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BIOLOGY

In vitro propagation and conservation of *Cattleya tigrina* A. Rich

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ABSTRACT: *Cattleya tigrina A. Rich has been suffering heavy losses in its natural habitat and it is now included in the list of plants that are vulnerable to extinction. The development of in vitro propagation and conservation methodologies, as well as acclimatization, are considered important for species at the risk of extinction, as they promote the multiplication and conservation of the species, hence avoiding the loss of their genetic variability. The present study established the protocol of micropropagation and the in vitro conservation of C. tigrina. For the in vitro propagation, the study tested two volumes of the MS medium and two medium consistencies (stationary liquid and semi-solid). For acclimatization, the substrate mixtures containing pine bark, charcoal, vermiculite, and coconut coir were analyzed. For the in vitro conservation, different concentrations of the salts were tested in the MS medium, together with the osmotic regulators (sucrose, mannitol, and sorbitol), and at two temperatures (18 and 25 °C). The results obtained inferred that the semi-solid medium was superior to the stationary liquid medium in the variables of survival and the presence of roots, while the liquid medium was superior to the semi-solid medium in the number of shoots. For acclimatization, pine bark was the substrate where the plants developed an improved height, with sprouting, and rooting. The conservation was satisfactory and the plants remained viable for a period of 730 days, with the MS medium with 25% of the salts, and at temperatures of 18 ºC or 25 ºC. The plants were propagated in the stationary liquid MS medium (10 mL) and the semi-solid medium (25 mL), while they were acclimatized in pine bark and preserved in the MS medium with 25% of the salts (18 ºC or 25 ºC).*
Key words: *in vitro propagation, acclimatization, slow growth, osmotic regulators.*

Propagação e conservação *in vitro* de *Cattleya tigrina* A. Rich

RESUMO: *A Cattleya tigrina A. Rich vem sofrendo grandes perdas no seu habitat natural, sendo assim foi inclusa na lista de vulneráveis a extinção. O desenvolvimento de metodologias de propagação e conservação in vitro, bem como de aclimatização, são consideradas importantes para espécies em risco de extinção, por promover a multiplicação e conservação da espécie, evitando a perda do seu material genético. Desta forma, o presente trabalho visou estabelecer protocolo de micropropagação e conservação in vitro de C. tigrina. Para propagação in vitro testou volumes de meio de cultura e duas consistências do meio MS (líquido estacionário e semissólido). Para aclimatização, analisou misturas de substratos contendo casca de pinus, carvão vegetal, vermiculita e pó de coco. Para conservação in vitro, foram testados diferentes concentrações de sais no meio MS, reguladores osmóticos (sacarose, manitol e sorbitol), e duas temperaturas (18 e 25 °C). Os resultados obtidos inferem que, o meio de cultura semissólido foi superior ao líquido nas variáveis sobrevivência e presença de raízes, enquanto que o meio líquido foi superior ao meio semissólido em números de brotos. Na aclimatização a casca de pinus foi o substrato em que as plantas se desenvolveram melhor em altura, brotação e enraizamento. A conservação foi satisfatória e as plantas permaneceram viáveis por um período de 730 dias, usando 25% dos sais MS e temperatura de 18 ºC ou 25 ºC. As plantas podem ser propagadas em meio MS líquido estacionário (10 mL) ou semissólido (25 mL), aclimatizadas em casca de pinus e conservadas em 25% dos sais MS (18 ºC ou 25 ºC).*
Palavras-chave: *multiplicação in vitro, aclimatização, crescimento lento, reguladores osmóticos.*

INTRODUCTION

The *Cattleya* genus is distributed in several tropical regions of South and Central America. The exuberance of the flowers makes the species of this genus the favorite one among decorators, collectors, orchidophiles, and extractivists (BARROS et al., 2015). This causes some species, including *C. tigrina* A. Rich, to be included by the Secretariat of the Environment in the list of plants vulnerable to extinction (SMA Resolution 48/2004). Researches aiming to improve the processes of propagation and the development of orchids are important, to multiply them and avoid their extinction. Before these

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particular researches, the techniques of plant tissue culture, micropropagation, and *in vitro* conservation were greatly important for the species.

