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Commercial fertilizers and organic additives in orchid micropropagation

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ABSTRACT

In vitro cultivation strategies promise conservation and micropropagation of vulnerable or economically exciting plants, especially in Orchidaceae. Numerous reports are available for an extensive contingent of orchids of the most varied genera, and the main issue is related to the behavior of species during the process of germination and growth concerning the nutrients used in the culture media. In Orchidaceae, several evidence support that each species responds differently and specifically according to the biotic and abiotic conditions available for *in vitro* growth. The main concern is to seek simplified culture media that promote germination and the development of healthy plants on a large scale. This study assessed the behavior of *Catasetum fimbriatum* and *Catasetum macrocarpum* in culture media containing commercial fertilizers supplemented with organic additives, compared with traditional media, an economically more viable strategy for micropropagation. Seeds were cultured on traditional nutrient media or with commercial fertilizer and organic additives in different combination. The analysis of plant growth after 120 days *in vitro* showed that the best treatment in both species was the medium containing commercial foliar fertilizer Peters NPK® 10:30:20 supplemented with potato and banana pulp.

Index terms: Tissue culture; Orchidaceae; simplified medium; *Catasetum.*

INTRODUCTION

The Orchidaceae is considered the most evolved family in the plant kingdom (Hossain et al., 2013). The diversity counts with 27,732 accepted species in 773 accepted genera (Shaw, 2016) and over 160,000 registered hybrids (RHS, 2020). This monocotyledons family is distributed all over the world, mostly in tropical regions. With low occurrence, the populations are restricted to small forest fragments (Parra Sánchez; Armenteras; Retana, 2016), so that seed prolificacy is restrained to a small population and degraded areas reduce the chances of seed dispersal and development, limiting the number of seedlings, increasing plantlets mortality (Diez, 2007).

The genus *Catasetum* (subfamily Epidendroideae, tribe Cymbidieae, subtribe Catasetinae) proposed in 1822 by C. S. Kunth possess around 180 species broadly distributed in Tropical America (Govaerts et al., 2019). One peculiar characteristic is the sexual dimorphism, presenting inflorescences with male, female, or mixed flowers (less frequently hermaphrodite flowers) combined with a complex pollination mechanism, which the male flowers have a remarkable technique for ejecting pollinia (Romero-González, 2018). Of relatively easy cultivation, Catasetum is known for its exotic beauty flowers, providing an essential economic position in the floristic market. However, *Catasetum* species with great economic potential have been extracted from their habitat, compromising their natural reproduction and survival (Ferreira et al., 2018).

Catasetum fimbriatum (C. Morren) Lindl. occurs in the Brazilian phytogeographic domains Amazon, Cerrado, Atlantic Forest, Pantanal, inhabiting the vegetation Cerrado (*lato sensu*), Ciliary Forest or Gallery, Seasonal Deciduous Forest, and Seasonal Semideciduous Forest, possessing great ecological plasticity. *Catasetum macrocarpum* Rich. ex Kunth, occurs in the Amazon, Cerrado

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and Atlantic Forest, vegetating Anthropic Area, Campinarana, Rupestre Field, Cerrado (*lato sensu*), Ciliary Forest or Gallery, Igapó Forest, Terra Firme Forest, Várzea Forest, Seasonal Semideciduous Forest, Forest Ombrófila, Restinga, Amazonian Savanna (Barros et al., 2015) and is subject to intense environmental pressure in some of these areas (Ferreira et al., 2018).

A feature about orchids is related to their small seeds that contain an undifferentiated embryo with insignificant reserve material, and the germination process depends exclusively on an association with a mycorrhizal fungus (Zhang et al., 2018). To substitute the natural process, the asymbiotic germination makes large-scale orchids cultivation possible for commercial and ecological purposes (Hossain et al., 2013).

Traditional culture media is used for *in vitro* propagation of numerous species, and different responses are observed due to the nutritional specificity in each orchid genus or even species (Cunha et al., 2011; De Freitas et al., 2014; Ferreira et al., 2018; Parthibhan; Rao; Kumar, 2015; Paul; Kumaria; Tandon, 2012).

