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Effect of Activated Charcoal for Seedlings Development of *Catasetum fimbriatum* Lindl (Orchidaceae)

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Abstract: The influence of activated charcoal for leaf, root and PLBs production from seedlings of *C. fimbriatum* has been analyzed. A higher number of expanded leaves occurred on media containing 0.5% of activated charcoal. No significant differences were reported in root and PLBs formation from seedlings of *C. fimbriatum* when seedlings grown on medium with different concentrations (0.25 and 0.5%) of activated charcoal were compared. Results revealed that activated charcoal might be important for the accumulation of root-derived cytokinins to reach an endogenous auxin/cytokinin rate adequate to leaf production in *C. fimbriatum*. Activated charcoal addition also affected the initial and final pH of KC medium. Moreover, pH change from 5.3 to 4.79 was detected at KC medium in the absence of activated charcoal, while pH of the medium with 0.25 and 0.5% activated charcoal significantly increased. After the initial relatively increase in pH, immediately after autoclaving, pH of media containing activated charcoal decreased according to duration of culture (10 months). Effect of activated charcoal after autoclaving process and in final pH of medium showed interference in the buffering capacity of KC medium after 10 months, but activated charcoal may be recommended to promote the development of aerial parts of *C. fimbriatum* replacing the use of the expensive exogenous growth regulators.

Key words: Activated charcoal, orchids, seedling development, *Catasetum fimbriatum*

Introduction

Catasetum fimbriatum is an epiphytic orchid from South America that is cultivated for commercial production and serves as a model plant for metabolic and developmental studies (Vaz *et al.*, 1998, Peres *et al.*, 1999; Majerowicz *et al.*, 2000). The orchid is easily propagated through *in vitro* root tip culture (Kerbaux, 1984), with a production of genetically uniform plants.

The regeneration of *Catasetum* plants from root tips involves an uncommon root-to-shoot meristem conversion (Kraus and Monteiro, 1989). Bud formation in root tip segments of *C. fimbriatum* is a direct process from protocorm-like bodies (PLBs) and does not induce formation of callus. Since shoot initiation and development have been reported in root tips of *Catasetum* cultured on hormone-free media (Peres and Kerbaux, 1999), competence for shoot development has been attributed to the establishment of an endogenous auxin/cytokinin ratio which favors cytokinins.

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Although this uncommon direct root-to-shoot conversion is adequate for the multiplication of the number of plants, it may delay the growth and development of plants expected by collectors and producers of orchids.

The present research aims at determining the influence of activated charcoal for leaf and root growth from seedlings of *C. fimbriatum*. Activated charcoal is commonly used in tissue culture media to improve growth and development. The beneficiary effect of activated charcoal on tissue responses *in vitro* has been attributed to different factors, such as adsorption of plant growth regulators and other organic compounds (Constantin *et al.*, 1977; Nissen and Sutter, 1990). Activated charcoal addition in culture medium of the *C. fimbriatum* seedlings may cause better development of root and aerial parts of plants without any addition of exogenous auxins and/or cytokinin.

Materials and Methods

The seeds of *C. fimbriatum* obtained from a producer in the region of Marialva, north-western region of the state of Paraná, Brazil, were germinated *in vitro* on Kundson-C medium (Knudson, 1943). After 9 months the seedlings (4.0-5.0 mm height) were placed in culture flasks containing 50 mL of KC medium solidified with 0.7% agar and 0.0, 0.25 and 0.5% of activated charcoal. Each flask contained twenty seedlings and experiments were repeated three times.

Cultures were incubated in a growth chamber at 25±2 °C under 14.9 µmolm⁻²s⁻¹, provided by light radiation with cold-white fluorescent lights (PPF), for a 15 h photoperiod.

After 10 months of culture, average number of leaves, roots and PLBs induced per explant was recorded for each concentration of activated charcoal. Difference among treatments was tested by one-way analysis of variance (ANOVA) and by Tukey-Kramer Multiple Comparison Test (The SAS System Copyright © 1999-2000 by SAS Institute Inc., Cary, NC, USA).

Results

A higher number of expanded leaves occurred on media containing 0.5% of activated charcoal (Table 1). On the other hand, root induction and PLBs formation from seedlings of *C. fimbriatum* showed no significant difference when seedlings grown on medium with different concentrations of activated charcoal were compared (Table 1). Effect of activated charcoal on multiple root and PLBs induction was not significantly different when submitted to Tukey's Student Range Test.

