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In vitro culture of *Vriesea cacuminis* L.B. Sm. (*Bromeliaceae*): an endemic species of Ibitipoca State Park, MG, Brazil

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ABSTRACT – This study aimed to establish a protocol for the micropropagation of *Vriesea cacuminis*. After the *in vitro* introduction from seeds, plantlets were propagated in medium supplemented with BA or GA_3 combined with NAA. The microcuttings were rooted in medium with NAA, IAA or IBA. The results showed that the cultures were efficiently established *in vitro*. Medium supplemented with 15 µM BA + 4.5 µM NAA provided the highest propagation rates. In response to 15 µM GA_3 + 1.5 µM NAA it was possible to obtain both shoots and roots. The highest microcutting rooting was found in response to the medium supplemented with NAA at 0.2 µM. After six months *in vitro*, the plantlets were acclimatized. This study's results showed that micropropagation is an efficient tool for *in vitro* genetic variability conservation and for large-scale multiplication of *V. cacuminis*, an endangered and endemic bromeliad.

Keywords: biodiversity conservation, acclimatization, micropropagation

RESUMO – Cultivo *in vitro* de *Vriesea cacuminis* L.B. Sm. (*Bromeliaceae*): uma espécie endêmica do Parque Estadual do Ibitipoca, MG, Brasil. Este estudo teve como objetivo estabelecer um protocolo para micropropagação de *Vriesea cacuminis*. Após introdução *in vitro* a partir de sementes, plântulas foram propagadas em meio suplementado com GA_3 ou BAP combinado com ANA. Microestacas foram enraizadas em meio de cultura suplementado com ANA, AIA ou AIB. As culturas foram eficientemente estabelecidas *in vitro*. O meio suplementado com 15 µM de BAP + 4,5 µM de ANA produziu taxas de propagação elevadas. Em resposta a 15 µM de GA_3 + 1,5 µM de ANA obteve-se brotos e raízes. Maior enraizamento das microestacas foi observado em resposta ao meio contendo ANA a 0,2 µM. Plântulas foram aclimatizadas após seis meses de cultivo *in vitro*. Os resultados deste estudo mostraram que a micropropagação é uma ferramenta eficiente para a conservação *in vitro* da variabilidade genética e para a multiplicação em larga escala de *V. cacuminis*, uma bromélia endêmica e ameaçada de extinção.

Palavras-chave: conservação da biodiversidade, aclimatização, micropropagação

INTRODUCTION

The Ibitipoca State Park, located in Lima Duarte, in the southern of Minas Gerais State, Brazil, was established in 1973. In the last decade, tourism has grown to more than 40,000 people visiting the area each year. The park presents different vegetation types, of which the *campos rupestres* (dry, rocky grasslands) are distinguished by the occurrence of endemic bromeliads (Rodela 1998). The common problems related to the intensive visitations on the *campos rupestres* are the development of erosion on trails and the extractivism of the visual appealing plant species (Versieux & Wendt 2006).

Bromeliaceae is one of the most important families of the Brazilian flora, accounting for around 40% of the described species (Martinelli *et al*. 2008). An important center of the bromeliads diversity is in the southeastern of Brazil, being the Atlantic Forest the ecosystem that has the highest biological richness and the highest level of specific endemism (Forzza & Wanderley 1998, Martinelli 2008). Even after the establishment of restrictive laws, bromeliads are extracted in the Atlantic Forest for commercial and ornamental purposes, resulting in a massive reduction in their population and a dramatic loss of biodiversity (Dal Vesco & Guerra 2010). Among the species subjected to illegal extraction, the ornamental plants, especially the bromeliads, have prominence because of their beauty, abundance and the fact that they are easily obtained in natural environments, in addition to their high commercial value (Negrelle *et al.* 2011). Studies performed in the states of Rio de Janeiro and São Paulo, showed that extractivism supplies around of 50% the market, whereas this number may reach 90% in some places (Andrade & Demattê 1999, Martinelli *et al*. 2008). These data are alarming because several bromeliads species have endemic and endangered populations (Versieux & Wendt 2006, Versieux 2011).