Micropropagation has been employed to increase the production of plantlets, reducing their cost, and collaborating to save several species of orchids from extinction (VILLA et al., 2014). In most protocols for the micropropagation of orchids, growth media with added agar are predominantly used, with or without the addition of growth regulators. The use of a semi-solid culture media has been implemented for several species, such as *Dendrobium nobile* Lindl (SU et al., 2012), *Brassocattleya pastoral x Laeliocattleya* Amber Glow (VILLA et al., 2014), and *Miltonia flavescens* (LEMES et al., 2016).

Another method for *in vitro* propagation is through a liquid medium, which is easy to prepare and handle, and this requires a smaller amount of the culture medium (SU et al., 2012). This type of medium has been assessed in some species, such as *Musa* spp. (ANDRADE et al., 2011), *Chrysopogon zizanioides* (SANTOS et al., 2012), and the orchids of *Cattleya loddigesii* (PASQUAL et al., 2009; GALDIANO JUNIOR et al., 2012), and *Oncidium baueri* Lindl (SORACE et al., 2008).

The development of methodologies for *in vitro* conservation is considered essential for those species at risk of extinction. The technique of slow growth is also important for allowing conservation of easy regeneration material. It has been used successfully for the conservation of several species, such as *Chrysopogon zizanioides* L. (SANTOS et al., 2012); *Epidendrum chlorocorymbos* Schltr. (LOPEZ-PUC, 2013); *Pogostemon cablin* Benth. (ARRIGONI-BLANK et al., 2015); and *Lippia alba* carvone chemotype (PEIXOTO et al., 2017).

During the technique of slow growth, the plants are subjected to conditions that reduce or suppress the metabolism of the plants *in vitro*, thus increasing the interval between the subcultures in the small to medium term. This procedure leads to a decrease in labor personnel, space, and costs for plant maintenance (MARCO-MEDINA; CASAS, 2012; SANTOS et al., 2012).

To slow the growth of the plants when employing the slow-growth technique, it is possible to use a reduction in temperature, light intensity, salt concentrations in the basal medium, with the addition of osmotic agents, such as mannitol, sorbitol, and growth inhibitors, such as acid abscisic (ABA) (MOOSIKAPALA; TE-CHATO, 2010). Sugar alcohols, such as mannitol and sorbitol are generally not metabolized by the plants, and they have been used to mimic the condition of osmotic stress. When added to the culture medium, these sugars reduce the water potential of the system, limiting the absorption of water and nutrients by the explant, and retarding its growth (MARINO et al., 2010; SILVA; SCHERWINSKI-PEREIRA, 2011).

The reduction of temperature and salt concentrations, as well as the osmotic stress that is caused by the addition of the alcoholic sugars mannitol and/or sorbitol to the culture medium, will reduce the growth *in vitro* of the *C. tigrina* seedlings. This allows for carrying out assays of *in vitro* conservation in the medium term while using this slow growth technique. Along with this technique, the consistency of the culture media will provide the *in vitro* propagation of the species, enabling the maintenance of the germplasm of the species.

Given the above information, this study established a protocol of micropropagation and *in vitro* conservation of *C. tigrina* A. Rich.

MATERIAL AND METHODS

Plant site and material

The specimens of *C. tigrina* A. Rich were collected and kept in an agricultural greenhouse. The project was registered with the number SisGen A53BE37. Ripe fruits *(capsules) were collected and they were used to establish the in vitro cultures.*

In vitro plant establishment

The seed capsules were washed with distilled water and neutral detergent. They were then disinfected with 70% (v/v) alcohol for 1 min in a laminar flow chamber. The material was next immersed in 5% sodium hypochlorite solution for 20 min and subjected to threefold washing with distilled water.