Supplementations and modifications of the classical culture media from Burgeff (1909), Knudson (1946) and Murashige and Skoog (1962), for example, have been evaluated to find out the nutritional needs of orchids and the addition of complex compounds like banana pulp, coconut water, silicon, banana and potato extract are used to increase the macro and/or micronutrients content, vitamins, amino acids or even growth regulators (Colombo et al., 2016; Herrmann; de Freitas; Périco, 2011; Pasqual et al., 2009; Vieira et al., 2009).

Several types of research explored the applicability of commercial fertilizers, combined or not with organic additives (simplified or alternative medium), as a strategy for preservation of vulnerable and endangered species or to reduce costs of *in vitro* commercial orchids production, such as *Catasetum fimbriatum* and *Cyrtopodium paranaensis* (Rego-Oliveira; Faria, 2005); *Cattleya loddigesii* (Moraes et al. 2009); *Laeliocattleya schilleriana* Rolfe (Cunha et al., 2011); *Cattleya trianaei* (Galdiano Junior; Mantovani; Macedo Lemos, 2012); *Cattleya walkeriana* Gardner (Rodrigues et al., 2012b); *Phalaenopsis* (Colombo; Favetta; Faria, 2012) and *Cattleya intermedia* (Freitas et al. 2014).

The present work aimed to evaluate the *in vitro* growth of two *Catasetum* species in media containing commercial fertilizers supplemented with organic additives compared to traditional culture media for *in vitro* micropropagation and seedlings production.

MATERIAL AND METHODS

Capsules of *Catasetum fimbriatum* (Morren) Lindl. and *Catasetum macrocarpum* Rich. *ex* Kunth before dehiscence and provided by orchidologists of Assis, São Paulo State, were the source of seeds for the present study. Capsules were initially immersed in 70% alcohol for two minutes, surface sterilized with 1.5% sodium hypochlorite for 15 minutes, followed by three 15 minutes rinses in distilled and autoclaved water before seed removal in flow chamber.

Different media were evaluated for *in vitro* germination and growth (Table 1): Murashige and Skoog (1962) medium with half the concentration of salts (MS/2), used as control [T1 – MS/2]; Knudson C (1946) [T2 - KC]; Burgeff (1909) [T3 – BU]; Murashige and Skoog medium with half the concentration of salts (MS/2) plus 100 g L^{-1} banana pulp (BP) [T4 – MS/2BP]; medium with 5.0 mL L^{-1} commercial fertilizer Biofert® Plus Universal plus 50 g L^{-1} potato (P) and 50 g L^{-1} banana pulp [T5 – Biofert®PBP]; medium with 5.0 g L^{-1} commercial leaf fertilizer JR Peters® Inc. Blossom Booster 10:30:20 plus 50 g L^{-1} potato and 50 g L^{-1} banana pulp [T6 – Peters®10:30:20PBP] and medium with 5.0 g L^{-1} commercial leaf fertilizer JR Peters® Inc. Orchid Special 30:10:10 plus 50 g L^{-1} potato and 50 g L^{-1} banana pulp [T7 – Peters®30:10:10PBP]. All media were supplemented with 20.0 g L^{-1} sucrose, 1.0 g L^{-1} activated charcoal, and 7.0 g L^{-1} agar. The pH was adjusted to 5.7 prior to autoclaving at 121 °C, 1 atm for 15 minutes.

For seeding, about 40 mg of seeds were disinfected with 0.4% sodium hypochlorite for five min and rinsed three times with sterile distilled water (Alvarez-Pardo; Ferreira; Nunes, 2006). The seeds were kept in water and distributed in 500 mL jars containing 100 mL of medium, four jars per treatment for each species. All cultures were maintained in a growth room at 25 ± 2 ºC under a 16/8 h (light/dark) photoperiod with a light intensity of 40 µmol m^{-2} s^{-1}. The *in vitro* germination and protocorms development were monitored in each treatment and visually evaluated for 180 days.

Table 1: Nutrient composition (mg L^{-1}*) of the culture media used for germination and seedling growth of *Catasetum macrocarpum* and *Ctsm. fimbriatum*.

