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ARTICLE

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In vitro propagation of *Cyrtopodium saintlegerianum* rchb. f. (orchidaceae), a native orchid of the Brazilian savannah

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Abstract – *In order to enable production of large quantities of plantlets for reintroduction programs, as well as economic exploration,* Cyrtopodium saintlegerianum *seeds were sown on Knudson culture medium. After seed germination, the protocorms were inoculated on Knudson culture medium supplemented with 6-benzyladenine (BA) and α-naphthaleneacetic acid (NAA). The obtained shoots were individually inoculated in Knudson supplemented with gibberellic acid (GA_3) in order to promote elongation. Seedlings were evaluated and then transplanted into trays containing commercial substrate Plantmax®-HT, or crushed Acuri leaf sheath. Auxin/ cytokinin ratio influenced in vitro propagation of C. saintlegerianum, resulting in increased shoot number when 2.0 mg L^{-1} BA was added to the culture medium in the absence or presence of 0.5 mg L^{-1} NAA. This species proved to be promising for massal in vitro multiplication. Despite having incremented in vitro shoots elongation, the use of GA_3 is unnecessary since it contributed negatively in the acclimatization of plants.*

Key words: Orchidaceae*, micropropagation, plant growth regulators, acclimatization.*

INTRODUCTION

The use of *in vitro* plant propagation methods in order to obtain large numbers of individuals for commercial purposes, for the plant conservation and protected area management, and for supporting programs of reintroduction of species in the native environment has proven to be successful for many species (Faria et al. 2002, Stewart 2008, Suzuki et al. 2012, Sabarimuthan et al. 2013). *In vitro* germination, unlike micropropagation, has the great advantage of generating genetically different individuals, which is a desirable factor for environmental programs. Thus, the seed germination is the most efficient method of propagating terrestrial orchids, and both symbiotic and asymbiotic germination methods have been used (Johnson et al. 2007, Aggarwal and Zettler 2010).

Cyrtopodium R. Br. is considered a neotropical genus and Brazil, with 39 species, is the country with the greatest diversity of this genus, and the Brazilian Savannah (Cerrado) is the most important diversity center (Menezes 2000, Romero-González et al. 2008). Cyrtopodium species have remarkable ornamental potential, and there are reports of pharmacological use of its pseudobulbs (Vieira et al. 2000). The search for unpublished species and increasingly exotic plants, in Brazil and in other parts of the world, leads native orchids trade to adopt extractive practices, which combined with the destruction of their natural habitat in the past 10 years due to the advancement of agriculture, and also leads to drastically reduction or to the extinction of the population of many species (Colombo et al. 2004, Flachsland et al. 2011), including those of the *Cyrtopodium* genus. These orchids are popular among collectors due to their high ornamental value because of their beautiful flowers (Menezes 2000).

Cyrtopodium saintlegerianum Rchb. f. is geographically well distributed in Brazil and found in the states of Pará, Tocantins, Piauí, Bahia, Mato Grosso, Goiás, Minas Gerais and Distrito Federal (Barros et al. 2010). This species has epiphytic habit, and in the Savannah regions, some palm species, such as acuri palm (*Scheelea phalerata* - Arecaceae), offer good conditions for its germination and development. In the northeast of the state of Mato Grosso do Sul, *C. saintlegerianum* has been intensively collected

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from its habitat, and additionally, the agricultural expansion has reduced the areas of its natural occurrence. Clements (1988) and Ferreira and Suzuki (2008) affirmed that the vulnerability of native orchids can also be related to their highly specialized life cycle, including a long vegetative period until the reproductive period, and seeds with little or no reserves, resulting in a dependence of the association with mycorrhizal fungi to germinate. These characteristics lead to a lower seminiferous propagation of orchids in nature, since less than 5% of the seeds will be successful in the development of new plant.