The capsules were opened and the seeds were isolated and inoculated in the MS culture medium (MURASHIGE and SKOOG 1962), with a half-strength concentration of macroelements, change supplemented with 30 g L^{-1} of sucrose, 7 g L^{-1} of agar (Sigma-Aldrich), and 1 g L^{-1} of activated charcoal, with the pH adjusted to 5.8 ± 0.1, before autoclaving (121±1 ºC, and 1.05 atm) for 15 minutes.

The flasks were kept in a temperature-controlled room (25 ± 2 ºC), with a 12 h-photoperiod when using LED lamps, and with a photosynthetic photon flux density (PPFD) of 40 µmol m^{-2} s^{-1}.

Leaf segments of the 1cm high plants were used as sources of explants for the propagation and conservation of the *in vitro* experiments.

In vitro conservation

Plants that were used in the *in vitro* conservation experiments were obtained from the *in vitro* cultivation maintenance of the species. The *in vitro* conservation under the slow growth technique was conducted in two experiments. In both of the *in vitro* experiments, a completely randomized design was used.

Reduction of the salts in the MS medium and the cultivation temperature

In the first experiment, a 4x2 factorial scheme was used, with four concentrations of the salts (macro and micronutrients) in the MS medium (100, 75, 50, and 25%), at two temperatures (18 and 25 °C), and with five replications; each one consisted of six tubes, with one plantlet in each.

Osmotic regulators and the cultivation temperature

In the second experiment, a 3x2 factorial scheme was used, with five replications. This process tested three combinations of the carbohydrate sources and the osmotic regulators [sucrose (20 g L^{-1}); sucrose (10 g L^{-1}) and mannitol (5 g L^{-1}); sucrose (10 g L^{-1}) and sorbitol (5 g L^{-1})], and at two temperatures (18 and 25 °C). Each replication was composed of six tubes with one plantlet in each. In both of the experiments, the plants were inoculated in test tubes and kept in the growth room, with a controlled temperature of 25 ± 2 °C, while under a photoperiod of 12 hours of light, and a photosynthetic photon flux density of 40 µmol m^{-2} s^{-1}, or BOD at a temperature of 18 °C.

Variables analyzed

The variables analyzed for the two *in vitro* conservation experiments were survival (%), the presence of a root (%), and the integrity of the plants, which were evaluated after 730 days of cultivation. The analysis was where 1 = totally green leaves and shoots; 2 = the beginning of the drying of the leaves; 3 = between 30 and 50% of dead leaves; 4 = more than 50% dead leaves; and 5 = totally dead leaves and shoots. The plant heights were evaluated according to the score scale of 1 = equal to the initial height; 2 = up to twice the initial height; 3 = up to three times the initial height; and 4 = more than three times the initial height, all according to the score scale that was adapted from LEMOS et al. (2002).

Results were submitted to an Analysis of Variance (ANOVA) and when necessary, the means were compared by Tukey's test at a 5% significance level when using Sisvar (FERREIRA, 2011), except for the concentrations of the salts of the MS medium, where polynomial regression was used. The data was used without transformation. The variables in percentages were transformed into the arcsine of the square root (X/100).

In vitro propagation, elongation, rooting, and acclimatization

In vitro propagation, elongation, and rooting

For the *in vitro* propagation, elongation, and rooting, a completely randomized design was used with a factorial scheme of 4×2, while testing four volumes (10, 15, 20, and 25 mL) and two MS medium consistencies (stationary and semi-solid liquid), with five replications. Each replication consisted of five flasks and each flask had two explants. The MS medium plus 30 g L^{-1} sucrose, with the pH adjusted to 5.8 ± 0.1, was used for all the experiments. For the semi-solid MS medium, 7 g L^{-1} of agar (Sigma-Aldrich) was used. The media were subjected to autoclaving (121 ± 1 °C, and 1.05 atm) for 15 minutes.