Strategies based on plant tissue culture allow for the large-scale propagation and germplasm conservation of endangered bromeliad species. Under natural conditions, bromeliads generally reproduce slowly, which makes the tissue culture an important alternative for propagation (Mercier & Kerbauy 1997, Carneiro *et al*. 1999, Barboza *et al*. 2004, Silva *et al*. 2009). The first studies of Bromeliaceae micropropagation were developed in the 70s (Mapes 1973). Nowadays, the literature presents several studies related to micropropagation of endangered and native species (Mercier & Kerbauy 1995, Carneiro *et al*. 1999, Pompelli & Guerra 2004, Rech *et al*. 2005, Alves *et al*. 2006, Pickens *et al*. 2006, Silva *et al*. 2009, Rech Filho *et al*. 2009, Guerra & Dal Vesco 2010, Pedroso *et al*. 2010). The patterns of *in vitro* morphogenic response observed in some bromeliads reveal specific features (Alves *et al*. 2006, Guerra & Dal Vesco 2010). This regenerative pattern, named as nodular culture (NC), presents distinct characteristics from organogenesis and somatic embryogenesis and it is recurrent in the *Vriesea* genus (Aranda-Peres *et al*. 2009, Silva *et al*. 2009, Dal Vesco & Guerra 2010, Guerra & Dal Vesco 2010, Pedroso *et al*. 2010).

The *Vriesea cacuminis* L.B. Smith is a rare bromeliad found only in two locations in Minas Gerais State, Brazil: the Ibitipoca State Park, a conservation unit, and at Serra Negra, an unprotected area (Versieux 2011). The species occurs as rupicola in *campos rupestres,* essentially at elevations above 1400 m (Versieux & Wendt 2006, Monteiro & Forzza 2008). Because of its endemism and threat of environmental degradation, this species was included on the Brazilian list of endangered plants as *vulnerable* (Martinelli & Moraes 2013). In order to enable the *in vitro* variability preservation as well as large-scale multiplication of *V. cacuminis*, this study aimed to develop a protocol for micropropagation and *ex vitro* acclimatization of this species.

MATERIAL AND METHODS

Plant material and *in vitro* establishment

Seeds of the *Vriesea cacuminis* L.B. Smith (*Bromeliaceae*) were collected in mature infructescences from a naturally developed specimen at Ibitipoca State Park (21°40’15” - 21°43’30”S and 43°52’35” - 43°54’15”W), Minas Gerais, Brazil, under license from regulatory agencies. The seeds were disinfested in a bleach solution at 0.6% (v/v) of active chlorine, for 20 min under stirring. After the surface disinfection, the seeds were inoculated on ½ MS medium (Murashige and Skoog 1962), supplemented with 7 g L^{-1} agar, 30 g L^{-1} sucrose and 2.22 *µM* GA_3. The culture media pH was adjusted to 5.7 ± 0.1, before autoclaving, performed for 20 min, at 120 °C at 1 atm pressure. The seeds were kept in test tubes (15 x 2.5 cm) containing 15 mL of culture media in a growth room at 25 ± 5 °C, illuminated with fluorescent 40 W lamps, with RFA of 45 µmol m^{-2} s^{-1} and a 16 hour photoperiod.

Multiplication and rooting phases

In the shoot multiplication phase, explants previously established *in vitro* from seeds were employed. The explants, consisting of one nodule cluster (0.5 ± 0.1 cm; containing 1.5 ± 0.5 microplants per cluster), which were toilette from the leaf blades on the sheath base, were inoculated on MS basal medium, supplemented with 6-benzyladenine (BA) or gibberellic acid (GA_3), at 0, 5, 10 or 15 µM, combined with α-naphthalene acetic acid (NAA), at 0, 1.5, 3 or 4.5 µM, in all of the possibilities, with 9 replications each, since these plant growth regulators are usually employed in the bromeliads micropropagation (Droste *et al*. 2005, Rech Filho *et al*. 2005, 2009). The explants were placed into test tubes (15 x 2.5 cm) containing 15 mL of culture medium in a growth room under the same conditions reported for the seed establishment. After 90 days of *in vitro* growth, the explants were evaluated regarding their shoot and root number and shoot number longer than 2 cm.