Storage of orchid seeds is important for providing seeds for a prolonged period strategy, considering that the species often produce flowers in specific months throughout the year; however, the storage results in seed viability loss. Thus, techniques that enable the use of seeds with low germination percentage is crucial to obtain greater seedlings number. Three in vitro pathways of protocorm cloning were revealed by Andronova et al. (2000), Batygina et al. (2003), Mahendran and Bai (2012) and Zeng et al. (2013): i) formation of a large shoot apexes number, with further endogenous production of adventitious roots; ii) formation of numerous secondary protocorms from epidermal cells of single protocorm; and iii) formation of numerous secondary protocorms derived from calluses.

Despite the economic importance to the floriculture and the therapeutic value of pseudobulbs, many Cyrtopodium species are commercially unknown (Batista and Bianchetti 2006), or do not have efficient propagation protocols. *In vitro* culture can be an interesting technique for both propagation and germplasm conservation of this species, and it provides justification for propagation and conservation of this valuable germplasm. In this respect, the aim of this study was to obtain a micropropagation protocol for *C. saintlegerianum*, aiming to enable production of plants for use in conservation programs, and for commercial exploration of the species.

MATERIAL AND METHODS

Plant material and asymbiotic germination of *C. saintlegerianum* seeds

Mature capsules of *C. saintlegerianum* were collected on expeditions in the Savannah area placed in Costa Rica – MS in 2012, supported by SISBIO 22570-2 authorization. This species has epiphytic habit and is commonly found in palm trees known as acuri (*Scheelea phalerata* - Arecaceae). For species identification procedures, exsiccates were prepared and deposited in the herbarium of the Botanical Institute, in São Paulo. Capsules were taken to laboratory and carefully washed. After that, the capsules were taken to the laminar flow and immersed in a solution of ethanol 70% (v/v) for two minutes, followed by a triple rinse in distilled autoclaved water. After fruit superficial sterilization, it was cut using a scalpel, and seeds were sowed on Petri dishes containing 30 mL of sterile culture medium.

The culture medium used was Knudson modified by Arditti and Ernst (1993), added with 20 g L^{-1} sucrose and solidified with 6 g L^{-1} agar (HiMedia®) in the absence and presence of 3 g L^{-1} activated charcoal. Media were adjusted to pH 5.8 ± 0.1, and were sterilized by autoclaving (121 °C and 104 kPa, 20 minutes), and 30 ml were poured into Petri dishes, which after seeds addition were subsequently sealed with PVC film (Dispafilm of Brazil Ltda) and kept in plants growth room under 16 h photoperiod with 36 μmol m^{-2} s^{-1} of photosynthetically active radiation, provided by two fluorescent lamps (luz especial do dia, 40 W, Osram) at 27 ± 2 °C.

Growth regulators on protocorms shoot induction medium

Thirty days after *C. saintlegerianum* seed inoculation, obtained protocorms were aseptically transferred to 250 mL flasks containing 40 mL of Knudson culture medium added with 30 g L^{-1} sucrose and solidified with 6 g L^{-1} agar. Media was supplemented with some combinations of plant growth regulators as 6-benzyladenine (BA) and α-naphthaleneacetic acid (NAA), resulting in seven treatments: 0.0; 1.0; 2.0, 0.5; 0.5; 2.0 and 0.0 mg L^{-1} BA combined with 0.0; 0.5; 0.5; 1.0; 2.0; 0.0 and 2.0 mg L^{-1} NAA, respectively. Fifteen protocorms were placed in each flask and each treatment consisted of five replicates (flasks), sealed with PVC film (Dispafilm of Brazil Ltda). The design of experiment was completely randomized and the flasks were kept in a growth room at the same conditions previously described. Evaluations and individualization of the shoots were carried out 60 days after inoculation, taking into account the following parameters: shoot length, root length, shoot number, root number and diameter, and root trichomes presence or absence.

Gibberellic acid in the elongation of vitroplants of *C. saintlegerianum*

Shoots resulted from protocorms multiplication and devoid of roots were isolated and transplanted into 250 mL flasks containing 40 mL of Knudson culture medium, with addition of 0.0; 5.0 and 10.0 mg L^{-1} gibberellic acid (GA_3) to stimulate elongation. Thus, for each treatment containing BA and or NAA on induction medium, shoots were used for inoculation in the three treatments of the development phase. In each GA_3 concentration (treatment), five flasks

containing three shoots (replications) were used. They were kept in a growth room under the same conditions previously mentioned. The design was completely randomized (7 x 3), with shoots originated from seven different media and three concentrations of GA_3. After 30 days on the development medium, plants were removed from the flasks, and shoot length, larger root length, lateral shoots number and root number were measured.

