

# Less light, more growth? Effects of the absence of light on roots of *in vitro* of *Catasetum fimbriatum* (Orchidaceae)

Regina A. Amadeu

Colégio Dante Alighieri, Al. Jaú 1061, São Paulo, SP, CEP 01420-003, Brazil

regina.amadeu@colegiodante.com.br

**ABSTRACT:** Due to the beauty of their flowers, orchids are extremely valued and commercialized. However, under natural conditions, the chances of reproduction are low. Thus, *in vitro* cultivation becomes an excellent option for rapid proliferation and for obtaining numerous plants with high genetic and phytosanitary quality. Among the many factors utilized to increase the efficiency and speed of production of these plants is the addition of activated carbon, which has been used due to its positive influence on the height and rooting in some species. The objective of this work was to find a way to improve the *in vitro* development of *Catasetum fimbriatum* in a simpler and cheaper way, without the use of activated carbon. My hypothesis is that the effect of light deprivation on the roots of the orchid could result in a development equivalent to that of the plant whose culture medium has activated charcoal, since I believe the growth of the orchid is not helped by the charcoal itself, but by the darkness it provides to the plant. Specimens of *Catasetum fimbriatum* were used as study material. The plants were studied in three groups, the first one (control) in conventional culture medium, the second with addition of activated carbon and the third with light deprivation in orchid roots. After three months, the specimens were evaluated according to the largest length of the root, length of the largest leaf, shoot fresh mass and root fresh weight. I found significant variations ( $p < 0.05$ ) in the different groups, and the one with greatest relevance (statistical significance) was the length of the largest root between group 1 (activated charcoal) and 2 (deprivation of light), which showed longer roots. We can conclude that light deprivation would be a good alternative to activated charcoal, since a longer root length may favor the rooting of the plant when transferred to the growth vessel, although the root length of the plant may also depend on the presence of phenols. Other studies should be performed to clarify the nutritional influence of activated carbon on the *in vitro* culture of *Catasetum fimbriatum*.

**KEYWORDS:** Orchids; *Catasetum fimbriatum*; Activated charcoal; *In vitro* culture

**Introduction.** Due to the beauty of its flowers, plants of the Orchidaceae family are extremely valued and commercialized. Orchidaceae comprises about 7% of all angiosperms, being considered one of the largest families of this group and presenting about 850 genera and 20,000 species distributed throughout the world. Moreover, its greatest diversity is found in the tropics. In Brazil alone, there are about 2,300 species distributed in 191 genera.<sup>1</sup>

Among these genera, *Catasetum*, from the subfamily Epidendroideae,<sup>2</sup> stands out due to its sexual dimorphism and a complex mechanism for pollination.<sup>3</sup> They are distributed throughout the Americas, especially in the tropical zone. The species *Catasetum fimbriatum* occurs only in South America, mainly in Brazil, Bolivia, Venezuela, and Argentina.<sup>4</sup>

Given the economic potential in their exploration, orchids have been extricated from their natural habitats. In addition, the advancement of agriculture has altered the ecosystems where they occur further hindering their survival and reproduction.

Under natural conditions, many angiosperms depend on the dissemination of seeds and require the association with mycorrhizal fungi for germination, consequently decreasing chances of success in multiplication.<sup>5</sup> Thus, *in vitro* techniques have

proven to be a reasonable alternative for the propagation of orchids, since the plants stop depending on the presence of fungi and exhibit faster proliferation, making it possible to obtain a large number of plants with a high genetic and phytosanitary quality.<sup>6</sup>

*In vitro* culture demands, for different species of Orchidaceae, specific culture media often modified with complex additives in order to provide the most favorable conditions of growth.<sup>7,8</sup> Activated charcoal is traditionally exploited to increase the efficiency and growth speed of *in vitro* cultures.<sup>9</sup> The use of charcoal may be beneficial to *in vitro* cultures of orchids due to its influence on the height and rooting of some species.<sup>5,10</sup>

