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In vitro propagation of *Vriesea reitzii*, a native epiphyte bromeliad from the Atlantic rainforest

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ABSTRACT. The induction of nodular culture (NC) and the subsequent development of microshoots of *V. reitzii* are considered an *in vitro* propagation model-system with high regenerative performance. Current research analyzed the determinant factors of the *in vitro* morphogenesis control of bromeliads. Seeds excised from mature capsules were grown on medium MS basic (MSB), liquid or gelled, supplemented or not with α-naphthaleneacetic acid (NAA), 6-benzilaminopurine (BAP) or thidiazuron (TDZ). The regeneration and elongation of microshoots were evaluated from NC sub-cultivated on MSB medium on liquid culture medium supplemented with different concentrations of indolyl-3-acetic acid (IAA) and gibberellic acid (GA_3). Plant growth regulators (PGR) supplemented into the medium MSB inhibited the germination of the seeds and induced NC in the second week of growth. The induced NC on MSB medium with NAA (4 μM) and sub-cultivated on MSB medium with NAA (2 μM) plus N^6(2-isopentenyl) adenine (2-iP) (2 μM) showed granular texture and high rate of proliferation. NC sub-culture in MSB medium with IAA (4 μM) provided a higher average number of microshoots (1,478 shoots g^{-1} of NC). Shoots over 3.0 cm resulted in more than 95% *ex vitro* survival.

Keywords: adventitious shoots, conservation, nodular culture, regeneration.

Propagação *in vitro* de *Vriesea reitzii*, uma bromélia epífita nativa da Mata Atlântica

RESUMO. A indução de culturas nodulares (CNs) e subsequente desenvolvimento de microbrotos de *V. reitzii* configuraram-se em um sistema de alta performance regenerativa *in vitro*. No presente trabalho foram estudados os fatores determinantes do controle da morfogênese *in vitro* das CNs. Sementes excisadas de cápsulas maduras foram cultivadas em meio básico MS (MSB) líquido ou geleificado e suplementado ou não com ácido naftalenoacético (ANA), 6-benzilaminopurina (BAP) ou thidiazuron (TDZ). A partir das CNs subcultivadas em meio MSB, foi avaliada a regeneração e o alongamento de microbrotos em meio de cultura líquido suplementado com diferentes concentrações de ácido indolil-3-acético (AIA) combinados com ácido giberélico (AG_3). A suplementação de fitorreguladores ao meio MSB inibiram a germinação das sementes e promoveram a indução de CNs na segunda semana de cultivo. As CNs induzidas em meio MSB suplementado com ANA (4 μM) e subcultivadas em meio MSB suplementado com ANA (2 μM) mais N^6(2-isopentenil) adenina (2-iP) (2 μM) apresentaram textura granular e alta taxa de proliferação. O cultivo destas CNs em meio MSB suplementado com AIA (4 µM) resultou no maior número médio de microbrotos (1.478 brotos g^{-1} de CN). Brotos maiores de 3,0 cm resultaram em mais de 95% de sobrevivência em ambiente *ex vitro*.

Palavras-chave: brotos adventícios, conservação, cultura nodular, regeneração.

Introduction

The Atlantic Rainforest is one of the 25 hotspots of biodiversity on Earth, featuring a combination of endemism and critical threat (METZGER, 2009; MYERS et al., 2000). Bromeliads are common components in this biome and belong to taxonomic groups with great richness and high specific and generic diversity (MARTINELLI et al., 2008). The microhabitats formed by bromeliads contribute towards the stability of forest ecosystem in establishing an interaction with several species, supporting the maintenance of diversity through adaptations and environment specializations (BENZING, 2000; MARTINELLI, 2000). However, threats against this group come from the fragmentation of the forest ecosystem and from the illegal extraction of plants due to their high ornamental and landscape value, or pharmacological interest (ARANDA-PERES; RODRIGUEZ, 2006; MARTINELLI et al., 2008).

The Bromeliaceous family has more than 3.172 species and subspecies (LUTHER, 2008), among which more than 50% of the species are epiphytes (MARTINELLI, 2000). The genus *Vriesea* comprises 261 species and 44 varieties and forms (LUTHER, 2008), distributed in the Americas and throughout the Caribbean (SMITH; DOWNS, 1977). The bromeliad epiphyte *Vriesea reitzii* Leme and Costa (Figure 1A) is native to the Atlantic Rainforest, distributed in the three southern states of Brazil at altitudes ranging between 750 and 1200 m, within the domains of the Araucaria Forest. The species is highly vulnerable because the *Araucaria angustifolia*, its primary epiphyte habitat, has had its natural populations extremely reduced (KLEIN, 1990; LEME; COSTA, 1991).

