Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9275
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v36i4.15492

Development of *cattleya amethystoglossa* x *nobilior* - orquidaceae in simplified culture media

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ABSTRACT. Three simplified, low-cost culture media, prepared with foliar fertilizers, were compared with the commonly used ½ MS-ban (Murashige Skoog medium ½ salt concentration, with banana fruit) for the *in vitro* multiplication of the orchid hybrid *Cattleya amethystoglossa* x *nobilior*. The simplified culture media tested were Hy-ban, which is made with 1.33 g L^{-1} of Hyponex fertilizer (NPK 6.5-6-19); KP-ban, which is made with 0.92 g L^{-1} of Kristalon™ (NPK 6-12-36) and 0.26 g L^{-1} of Peters (NPK 30-10-10); and Kcal-ban, which is made with 0.92 g L^{-1} of Kristalon™ (NPK 6-12-36) and 0.51 g L^{-1} of Calcinit (NPK 15.5-0-0+Ca 19.0%). Each medium was supplemented with 20 g L^{-1} of sucrose, 1 g L^{-1} of activated charcoal, 7 g L^{-1} of agar-agar and 40 g L^{-1} of banana fruit, pH 5.6. Plantlets were evaluated after 80 days for the following parameters: number of shoots, shoot length, number of roots, longest root length and fresh weight. While all four media can be used for the studied hybrid, the best medium was Kcal-ban.

Keywords: orchids, simplified culture medium, explant production.

Crescimento de *Cattleya amethystoglossa* x *nobilior* – orquidaceae em meios de cultura simplificados

RESUMO. Três meios de cultura simplificados, de baixo custo, preparados com adubos foliares foram comparados ao meio comumente usado ½ MS-ban (Murashige Skoog com ½ da concentração dos sais e polpa de banana) na multiplicação *in vitro* da orquídea híbrida *Cattleya amethystoglossa* x *nobilior*. Os meios simplificados testados foram: Hy-ban, feito com 1,33g L^{-1} do fertilizante Hyponex (NPK 6,5-6-19), o KP-ban, com 0,92 g L^{-1} de Kristalon™ (NPK 6-12-36) e 0,26 g L^{-1} de Peters (NPK 30-10-10) e o meio Kcal-ban, com 0,92 g L^{-1} de Kristalon ™ (NPK 6-12-36) e 0,51 g L^{-1} de Calcinit (NPK 15,5-0-0+Ca 19,0%). Cada meio recebeu 20 g L^{-1} de sacarose, 1 g L^{-1} de carvão ativado, 7 g L^{-1} de ágar-ágar e 40 g L^{-1} de polpa de banana, pH 5,6. Os explantes foram avaliados 80 dias após nos seguintes parâmetros: número de brotos, comprimento da parte aérea, número de raízes, comprimento da maior raiz e peso fresco. Para o híbrido estudado, os quatro meios de cultura podem ser usados, sendo o melhor deles o Kcal-ban.

Palavras-chave: orquídeas, meios de cultura simplificados, produção de explantes.

Introduction

Brazilian exports of live plants and floricultural products have been increasing (JUNQUEIRA; PEETZ, 2008). Brazilian orchid seedling exports were 78.8% higher in 2009 than in 2004. Even so, the total exported seedlings 2009 accounted for only 0.7% of live plant and floricultural product exports. Imports of orchid plantlets increased over 1,000% between 2004 and 2009, reaching nearly US$ 2.9 million in 2009, which shows that there is great domestic demand for these products and that the increased production of orchids as potted plants in Brazil has depended on imported plantlets (MDIC/SECEX, 2010).

Orchids are attractive plants for consumers, but their price is high because of the cost of production (STANCATO et al., 2001). Reducing these costs, and thereby decreasing the cost to consumers, may stimulate demand in a way that could leverage the latent potential of Brazilian biodiversity, resulting in significant income increases for farmers.