Acclimatization of *C. saintlegerianum plants*

Uniform plants in accordance with the mean length were selected from each *in vitro* treatment after elongation phase and transplanted into expanded polystyrene trays with 32 cells (7.5 x 7.5 cm, 4.5 cm depth) containing a mix of Plantmax®-HT and crushed acuri leaf sheath (1 : 1) as substrate. The trays were transferred to greenhouse with retention of 50% of the solar radiation flux and intermittent irrigation with micro sprinklers. After 108 days of the transplanting, plants were evaluated using the same parameters of the previous assessment, and data were statistically analyzed.

Data from each phase of micropropagation and acclimatization were subjected to statistical analysis using the statistical software Sisvar (Ferreira 2011). Percentage data were transformed into arcsen$\sqrt{(x/100)}$, and means were compared by Tukey test at 5% probability, and Student test (t test) was used for germination data.

RESULTS AND DISCUSSION

Asymbiotic germination of *C. saintlegerianum* seeds

Activated carbon (AC) positively influenced the formation of protocorms, and twenty days after inoculation, it was observed that 100% of seeds had germinated and were in the protocorm phase. This percentage was significantly higher than that found on AC-devoid medium (30%) (Table 1). Similar results were found by Schneiders et al. (2012), who obtained that, at 30 days after sowing, both treatments had germinated; however, the medium containing AC promoted higher germination of seeds (90%) compared to that with no AC (45%). Thomas (2008) affirmed that not all species undergo positive influence by the presence of AC; therefore, to establish a suitable protocol for each kind of orchid, specific tests are necessary. Corroborating this assertion, results contrary to those presented here were found by Hossain et al. (2010), when researching the orchid *Cymbidium giganteum,* which presented no positive relationship between the presence of AC and seed germination, but had protocorms formed in the presence of AC, being considerably larger than those from AC-absence medium. Many authors attribute to AC the property of adsorbing large variety of harmful substances and toxic gases released into the medium by plants and also during the autoclaving process (Pan and Staden 1998, Sáenz et al. 2010).

Table 1. Percentage of *Cyrtopodium saintlegerianum* seeds converted into protocorms on Knudson medium (KC) added or not with activated carbon (AC)

Medium	**Germination** (%)	
	15*	**30***
KC + AC (3 g L^{-1})	90 aB	100 aA
KC	5 bB	30 bA

Means followed by different lowercase letter in the same column, and means followed by different uppercase letter in the same row differ by Student's t test at 5% probability. * Days after sowing.

Table 2. *In vitro* evaluation of *Cyrtopodium saintlegerianum* protocorms grown for 60 days on Knudson medium, supplemented with different combinations of 6-benzylaminopurine (BA) and α-naphthalene acetic acid (NAA)

Phytohormones (mg L^{-1})		**Shoot number**	**Shoot length** (cm)	**Root number**	**Root length** (cm)	**Root diameter** (mm)	**Root pilosity***
BA	NAA						
0	0	2.10 c	2.30 a	4.60 c	2.75 a	0.90 d	+
1.0	0.5	7.70 b	0.84 bc	8.55 a	1.17 cd	1.42 abc	+
2.0	0.5	11.40 ab	0.66 c	5.20 bc	0.59 de	1.14 cd	–
0.5	1.0	8.65 b	1.22 b	8.30 ab	1.69 bc	1.72 ab	+
0.5	2.0	11.80 ab	1.20 b	5.45 abc	0.51 de	1.24 bcd	+
2.0	0	15.20 a	1.09 bc	3.00 c	0.44 e	1.36 abcd	–
0	2.0	3.10 c	2.60 a	5.25 bc	1.92 b	1.81 a	+
CV (%)		11.92	11.49	14.68	14.19	8.74	

Means followed by the same letter in the same column do not differ by Tukey test at 5% probability. * (–) Root without trichomes; (+) Root with trichomes.