Nutrients (mg L^{-1})	T1	T2	T3	T4	T5	T6	T7
Total Nitrogen	1411.62	609.30	446.13	1635.62	259.0	256.5	257.5
NH_4^+	189.92	136.51	68.26	189.92	-	0.25	0.054
NO_3^-	1221.70	472.79	377.88	1221.70	-	0.25	1.446
$H_2PO_4^-$	60.58	178.17	315.94	87.58	32.95	34.25	33.25
K^+	392.00	71.83	184.07	768.50	339.2	340.3	339.8
Mg^{2+}	37.36	24.65	50.48	65.16	21.45	21.3015	21.2
H_3BO_3	6.30	-	-	6.30	0.01	0.00034	0.001
Fe^{2+}	5.58	5.02	7.35	5.98	0.45	0.4025	0.405
Zn^{2+}	3.48	-	-	3.68	0.25	0.200125	0.2025
Cu^{2+}	0.06	-	-	0.06	0.03	0.00118	0.0035
Mn^{2+}	5.49	1.85	-	5.59	0.13	0.10125	0.1025
MoO_4^{2-}	0.18	-	-	0.18	0.01	0.000045	0.000045
Ca^{2+}	59.97	169.72	244.25	63.37	3.50	3.5	3.5
SO_4^{-2}	172.07	472.79	393.91	172.07	-	-	-
I	0.63	-	-	0.63	-	-	-
Co	0.10	-	-	0.10	0.01	-	-
Cl^-	106.23	-	-	106.23	0.15	-	-
Na^+	4.63	-	-	4.63	-	-	-

* Composition of the nutrients obtained from the compilation of data from the manufacturers for commercial fertilizers and the Table TACO – Tabela Brasileira de Composição de Alimentos (UNICAMP, 2011) for banana (BP) and potato (P). T1 – half salts concentration Murashige and Skoog (MS/2); T2 – Knudson C medium (1946); T3 – Burgeff medium (1909); T4 – half salts concentration Murashige and Skoog (MS/2) added 100 g L^{-1} banana pulp; T5 – medium containing 5.0 mL L^{-1} commercial fertilizer Biofert® Plus Universal added 50 g L^{-1} potato and 50 g L^{-1} banana pulp; T6 – medium containing 5.0 g L^{-1} commercial leaf fertilizer JR Peters Inc. Blossom Booster NPK 10:30:20 added with 50 g L^{-1} potato and 50 g L^{-1} banana pulp; T7 – medium containing 5.0 g L^{-1} commercial leaf fertilizer JR Peters Inc. Orchid Special NPK 30:10:10 added with 50 g L^{-1} potato and 50 g L^{-1} banana pulp.

For *in vitro* growth assessment, selected seedlings with 4 cm length are transferred to new jars (14 plantlets/jar) containing the same media from germination to grow for 120 days. Monthly, for four months, the seedlings were transferred to new jars containing the same media. From each jar, 10 seedlings were randomly selected for evaluation.

Experiments for *in vitro* growth evaluation were performed in a completely randomized design with six treatments of 10 replicates each and were set up wherein each replicate consisted of 10 seedlings.

In vitro response was evaluated based on growth parameters: number of roots (NR); longest leaf length (LLL); number of shoots (NS); longest root length (LRL), and largest root diameter (LRD). The variables were subjected to analysis of variance (ANOVA) and the differences among means compared by Tukey's test ($P \leq 0.05$) using the statistical analysis software Sisvar 5.6.

RESULTS AND DISCUSSION

Several epiphytic orchid species are widely explored for in vitro cultivation, and numerous nutrient formulations have been evaluated for germination, stages of development and growth. Although there is no consensus, several reports indicate that MS medium (Murashige and Skoog) with half the nutrients concentration (MS/2) and formulations containing organic additives and/or commercial fertilizers represent a promising strategy for *in vitro* germination and growth of orchid seedlings, probably due to the lower nutritional requirements of each species (Galdiano Junior; Mantovani; Macedo Lemos, 2012; Galdiano Junior et al. 2014).