In the analysis of *in vitro* rooting, explants from culture media containing BA or GA_3 were inoculate on MS basal medium, supplemented with 0, 0.1, 0.2, 0.3 or 0.4 µM of NAA, indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA), with 10 replications each, since these plant growth regulators are the auxins most widely employed aiming at cell division and root induction in the *in vitro* systems (Krikorian 1995). The cultures were maintained in a growth room under the same conditions as reported for the previous assays. The cultures were also kept in test tubes (15 x 2.5 cm) containing 15 mL of culture medium. After 90 days of *in vitro* growth, the explants were evaluated regarding their root number and root length.

Acclimatization

The plantlets obtained in *in vitro* rooting phase were acclimatized in a shade environment using polystyrene trays covered with transparent plastic, totally enclosed, to simulate the moist environment found inside of the test tubes. Plantmax Hortaliças HT® (Eucatex Agro, SP) was used as an acclimatization substrate. After 30 days, the trays, without a plastic cover, were transferred to the greenhouse and kept under a microsprinkler irrigation system (twice a day, for 5 min) for five months. Then the fully acclimatized plants were transplanted in 5 L vessels containing a substrate prepared with vegetable-soil/manure/sand/coconut fiber dust in the ratio of 4:3:2:1 (v/v) and maintained in field conditions under periodical watering (twice a week, until field capacity), without further fertilization. At the end of this phase, the survival rate of the transplanted plants was registered.