Orchids of the genus Cattleya are considered to be the 'Queens of Orchids' because of their showy, large and colorful flowers. They are found exclusively in tropical Central and South America. *Cattleya amethystoglossa* Lindl. & Rchb. is native to Bahia, Brazil, with flowers up to 8 cm in diameter and light pink petals sprinkled with pink-lilac macules (SUTTLEWORTH, 1994; WATANABE, 2002). *Cattleya nobilior* Rchb. f. is a species native of central Brazil, with flowers up to 15 cm in diameter and colors ranging from white to pinkish-purple (WATANABE, 2002).

Academic culture media tested for the *in vitro* cultivation of Cattleyas in Brazil include MS (PASQUAL et al., 2009; UNEMOTO et al., 2007), WPM (ARAÚJO et al., 2009) and KC (ARAÚJO et al., 2006, MORAES et al., 2009). Simplified culture media (i.e. made with preformulated commercial fertilizers) were also tested for *C. forbesii* (UNEMOTO et al., 2007). Simplified culture media were also tested in other orchid genera and species: *Oncidium nanum* (UNEMOTO et al., 2007); *Catasetum fimbriatumi* and *Cirtopodium paranaensis* (REGO-OLIVEIRA; FARIA, 2005); and *Laelia longipes*, *L. tenebrosa* and *Miltonia spectabilis* (STANCATO et al., 2008). In all cases, simplified media were superior, with the exception of one that gave results similar to academic culture media.

Material and methods

Plantlets of *Cattleya amethystoglossa* Lindl. Rchb.f. x *nobilior* Rchb.f. hybrid (with leaves of 0.4 ± 0.1 cm and up to two roots of 0.4 ± 0.1 cm) were obtained from germinated seeds in ½ KP-ban (see protocol below). Plantlets were transplanted to one of four different culture media, including the control medium ½ MS-ban (a modified MS medium with half the concentration of macronutrients, with banana fruit added), currently used for Cattleya cultivation by commercial orchid growers (MURASHIGE; SKOOG, 1962). The others media, considered simplified because commercial fertilizers were used as a source of salts, were as follows: Hyponex-banana, referred as Hy-ban, containing 1.33 g L^{-1} of Hyponex fertilizer (NPK 6.5-6-19); Kristalon™ Peters-banana, referred as KP-ban, containing 0.92 g L^{-1} of Kristalon™ fertilizer (NPK 6-12-36) and 0.26 g L^{-1} of Peters fertilizer (NPK 30-10-10); and Kristalon™ Calcinit-banana, referred as KCal-ban, containing 0.92 g L^{-1} of Kristalon™ fertilizer (NPK 6-12-36) and 0.51 g L^{-1} of Calcinit fertilizer (NPK 15.5-0-0 + Ca 19.0%). All media were supplemented with 20 g L^{-1} of sucrose, 1 g L^{-1} of activated charcoal, 7 g L^{-1} of agar-agar, and 40 g L^{-1} of banana cv. Nanica, pH adjusted to 5.6. Each simplified medium was also supplemented with 2 mL L^{-1} of hydrogen peroxide. After homogenization, 50 mL of medium was poured into 500 mL flasks and sterilized in a microwave oven for 3 min (VENTURIERI et al., 2013). The ½ MS-ban medium was autoclaved at 121ºC and 1.0 kg cm^{-2} pressure for 20 min. because of its inability to gel in presence of hydrogen peroxide (necessary for microwave sterilization). Subcultures were made under sterile conditions in a laminar flow hood, with 6 plantlets per flask and 4 flasks per treatment. Flasks were incubated on shelves at 26 ± 3°C and illuminated by 32 W fluorescent bulbs that generated 60 μmol m^{-2} s^{-1} during 16 hours of light per day cycle. After 80 days, plantlets were evaluated. At this age, plantlets had reached approximately one-half of the size normally used for *ex-vitro* cultivation, an appropriate size to evaluate the relative efficiency of different culture media, while minimizing any artifacts caused by nutrient depletion. The following parameters were evaluated: shoot length, number of shoots, number of roots, longest root length and fresh weight. The design adopted was completely randomized. Each plant was considered a repetition. The results were analyzed with an ANOVA, and the Tukey test at 5% was used to compare means (BANZATTO; KRONKA, 2006).