Since tissue culture techniques are a conservation strategy for threatened species (GUERRA; DAL VESCO, 2010), they have been applied to several species of bromeliads native to the State of Santa Catarina, Brazil, such as *V. friburguensis* var. *paludosa* (ALVES; GUERRA, 2001), *Dyckia distachia* (POMPELLI et al., 2005), *V. reitzii* (RECH FILHO et al., 2005), *V. gigantea* and *V. philippocoburguii* (DROSTE et al., 2005) and *Billbergia zebrina (*DAL VESCO et al., 2011). In the case of bromeliads, they are regenerative *in vitro* systems based on morphogenetic patterns associated with the induction of nodular culture (NC) (ALVES et al., 2006; DAL VESCO; GUERRA, 2010; RECH FILHO et al., 2009). Nodular cultures are defined as groups or conglomerates of organogenic nodules with high regenerative competence. According to Gahan and George (2008), the high competence of the morphogenetic system *in vitro* culminates in the multiple productions of adventitious shoots under adequate growth conditions.

Different strategies are employed for the induction of NC in bromeliads, among which may be mentioned the use of floral segment organs which induce the formation of callus similar to the NC in *Aechmea fasciata* (HUANG et al., 2011a) and in *Guzmania* "Hilda" (HUANG et al., 2011b). Dal Vesco et al. (2011) reported the induction of NC from the nodal segments of *B. zebrina*. However, the induction of NC in bromeliads is mainly based on leaf segments (ALVES et al., 2006; DAL VESCO; GUERRA, 2010; RECH FILHO et al., 2009). To the best of our knowledge, the induction and development of NC from seeds has not yet been reported. Current study establishes a system of NC induction and regeneration of adventitious shoots from seeds of *V. reitzii* in response to different types and combinations of PGR.

Material and methods

Growth conditions

The basic culture medium comprised the saline formulation MS (MURASHIGE; SKOOG, 1962), supplemented with Morel vitamins, sucrose (30 g L^{-1}), henceforth defined medium MS basic (MSB), liquid or gelled with Agar-agar (Sigma®). Culture medium´s pH was adjusted to 5.5 before autoclaving, for 15 min., at 121°C and 1.3 kgf cm^{-2}. Growths were maintained in growth room at 25 ± 2ºC, photoperiod of 16 hours, with luminous intensity 50 - 60 μmol m^{-2} s^{-1} by clear fluorescent light from Sylvana® lamps (40 - 60 W).

Induction of NC

Seeds extracted from mature capsules were excised from matrix plants of *V. reitzii* maintained at the Epagri Training Center of São Joaquim, S. Joaquim, Santa Catarina State, Brazil, altitude 1470 m (Figure 1A), and used as explants sources. De-infestation process and growth incubation conditions followed procedures by Alves et al. (2006).

The experimental design consisted of a factorial scheme (4 x 2) with eight treatments. Four growth environments: 1) MSB free of PGR; 2) MSB + NAA (4 μM); 3) MSB + BAP (4 μM); 4) MSB + TDZ (0.1 μM), combined with two growth conditions: 1) in a test tube (22 x 150 mm) containing 15 mL of liquid culture medium, on filter paper bridge and 2) in 340 glass bottles containing 25 mL of gelled culture medium. Each experimental unit comprised 8 test tubes containing 2 - 3 seeds $tube^{-1}$ and 3 glass bottles containing 6 - 7 seeds, a total of 20 ± 1 seeds per experimental unit, arranged in a completely randomized block (RCB), with three replicates. Percentage data of NC induction and germination were collected after six weeks of growth.

Maintenance of NC

Nodular cultures and microshoots regenerated in all induction treatments were sub-cultivated in MSB medium free of PGR and supplemented with 2 μM of NAA plus 2-iP, during 15 weeks. Gelled media in test tubes and in 340 mL glass bottles containing 15 and 25 mL of culture medium, respectively, were used during the maintenance phase.