Growth regulators on protocorms shoot induction medium

The presence of BA in combination or not with NAA resulted in increased shoot proliferation in comparison with medium devoid of growth regulators (control). On the other hand, isolated presence of 2.0 mg L^{-1} NAA resulted in a mean of 3.10 shoots and did not differ from control. Among the treatments with the presence of BA, one that contained 2.0 mg L^{-1} induced a mean of 15.20 shoots, being considered the best treatment for this parameter (Table 2, Figure 1). The data confirm the attribute of cytokinins to induce cell division and differentiation of shoots. Similarly, Ramos and Carneiro (2007) reported that treatments containing 0.5 or 2.0 mg L^{-1} BA combined with 0.1 mg L^{-1} NAA induced higher production of shoots at the base of the *Cattleya* x M*esquitae* explants.

For shoots length mean, treatment with 2.0 mg L^{-1} NAA had higher measures (2.60 cm) when compared with those derived from the other treatments containing growth regulators; however, they did not statistically differ from the control treatment (2.30 cm) (Table 2). Different response was obtained when *in vitro* multiplication of *Cattleya* x *Mesquitae* was done on etiolating conditions, with no significant difference to the height of the main budding in different tested concentrations of NAA and BA (Ramos and Carneiro 2007). Thus, it is evident that cytokinin presence resulted in both shoot proliferation and inhibition in the shoot elongation. The shorter length of the main shoot was found in the treatments with 1.0 and 2.0 mg L^{-1} BA with 0.5 mg L^{-1} NAA (Table 2). This result can be attributed to the effects of BA on breaking correlative inhibition, stimulating the development of a greaterside shoots number, resulting in competition between them. Suzuki and Kerbauy (2006) argue that cytokinins significantly inhibited shoot and root elongation of *in vitro* plants of *Catasetum fimbriatum*.

Regarding the root number in *C. saintlegerianum* vitroplants, those treatments containing lower doses of auxin NAA (0.5 or 1.0 mg L^{-1}) in combination with lower doses of cytokinin BA (0.5 or 1, 0 mg L^{-1}) (Table 2, Figure 1) showed better results. On the other hand, higher doses of auxin and cytokinin (2.0 mg L^{-1}) resulted in reduction in the root number mean compared to the treatments mentioned above. Furthermore, results demonstrate that the presence of auxin was essential for the induction of roots, and all treatments containing auxin showed a superior root number in relation to control vitroplants. Souto et al. (2010) found that in *Cattleya bicolor* vitroplants, using 0.5 or 1.0 mg L^{-1} NAA, rooting response was significantly better when compared to those cultivated on NAA free medium.

Regarding root length, it was found that roots from vitroplants cultivated on medium devoid of growth regulators (control) were statistically higher than the other treatments (Table 2). This indicates that the concentrations used were inhibitory to root elongation. Rafique et al. (2012) showed similar behavior to *Dendrobium sabin*, with reduction in both length and root number when auxin concentration was supra optimal. In the present study, the best responses to root number also occurred in lower concentrations of NAA and

