In vitro multiplication of *Codonanthe devosiana* [1]

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ABSTRACT

Codonanthe devosiana is a species endemic to Brazil belonging to the family Gesneriaceae. It features ornamental potential for use in hanging pots and shaded environments. The aim is to evaluate the effects of the combination of different concentrations of BAP and GA_3 on the multiplication and development of *in vitro* explants of *C. devosiana*. We used MS media supplemented with concentrations of 0.0, 0.2 and 0.4 mg L^{-1} of BAP and 0.0, 0.25 and 0.50 mg L^{-1} of GA_3. The number, bud dry matter and height, callus dry matter and presence of roots were evaluated in agglomerates with multiple buds. The interaction between BAP and GA_3 concentrations was verified for number of shoots, shoot dry matter and callus dry matter. For shoot height and root formation, only BAP concentrations were observed. The highest number of shoots (4.8) occurred when combined with the concentration of BAP 0.2 mg L^{-1} and 0.5 mg L^{-1} GA_3. The lowest shoot height was observed without the use of BAP, as well as the greatest rooting. It is concluded that the combination of 0.2 mg L^{-1} of BAP with 0.5 mg L^{-1} of GA_3 allows for a more efficient multiplication of *C. devosiana* explants.

Keywords: floriculture, Gesneriaceae, growth regulators.

RESUMO

Multiplicação *in vitro* de *Codonanthe devosiana*

Codonanthe devosiana é uma espécie endêmica do Brasil pertencente à família Gesneriaceae. Apresenta potencial ornamental para uso em vasos suspensos e ambientes sombreados. O objetivo foi avaliar o efeito da combinação de diferentes concentrações de BAP e GA_3 na multiplicação e desenvolvimento de explantes *in vitro* de *C. devosiana*. Utilizou-se meio MS suplementado com as concentrações de 0,0; 0,2 e 0,4 mg L^{-1} de BAP e 0,0; 0,25 e 0,50 mg L^{-1} de GA_3. Foram avaliados o número, massa seca e altura de brotações, massa seca de calos e presença de raízes nos aglomerados com múltiplas brotações. Foi verificada interação entre as concentrações de BAP e GA_3 para número de brotações, massa seca de brotações e massa seca de calo. Para altura de brotações e formação de raízes houve efeito apenas das concentrações de BAP. O maior número de brotações (4,8) ocorreu quando combinada a concentração de 0,2 mg L^{-1} de BAP e 0,5 mg L^{-1} de GA_3. A menor altura de brotações foi constatada sem o uso de BAP, assim como o maior enraizamento. Conclui-se que a combinação de 0,2 mg L^{-1} de BAP e 0,5 mg L^{-1} de GA_3 permite a multiplicação mais eficiente de explantes de *C. devosiana*.

Palavras-chave: floricultura, Gesneriaceae, reguladores de crescimento.

1. INTRODUCTION

Codonanthe devosiana Lem. is a species endemic to Brazil belonging to the family Gesneriaceae, occurring naturally in the areas of the Atlantic Forest (ARAÚJO and CHAUTEMS, 2015) in Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul States. It growths at altitudes ranging from 20 to 800 m, on humid rocks or tree trunks in shady environments, and may also occur in places with higher insolation (LOPES et al., 2005). It is classified as a characteristic holoepiphyte, that is, normally it is germinated and grows on other vegetables (KERSTEN and SILVA, 2001), pollination and dispersion strategies were entomophilous and autochorous (PADILHA et al., 2015). It has an herbaceous bearing, hanging stems and flowering throughout the year, presenting ornamental potential, mainly for use in hanging pots and shaded environments (SAUERESSING, 2016).

One of the main obstacles to the greater use of native species such as ornamental plants is the lack of quality and quantity seedlings for commercialization. In this sense, micropropagation allows a large number of plants to be obtained in a short period, with high uniformity and sanitary quality, independent of seasonal factors (CID, 2014), and may be an important alternative for the propagation of native species and their commercial cultivation, avoiding extractivism. However, for the viability of this type of culture, the composition and concentration of growth regulators in the culture medium are determinant to the growth and induction of physiological responses, such as root formation, calluses and buds, and internode elongation (CID, 2014). Cytokinins are, along with auxins, the most

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used growth regulator class in *in vitro* culture, while the use of gibberellin is less frequent. Cytokinins are used to induce bud formation and high multiplication rates, while gibberellins are used in *in vitro* cultivation for bud elongation (CID, 2014).