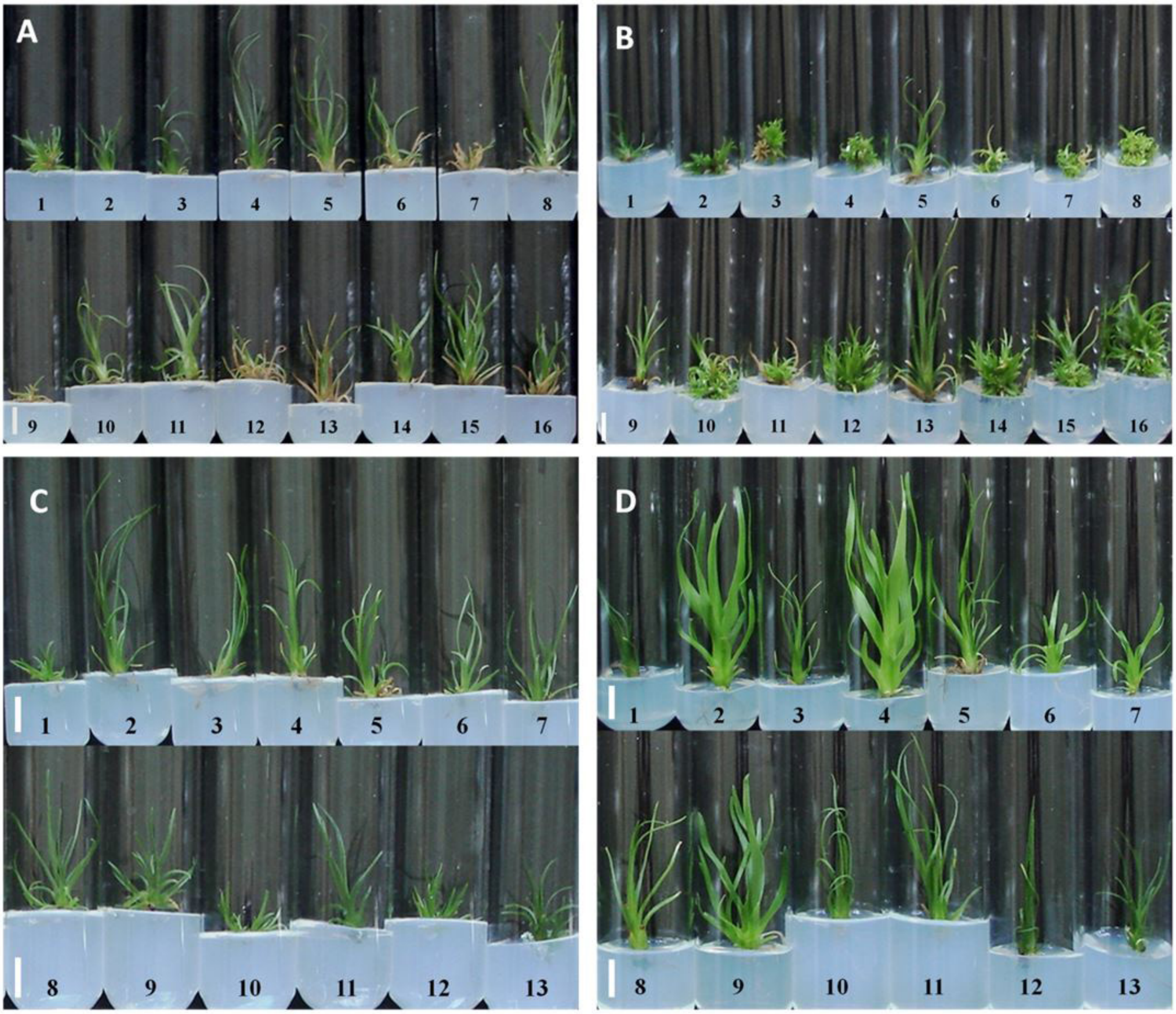
Statistical Analyses

The data were normalized by use of $\sqrt{x+0.5}$ and subjected to analysis of variance (ANOVA). Polynomial regression was used in accordance with residual requirements in the essays related to multiplications and rooting experiments. The averages were clustered by the Scott-Knott test at 5% of probability, using the SAEG program (version 9.1).