Results and discussion

For the hybrid *C. amethystoglossa* x *nobilior*, there was no statistical difference among the four tested culture media in terms of number of shoots, number of roots, longest root length or fresh weight. There was a significant difference only for shoot length, for which KCal-ban medium showed the highest average (14.61 mm, see Table 1). The lowest averages were observed using ½ MS-ban (12.04 mm) and Hy-ban (11.40 mm). These results were similar to those obtained by Moraes et al. (2009), on *in vitro* growth of *C. loddigesii*, where the longest shoot length aveage was obtained in media containing Kristalon™ commercial fertilizer, and media containing Hyponex showed a lower average, similar to ½ MS medium. Rego-Oliveira and Faria (2005), working with *in vitro* cultures of *C. fimbriatum* and *C. paranaensis*, reported better shoot development in MS and ½ MS culture media, compared with media made with commercial fertilizers (NPK 10-5-5 and NPK 10-30-20), an inverse of the effect found in our study. A possible explanation for this difference is that Kristalon™, the salt base of the alternative culture medium used in the present experiment, contains micronutrients not available in the minimal NPK formulas used by Rego-Oliveira and Faria (2005).

Table 1. Results of the average number of shoots, shoot height, number of roots, longest root length and fresh weight for different culture media, for the hybrid *Cattleya amethystoglossa* x *nobilior*.

Culture media	number of shoots	shoot length (mm)	number of roots	longest root length (mm)	fresh weight (mg)
KP-ban	3.96 a*	12.04 b	5.83 a	25.25 a	468.00 a
KCal-ban	3.46 a	14.61 a	5.46 a	21.71 a	442.79 a
Hy-ban	3.63 a	11.40 b	6.83 a	23.50 a	400.29 a
½ MS-ban	4.08 a	10.56 b	6.25 a	20.73 a	417.04 a
CV** (%)	44.12	22.29	35.20	27.74	43.01

*Means followed by same letters vertically do not differ statistically by the Tukey test at 5% significance level. Highest means are in bold. **CV - Coefficient of Variation (%).

The highest average number of shoots was produced using ½ MS-ban (4.08) while Hy-ban yielded the lowest (3.63). Hy-ban medium produced highest average number of roots (6.83) while KCal-ban produced the lowest (5.46). For *in vitro* cultivation of *Cattleya loddigesii*, Moraes et al. (2009) found that Hyponex produced a higher number of roots than media containing Kristalon™, as in the present study. A higher number of roots per plant in medium prepared with Hyponex was also observed for *in vitro* cultivation of *Phalaenopsis* hybrids, with or without the addition of micronutrients (CARDENAS; WANG, 1998). Villa et al. (2007) found that the addition of calcium nitrate and potassium chloride to Knudson culture medium increased the number of roots produced during micropropagation of grapevine rootstock. However, calcium nitrate is not sufficient to increase the number of roots produced in our culture medium. KCal-ban, which has a lower concentration of calcium nitrate than that found in Hy-ban or ½ MS-ban, produced fewer roots than the latter two media (albeit without a statistically significant difference). In orchids, the numbers of roots and shoots produced are a function of the nitrogen concentration in the culture medium (unpublished data). High numbers of roots and shoots indicate that the plants are allocating more energy to split themselves than to grow as a single plant, a function suitable for formation of a greater number of plants. Low number of roots and shoots, in contrast, indicate that energy is being directed to the formation of larger plants, which would more suitable for *ex vitro* growth.