For *Sinningia speciosa*, Lood. Hiern. *Saintpaulia ionantha* Wendl. *Chirita moonii* Gardn. species belonging to Gesneriaceae family, there are significant variations in the response of the application of BAP for the multiplication of explants (ARAÚJO et al., 2004; LUCAS et al., 2007; HERATH, 2013), confirming the need for adjustments in the concentration of phytoregulators for each species.

As a native species with ornamental potential not yet explored by floriculture, the use of *in vitro* propagation may be an alternative to obtain clones of selected specimens, guarantee the health of seedlings produced and enable its cultivation. In this context, the effects of the combination of different concentrations of BAP (6-Benzylaminopurine) and GA_3 (Gibberellic acid) on the multiplication and development *in vitro* of explants of *C. devosiana* was evaluated.

2. MATERIALS AND METHODS

Cultivated explants *in vitro* obtained from seeds collected in the municipality of São Francisco de Paula, located at the coordinates 29°27'46" S and 50°36'13" W, were used.

We used a MS culture medium (MURASHIGE and SKOOG, 1962) supplemented with 30 g L^{-1} of sucrose and 7 g L^{-1} of agar. Concentrations and combinations of growth regulators cytokinin and gibberellin were tested. As a source of cytokinin, BAP (6-Benzylaminopurine) was used at concentrations of 0.0, 0.2 and 0.4 mg L^{-1} and, as a source of gibberellin, GA_3 (Gibberellic Acid) at the concentrations 0.0, 0.25 and 0.50 mg L^{-1} was used.

The pH of the culture medium was adjusted to 5.8 ± 0.1, then poured into 300 mL glass vials and filled with approximately 30 mL of medium; then, the vials were closed with double aluminum paper sheets. After this procedure, they were autoclaved at 121 °C, at a pressure of 1.2 atm, for 15 minutes.

Apical segments standardized to 0.5 cm in height and four leaves were used as explants. After incubation of explants in a flow chamber, the flasks were kept in a growth room with a 16-hour photoperiod and temperature of 25 ± 2 °C, and light intensity from 27 to 33.75 μmol m^{-2} s^{-1}.

The evaluations were carried out after 108 days regarding number, bud dry matter and height, callus dry matter and presence of roots in agglomerates with multiple buds. The presence of roots was assessed by visually assessing the tissues with apparent roots. Subsequently, the number of buds was counted, separating shoots from roots or from the callus in which they were inserted. The multiplication rate was obtained by dividing the total number of buds by the initial number of explants of each replicate. The height of the buds was measured using a millimeter ruler. Twenty buds were selected at random for each replication.

After these procedures, shoot and calluses were placed in a 65 °C oven, separately, until constant weight, and weighed to obtain the dry matter, which was divided by the total number of buds of the replication to obtain the individual mass of buds and the mass of callus formed to produce a bud (mg).

The experiment was completely randomized in a 3x3 factorial design (three concentrations of BAP and three concentrations of GA_3). Four replicates were made composed of three flasks containing six explants each. Data were subjected to analysis of variance and means were compared by Tukey test at 5% probability.

3. RESULTS AND DISCUSSION

We verified an interaction between BAP and GA_3 concentrations for number of buds, bud dry matter and callus dry matter. For shoot height and root formation, only BAP concentrations were observed. The highest number of buds occurred when combined 0.2 mg L^{-1} of BAP and 0.5 mg L^{-1} of GA_3, with the production of 4.8 shoots per initial explant (Table 1). Thus, through three subcultures, it is possible to obtain more than 100 plants from a single initial explant, demonstrating with this the high capacity of multiplication of the species.

Table 1. Number of buds per explant, dry mass of callus and dry buds mass of *Codonanthe devosiana* cultivated *in vitro*.

	Number of Buds			Dry Mass of Buds (mg)			Dry Mass of Callus (mg)		
	BAP			BAP			BAP		
GA_3	0	0.20	0.40	0	0.20	0.40	0	0.20	0.40
0	1.1 Ac	2.7 Bb	4.0Aa	8.2 Aa	2.8 Ab	3.4 Bb	17.2 Ba	11.8 Aab	08.1 Ab
0.25	1.2 Ab	2.7 Ba	3.4 Aa	4.8 Ba	3.5 Aa	3.8 Ba	19.9 Ba	09.3 Ab	08.0 Ab
0.50	1.4 Ac	4.8 Aa	3.1 Ab	5.0 Bab	3.0 Ab	6.7 Aa	33.1 Aa	06.7 Ab	06.7 Ab
CV	24.29			23.72			22.50		

Means followed by distinct letters, upper case in the column and lower case in the line, differ by Tukey test at 5%.