RESULTS

***In vitro* establishment and shoot propagation**

The *in vitro* establishment of the *V. cacuminis* cultures

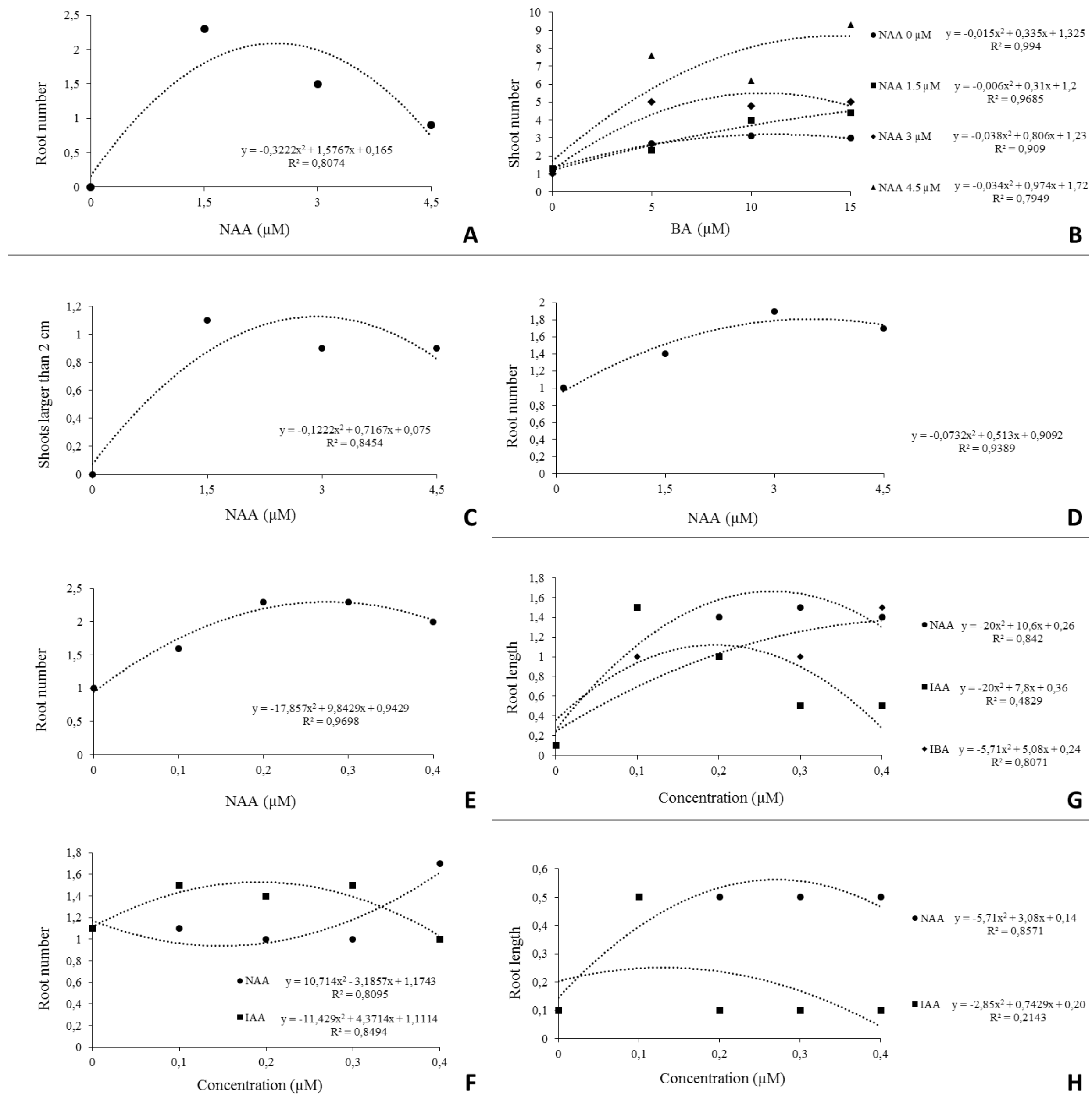


Figs. 1A-D. *In vitro* culture of *Vriesea cacuminis*. **A.** In multiplication media supplemented with GA_3 and NAA, 180 days after inoculation. (Treatments: **1**. 0 µM GA_3 + 0 µM NAA; **2**. 0 µM GA_3 + 1.5 µM NAA; **3**. 0 µM GA_3 + 3 µM NAA; **4**. 0 µM GA_3 + 4.5 µM NAA; **5**. 5 µM GA_3 + 0 µM NAA; **6**. 5 µM GA_3 + 1.5 µM NAA; **7**. 5 µM GA_3 + 3 µM NAA; **8**. 5 µM GA_3 + 4.5 µM NAA; **9**. 10 µM GA_3 + 0 µM NAA; **10**. 10 µM GA_3 + 1.5 µM NAA; **11**. 10 µM GA_3 + 3 µM NAA; **12**. 10 µM GA_3 + 4.5 µM NAA; **13**. 15 µM GA_3 + 0 µM NAA; **14**. 15 µM GA_3 + 1.5 µM NAA; **15**. 15 µM GA_3 + 3 µM NAA; **16**. 15 µM GA_3 + 4.5 µM NAA); **B.** In multiplication media in response to BA and NAA, 180 days after inoculation. (Treatments: **1**. 0 µM BA + 0 µM NAA; **2**. 0 µM BA + 1.5 µM NAA; **3**. 0 µM BA + 3 µM NAA; **4**. 0 µM BA + 4.5 µM NAA; **5**. 5 µM BA + 0 µM NAA; **6**. 5 µM BA + 1.5 µM NAA; **7**. 5 µM BA + 3 µM NAA; **8**. 5 µM BA + 4.5 µM NAA; **9**. 10 µM BA + 0 µM NAA; **10**. 10 µM BA + 1.5 µM NAA; **11**. 10 µM BA + 3 µM NAA; **12**. 10 µM BA + 4.5 µM NAA; **13**. 15 µM BA + 0 µM NAA; **14**. 15 µM BA + 1.5 µM NAA; **15**. 15 µM BA + 3 µM NAA; **16**. 15 µM BA + 4.5 µM NAA); **C.** and **D.** In rooting media, explants precultured in media with GA_3 or BA, respectively, in response to NAA, IAA or IBA, 140 days after inoculation. (Treatments: **1**. control; **2**. 0.1 µM NAA; **3**. 0.2 µM NAA; **4**. 0.3 µM NAA; **5**. 0.4 µM NAA; **6**. 0.1 µM IAA; **7**. 0.2 µM IAA; **8**. 0.3 µM IAA; **9**. 0.4 µM IAA; **10**. 0.1 µM IBA; **11**. 0.2 µM IBA; **12**. 0.3 µM IBA; **13**. 0.4 µM IBA). Scale bars = 1 cm.

was followed by the assessment of fungal and/or bacterial contaminations, and mainly, by the recording of seed germinations. The germination began five days after the *in vitro* seed inoculations, reaching a maximum germination percentage (95%) after 15 days. The culture contaminations were low, with less than 2% of microbiological infection.