Different formulations can reduce *in vitro* production costs; however, attention should be given to the differential behavior for species germination and growth concerning the concentration of the nutrients of the culture medium, especially nitrogen and potassium contents, which differently affect the seedling formation and root system development.

The qualitative analysis of the seed's germination of *Catasetum macrocarpum* showed that the media T1 (MS/2 – control) and T6 (Peters®10:30:20PBP) were more effective in inducing embryos to develop into protocorms, which later differentiate into shoots and roots, thus forming the seedlings. The media T4 and T7 (MS/2BP and Peters®30:10:10PBP, respectively) produced fewer protocorms, but the seedlings were more vigorous, while the T5 (Biofert®PBP) showed the smallest germinability for this species, whose protocorms showed necrosis still in the early stage of the differentiation process. In the traditional media T2 (KC) and T3 (BU), it was possible to observe high germination but with slow differentiation of protocorms onto seedlings.

The qualitative analysis of *Catasetum fimbriatum* germination indicated that the media T5 (Biofert®PBP) and T6 (Peters®10:30:20PBP) showed a large amount of germinated and differentiated seeds with accelerated seedlings growth as well as the medium T4 (MS/2BP). The traditional media T1 (MS/2 – control), T2 (KC), and T3 (BU) showed high germination but slow differentiation of protocorms onto seedlings. The lower ability to initiate germination observed for this species occurred in material inoculated in the medium T7 (Peters®30:10:10PBP). However, the protocorms developed relatively well in shoots, according to data obtained from the growth parameters.

It is interesting to observe in Table 1 that the main source of nitrogen, potassium, magnesium and calcium of the T5 – Biofert®PBP, T6 – Peters®10:30:20PBP and T7 – Peters®30:10:10PBP medium comes essentially from the organic additives banana pulp and potato and other nutrients such as H_2PO_{4-}, K^+, H_3BO_3, Fe^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}, MoO_4^{2-}, Co and Cl^- are supplemented from the fertilizers Biofert® Plus Universal, JR Peters® Inc. NPK 10:30:20 and JR Peters® Inc. NPK 30:10:10.

The effects of culture media on the *in vitro* multiplication and growth of *Catasetum macrocarpum* for 120-days-old seedlings are in Table 2. The growth parameter number of shoots (NS) showed a more efficient response in media T1 [MS/2 – control] (1.87) and T6 [Peters®10:30:20PBP] (2.49). The treatments involving traditional media T2 (KC) and T3 (BU) provided the lowest shoots formation rates (0.58 and 0.39, respectively), though they did not show significant differences from T4 (MS/2BP), T5 (Biofert®PBP), and T7 (Biofert®PBP) (0.76, 0.62 and 0.74, respectively) and was observed a qualitative variation the development of the shoots.

The parameter longest leaf length (LLL) reflects the development and growth of the shoot, showed a more effective response in media based on fertilizers and supplemented with organic additives: T4 [MS/2BP] (11.00 mm), T5 [Biofert®PBP] (11.77 mm), T6 [Peters®10:30:20PBP] (13.79 mm) and T7 [Peters®30:10:10PBP] (10.68 mm). The T2 (KC) and T3 (BU) differed significantly concerning the other media and contributed less to the development and shoots growth (5.44 and 3.63 mm, respectively), while the control T1 (MS/2 – control) showed intermediate growth of shoots (9.28 mm).

The evaluation of the root development for *Catasetum macrocarpum* showed that the variation in media composition did not significantly interfere in the establishment of the number of roots (NR), even though the T4 (MS/2BP), T6 (Peters®10:30:20PBP) and T7 (Peters®30:10:10PBP) media that contain banana pulp showed the best response (7.81, 7.36 and 7.40, respectively) as well as traditional media T1 [MS/2] (5.77) and T2 [KC] (6.18).

For the parameter longest root length (LRL), the best growth response was observed in the T6 [Peters®10:30:20PBP] (11.75 mm) and T4 [MS/2BP] (9.25), but there was no significant variation in relation to the largest root diameter (LRD) for any of the evaluated media (LRD variation from 0.10 to 0.16 mm between all media). The T3 medium (BU) contributed less effectively to the formation of the root system (3.52 mm), although the length of the main roots and their respective diameters do not vary significantly concerning the other media (Table 2).