However, the real cause of the benefits of the addition of charcoal to the culture medium remains unclear. Such effects have been attributed to the formation of a dark environment in the medium or to the adsorption of substances such as phenols, ethylene, growth regulators, vitamins and other organic compounds.<sup>11</sup>

According to Pan and Staden<sup>11</sup>, a problem frequently encountered during the early stages of *in vitro* culture is the eventual tissue death due to excessive production of polyphenols, possibly triggering defense reactions. Polyphenols often

have the connotation as being inhibitory substances that should be avoided or eliminated from *in vitro* environments. Although there are several methods of preventing the accumulation of such compounds, incorporation of charcoal or polyvinylpyrrolidone into crops can prevent phenolic adsorption and render polyphenol oxidase and peroxidase inactive.<sup>11</sup>

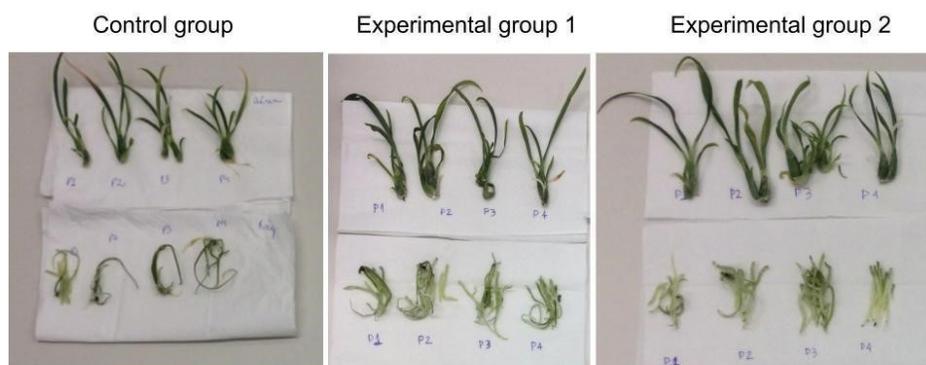
Auxins and cytokinins are plant growth regulators frequently used in *in vitro* cultures, and their concentrations and combinations in the culture medium is generally an important factor that determines successful plant regeneration. The use of activated charcoal for the adsorption of toxic plant metabolites is known. Activated charcoal is able to adsorb high concentrations of some growth regulators in liquid and solid media.<sup>10</sup>

Pan and Staden<sup>11</sup> also suggest that it is possible for activated charcoal to excrete growth promoting substances, but they do point out that it requires more studies

According to Moraes and colleagues<sup>12</sup>, activated charcoal has been used to stimulate rooting because of its high capacity to exclude light from the culture medium and to reduce crop oxidation by the presence of phenols produced by the tissues themselves.

In addition, according to the theory of positive and negative phototropism, the aerial part of the plant grows toward the light and the root develops in the opposite direction. Therefore, the objective of the study was to verify if the effect of light deprivation on the *in vitro* culture of *Catasetum fimbriatum* could result in an equivalent development to that of plants growing on culture medium with activated charcoal. Accordingly, we believed the factor that would aid in orchid growth would not be the charcoal itself, but the darkness it provides to the plant.

**Results and Discussion.** After three months of *in vitro* culture, all the plants were analyzed (Fig. 4) and the measurements were taken (Table 1).



**Figure 4.** Examples of some individuals for three groups of plants after growth. The shoots and the roots for each plant were detached to proceed with the measurements.

The mean of the shoot fresh mass of the control group was significantly smaller than that of the experimental group 1. The mean of the root fresh mass of the experimental group 2 was significantly heavier than that of the control group, although experimental group 2 showed significantly longer root length mean compared to experimental group 1 (Table 2).

Groups	Shoot fresh mass (g)	Root fresh mass (g)	Largest leaf length (cm)	Largest root length (cm)
Control	0.43±0.23	0.33±0.18	4.85±2.09	5.18±2.59
Experimental group 1	<b>0.76±0.63</b>	0.54±0.47	5.01±2.09	4.33±1.07
Experimental group 2	0.58±0.41	<b>0.55±0.41</b>	5.36±2.49	5.95±2.35

**Table 1.** Mean values for the largest leaf length, largest root length, shoot fresh weight and root fresh mass of *Catasetum fimbriatum* individuals after 112 days of cultivation, in the three experimental groups. Means in bold were significantly different ( $p < 0.05$ ) from means of the control group.