In the laminar flow cabinets, the plants standing at 1 cm in height were inoculated in 250 mL flasks, which contained the medium volumes that were predetermined for each treatment. The flasks were kept in the growth room at a controlled temperature of 25 ± 2 °C, with a photoperiod of 12 hours, and with a photosynthetic photon flux density of 40µmol m^{-2} s^{-1} that was provided by cold white fluorescent lamps. After 90 days of cultivation, the variables of survival (%), the presence of a root (%), and the number of shoots were all evaluated.

Acclimatization

The plants that were used in acclimatization came from the *in vitro* propagation experiments. A completely randomized design was used, while testing seven substrates: [S1) crushed pine bark; S2) coconut coir; S3) crushed pine bark: coconut coir (2:1 v/v); S4) crushed pine bark: vermiculite (2:1 v/v); S5) crushed pine bark: charcoal (2:1 v/v); S6) crushed pine bark: coconut coir: charcoal: vermiculite (2:1:1:1 v/v), and S7) crushed pine bark: charcoal: vermiculite (2:1:1 v/v)]. The experiment was carried out when using six replications, with each replication consisting of five plantlets. 1 g L^{-1} of dolomitic limestone was added to the treatments, together with coconut coir, to neutralize the effect of the tannins that were present in the coconut coir. The acclimatization experiment was carried out in a protected environment, with 50% netted shade and intermittent irrigation, with a misting system to ensure high relative humidity. The variables of survival (%), the plant height (cm), the

number of shoots, and the root length (cm), were all evaluated, 120 days after transplanting.

RESULTS AND DISCUSSION

In vitro conservation

Reduction of the salts in the MS medium and the cultivation temperature.

After 730 days of cultivation under the slow growth (Figure 1), the survival of the plantlets showed mean values of 80%, regardless of the temperature and the concentration of the salts in the MS medium, while showing no significant differences (Table 1). Since *C. tigrina* A. Rich is an epiphytic species, which presents endoreduplication, and its physiological and morphological adaptations can likely be adjusted, depending on the number of nutrients that are available *in vitro* or *ex vitro*. This would allow for their conservation for a longer period, without the need for exchanging the medium (SILVA et al., 2017). Given this, one can infer that *C. tigrina* A. Rich tolerates different concentrations of salts and temperature variations. In its natural habitat, this species feeds on plant litter and animal and/or insect droppings (KERSTEN, 2010). This nutrient availability is variable throughout the year, making this species adapting to a lower or higher supply of nutrients.

The integrity of the plants was indicative of their nutritional status during the process of conservation. At both temperatures, as the salt concentrations increased, the leaves showed an early dryness, with 30 to 50% of dead leaves (Figure 2A). There were no significant differences when using a volume of 25 mL of the MS medium, at both of the temperatures (Table 1). Results can be explained by an increasing linear equation for both temperatures.

Figure 1 - *C. tigrina* plantlets at 730 days of the *in vitro* conservation, as a function of the different salt concentrations (25%, 50%, 75%, and 100%) of the MS medium at 18 °C (A) and 25 °C (B), and the osmotic regulators [T1 - sucrose (20 g L-1); T2 - sucrose (10 g L-1) and mannitol (5 g L-1); T3 - sucrose (10 g L-1) and sorbitol (5 g L-1)], at 18 °C (C) and 25 °C (D).

Table 1 - Survival (%), integrity (scores 1 to 5[**]), and height (scores 1 to 4[***]) of the *C. tigrina* A. Rich plantlets at 730 days of *in vitro* conservation, as a function of the different salt concentrations of the MS medium and temperature.

