

## Comparative *in vitro* initial development of *Cattleya coccinea* in different culture media

Cleber da Silva Costa\*

Andreia Gazeffi Moraes Bacelar

Alessandro José da Silva

Universidade Nove de Julho, São Paulo, SP, Brazil.

Regina Mayumi Hamasaki

Universidade Braz Cubas, Mogi das Cruzes, SP, Brazil.

### Workshop Information

I Workshop of Plant Biology (I Workshop de Biologia Vegetal) was held in the Bioscience Institute – UNESP, campus of Rio Claro, Brazil, during August 20 and 21, 2012. Workshop was a scientific event organized by Post-graduate students from that Institute aiming to integrate Post-graduate and Graduate students from different areas related to Plant Biology (Anatomy, Ecology, Evolution, Morphology, Physiology, and transitional areas) from different Universities. Workshop Organization offered a large number of speaking activities, scientific discussions, and extra short-courses to improve the knowledge and formation of students in Plant Biology.

### Scientific Committee

Alessandra Tomaselli Fidelis, Anna Carolina Bressan, Daniela de Oliveira Dinato, Diogo Amorim, Elaine Lopes, Letícia Peres Poli Luis Felipe Daibes, Marcelo Claro de Souza, Milene Amâncio Alves Eigenheer, Naiara Lopes de Sousa, Nara Oliveira Vogado, Natalia Costa, Paulo Roberto de Moura Souza Filho, Rafael Marques Guimarães Konopczyk, Rita de Cássia Andreotta, Tiago Haruo Ishibashi, Vitor de Andrade Kamimura, and Yuri Brenn.

\*Corresponding author: costacs@uninove.br

Received on August 14, 2012. Accepted on August 21, 2012. Online published on December 13, 2012.

## INTRODUCTION

In nature, germination of orchid seeds yields very few (Stoutamire 1964), this feature had led to the production of plants by *in vitro* (Knudson 1922). *Cattleya coccinea* Lindley is an epiphytic orchid that occurs in Brazil and Argentina (Govaerts et al. 2010). There is a lack of information concerning *in vitro* germination of this plant.

In Brazil, nitrate salts used in culture media are controlled by the Army and methods that could facilitate the making of media would be useful for agricultural purposes (Brasil 2000). Many works have been made to evaluate which media is better for *in vitro* culture of many orchid species. Many of those researches use variations of Murashige and Skoog (1962) proposed media and/ or Knudson (1946) some of them use commercial fertilizers as nutrients to elaborate a medium (Bilce and Karsburg 2009, Moraes et al. 2009, Rodrigues et al. 2012).

The present work aims to compare the initial development of *C. coccinea* in commercial fertilizer, Murashige and Skoog (1962) and Knudson (1946) media with and without banana pulp.

## MATERIAL AND METHODS

The experiment consisted of six different media. Three types of media were complemented with 30 g L<sup>-1</sup> of sucrose. They were complemented as following: Murashige and Skoog (1962) (MS), Knudson (1946) (KN) and composed of 3 g L<sup>-1</sup> of Hyponex commercial fertilizer (N-6 P-6 K-19) (HP). Other three media based on the previous formula described, received 150 g L<sup>-1</sup> of banana pulp and 15 g L<sup>-1</sup> of sucrose instead of 30 g L<sup>-1</sup> (MSB, KNB and HPB, respectively). 1.5 g L<sup>-1</sup> of activated charcoal was added in all media. The pH of the solutions was adjusted to 5.8 ± 0.1 and 4.5 g L<sup>-1</sup> of agar were added for gelling purpose prior to sterilization in autoclave at 1.5 atm for 15 minutes at 127 °C.

The experiments were conducted as following described: *C. coccinea* seeds were sterilized with 0.6 % sodium hypochlorite solution for 10 minutes. Then 1 mL of the solution with seeds was readily deposited in each of the 10 flasks of HPB medium. After 120 days protocorms grown in this medium were transferred to the six different media each type of media were represented by three flasks. For each flask

10 protocorms were deposited performing 30 individuals per treatment.

The experiments were conducted under controlled conditions at  $25 \pm 2$  °C, 16 h photoperiod and illumination by white fluorescent lamps.

After 240 days of culture, it has been performed measures of growth parameters such as number of leaves and roots, major leaf and root length, fresh and dry mass of the role plants.

The data were submitted to analysis of variance (ANOVA) followed by Duncan test with 5% significance.

## RESULTS AND DISCUSSION

The germination of *C. coccinea* seeds was observed after 70 days of culture in HPB medium. The protocorms were ready to be transferred to the six different media within 120 days of culture and after 240 days the biometrical data were collected. The comparison between these data concerning different media is shown in Figure 1.