In the analysis of the GA_3 and NAA effects on the shoot number and on the shoot number larger than 2 cm a significant difference was not found among the treatments (Table 1). Regarding the rooting, the analysis showed significant effects for the interaction between these growth regulators. Although we have found differences regarding to the control, increases in NAA concentration did not result in significant increases in the root number up to 10 µM GA_3. However, at the higher GA_3 level, the addition of 1.5 µM NAA promoted a higher root number than those found in response to the other treatments with NAA (Fig. 1A; Fig. 2A).

In the absence of BA, increases in NAA concentrations did not result in a significant increase in shoot numbers (Table 2; Fig. 1B). In contrast, under BAP effects, the shoot multiplications were improved in response to the increment in the NAA concentration, especially at the highest ones. The increase in BAP concentrations resulted



Figs 2 A-H. Regression adjustment to *in vitro* cultures of *Vriesea cacuminis*, after 90 days of evaluation. **A.** Number of roots in response to NAA concentrations, in medium supplemented with 15 µM GA_3; **B.** Number of shoots in response to BA and NAA combinations; **C-D.** Number of shoots larger than 2 cm (**C**) and number of roots (**D**) in response to NAA concentrations, in culture medium without BA; **E-F.** Number of roots in microcuttings previous cultured *in vitro* in media containing GA_3 (**E**) or BA (**F**) in response to different concentrations of NAA and/or IAA; **G-H.** Root length (cm) in microcuttings previous cultured *in vitro* in media containing GA_3 (**G**) or BA (**H**) in response to different concentrations of NAA, IAA or IBA.

in an adjustment of a quadratic polynomial regression in response to all NAA concentration (Fig. 2B). The highest propagation rate (9.3 shoots/explant) was found in response to 15 µM BA + 4.5 µM NAA. Regarding to the shoots number larger than 2 cm, no significant effects were observed for interaction between BA and NAA, although, compared to the control, there were significant responses to the addition of NAA, in the medium without BA (Table 2; Fig. 2C). For the rooting, when compared to the control, significant effects were found only in the absence of BA (Table 1; Fig. 2D).

Root formation and plantlets acclimatization

In the absence of auxins, explants from the culture media supplemented with GA_3 did not demonstrate a suitable rooting, which was also observed for those from the media with BA (Table 3; Fig. 1C-D). The addition of NAA in the culture media stimulated the rooting more efficiently than other auxins. Improvement in the rooting was found at 0.2 and 0.4 µM NAA, respectively for plantlets from the culture media supplemented with GA_3 and BA (Fig. 2E-F). The root size was also evaluated. Regardless of their origin, explants from the culture media containing

Table 1. Average shoot number, shoots larger than 2 cm and root number of *V. cacuminis* cultured *in vitro* in response to different combinations of GA_3 and NAA, after 90 days of treatment.

GA³ (µM)	shoot number				shoots larger than 2 cm				root number			
	NAA (µM)				NAA (µM)				NAA (µM)			
	0	1.5	3.0	4.5	0	1.5	3.0	4.5	0	1.5	3.0	4.5
0	1.3 aA*	1.0 aA	1.0 aA	1.0 aA	1.2 aA	1.1 aA	0.9 aA	0.9 aA	0.0 bA	1.9 aB	1.4 aA	1.9 aA
5	1.0 aA	1.0 aA	1.1 aA	1.0 aA	0.9 aA	1.2 aA	1.1 aA	0.9 aA	0.0 bA	1.4 aB	1.7 aA	1.4 aA
10	1.3 aA	1.3 aA	1.0 aA	1.0 aA	1.2 aA	0.9 aA	0.9 aA	0.9 aA	0.0 bA	1.8 aB	1.2 aA	1.4 aA
15	1.1 aA	1.0 aA	1.1 aA	1.0 aA	1.1 aA	0.9 aA	1.1 aA	0.9 aA	0.0 cA	2.3 aA	1.5 bA	0.9 cA

* For each parameter, means followed by the same small (in lines) or capital (in arrows) letters are not different according to Scott-Knott's test at 5% of probability.

Table 2. Average shoot number, shoots larger than 2 cm and root number of *V. cacuminis* cultured *in vitro* in response to different combinations of BA and NAA, after 90 days of treatment.