MS Salts (%)	Temperature (ºC)	
	25	18
	Survival (%)	
25	90.0 a	86.7 a
50	90.0 a	86.6 a
75	86.7 a	73.3 a
100	83.3 a	73.3 a
CV (%)	20.85	
	Plant Integrity	
25	1.8 a	2.2 a
50	2.0 a	2.8 b
75	2.3 a	3.4 b
100	2.7 a	3.4 b
CV (%)	16.49	
	Height (cm)	
25	2.3 a	2.3 a
50	2.9 a	2.6 a
75	2.9 a	2.9 a
100	2.5 a	2.6 a
CV (%)	15.74	

[*]Means followed by the same letter in the rows did not differ from each other by Tukey's test at a 5% significance level. [**]Integrity scores: 1 = totally green leaves and shoots; 2 = beginning of the drying of the leaves; 3 = between 30 and 50% of dead leaves; 4 = more than 50% of dead leaves; and 5 = totally dead leaves and shoots. [***]Height scores: 1 = equal to the initial height (1-2 cm); 2 = up to twice the initial height; 3 = up to three times the initial height; and 4 = more than three times the initial height.

It is believed that the senescence of leaves took place during the long period that the plants were *in vitro*. Even so, no physiological damage was caused.

A root presence was observed in all of the treatments (100%), regardless of the temperatures and the concentrations of the salts in the growth medium. The presence of roots may have indicated that the plants were developing satisfactorily and that the *in vitro* conditions did not limit the resumption of their growth (CAMOLESI et al., 2010).

Regarding the height of the plants, the two growing temperatures showed a similar performance, with values not exceeding 2.9 cm, showing no significant differences (Table 1). The results can be explained by a quadratic equation for both temperatures (Figure 2B). This is beneficial for conservation, whose primary purpose is reducing the growth of the plant but by keeping their viability for a longer period.

Osmotic regulators and the cultivation temperature.

The survival variable obtained percentages of 86.7 to 96.7% at 730 days of the *in vitro* conservation, with different carbon sources, osmotic regulators, and temperatures (Table 2). Having said that, there were no significant differences in any of the treatments at both of the temperatures. The sugar alcohols, such as mannitol and sorbitol, are generally not metabolized by the plants but they have been used to simulate the condition of osmotic stress when added to the growth medium, decreasing the water potential (SILVA and SCHERWINSKI-PEREIRA, 2011). Since the genus *Cattleya*

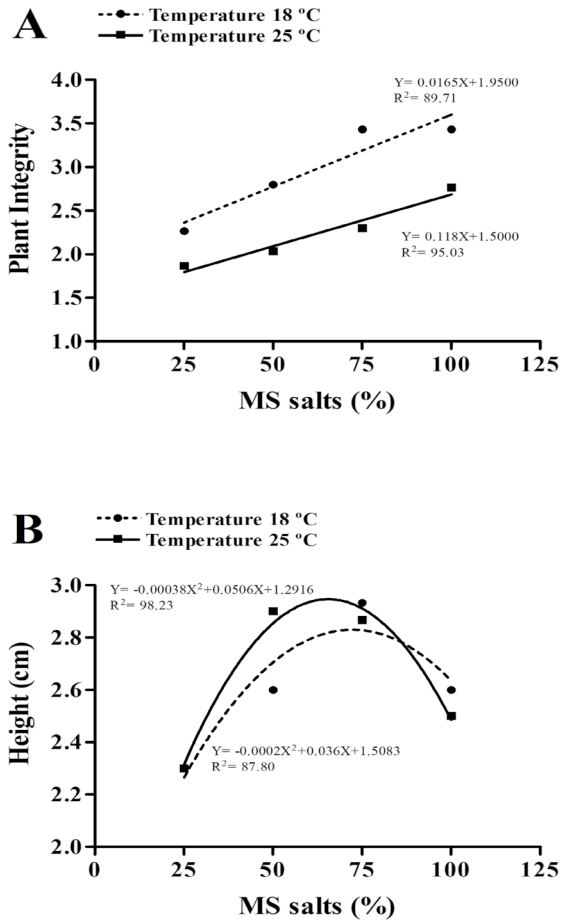

Figure 2 - Plant integrity (scores 1 to 5**) (A) and height (scores 1 to 4***) (B) of the *C. tigrina* A. Rich plantlets at 730 days of the *in vitro* conservation, as a function of the different salt concentrations of the MS medium and temperature.

has CAM photosynthesis (HE et al., 2013), the concentrations of mannitol and sorbitol probably did not affect the use of water by the plants; therefore, providing for their survival.