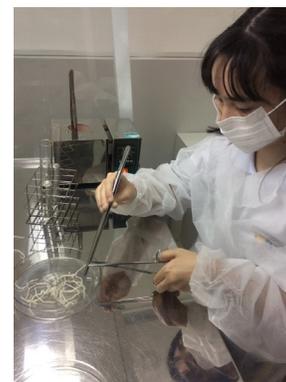
Groups	Shoot fresh mass	Root fresh mass	Largest leaf length	Largest root length
Control / Experimental 1	<b>0.03</b>	0.07	0.83	0.20
Control / Experimental 2	0.17	<b>0.04</b>	0.53	0.36
Experimental 1 / Experimental 2	0.36	0.97	0.52	<b>0.01</b>

**Table 2.** Results of the ANOVA for the means of the largest leaf length, largest root length, shoot fresh mass and root fresh mass of individuals in *Catasetum fimbriatum* ( $\alpha = 0.05$ ).

**Conclusion.** Based on the observed results, we can conclude that, depending on the objective of the producers, light deprivation would be a good alternative as a substitute for activated charcoal, since a longer root length may favor the rooting of the plant when transferred to a vessel, although the root length of the plant may also depend on the presence of phenols. Despite all this, the presented information is useful to guide further studies on this subject.

**Methods.** The experiment was carried out at the Biotechnology Laboratory of the Dante Alighieri School (São Paulo, Brazil). The species used as study material was *Catasetum fimbriatum* (Orchidaceae).

The nodal segments of an etiolated plant were used for micropropagation and inoculated in 22 mm x 200 mm test tubes containing 20 mL of the culture medium Universidade de São Paulo (USP) (Fig. 1).<sup>13</sup> The culture medium pH was adjusted to  $5.8 \pm 0.1$  before autoclaving.

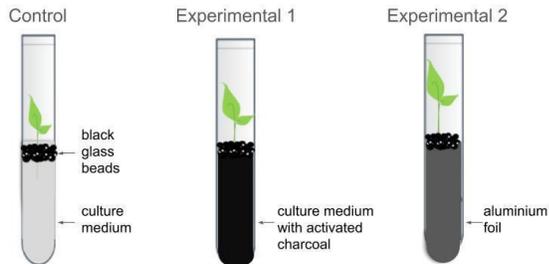


**Figure 1.** Nodal segments being inoculated in the culture medium by the author.

The specimens were separated into three experimental groups: control group with culture medium without activated charcoal in test tubes without any cover; experimental group

1 with activated charcoal added to the culture medium (5.0 g/L) in test tubes without any cover; and experimental group 2, with culture medium without activated charcoal and test tube wrapped with aluminum foil at the bottom, in order to seal the plant root of light. A layer of pre-autoclaved black glass beads was added in all experimental groups to block light from above (Fig. 2).

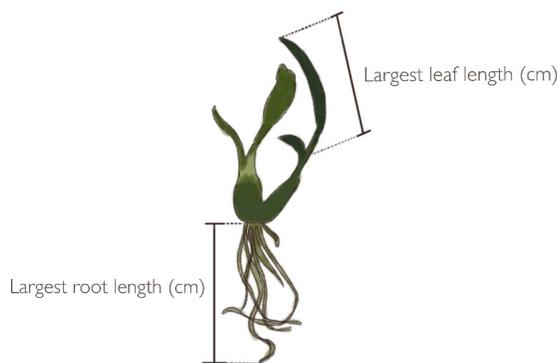
## Metodologia



**Figure 2. Scheme of test tubes for the three experimental groups.**

Each experimental group consisted of 20 tubes with a nodal segment, totaling 60 individuals.

The test tubes were kept in a growth room for 112 days at a temperature of  $22 \pm 3$  °C, photoperiod of 12 hours and a light intensity of 1300 lux. The analyzed variables were: root length, leaf length (one per plant), shoot fresh mass and roots fresh mass (Fig. 3).