KP-ban medium produced plantlets with the highest average root length and fresh weight in our experiment. Rego-Oliveira and Faria (2005), studying *in vitro* development of *C. fimbriatum*, obtained the best growth of the main root using a medium prepared with NPK 10-5-5, a commercial fertilizer, while MS and ½ MS produced lower averages. However, the treatment with the highest average number of roots was ¼ MS culture medium

and the medium prepared with NPK 10-5-5 commercial fertilizer ranked second. Using MS, ½ MS and a medium prepared with NPK 6-6-8 fertilizer for *in vitro* cultivation of *C. forbesii* and *O. nanum* species, Unemoto et al. (2007) obtained similar results for number of roots and lengths of the longest root as reported in the present study. Thus, for root length, the performance of culture media made with commercial fertilizer varies with both species and formulation. For fresh weight, the highest value in our experiment was observed using KP-ban and KCal-ban media. This finding is similar to the findings of Moraes et al. (2009) for *in vitro* cultivation of *C. loddigesii*, where the highest fresh weight was produced using medium made with Kristalon™ while MS and Hyponex produced lower fresh weights. Unemoto et al. (2007) found that the average fresh weight of *O. nanum* was higher in media made with NPK 6-6-8 fertilizer than with MS or ½ MS. However, in contrast to the results of our study, their results with *C. forbesii* were statistically similar to ½ MS and NPK 6-6-8 fertilizer. Cardenas and Wang (1998) obtained better accumulation of fresh weight in *Phalaenopsis* with the use of Hyponex. Unlike the numbers of roots and shoots, high values for root length and fresh weight are desirable when an *ex vitro* phase is the goal. Figueiredo et al. (2007), while studying three different orchid hybrids, stated that the ideal culture medium must be empirically determined.

In general, better results are achieved with culture media made with commercial fertilizers than with academic culture media. For instance, Kristalon commercial fetilizer was among the most appropriate media for Cattleyas.

Figure 1 shows that all culture media produced good results in terms of root and shoot development and overall seedling growth while KP-ban did promote greater root development. Thus, for the studied hybrid, all four media may be used; with the best being was Kcal-ban.

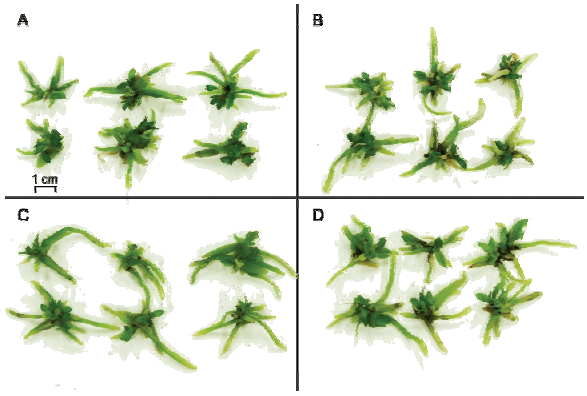

Figure 1. Growth of hybrid *C. amethystoglossa* x *C. nobilior* plantlets on (A) ½ MS medium, (B) Hy-ban medium, (C) KP-ban medium and (D) KCal-ban medium. Plantlets shown are the six closest to each culture average.

The *in vitro* production of orchids is expensive, mainly because of labor and reagent costs. The present study demonstrates that culture media formulated with commercial fertilizer can substantially reduce reagent costs but produce high quality seedlings. These reduced costs may create opportunities for small entrepreneurs. Many

endangered orchid species can be found in hobbyist orchid grower collections. It is essential that simplified technology for the micropropagation of orchids, as demonstrated here, be made available, at amateur level, to facilitate their hobby.

Conclusion

For the studied hybrid, all four media may be used; with the best being was Kcal-ban. The simplified methodology for micropropagation of orchids, as demonstrated here, can be made available, even at amateur level, under a considerable lower cost and labor.

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Received on December 12, 2011.
Accepted on March 3, 2012.