro* propagation of the Brazilian orchid *Cattleya walkeriana*.

MATERIAL AND METHODS

Cattleya walkeriana seeds, obtained from flowers artificially pollinated in a greenhouse, were germinated in M & S culture medium (Murashige and Skoog, 1962), modified to half the concentration of macronutrients. Plantlets approximately 1.0 cm tall were selected and subcultivated to the same culture medium supplemented with 0, 1, 2, 4 and 6 g/L activated charcoal and having the pH adjusted to 6.0 with the addition of potassium hypo chloride. The culture media were distributed in glass flasks and sterilized in autoclave at 120 ºC, 1 atm for 20 minutes. The experiment was kept in a growth chamber with 16 hours light at 26 ºC.

A randomized complete block design was used, with 45 replications per treatment. The canopy length was measured after 30, 150 and 210 days from the start of the experiment. At the last assessment, the greatest root length, number of roots, number of shoots and plantlet fresh matter weight data were collected. The canopy length data was assessed using linear regression analysis. The data for greatest root, number of roots, number of shoots and total fresh plantlet weight were submitted to analysis of variance using the Tukey test at the 5 % level of significance.

RESULTS AND DISCUSSION

The treatments supplemented with 4 and 6 g/L activated charcoal did not gel and prevented plantlet inoculation and thus they were discarded from the experiment. Druart and Wulf (1993) found that saccharin hydrolysis, which normally reaches 10% during autoclaving, increased to 95 % in the presence of 1 % activated charcoal. This acidifies the solution because of a specific reaction in fructose formation. The change in the first source of carbon available from saccharine to a fructose mixture increased osmosis and prevented the agar from gelling. *C. walkeriana* plant development increased with the increase in the activated charcoal concentration and cultivation time (Figure 1).

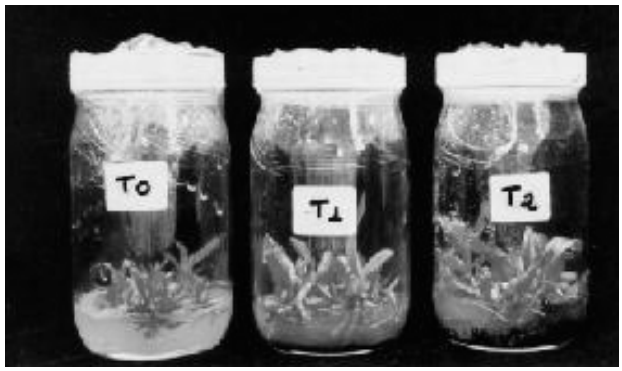

Figure 1. Plantlets growth of the *Cattleya walkeriana* orchid developed in M&S medium supplemented with activated charcoal different concentrations: T_0= without activated charcoal; T_1=1 g / L e T_2=2 g/L, after 210 days of the experiment beginning.

The regression study showed a direct relationship between the increasing doses of activated charcoal and increase in canopy length (Figure 2). The analysis of variance (Table 1) showed that the treatment without activated charcoal differed significantly from the treatments 1 and 2 g/L for the variables greatest root length (2.14, 3.12 and 33.52 cm, respectively) and total fresh plantlet weight (0.71, 1.58 and 1.54g, respectively).

Pan and Staden (1998) concluded that the addition of activated charcoal to culture medium can influence plant development positively or negatively, especially rooting, shoot growth and embryogenesis, darkening the substrate and adsorbing substances that are

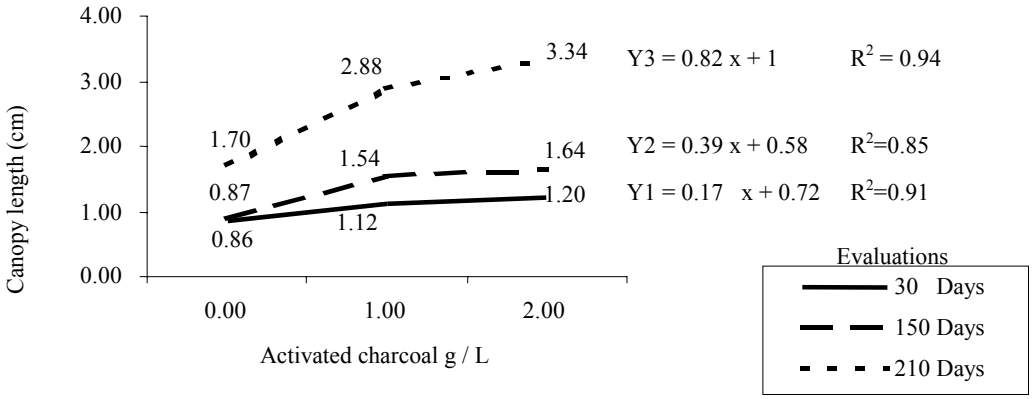

Figure 2. Mean values for canopy length of *Cattleya walkeriana* plantlets developed in M&S medium supplemented with activated charcoal on three assessments, after 210 days of the experiment beginning.