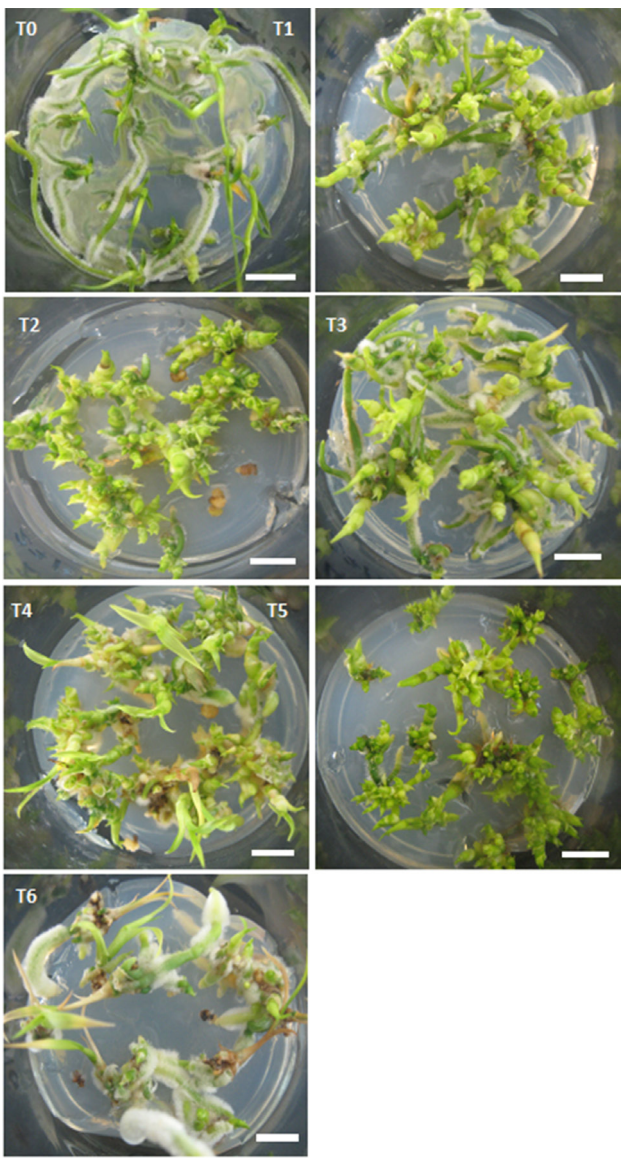

Figure 1. *Cyrtopodium saintlegerianum* vitroplants obtained from protocorms after 60 days on Knudson medium supplemented with 6-benzylaminopurine (BA) and α-naphthalene acetic acid (NAA). Treatment (mg L^{-1}): T0 - Control; T1 - 1.0 BA + NAA 0.5; T2 - 2.0 BA + NAA 0.5; T3 - 0.5 BA + NAA 1.0; T4 - 0.5 BA + NAA 2.0; T5 - 2.0 BA; T6 - ANA 2.0. Bar = 1cm.

BA. The long exposition period of plant material to growth regulators presence may have resulted in this inhibition.

Basal diameter of the higher root found in vitroplants of *C. saintlegerianum* was influenced by NAA concentration, and in the control treatment, the diameter mean was 0.9 mm, statistically differing from that with only 2.0 mg L^{-1} NAA, which presented mean diameter of 1.81 mm (Table 2). Root pilosity was influenced according to presence and concentration of BA and NAA. For example, 2.0 mg L^{-1} BA presence on culture medium resulted in lower pilosity in the roots, i.e., little or no pilosity (Table 2, Figure 1). Considerable pilosity in the roots of vitroplants of *C. saintlegerianum* was obtained on growth regulators free-medium or in those with higher concentrations of NAA. This behavior was also observed by Mayer et al. (2008) in vitroplants and argued that ethylene promoted the formation of these trichomes.

Gibberellic acid in the elongation of vitroplants of *C. saintlegerianum*

C. saintlegerianum vitroplants derived from medium added with 0.5 mg L^{-1} BA and 1.0 mg L^{-1} NAA showed greater shoot growth during the elongation phase when they were put on media containing 5.0 or 10.0 mg L^{-1} GA_3. Similarly, those vitroplants from medium containing 2.0 mg L^{-1} NAA and elongated on a medium containing 10 mg L^{-1} GA_3 also showed increased growth. In general, *C. saintlegerianum* vitroplants originated from medium with high auxin/cytokinin ratio presents increased growth during elongation phase when placed in the presence of 10 mg L^{-1} GA_3 (Table 3). Cytokinins regulate several plant growth aspects and developmental processes, including cell division, apical dominance, photomorphogenic development and shoot differentiation (Mok and Mok 2001). GA_3 is well known for its role in the elongation of axial organs such as stems,

Table 3. Morphological parameters of *Cyrtopodium saintlegerianum* vitroplants obtained from protocorms cultivated on Knudson medium with different combinations of 6-benzylaminopurine (BA) and α-naphthaleneacetic acid (NAA), and transferred to Knudson medium containing gibberellic acid (GA_3) for 30 days