Concerning the integrity of the plants, they showed fully green leaves and shoots for both temperatures (Table 2). By using temperatures lower than those that were used for the *in vitro* cultivation, this decreased the plant metabolism, causing a reduction in the enzyme activity (LIMA-BRITO et al., 2011), which was probably decisive in the viability of the plants during the *in vitro* conservation.

During the period of conservation, 100% of the plants presented roots (Table 2). All the same, there were no significant differences in any of the treatments at both of the temperatures. The presence of roots may have indicated good growing conditions, being positive for the resumption of growth, whenever it was necessary and beneficial for the eventual acclimatization (CAMOLESI et al., 2010).

Table 2 - Survival (%), integrity (scores 1 to 5[**]), root presence (%), and height (scores 1 to 4[***]) of the *C. tigrina* A. Rich plantlets at 730 days of the *in vitro* conservation, as a function of the osmotic regulators and temperature.

Temperature	Osmotic Regulators (g L^{-1})		
(ºC)	Sucrose (20)	Sucrose (10): Mannitol (5)	Sucrose (10): Sorbitol (5)
		Survival (%)	
18	93.3 aA	86.7 aA	93.3 aA
25	90.0 aA	96.7 aA	90.0 aA
CV (%)		11.15	
		Plant Integrity	
18	1.4 bA	1.8 aA	1.5 aA
25	1.0 aA	1.5 aB	1.9 bB
CV (%)		21.95	
		Height	
18	2.0 aA	1.9 aA	2.0 aA
25	2.3 aA	2.2 aA	2.1 aA
CV (%)		12.15	

[*]Means followed by lowercase letters in the column and uppercase letters in the row for each variable did not differ statistically by Tukey's test, at a 5% significance level. [**]Integrity scores: 1 = totally green leaves and shoots; 2 = beginning of the drying of the leaves; 3 = between 30 and 50% of dead leaves; 4 = more than 50% of dead leaves; and 5 = totally dead leaves and shoots. [***]Height scores: 1 = equal to the initial height (1-2 cm); 2 = up to twice the initial height; 3 = up to three times the initial height; and 4 = more than three times the initial height.

Concerning the height, the plantlets showed little growth, as this was the main purpose of slow growth, regardless of the temperatures and the osmotic regulators (Table 2). It was assumed that this plant, which has CAM photosynthesis, epiphytic growth, and endoreduplication, was adapting to the various conditions, showing slow growth, as is confirmed by the literature (KERSTEN, 2010).

In vitro conservation requires the reduction of the plant growth, to avoid consecutive subcultures, and to not lose the propagation capacity of the species, while aiming to resume the growth. The viability test was indicative of the success of the development of a conservation protocol.

After a period of 730 days of *in vitro* conservation, all of the plantlets were transferred to the complete MS medium and they resumed their normal growth. This procedure was required to show that the plants that were conserved *in vitro*, and maintained their vigor and their propagation potential. The viability test showed that the different concentrations of the MS salts and the osmotic regulators that were used in the conservation did not affect the responsive capacity of the culture. It can be inferred that the *in vitro* conservation of *C. tigrina* under slow growth for a period of 730 days was possible without any visible disturbances, regardless of the treatments used.

Propagation, elongation, enraization in vitro, and acclimatization.