**Figure 3. Illustration of how the measurements were taken.**

The measurement means of each of the groups were compared in a one-way ANOVA test, with a significance level of 5%, after being submitted to the Doornik-Hansen normality test.

**Acknowledgements.** To my advisors Nilce de Angelo and Fernando C. de Domenico and to my co-advisor Sandra Maria R. Tonidandel. Also, I would like to thank all the team responsible for the Programa de Pré-iniciação Científica Cientista Aprendiziz (a scientific pre-initiation program) of Dante Alighieri School for the valuable support.

## References.

- Barros, F. Diversidade taxonômica e distribuição geográfica das Orchidaceae brasileiras. *Acta Bot. Bras.* **1990**, *4*, 177-187
  - Machnicki-Reis, M.; Engels, M. E.; Petini-Benelli, A.; Smidt, E. C. O gênero *Catasetum* Rich. ex Kunth (Orchidaceae, Catasetinae) no Estado do Paraná, Brasil. *Hoehnea*, **2015**, *42*, 185-194.
  - Romero, G. A. Non-functional flowers in *Catasetum fimbriatum* orchids (Catasetinae, Orchidaceae). *Bot. J. Linn. Soc.* **1992**, *109*, 305-313.
  - Ferreira, A. W. C. 2005. Fenologia de *Catasetum fimbriatum* (Morren) Lindl. (Catasetinae, Orchidaceae) e sua polinização por abelhas Euglossini (Hymenoptera, Apidae) na região de São Carlos-SP, Brasil. M.Sc. Thesis, Universidade Federal de São Carlos, 2005.
  - Soares, J. S.; Rosa, Y. B. C. J.; Macedo, M. C.; Sorgato, J. C.; Rosa, D. B. C. J.; Rosa, C. B. C. J. Cultivo *in vitro* de *Brassavola tuberculata* (Orchidaceae) em meio de cultura alternativo suplementado com diferentes concentrações de açúcar e carvão ativado. *Magistra* **2012**, *24*, 226-233.
  - Figueiredo, M. A.; Pasqual, M.; Araujo, A. G.; Junqueira, K. P.; Santos, F. C.; Rodrigues, V. A. Fontes de potássio no crescimento *in vitro* de plantas de orquídea *Cattleya loddigesii*. *Ciênc. Rural* **2008**, *38*, 255-257.
  - Campos, D. M. *Orquídeas: reprodução por sementes em laboratório caseiro*; 2010.
  - Morales, S.; Mulaneze, M. A. G.; Machado, M. F. P. S. Effect of Activated Charcoal for Seedlings Development of *Catasetum fimbriatum* Lindl (Orchidaceae). *J. Plant Sci.* **2006**, *4*, 87-93.
  - Arditti, J.; Ernst, R. *Micropropagation of orchids*; J. Wiley: New York, 1993.
  - Prizao, E. C. Efeito de carvão ativado e do grafite no crescimento *in vitro* de orquídeas. M.Sc. Theses, Universidade Estadual de Maringá, 2007.
  - Pan, M. J.; Staden J. The use of charcoal in in vitro culture - A review. *Plant Growth Regul.* **1998**, *26*, 155-163.
  - Moraes, A. P.; Tacuatiá, L. O.; Carvalho, F. I. F.; Kalchuk-Santos. 2009. Androgênese em milho: cultura *in vitro* e desenvolvimento dos micrósoros em genótipos brasileiros. *Plant Cell Cult. Micropropag.* **2009**, *5*, 79-86.
  - Chaer, L. Estudo para o estabelecimento de uma nova estratégia de clonagem *in vitro* de *Cattleya* e *Cymbidium* (Orchidaceae) por meio da utilização de gemas laterais de caules estiolados. M.Sc. Thesis, Universidade de São Paulo, 2012.
- Authors.** I'm a 15- year-old scientist who has been conducting my own scientific studies since I was 12. At the present, I'm involved with a new study about Tourette syndrome.