Ferreira et al. (2018) indicated to *Ctsm. macrocarpum* that each developmental stage for this specie requires a different culture medium with specific nutrient concentrations. The authors report that culture media containing higher levels of nitrogen (MS and MS/2) had lowest germination percentages and demonstrated that germination occurs better in the Knudson medium. At the same time, protocorms development is

better in Vacin & Went medium and seedling multiplication and growth is more effective in the MS/2 medium supplemented with 1.0 and 0.5 mg L^{-1} of benzyladenine, respectively.

The *in vitro* behavior of this species after 450 days of cultivation was also evaluated from seedlings submitted to four salts concentrations of MS (25%, 50%, 75%, 100%), two temperatures and three combinations of carbon sources and osmotic regulators (sucrose, mannitol or sorbitol) and the recommendation was that MS medium with 25% salt concentration supplemented with 20 g L^{-1} sucrose at a temperature of 25 ºC is suitable for *in vitro* conservation this species, where originated plants maintain their vigor and development during long periods of cultivation (Menezes-Sá et al., 2019).

In comparison to *Ctsm. macrocarpum*, the species *Ctsm. fimbriatum* had more variable behavior for all growth parameters concerning the culture media used, especially regarding the number of shoots and roots (Table 2, Figure 1a and 1b).

The growth parameter number of shoots (NS), *Ctsm. fimbriatum* was equivalent in all evaluated media (Table 2). For the LLL growth parameter, which reflects the leaf development, there was no significant difference between the conventional media T1 (MS/2) and T2 (KC) (8.65 and 6.48 mm), the medium supplemented with organic additives (T4–MS/2BP, 8.28 mm) or the media containing fertilizers plus organic additives (T5–Biofert®PBP and T7–Peters®30:10:10PBP, LLL 9.03 and 7.69 mm, respectively). However, leaf development was favored in these media, compared to T3 (BU), which presented a less expressive result, while the T6 (Peters®10:30:20PBP) media showed intermediate growth.

Table 2: Mean values for growth parameters: number of shoots (NS); longest leaf length (LLL); number of roots (NR); longest root length (LRL); largest root diameter (LRD), for seven culture media in the development of *Catasetum macrocarpum* and *Ctsm. fimbriatum* after 120 days of cultivation.

Species	Treatments *	Parameters				
		NS	LLL (mm)	NR	LRL (mm)	LRD (mm)
Catasetum macrocarpum	[T1–MS/2]	1.87 a**	9.28 b	5.77 ab	6.10 b	0.12 ab
	[T2–KC]	0.58 b	5.44 c	6.18 ab	6.87 b	0.10 b
	[T3–BU]	0.39 b	3.63 c	3.52 c	7.79 b	0.12 ab
	[T4–MS/2BP]	0.76 b	11.00 ab	7.81 a	9.25 ab	0.12 ab
	[T5–Biofert®PBP]	0.62 b	11.77 ab	5.24 bc	7.79 b	0.14 ab
	[T6–Peters®10:30:20PBP]	2.49 a	13.79 a	7.36 ab	11.75 a	0.12 ab
	[T7–Peters®30:10:10PBP]	0.74 b	10.68 ab	7.40 a	7.60 b	0.16 a
Catasetum fimbriatum	[T1–MS/2]	0.54 ab	8.65 a	3.00 d	6.59 bc	0.15 bcd
	[T2–KC]	0.28 b	6.48 ab	3.06 d	5.69 bc	0.13 d
	[T3–BU]	0.57 ab	3.62 c	3.07 d	8.22 ab	0.14 cd
	[T4–MS/2BP]	0.89 ab	8.28 a	6.28 a	4.50 c	0.14 cd
	[T5–Biofert®PBP]	1.13 a	9.03 a	5.06 ab	10.70 a	0.19 a
	[T6–Peters®10:30:20PBP]	0.91 a	4.81 bc	4.67 bcd	7.59 ab	0.17 ab
	[T7–Peters®30:10:10PBP]	0.67 ab	7.69 a	4.95 abc	7.51 ab	0.17 ab