Parameter	**Induction medium** (mg L^{-1})		**Elongation medium - GA_3** (mg L^{-1})		
	BA	NAA	0	5	10
Shoot length (cm)	0.0	0.0	3.31 aA	5.35 abcA	4.40 bcA
	1.0	0.5	2.84 aB	5.99 abA	5.24 abcAB
	2.0	0.5	2.29 aA	3.86 bcA	3.01 cA
	0.5	1.0	4.53 aB	7.74 aA	8.06 abA
	0.5	2.0	3.21 aA	3.78 bcA	5.63 abcA
	2.0	0.0	2.36 aA	4.11 bcA	4.21 cA
	0.0	2.0	3.74 aB	2.87 cB	8.10 aA
Root length (cm)	0.0	0.0	2.76 aAB	4.62 aA	2.38 abB
	1.0	0.5	3.87 aA	4.09 aA	3.00 aA
	2.0	0.5	3.90 aA	3.14 aA	3.14 aA
	0.5	1.0	2.11 aA	2.95 aA	3.03 aA
	0.5	2.0	3.74 aA	2.94 aA	2.69 aA
	2.0	0.0	2.17 aA	2.37 aA	3.17 aA
	0.0	2.0	3.55 aA	3.22 aA	1.01 bB
Tiller number*	0.0	0.0	0.93 aA	0.47 aB	0.73 abA
	1.0	0.5	1.00 aA	0.00 bB	0.87 aA
	2.0	0.5	0.93 aA	0.53 aB	0.93 aA
	0.5	1.0	0.80 aA	0.00 bC	0.47 bcB
	0.5	2.0	0.93 aA	0.00 bB	0.00 dB
	2.0	0.0	1.07 aA	0.33 aB	0.40 cB
	0.0	2.0	0.40 bA	0.00 bB	0.00 dB
Root number	0.0	0.0	4.53 abA	1.87 aB	2.67 aAB
	1.0	0.5	6.60 aA	1.93 aB	1.73 aB
	2.0	0.5	5.07 abA	1.47 aB	1.67 aB
	0.5	1.0	5.00 abA	1.73 aB	2.40 aB
	0.5	2.0	6.07 abA	1.53 aB	2.40 aB
	2.0	0.0	5.07 abA	1.73 aB	1.80 aB
	0.0	2.0	3.73 bA	1.07 aB	0.27 bC

Means followed by the same lowercase letter in the same column, and uppercase letter in the same row do not differ by Tukey test at 5% probability. *Tiller number, except for main shoot.

Table 4. Evaluation of acclimatized plants *Cyrtopodium saintlegerianum*, with 108 days of transplanting, resulting in induction of shoots protocorms with different combinations of 6-benzylaminopurine (BA) and α-naphthalene acetic acid (NAA), and elongated in different concentrations of gibberellic acid (GA_3). Shoot length (SL); Root length (RL); Shoot number (SN), root number (RN) and fresh weight (FW)

Phytoregulators (mg L^{-1})		**SL** (cm)	**RL** (cm)	**SN**	**RN**	**FW** (g)
BAP	**NAA**					
0	0	5.34 b	5.74	0.48	3.44	0,69
1.0	0.5	7.02 a	6.00	0.13	4.22	0,82
2.0	0.5	6.32 a	6.53	1.22	3.73	0,94
0.5	1.0	7.14 a	5.54	0.34	4.18	0,76
0.5	2.0	6.71 a	5.67	0.36	3.78	0,79
2.0	0	6.73 a	7.94	0.14	3.18	0,56
0	2.0	7.76 a	6.21	0.00	2.94	0,87
GA_3 (mg L^{-1})						
0		7.27 a	7.81 a	0.62	6.10 a	1.56 a
5		6.29 b	5.58 b	0.25	2.43 b	0.39 b
10		6.59 b	5.30 b	0.27	2.38 b	0.37 b
Phytoregulators (F)		3.4209 **	0.7647	1.42	1.37	0.99
AG_3 (AG)		3.5183 *	4.9528 *	0.91	62.92 **	73.82 **
F*AG		1.1685	0.5109	1.41	1.18	1.83
CV (%)		18.20	45.44	25.87	33.80	46.71

* Means followed by the same letter in the same column do not differ by the Scott-Knott test at 5% probability.