Table 1. Mean values for canopy length, greatest root length, total fresh plantlet weight, number of shoots and number of roots of the *Cattleya walkeriana* orchid developed in M&S medium supplemented with activated charcoal different concentrations, after 210 days of the experiment beginning.

Activated charcoal (g/L)	Canopy length (cm)	Greatest root length (cm)	Fresh weight (g)	Number of shoots	Number of roots
0	1.70[1/] b[3/]	2.14[1/] b	0.71[1/] b	5.36[2/] a	12.57[2/] a
1	2.88 b	3.12 a	1.58 a	6.00 a	14.69 a
2	3.34 a	3.51 a	1.54 a	4.71 a	15.43 a
CV (%)	10.25	18.59	28.81	14.37	11.41

[1/]Original data are averages of 45 replications; [2/] Data was transformed in $\sqrt{x+0.5}$ for the statistical analysis; [3/] Means followed by the same letter, in the same column did not differ at the 5 % level of probability by the Tukey test.

nutritive or toxic to the plant and acting as an ion collector. Druart and De Wulf (1993) attributed the frequent *in vitro* growth and organogenesis of plant tissues to aborption of components from the culture medium and/or the atmosphere of the cultivation recipient. Gangaprasad et al. (2000) obtained 4 to 6 cm sprout rooting in medium containing NAA (2.7 mm) and activated charcoal (0.2 %). According to Paek et al. (1998) activated charcoal was necessary for healthy plantlet production of *Cymbidium* species and to stimulate shoot growth at the level between 0.1-0.3 % but concomitantly, the rhizome growth decreased.

The best response for *in vitro* propagation of *Dendrobium lindleyi* Steud. was reported in M&S culture supplemented with NAA (2 mg/mL), IBA (1 mg/mL) and quinetine (1 mg/mL) in the presence of 0.2% activated charcoal (Satinder et al., 1997). Seeni and Latha (1992) obtained immediate rooting in shoots of *Renanthera imschootiana* Rolfe transferred to culture medium containing 4.4 ¼M AB, 10 ¼M NAA and 1% activated charcoal. Rhizome growth and organogenesis of *Cymbidium lancifolium* were promoted with the addition of 1.0 mg of activated charcoal/liter of modified M&S culture medium (Kim and Lee, 1992). The activated charcoal promoted rhizome growth *C. forrestii*, but inhibited shoot formation (Paek and Yeung, 1991).

No significant differences were observed for the number of shoots and roots among the treatments with mean sprouting number equal to 5.36 ± 0.65 and roots 14.69 ± 2.2 (Table 1). In a similar experiment, Collins and Dixon (1992) obtained better results for root formation of *Diuris longifolia* in treatments with saccharine and added 0.05 % activated charcoal.

CONCLUSIONS

The results obtained in this experiment enabled us to conclude that the addition of 2 g/L activated charcoal to culture medium were beneficial for *in vitro Cattleya walkeriana* propagation.

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RESUMO

Preservação da orquídea brasileira *Cattleya walkeriana* Gardner usando a propagação *in vitro*

Cattleya walkeriana é encontrada em diferentes regiões do Brasil, crescendo sobre pedras ou árvores, em áreas próximas a lagos, rios e pântanos. Esta orquídea devido à beleza de suas flores é muito cobiçada pelos orquidófilos e está correndo risco de extinção devido às coletas predatórias e destruição de seu hábitat. O meio nutritivo utilizado foi o Murashige and Shoog (M&S, 1962) modificado com a metade da concentração dos macronutrients suplementado com 1, 2, 4 e 6 g/L de carvão ativado e pH 6,0. Foram inoculadas cinco plântulas de *C. walkeriana* com, aproximadamente, 1,0 cm de altura em cada frasco. O delineamento experimental utilizado foi o inteiramente casualizado, com 45 repetições por tratamento. Sete meses após o início do experimento foram realizadas as avaliações do comprimento da parte aérea e da maior raiz, número de raízes e de brotações e peso fresco das plântulas. O desenvolvimento vegetativo

das plântulas de *C. walkeriana* aumentou com o incremento da concentração de carvão ativado e com tempo de cultivo. Os resultados obtidos neste experimento nos permitem concluir que a adição de 2 g/L de carvão ativado ao meio de cultura foi benéfica para a propagação de *Cattleya walkeriana in vitro*.

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