Regarding the survival of the plants and the root presence at 90 days of *in vitro* cultivation, the results can be explained by an increasing linear equation for the semi-solid medium, and by a decreasing linear equation for the liquid medium (Figure 3A). There were no significant differences when using a 10 mL volume of the MS medium in the two consistencies of the medium. As the volume of the medium increased, the survival rate and the root presence decreased (Table 3). The low survival rate and the rooting of the plants, except in the 10 mL volume, may have occurred because the plantlets were immersed in the medium, which makes the gas exchange difficult. For these variables, the semi-solid medium was more adequate, providing for survival and rooted plant values above 90% (Figure 3B).

The number of shoots per explant ranged from 2.3 to 5.5 in the liquid medium, with the lowest volume allowing for the highest number of shoots. There was a significant difference when using the volumes of 10 and 15 mL of the MS medium in the two consistencies of the medium (Table 3).

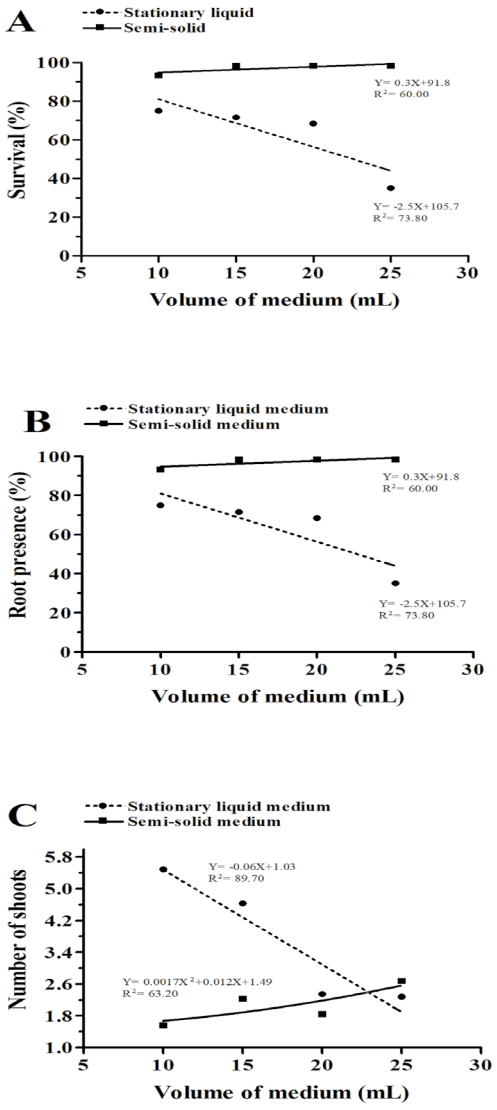

Figure 3 - Survival (A), root presence (%) (B), and the number of shoots (C) per the plants of the *C. tigrina* A. Rich plantlets at 90 days of the *in vitro* cultivation, as a function of the volume of the stationary liquid medium and the semi-solid medium MS.

When compared to the liquid medium, the semi-solid medium had lower performance, obtaining no more than 2.7 shoots/explant (Figure 3C). The use of agar as a solidifying agent of the medium may have contributed to the existence of a barrier in the processes of diffusion and absorption of the nutrients, providing a low number of shoots per plant (GALDIANO-JÚNIOR et al., 2012).

Concerning elongation and rooting, the shoots grown *in vitro* did not show differences

Table 3 - Survival (%), root presence (%), and the number of shoots per plant of the *C. tigrina* A. Rich plantlets at 90 days of the *in vitro* cultivation, as a function of the volume of the stationary liquid medium and the semi-solid medium MS.