* T1 – half salts concentration Murashige and Skoog (MS/2); T2 – Knudson C medium (1946); T3 – Burgeff medium (1909); T4 – half salts concentration Murashige and Skoog (MS/2) added 100 g L^{-1} banana pulp; T5 – medium containing 5.0 mL L^{-1} commercial fertilizer Biofert® Plus Universal added 50 g L^{-1} potato and 50 g L^{-1} banana pulp; T6 – medium containing 5.0 g L^{-1} commercial leaf fertilizer JR Peters Inc. Blossom Booster NPK 10:30:20 added with 50 g L^{-1} potato and 50 g L^{-1} banana pulp; T7 – medium containing 5.0 g L^{-1} commercial leaf fertilizer JR Peters Inc. Orchid Special NPK 30:10:10 added with 50 g L^{-1} potato and 50 g L^{-1} banana pulp.
** Equal letters in the same column for each species do not differ in Tukey's test at 5%.

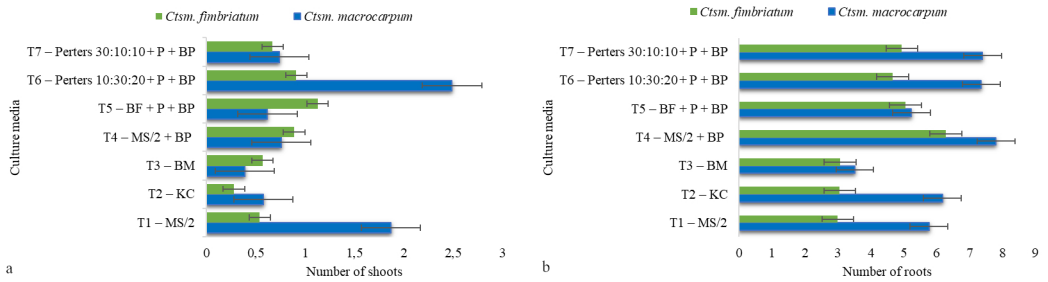

Figure 1: Comparison of growth parameters number of shoots (a) and number of roots (b) between *Catasetum macrocarpum* and *Ctsm. fimbriatum* as a function of the evaluated culture media. Error bars represent standard deviation (n = 100). Statistical significance was assessed by performing ANOVA and means compared by Tukey's test ($P \leq 0.05$).

The evaluation of the root development for *Ctsm. fimbriatum* showed that the conventional medium T4 (MS/2BP) induced the formation of a higher number of roots (6:28), but this behavior did not differ significantly from T5 [Biofert®PBP] (5:06) and T7 [Peters®30:10:10PBP] (4.95) media. The traditional culture media (T1 – MS/2, T2 – KC and T3 – BU) showed significant differences in relation to the media containing fertilizers and, or supplemented with organic additives (3.0, 3.06 and 3.07, respectively), except for the T6 – Peters®10:30:20PBP medium, reflecting less effective behavior in inducing root development.

For the parameter longest root length (LRL), the behavior was quite different compared to *Ctsm. macrocarpum*. The development of the root system of *Ctsm. fimbriatum* was more accentuated in the medium T5 – Biofert®PBP (10.70 mm and 0.19 mm, respectively).

Despite not differing from each other, the T1 (MS/2), T2 (KC), and T4 (MS/2BP) media contributed less to root elongation (6.59, 5.69 and 4.50 mm). Concerning the largest root diameter (LRD), although low differences were detected among the media, those containing fertilizers exclusively and organic additives (T5 – Biofert®PBP, T6 – Peters®10:30:20PBP and T7 – Peters®30:10:10PBP) showed better response to this parameter (0.19, 0.17 and 0.17 mm, respectively).

Regarding the germination process and the development of protocorms, the use of organic additives like potato extract has expressed significant results for species such as Vanda Kasem's Delight (Gnasekaran et al., 2012) where supplementation with potato in the initial culture medium favored germination and promoted results positive in the protocorms formation.