** Means followed by the same letter in the same column do not differ by the Scott-Knott test at 1% probability.

petioles and inflorescences (DeMason 2005). However, in some cases, GA_3 can be ineffective (Soares et al. 2009) or inhibitory (Araujo et al. 2009) to the development of shoots of *in vitro* orchids.

In respect to length of higher root in the *C. saintlegerianum* vitroplants after elongation phase, the results did not differ between treatments, showing little influence of the shoots induction medium on elongation phase. However, in absolute numbers, vitroplants that showed better response were those derived from control treatment in shoots induction phase and elongated on medium with 5.0 mg L^{-1} GA_3 (Table 3). So, as demonstrated here, this growth regulator was not decisive to roots elongation in *C. saintlegerianum* vitroplants.

Acclimatization of *C. saintlegerianum* vitroplants

In acclimatized plants assess, it was observed that the absence of GA_3 in the elongation phase, regardless of the medium used for shoot induction, has favored greater plant survival during acclimatization, with survival rate of 90%. On the other hand, plants derived from media with maximum GA_3 concentration (10 mg L^{-1}) resulted in higher mortality, close to 50% (data not shown). This failure in the acclimatization of plants from higher GA_3 concentration during elongation phase can be explained by the higher shoot growth at the expense of diameter, resulting in vitroplants with etiolating appearance, and therefore more susceptible to tumbling. Additionally, the smaller diameter may have negatively influenced the hardiness of vitroplants with respect to dehydration during acclimatization.

Despite being an epiphytic species, *C. saintlegerianum* vitroplants showed good development on substrate containing Plantmax®-HT and crushed acuri leaf sheath. Moraes et al. (2002) also reported the use of Plantmax®-HT with good results in the cultivation of *Dendrobium nobile*, an epiphytic orchid.

Massal *in vitro* propagation of *C. saintlegerianum* demonstrated to be viable from protocorms grown in the presence of 6-benzylaminopurine (2.0 mg L^{-1}), and in the absence or presence of α-naphthaleneacetic acid (0.5 mg L^{-1}), with significant influence of auxin and cytokinin ratio. The use of gibberellic acid promoted elongation of vitroplants, but contributed negatively in its acclimatization, making it unnecessary the use of this plant regulator in the production of *C. saintlegerianum* vitroplants.

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Propagação in vitro de *Cyrtopodium saintlegerianum* rchb. f. (orchidaceae), uma orquídea nativa do cerrado brasileiro

Resumo – *Visando à obtenção de grande quantidade de mudas para programas de reintrodução, bem como viabilizar sua exploração comercial, sementes de* Cyrtopodium saintlegerianum *foram incubadas em meio Knudson. Após a germinação, os protocormos foram inoculados em meio Knudson suplementado com 6-benzilaminopurina (BA) e ácido α-naftalenoacético (ANA) para indução de brotações, que foram posteriormente individualizadas e inoculadas em meio Knudson acrescido com ácido giberélico (AG_3), para promover alongamento. As plantas resultantes do processo in vitro foram avaliadas e transplantadas para bandejas contendo Plantmax® e casca de Acurí triturada. A relação auxina citocinina influencia na propagação in vitro de* C. saintlegerianum*, induzindo um incremento no número de brotações quando o meio de cultura foi acrescido de 2,0 mg L^{-1} de BA, na ausência ou presença de 0,5 mg L^{-1} de ANA. A espécie demonstrou-se promissora na multiplicação massal in vitro. O uso do ácido giberélico favoreceu o alongamento das brotações in vitro, mas contribuiu negativamente na aclimatização das plantas, tornando desnecessária a utilização deste fitorregulador na produção de mudas de* C. saintlegerianum.

Palavras-chave: *Orchidaceae, micropropagação, fitorreguladores, aclimatização.*

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