Sinningia speciosa, a species belonging to the same family, the highest bud production (3.5) also occurred when using the BAP concentration of 2 mg L^{-1} (ARAÚJO et al., 2004). For *Saintpaulia ionantha*, also belonging to the family Gesneriaceae, the concentration 0.4-1.0 mg BAP L^{-1} provided the highest multiplication rate for the tested cultivars (LUCAS et al., 2007). *Chirita moonii* produced 12 buds per initial explant using 3 mg BAP L^{-1} in a two-month period (HERATH, 2013). Thus, as can be observed, species of the same family present varied responses to the application of phytoregulators, being important the adjustment for each species worked. For *Mentha* x *Piperita* L., the combination of 4.0 mg BAP L^{-1} and 0.5 mg GA_3 L^{-1} was the one that promoted the highest number of buds: 6.08 per explant, indicating that GA_3 can stimulate multi-budding when combined with BAP (MORAIS et al., 2014). A similar result was found for *Ananas comosus* L., where the highest shoot production (6.1) occurred when the concentration 1.0 BAP mg L^{-1} was combined with 0.5 mg GA_3 L^{-1} (DIAS et al., 2011).

In another ornamental plants the effect of BAP and GA_3 are described, for example, in micropropagation of *Begonia homonyma* Steud. the combination of 5 µM GA_3 and 0.5 µM BA (Benzyladenine) produced the highest number of adventitious shoots (37.2 shoots per explant, 44.0 mm in length) after 12 weeks culture (KUMARI et al., 2017) and in *Magnolia* the presence of 0.1 mg L^{-1} of GA_3 have obtained a highest multiplication rate (5.9 shoots/explants) and the highest number of leaves (25.7 leaves per multiplied clumps) (WOJTANIA et al., 2016).

The combination of BAP and GA_3 was also favorable for the multiplication of *C. devosiana* explants. Cytokinins promote cell division and are used to promote the induction of adventitious buds from callus or to induce multi-budding from axillary or apical buds, and are frequently used in culture *in vitro.* However, gibberellins are not considered essential for micropropagation, but can be used to promote shoot lengthening in some species (HARTMANN et al., 2011), as well as having positive effects on explant multiplication.

The adjustment of phytoregulator concentrations should be favorable for multiplication, but should also provide buds with satisfactory development for later acclimatization. In this sense, we verified that, in the absence of BAP, there was a decrease in shoot dry mass with the use of GA_3. The inverse was verified at the concentration 0.4 mg BAP L^{-1}, where we observed an increase when the concentration of 0.5 mg GA_3 L^{-1} was used. Thus, although the combination of 0.4 mg BAP L^{-1} with 0.5 mg GA_3 L^{-1} produced fewer buds, they were more developed, as verified by the data of bud dry mater (6.7 mg). A minimal development of buds is necessary for a rapid and uniform multiplication in a posterior subculture (HARTMANN et al., 2011), and to facilitate seedlings acclimatization.

Another characteristic verified and evaluated in this study was the presence of callus, which is quite expressive in some treatments. In the absence of BAP, there was a higher dry matter of callus in relation to the other concentrations tested. When the absence of BAP was combined with the use of 0.5 mg GA_3 L^{-1}, 33 mg of callus was produced per bud produced, whereas, for example, in the presence of 0.2 mg BAP L^{-1}, at the same concentration of GA_3 (0.5 mg L^{-1}), the ratio was 6 mg of callus for a bud.

In general, in genotypes of *Eucalyptus dunni* Maiden, the addition of GA_3 to the culture medium was also not favorable for bud elongation and the increase of their concentration increased the formation of callogenic structures (NAVROSKI et al., 2013). A similar result was found for *Mentha x Piperita*, in which there was a calogenesis above 77% of the explants when using concentrations of 0.5 mg GA_3 L^{-1} of and 2 mg BAP L^{-1} (MORAIS et al., 2014). For *Saintpaulia ionantha*, the presence of callus was observed only at concentrations higher than 0.4 mg BAP L^{-1} (LUCAS et al., 2007). Thus, the composition of the medium and the concentrations of phytoregulators are essential to obtain high multiplication rates and quality explants. Both cytokinin and gibberellin, when used in excess, may cause callus formation, which in the case of multiplication of explants *in vitro* is not interesting, since these structures may compromise the proliferation of axillary buds and the lengthening of budding, affecting its development (GRATTAPAGLIA and MACHADO, 1998). In addition, the nutrients used in callus formation could be used for the development of new shoots in explants.

For the height of bud of *C. devosiana*, there were only effects of BAP concentrations, in which the lowest height was observed without the use of the phytoregulator, there being no effects of GA_3, contrary to what was expected (Table 2).

Table 2. Buds height and rooting percentage of agglomerates with multiple buds of *Codonanthe devosiana* cultivated *in vitro*.