BA (µM)	shoot number				shoots larger than 2 cm				root number			
	NAA (µM)				NAA (µM)				NAA (µM)			
	0	1.5	3.0	4.5	0	1.5	3.0	4.5	0	1.5	3.0	4.5
0	1.3 aB*	1.3 aC	1.0 aB	1.1 aC	0.0 bA	1.1 aA	0.9 aA	0.9 aA	1.0 bA	1.4 aA	1.9 aA	1.7 aA
5	2.7 bA	2.3 bB	5.0 aA	7.6 aB	0.0 aA	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB
10	3.1 bA	4.0 bA	4.8 aA	6.2 aB	0.0 aA	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB
15	3.0 cA	4.4bA	5.0 bA	9.3 aA	0.0 aA	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB	0.0 aB

* For each parameter, means followed by the same small (in lines) or capital (in arrows) letters are not different according to Scott-Knott's test at 5% of probability.

Table 3. Average root number in explants of *V. cacuminis*, precultured *in vitro* in media containing GA_3 or BA, in response to different concentrations of NAA, IAA or IBA, 90 days after the beginning of the experiment.

from GA³ culture media				from BA culture media			
µM	NAA	IAA	IBA	**µM**	NAA	IAA	IBA
0.0	1.0 aB*	1.0 aA	1.0 aA	0.0	1.1 aB	1.1 aB	1.1 aA
0.1	1.6 aB	1.3 bA	1.0 bA	0.1	1.1 bB	1.5 aA	1.0 bA
0.2	2.3 aA	1.1 bA	1.0 bA	0.2	1.0 bB	1.4 aA	1.0 bA
0.3	2.3 aA	1.1 bA	1.0 bA	0.3	1.0 bB	1.5 aA	1.0 bA
0.4	2.0 aA	1.0 bA	1.1 bA	0.4	1.7 aA	1.0 bB	1.0 bA

* For each previously growth regulator utilized (BA or GA_3), means followed by the same small (in lines) or capital (in arrows) letters are not different according to Scott-Knott's test at 5% of probability.

Table 4. Average root length (cm) in explants of *V. cacuminis*, precultured *in vitro* in media containing GA_3 or BA, in response to different concentrations of NAA, IAA or IBA, 90 days after the beginning of the experiment.

from GA_3 culture media				from BA culture media			
µM	NAA	IAA	IBA	**µM**	NAA	IAA	IBA
0.0	0.1 aB*	0.1 aD	0.1 aC	0.0	0.1 aB	0.1 aB	0.1 aA
0.1	1.5 aA	1.5 aA	1.0 aB	0.1	0.5 aA	0.5 aA	0.1 bA
0.2	1.4 aA	1.0 bB	1.0 bB	0.2	0.5 aA	0.1 bB	0.1 bA
0.3	1.5 aA	0.5 cC	1.0 bB	0.3	0.5 aA	0.1 aB	0.1 aA
0.4	1.4 aA	0.5 bC	1.5 aA	0.4	0.5 aA	0.1 bB	0.1 bA

* For each previously growth regulator utilized (BA or GA_3), means followed by the same small (in lines) or capital (in arrows) letters are not different according to Scott-Knott's test at 5% of probability.

GA_3 or BA showed the longest roots in response to the NAA and IAA, both at 0.1 µM (Table 4; Fig. 2G-H). More elongated roots were found from the explants previously maintained in the culture media containing GA_3.

The survivor rate to *ex vitro* acclimatization of plantlets of *V. cacuminis* was quite high, reaching, on average a 90% success rate for transplantation, regardless of the previous source of the explants.

DISCUSSION

The disinfection procedures used in this study were quite effective from the *V. cacuminis* seeds, with a very low fungal and bacterial contamination (less than 5%). Some species of the genus *Vriesea* produces seeds with a low germination capacity (Mercier & Kerbauy 1995, Droste *et al*. 2005, Silva *et al*. 2009) while in others the germination reaches values close to 100% (Droste *et al.* 2005, Silva *et al.* 2008). The germination found in this study from the *V. cacuminis* seeds were quite high (95%), showing that, at least for this species, the micropropagation is an efficient and useful propagation method, because the *in vitro* germination ensures a substantial amount of aseptic explants for subsequent *in vitro* culture phases and the maintenance of the genetic variability (Mercier & Kerbauy 1997).