The overall performance of the T6 medium (Peters® NPK 10:30:20 and potato and banana pulp) in the growth of *Ctsm. macrocarpum* and *Ctsm. fimbriatum* also may be related especially to the potato's presence as an additive which also contains vitamins like B6 that could provide essential amino acids and induces better seed germination (Gnasekaran et al., 2012) and the banana pulp that induces the roots multiplication and elongation of shoots.

In cultivation of *Dendrobium lituiflorum* Lindl. has been proposed that banana can promote rapid plants regeneration without causing any deformation in growth and, considering that it is an available and low-cost organic additive, has immense potential to be exploited in the large-scale production of tropical orchids, in addition to promoting the development in rural populations and preserving biodiversity (Vyas et al., 2009).

A similar result was obtained in the *in vitro* cultivation of *Dendrobium nobile* Lindl. using a fertilizer NPK supplemented with banana pulp that showed a better development of seedlings and emphasized that the alternative culture media proves to be viable for the orchid's growth and development by its simplicity of use, availability of products and low final cost (Su et al., 2012).

The analysis of seedling growth of *Ctsm. macrocarpum* and *Ctsm. fimbriatum* in different treatments (Table 2) demonstrates the potential use of the combination of commercial fertilizers and organic additives. A different response for the *in vitro* growth of *Catasetum fimbriatum* (E. Morren) Lindl. & Paxton has been obtained in the MS culture media MS/2, MS/4, Vacin & Went, Knudson C and media with NPK (10-5-5) and NPK (10-30-20) reinforcing the need to determine

culture media with nutritional formulations suitable for optimization of plant quality produced *in vitro* (Rego-Oliveira; Faria, 2005).

Evidence from other genera of Orchidaceae demonstrates the potential action of commercial fertilizer Peters® NPK 10-30-20 at *in vitro* orchid development. Evaluation of *in vitro* growth of *Cattleya trianaei* seedlings in MS, MS/2 media and formulated with Peters® NPK 10-30-20 in different doses recommended the use of the simplified medium with fertilizer Peters® 3 g L^{-1}, which presents more balanced amounts of the two sources of nitrogen (NH_4 and NO_3) as an alternative and low-cost strategy for *in vitro* growth of this species (Galdiano Junior; Mantovani; Macedo Lemos, 2012). For seedlings of the hybrid *Cattleya walkeriana* x *C. loddigesii* cultivated with different formulations, including Peters® NPK fertilizer 10-30-20 and NPK 30-10-10 the pieces of evidence shows that the addition of this fertilizer results in a higher yield of a fresh matter of seedlings (Rodrigues et al., 2012a).

In vitro growth of *Cattleya walkeriana* Gardner seedlings under different doses of NPK fertilizer 10-30-20 supplemented with Mg and micronutrients demonstrated low concentrations of this fertilizer (3.55 g L^{-1}) could lead to maximum plants production. In contrast, high concentrations induce a significant reduction of root growth. The influence of N, P, K, Ca, Mg, S, Fe, Mn, B and Zn on plant growth is discussed and suggest that considering the root structure of epiphytic orchids, nutrient supplementation should be continuous and in low concentrations (Rodrigues et al., 2012b).

Supplementation with organic additives such as banana pulp has been evaluated in the *in vitro* growth of *Cattleya* hybrid (Araújo et al., 2006), *Cattleya loddigesii* (Pasqual et al. 2009), *Dendrobium aqueum* (Parthibhan; Rao; Kumar, 2015) and *Paphiopedilum venustum* (Kaur; Bhutani, 2016), evidencing a positive relationship between the presence of this organic additive and the increase in the number of shoots and healthy seedlings growth, corroborating the results obtained here with the T6 – Peters®10:30:20PBP medium.

The question remains whether the presence of banana pulp as a natural additive could produce similar or better effects to the auxins (IBA or ANA) in roots number and length than the components of medium (Menezes Gonçalves et al., 2016). Bananas are a good source of K, Mg, Cu and Mn, generally contain more carotenoid type lutein than provitamin A pigments, β-carotene and α-carotene and low amounts of vitamin C or A (Wall, 2006). The banana pulp could supplement the amount of sugar, vitamins, amino acids, antioxidants, minerals, organic acids and growth regulators in culture media (George; Hall; De Klerk, 2008; Martínez; García, 2007) and therefore it could explain the relation between banana pulp concentration and root growth, leaf growth and fresh weight (Araújo et al., 2006).