Medium volume (mL)	Consistencies of the medium	
	Stationary liquid	Semi-solid
	Survival (%)	
10	75.0 a	93.3 a
15	71.6 b	98.3 a
20	68.3 b	98.3 a
25	35.0 b	98.3 a
CV (%)	20.83	
	Root presence (%)	
10	75.0 a	93.3 a
15	71.6 b	98.3 a
20	68.3 b	98.3 a
25	35.0 b	98.3 a
CV (%)	20.83	
	Number of shoots	
10	5.5 a	1.5 b
15	4.5 a	2.2 b
20	2.3 a	1.8 a
25	2.3 a	2.7 a
CV (%)	41.67	

*Means followed by the same letters in the lines did not differ from each other by Tukey's test, at a 5% significance level.

between treatments, with both being efficient to lengthen the roots of the plants. Root production is easily achieved with some species in an MS medium, with or without the addition of extra auxins (CAMOLESI et al., 2010).

In plant acclimatization, the different substrate mixtures did not affect the survival, showing 100% survival, while there were no significant differences for any of the substrates (Table 4). This high survival rate may be related to the fact that all of the

Table 4 - Effect of the different substrates on the plant height (cm), the number of shoots, and the root length (cm) of the acclimatized *C. tigrina* plantlets.

Substrates	Plant height (cm)	Number of shoots	Root length (cm)
S1	5.5 ab	1.0 b	6.1 a
S2	5.6 a	1.1 ab	5.8 ab
S3	5.6 a	1.4 ab	5.5 ab
S4	5.4 ab	1.3 ab	5.2 ab
S5	5.3 b	1.5 a	4.4 ab
S6	5.3 b	1.3 ab	4.3 b
S7	5.4 ab	1.4 ab	4.5 ab
CV (%)	2.29	20.14	18.11

*Means followed by the same letters in the column did not differ from each other by Tukey's test, at a 5% significance level. S1) crushed pine bark; S2) coconut coir; S3) crushed pine bark : coconut coir (2:1); S4) crushed pine bark : vermiculite (2:1); S5) crushed pine bark : charcoal (2:1); S6) crushed pine bark : coconut coir : charcoal : vermiculite (2:1:1:1); S7) crushed pine bark : charcoal : vermiculite (2:1:1).

substrates that were used had a relatively thick texture, allowing for the gas exchange between the roots and the environment (ZANDONÁ et al., 2014). These results are similar to those reported for *Miltonia flavescens* Lindl. when it was grown on substrates based on coconut coir and pine bark (STEFANELLO et al., 2009).

Regarding the height of the plants, a significant difference between the substrates was noticed, with substrates S2 and S3 being the ones that caused the highest heights (5.6 cm), while the lowest heights were the substrates S5 and S6 (5.3 cm). These four substrates did not differ statistically from each other (Table 4). The good physical properties of coconut coir and pine bark are promising for the proper growth of the plants, providing good conditions for their growth and development. The substrates that were used had a beneficial physical structure, providing high porosity, a high potential for moisture retention, and a buffering power for the pH values (ZANDONÁ et al., 2014).

For the number of shoots, there was a statistical difference, with the substrate S5 (1.5) being superior to the substrate S1 (1.0) but they did not differ statistically from the other treatments (Table 4). Because *Cattleya tigrina* A. Rich is a plant of epiphytic growth, its slow growth and development may be related to the low number of shoots produced (ENDRES-JÚNIOR et al., 2015).

Regarding the length of the root, the highest average (6.1 cm) was provided by substrate S1, while not differing from the other substrates, except from substrate S6, which produced the lowest root length (4.3) (Table 4). The presence of roots may have indicated that the plants were vegetating normally, or that the growing conditions were not causing restrictions that may have hindered their growth and development (CAMOLESI et al., 2010).

CONCLUSION

For the *in vitro* propagation of the plants, the stationary liquid and the semi-solid MS culture media were used, with 10 mL for the liquid, and 25 mL for the semi-solid medium. The acclimatization of the plants was carried out when using only pine bark. The species was conserved under slow growth for a period of 730 days when using the growth medium with 25% of the MS salts, and temperatures of 18 ºC or 25 ºC.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the manuscript design and writing. All authors critically revised the manuscript and approved the final version

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