The propagation rate found in response to 15 µM BA + 4.5 µM NAA was close to those observed by Fitchet (1990), studying pineapple micropropagation, and by Silva *et al.* (2009), with the *Vriesea scalaris*, species which a high BA concentration promoted extensive shoot proliferation, despite the shoot, as observed in this study, have become small and compact. The gibberellins are associate with the *in vitro* explant elongation in bromeliads (Rech Filho *et al.* 2005, Dal Vesco & Guerra 2010) and this effect was also noticed in this study for *V. cacuminis* by the high number of shoots larger than 2 cm.

In bromeliads, the basal part of the leaves shows vascular elements that may have competent cells for re-differentiation when activated by plant growth regulators (Hosoki & Asahira 1980). Several studies show that in bromeliads, the *in vitro* regenerative route in response to cytokinins follows a specific pattern associated with nodular cultures (NC) (Dal Vesco *et al.* 2011). NCs are globular in form, translucent to yellowish in color, and compact in texture. Under appropriate conditions, the NCs develop radially into a number of small shoots called microshoots, which elongate to form shoots (Guerra & Dal Vesco 2010). This morphogenic pattern seems to be a common characteristic in the Bromeliaceae and recurrent in *Vriesea* genus (Rech Filho *et al.* 2005, Alves *et al.* 2006, Guerra & Dal Vesco 2010). Morphogenic structures with regenerative potential similar to those found in this study for the *V. cacuminis* were also observed for different Brazilian bromeliads, *Vriesea friburgensi* var. paludosa (L.B. Sm.) L.B. Sm. (Alves & Guerra 2001), *Vriesea fosteriana* L.B. Sm. (Mercier & Kerbauy, 1997) and *Vriesea reitzii* Leme & Costa (Rech Filho *et al.* 2005). Based on the regenerative competence of the NCs, they constituted an important source to the mass production of plantlets at a low cost, mainly when associated with the use of bioreactors (Guerra & Dal Vesco 2010).

The new adventitious roots formation in the *in vitro* multiplication phase allows the achievement of complete *V. cacuminis* plantlets for *ex vitro* acclimatization, increasing the efficiency of the micropropagation procedure. According to Kochiba *et al.* (1974), the GA_3 action on rooting is due that stimulates the emergence and/or the development of a meristematic root zone, allowing the emergence of new adventitious roots. In this study, the positive effects of GA_3 on rooting were observed when associated to NAA. Although some species develop adventitious roots stimulated only by endogenous auxin, the additional gibberellins requirement is common for stimulating the rhizogenesis (Hartmann *et al.* 2002). The importance of NAA on *in vitro* rooting was also found by Nilssen & Sutter (1990), showing that this auxin is more stable in the culture than IAA and IBA (Mercier & Kerbauy 1992, Silva *et al.* 2008, 2009). The analysis of the results presented in Tables 2 and 3 showed that rooted plantlets can be produced using culture media supplemented with 0.2 µM NAA or even 15 µM GA_3 + 1.5 µM NAA, since the root number in these treatments was the same (2.3 roots/explant). It should be emphasized, however, that the characteristics of the plantlets obtained after the acclimatization was better when they were previously cultured in the presence of GA_3. Although the root number was low in the evaluated conditions, the rooting founded here were similar to those achieved with another *Vriesea* species (Mercier & Kerbauy 1995, Droste *et al.* 2005, Rech *et al.* 2005).

The survival of *ex vitro* conditions depends on the features of plantlets produced *in vitro* (Hartmann *et al.* 2002). In this work, the survival to acclimation was quite high, with average of 95% for plantlets from culture media containing GA_3 and 85% for plantlets from BA, what is commonly observed for most species of bromeliads grown *in vitro* (Mercier & Kerbauy 1995, Rech *et al.* 2005, Alves *et al.* 2006, Rech Filho *et al.* 2009, Guerra & Dal Vesco 2010). Although the explants (seeds) used to establish the *in vitro* cultures of the *V. cacuminis* does not allow obtaining clones, the plantlets produced *in vitro* are suitable for maintenance of genetic variability in field conditions, which is important for wild species.

The results of this study allowed for the establishment of an efficient protocol for *in vitro* propagation and the maintenance of the genetic variability of the *V. cacuminis*, an endangered and endemic bromeliad.

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