The use of potato as an organic additive in the culture media was reported for *Dendrobium* as beneficial in protocorm developing and seedlings formation (Tharapan; Thepsithar; Obsuwan, 2014). Apart from occasional reports of potato used as an additive for orchid propagation (George; Hall; De Klerk, 2008), little is known about its influence and benefits to orchids *in vitro* development. This contains carbohydrates, vitamins, proteins, phenolic compounds and some amino acids and fatty acids and one of these compounds, alone or in combination, might increase orchids growth (Tharapan; Thepsithar; Obsuwan, 2014). However, potato powder was inefficient in initiating shoot multiplication and harmful to the *in vitro* culture of *Paphiopedilum venustum* (Wall. Ex Sims.) by reducing the osmotic potential of culture medium from the hydrolysis of the starch during autoclaving (Kaur; Bhutani, 2016).

The results here obtained revealed that the nutrient-rich media are not the most suitable for germination and growth of *Catasetum* species and that this probably reflects the actual demand for growth of the epiphyte species, i.e., low quantities of nutrients as proposed by Knudson (1946) and Arditti (2009). Differences in the growth of shoots and roots occur in response to the availability of nutrients and sources of nitrogen and may be related how plants maximize the acquisition and use of such elements for development.

The composition of nutrients in the culture media indicated in Table 1 shows that, for the T6 medium, which had the best result in general, the main source of total nitrogen was available by the additives potato (144 mg L^{-1}), followed by banana pulp (112 mg L^{-1}). At the same time, JR Peters® Inc. NPK 10:30:20 fertilizer provided a relatively low but balanced amount of different forms of nitrogen (0.25 mg L^{-1} NH_4^+ and 0.25 mg L^{-1} NO_3^-) and the combination of these elements in association with the other nutrients in the culture media, even in small quantities, would be sufficient to favor the process of morphogenesis and regeneration.

Comparison between the nutrient amounts of the evaluated media (Table 1) shows that, for stimulate the germination process and the subsequent differentiation stages of protocorms until the full development of seedlings of both *Catasetum* species, that are epiphytes and inhabit nutrient-poor environments, a minimum macronutrients supplementation is necessary. This simplification of the culture media can contribute to reducing the commercial production costs of these orchids, since producing one liter of simplified medium formulation generates savings of 75-80% compared to traditional media (Su; Ribeiro; de Faria, 2012).

There are suggestions that glutamine is a better form of nitrogen for the growth of *Ctsm. fimbriatum* than inorganic forms and free amino acids may be important natural sources of nitrogen for this plant species (Majerowicz et al., 2000). Studies on the effects of forms of organic nitrogen (urea and glutamine) or inorganic (nitrate and ammonium) on dry matter accumulation in shoots and roots and on nitrogen assimilatory enzyme activities demonstrated inverse patterns for two genotypes of *Ctsm. fimbriatum* and evidenced that growth pattern was influenced but not altered by any nitrogen source (Majerowicz; Kerbauy, 2002).

The question about the real contribution of nutrients from organic additives and commercial fertilizers to germination and morphogenesis is not yet fully elucidated and opens future research perspectives. However, this study demonstrated that combination of additives banana pulp and potato, which generally have low cost for the *in vitro* culture process, associated with commercial fertilizers like Peters® NPK 10:30:20, even in minimal quantities, proved to be more efficient for germination, early development of protocorms and complete seedling formation compared to traditional culture media to *Catasetum macrocarpum* and *Ctsm. fimbriatum*.

CONCLUSIONS

From germination to complete seedling development of *Catasetum macrocarpum* and *Ctsm. fimbriatum* it is possible to use a basal medium, of simple composition and low cost, containing commercial fertilizers and supplemented with organic additives. We assume that the media containing commercial leaf fertilizer Peters® NPK 10:30:20, supplemented with banana pulp and potato, were the most efficient for growth in both species under analysis, providing appropriate nutrients quantities for the development of protocorms and formation of shoots and roots.

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