BAP	Height (mm)	Rooting (%)
0	09.19 b	70.83 a
0.20	13.47 a	66.66 ab
0.40	13.08 a	45.37 b
CV	17.42	41.18

Means followed by distinct letters, upper case in the column and lower case in the line, differ by Tukey test at 5%.

Gibberellins act in the stem growth, promoting cellular stretching, mainly in young cells (KERBAUY, 2013). The GA_3 is used in propagation *in vitro* to promote shoot elongation, during the pre-rooting phase, in cultures that form budding agglomerates, when shoots cannot be separated due to their size. However, there is usually no uniform response to shoot lengthening (GRATTAPAGLIA and MACHADO, 1998), which may explain the absence of gibberellin effects in this work. In addition, the higher multiplication rates with the increase of BAP concentration may have led to the grouping of shoots, causing them to grow and increase their height, which in this work proved to be favorable, since there were no damages to the multiplication of shoots and their development.

In *Egletes viscosa*, the highest height of the plants occurred with the use of 0.5 mg GA_3 L^{-1} of, and absence of BAP in the culture medium, with the addition of cytokinin, causing a reduction in height growth of plants (DINIZ et al., 2003). The increase in the concentration of cytokinin in the medium causes an increase in the number of shoots and a decrease in their height (LUCAS et al., 2007; MORAIS et al., 2014). In contrast to that observed in the present study, in *Sinningia speciosa*, the height of shoots linearly decreased with increasing BAP concentrations in the culture medium (ARAÚJO et al., 2004), similar to that observed for *Saintpaulia ionantha*, where the highest shoot height occurred when there was no addition of BAP to the culture medium (LUCAS et al., 2007). The same occurred for *Ananas comosus*, where the largest shoot length was obtained in the absence of BAP and with an GA_3 concentration of up to 2 mg L^{-1}, there being no interaction between growth regulators for this evaluation (DIAS et al., 2011). It may be inferred in this case that the endogenous concentration of gibberellin produced by *in vitro* explants of *C. devosiana* was not limiting to promote elongation of stems and therefore their use is not indicated for this purpose. However, due to its action on the increase in sprout production, its use may be restricted to the initial stage for multiplication of explants.

Bud size decreased with the addition of GA_3 and the absence of BAP for *Mentha x Piperita*, differently from that observed for *C. devosiana.* The height of buds was also shorter when the concentration of cytokinin applied in combination with gibberellin was higher, and the larger size was obtained in the absence of both phytoregulators (MORAIS et al., 2014). These results, according to the authors, indicate that the endogenous concentration of hormones of the species is sufficient to stimulate the growth of the formed shoots, which may also have happened in this study.

In the case of rooting, there was also only effect of the BAP, and for the higher concentrations, a lower root formation was verified (45%). In the absence of BAP, rooting was greater than 70%. High concentrations of cytokinin to stimulate bud formation may lead to inhibition of induction or to a stall of root growth (CID, 2014). The results of this work were similar to those reported for *Ananas comosus*, in which the highest number of roots was obtained in the absence of BAP with no influence of the concentrations of GA_3 used (DIAS et al., 2011), and for *Saintpaulia ionantha*, where root formation was observed only in the absence of BAP (LUCAS et al., 2007). For *Egletes viscosa***,** a higher rooting was observed when increasing concentrations of GA_3 were used. The opposite was observed for BAP concentrations, where there was a reduction in the number of explants with roots at higher concentrations of this regulator (DINIZ et al., 2003).

Thus, for *C. devosiana*, even in the absence of BAP, when there is a greater rooting (70.8%), a subsequent step of *in vitro* cultivation would be necessary for the rooting of explants to obtain a greater success in the process of acclimatization of plants.

Although studies for the species are still at an initial phase and data have been obtained from seedlings, they may serve as the basis for enabling the cloning of adult individuals with previously known potential or selected varieties, since *C. devosiana* presented considerable explant multiplication capacity, being able to be easily cultivated *in vitro*, thus obtaining standardized plants with sanitary quality assurance.

4. CONCLUSIONS

For *C. devosiana*, the combination of 0.2 mg L^{-1} of BAP and 0.5 mg L^{-1} of GA_3 allows a more efficient multiplication of explants, as it causes more budding developed and with less callus formation.

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AUTHORS CONTRIBUTIONS

A.A.E.: Responsible for the implementation, evaluation and writing of the article; **M.C.W.**: assisted in the implementation of the experiment and writing of the article; **G.T.G.**: assisted in the implementation and evaluation of the experiment; **C.S.F.**: assisted in the preparation of the study and correction of the article; **G.S.**: assisted in the preparation of the study and the correction of the article.

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