Activated charcoal addition also affected the initial and final pH of KC medium. The pH of KC medium without and with activated charcoal was adjusted to 5.3 prior to autoclaving. After autoclaving, media were cooled to room temperature and pH recorded: pH changes from 5.3 to 4.79 were detected at KC medium in the absence of activated charcoal, while pH of medium with 0.25 and 0.5% activated charcoal was significantly increased (Table 1).

Table 1: Comparison for leave, root and PLBs formation in *C. fimbriatum* seedlings, cultured for 10 months in 0.0, 0.25 and 0.5% of Activated Charcoal (AC), in a randomized design, with three replicates and 20 seedlings per flask and pH variations after autoclaving and after 10 months culture when activated charcoal was added on KC medium prior to autoclaving (pH 5.3)

Culture Medium	Mean No.			pH	
	Leaf/seedling	Root/seedling	PLBs/seedling	After autoclaving	After 10 months culture
KC	3.49±0.10b	1.73±0.08a	3.77±0.28ab	4.79±0.09b	4.05±0.74a
KC + 0.25% AC	3.53±0.11b	1.57±0.09a	4.66±0.32a	5.63±0.06a	3.91±1.72ab
KC + 0.5% AC	3.97±0.11a	1.59±0.09a	3.65±0.30b	5.89±0.10a	3.73±2.16b
CV (%)	26.01	47.90	66.30	3.16	3.05

Means followed by same letters are not significantly different at 5% probability by Tukey-Kramer Multiple Comparison Test

Discussion

Results of current research indicate that activated charcoal may be important for accumulation of root-derived cytokinins so that an endogenous auxin/cytokinin rate, adequate for leaves production in *C. fimbriatum*, could be reached. In *C. fimbriatum*, growth of aerial parts, such as shoots, has been correlated with higher cytokinin level (Peres *et al.*, 2001); genotypes with cytokinin accumulation in shoot showed a high shoot formation and root inhibition. Although the effect of activated charcoal on multiple root induction was not significantly different when submitted to Tukey's Student Range Test, actually, adsorption capacity of activated charcoal should reduce the endogenous auxin level.

High coefficient of variation detected for number of root and PLBs per seedlings (47.9 and 66.3%, respectively) indicates a great variation of results within each treatment for each activated charcoal concentration. Different results within each treatment may be due to genetic diversity in *C. fimbriatum*. Differences in the endogenous content of cytokinins and auxin have been described for different *C. fimbriatum* genotypes (Peres *et al.*, 2001). Thus, differentiated effect of activated charcoal should be expected for different genotypes.

Addition of activated charcoal seems to make up an alkaline media after the autoclaving stage. Contrastingly, pH of different culture media decreased by about 1-2 units after autoclaving when different concentrations of activated charcoal were present (Pan and Staden, 1999). Differential effect of activated charcoal in different culture media could be attributed to activated charcoal types. Different results were obtained when activated charcoal of different quality (purity) was used (Hinnen *et al.*, 1989).

On the other hand, in the wake of an initial relatively increase in pH immediately after autoclaving, the pH of media containing activated charcoal decreased according to culture duration (10 months). The effect of activated charcoal after autoclaving process and in final pH of the medium interferes in the buffering capacity of KC medium. This fact may be seen after 10 months of *C. fimbriatum* seedlings culture (Table 1). The pH of KC medium containing activated charcoal decreased by about 1-2 units during the *in vitro* seedling culture. A low capacity of the activated charcoal to buffer the pH of the medium has been also reported for *in vitro Phalaenopsis* hybrids culture (Hinnen *et al.*, 1989).

Alterations of average pH to an optimum level for morphogenesis has been reported as a beneficial effect of activated charcoal by Owen *et al.* (1991) and its addition in KC medium of the *C. fimbriatum* seedlings induced a higher number of leaves. Therefore, activated charcoal may be recommended to promote the development of aerial parts of *C. fimbriatum* replacing the use of the expensive exogenous growth regulators.

This is an important conclusion benefiting *Catasetum* collectors and producers that could be increasing the aerial growth and development of *C. fimbriatum* seedlings without to decrease the root and PLBs development, by charcoal activated addition in original Kundson-C culture medium.

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