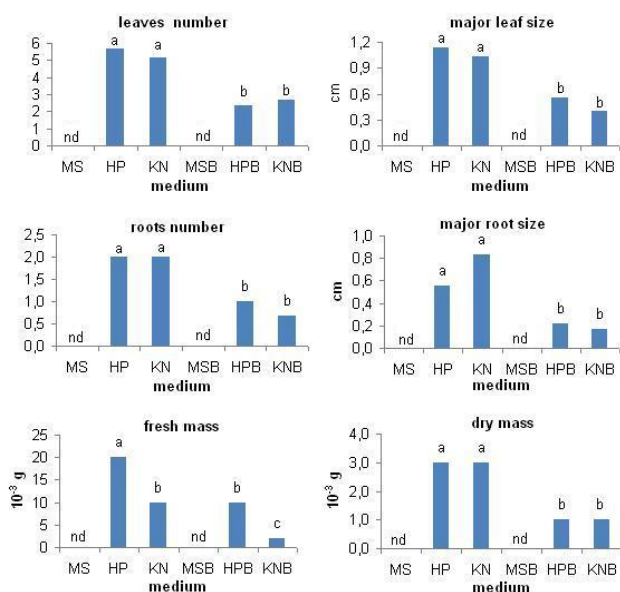


Figure 1. Effects of different media in the initial development of *C. coccinea* seedlings after 240 days of culture. Bars followed by different letters present significant variance between treatments as predicted by Tukey test ( $P < 0,05$ ). nd: no data due to death of the seedlings.

By the analysis of data collected HP and KN were the best media for growth and development of *C. coccinea* seedlings. The measured parameters of the plants drop when it is used HPB or KNB medium which means that banana pulp is not suitable for this specie (Figura 1). Different from the observed on the present work, Araújo et al. (2006) obtained good results on *in vitro* growth of *Cattleya loddigesii* using banana pulp and/ or coconut water. Similar results were obtained by Pasqual et al. (2009). As HP medium has commercial

fertilizer in its formula, so it can be an advantage for orchid plant growers.

In the period in which the protocorms were grown in the six different media, all those cultivated in MS and MSB perished (Figura 1). Moraes et al. (2009) studying *in vitro* development of *Cattleya tigrina* tested MS and different commercial fertilizers (Hyponex and Krystalon); in this study Krystalon showed the best results and Hyponex media had best results than MS. Araújo et al. (1999) compared the *in vitro* development of *Cattleya walkeriana* and *Cyrtopodium palmifrons* in MS and KN media and found that KN was the best for both orchids due to its minor salt concentration. In our work the death of plants in MS and MSB media can be explained by the salt concentration of this media as attested by Araújo et al. (1999).

## CONCLUSIONS

HP medium can be used comfortably replacing KN medium on *in vitro* culture of *C. coccinea*. It has to be avoided the use of banana pulp for the *in vitro* culture of this orchid since it reduces plant development as well as use MS medium since all the plants perished in this medium.

## References

- Araújo LG, Carneiro IF, Prabhu AS. 1999. Produção *in vitro* de mudas de *Cattleya walkeriana* e *Cyrtopodium palmifrons* a partir de sementes. *Pesq Agropec Trop* 29:67–71.
- Araújo AG, Pasqual M, Villa F, Costa FC. 2006. Água de coco e polpa de banana no cultivo *in vitro* de plântulas de orquídea. *Rev Ceres* 53:608–613.
- Bilce T, Karsburg IV. 2009. Germinação *in vitro* de sementes de *Cattleya nobile* em meio de cultura alternativo. In: 2ª Jornada Científica da Unemat. Mato Grosso.
- Brasil. 2000. Ministry of Defence. Decree n.3665. Available at [www.planalto.gov.br/ccivil\\_03/decreto/D3665.htm](http://www.planalto.gov.br/ccivil_03/decreto/D3665.htm). Accessed at Jul. 03, 2012.
- Govaerts, R, Pfahl J, Campacci MA, Baptista DH, Tigges H, Shaw J, Cribb PJ, George A, Kreuz K, Wood J. 2010. The board of trustees of the Royal Botanic Gardens, Kew. Available at: <http://apps.kew.org/wcsp/home.do>. Accessed at Jun. 30 2012.
- Knudson L. 1922. Nonsymbiotic germination of orchid seeds. *Bot Gaz* 73:1–25
- Knudson L. 1946. A new nutrient solution for germination of orchid seeds. *Am Orc Soc Bull* 15:214–217.
- Moraes CP, Diogo JA, Pedro NP, Canabrava RI, Martini GA, Marteline MA. 2009. Desenvolvimento *in vitro* de *Cattleya loddigesii* Lindley (Orchidaceae) utilizando fertilizantes comerciais. *Rev Bras Bioc* 7:6–69.
- Moraes CP, Santos NS, Massaro R, Cordeiro GM, Leal TS. 2009. Desenvolvimento *in vitro* de *Cattleya tigrina* A. Richards (Orchidaceae) utilizando fertilizantes comerciais. *Ens Ci* 13:57–65.
- Murashige T, Skoog, FA. 1962. A Revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* 15:473–497.
- Pasqual M, Figueiredo MA, Rezende JC, Araújo AG, Santos FC, Ferreira EA, Junqueira KP. 2009. Fontes de nitrogênio, polpa de banana e ágar no desenvolvimento *in vitro* de plântulas de orquídea. *Hort Bras* 27:211–216.

Rodrigues DT, Novais RF, Alvarez VH, Dias JMM. 2012. Cultivo *in vitro* de orquídea em meios com diferentes concentrações de fertilizante mineral. Rev Ceres 59:9–15.

Pasqual M, Figueiredo MA, Rezende JC, Araújo AG, Santos FC, Ferreira EA, Junqueira KP. 2009. Fontes de nitrogênio, polpa de

banana e ágar no desenvolvimento *in vitro* de plântulas de orquídea. Hort Bras 27:211–2116.

Stoutamire WP. 1964. Seeds and seedlings of native orchids. Mich Bot 3:107–119.