Regeneration of microshoots

NC and cultures of microshoots maintained in culture medium MSB were used as sources of explants so that the regenerative efficiency of different combinations of PGRs could be evaluated. Supplementation to medium MSB with five

different combinations of PGRs was tested: 1) MSB free of PGRs; 2) IAA (4 μM) 3) IAA (4 μM) plus GA_3 (4 μM); 4) GA_3 (4 μM); 5) NAA (1 μM) plus BAP (2 μM). Each experimental unit consisted of five test tubes (22x150 mm) containing 15 mL of liquid culture medium and inoculated with 0.27 ±0.004 g of fresh mass of NC per tube, on filter paper bridge, with four replicates in RCB design. Fresh mass data (g) of nodular culture and number of microshoots regenerated were collected after nine weeks of growth.

Elongation of shoots

NC from medium MSB culture was used as explants source. The experimental delineation was a bi-factorial scheme (4 x 3) with 12 treatments: four concentrations of IAA (0, 4, 8 and 12 μM) combined with three of GA_3 (0; 5 and 10 μM) supplemented in medium MSB culture. Each experimental unit comprised three glass bottles with 18 mL of liquid culture medium. Each bottle was inoculated with 2.0 ± 0.04 g of fresh NC mass, ordered in RCB, with three replicates. Data on elongated shoots per height sprout class and fresh mass (g) of non-elongated cultures were collected after 20 weeks of growth.

Acclimatization

Elongated shoots over 3.0 cm were transferred to substrates composed of a mixture of carbonized rice coat, pine bark and mixture of commercial Plantmax® (2:2:1 v/v) disposed in trays with 128 cells (60 cm^3 each). The plantlets were kept in a nebulizer tunnel with intermittent irrigation. Survival percentage data were collected after 5 and 15 weeks. The plantlets were later transplanted to 350-mL pots with the same substrate as described above.

Data analysis

The collected data of each parameter were submitted to verify F_{max} heterogeneity of the variances (S^2). When necessary, the original data were transformed into $(x+0.5)^{1/2}$. Data were submitted to analysis of variance (ANOVA), to Student-Newman-Keuls´ test (SNK-5%) of means separation and regression analysis with Statgraphics software 7.0, following Compton (1994).

Results and discussion

Induction of NC

Seeds of *V. reitzii* grown on MSB culture medium and supplemented with different types and concentrations of PGRs were induced to form NC with a yellowish green color after two weeks in culture (Figure 1B). Further, MSB seeds grown in a PGRs-free medium germinated and formed seedlings (Figure 1C). Inhibition of normal germination of seeds and callus proliferation and induction of adventitious buds were also observed in the seeds of *Tillandsia eizii* (PICKENS et al., 2006). Thus, this type of explants is constituted by competent cells to recognize the induction signs (HICKS, 1994) and may be redirected to new regenerative routes (GAHAN; GEORGE, 2008).

The highest significant ($p < 0.001$) percentages of NC induction resulted from medium MSB liquid culture supplemented with 4 μM NAA (81.8%) or with 0.1 μM TDZ (80.9%) after six weeks in test tubes, on filter paper bridge (Figure 2). Nevertheless, different morphological characteristics associated with different PGRs were observed (Figure 1D-G). When induced from culture medium supplemented with NAA, NC showed granular texture and high capacity for proliferation (Figure 1D) when compared to the most compact cultures which originated in MSB supplemented with TDZ (Figure 1E) and evolved towards the formation of NC (Figure 1F). Moreover, the culture medium supplemented with 4 μM BAP caused the simultaneous induction of NC and microshoots (Figure 1G).

In current study, the highest ($p < 0.01$) percentage of seed germination occurred in response to the MSB culture medium (Figure 2), either liquid (78.1%) or gelled (79.6%). The culture in 340 mL glass bottles with gelled medium resulted in a higher germination rate when compared to that in liquid medium. Germination and seedling development was inversely proportional to NC induction. Therefore, when the NAA was supplemented in the MSB culture medium inhibited the germination of the seeds (Figure 2).

NAA supplemented to the culture medium Knudson inhibited the growth of seedlings in *T. eizii*, but did not reduce the rate of seed germination (PICKENS et al., 2003). In *V. reitzii*, the induction of nodular cultures occurred from the basal region of seedling explants cultured on liquid BM medium supplemented with NAA plus BAP (RECH FILHO et al., 2005). Nodular cultures were also obtained from the basal region of leaf segments in response to the culture medium supplemented with 2,4-D plus Kinetin (ALVES et al., 2006), as well as in liquid MSB medium supplemented with NAA plus 2-iP (DAL VESCO; GUERRA, 2010). In *A. fasciata*, the highest rate of induction of structures similar to nodular cultures were obtained on half-strength MS basal medium supplemented with 2,4-D plus NAA, using floral organs as explants (HUANG et al., 2011a).

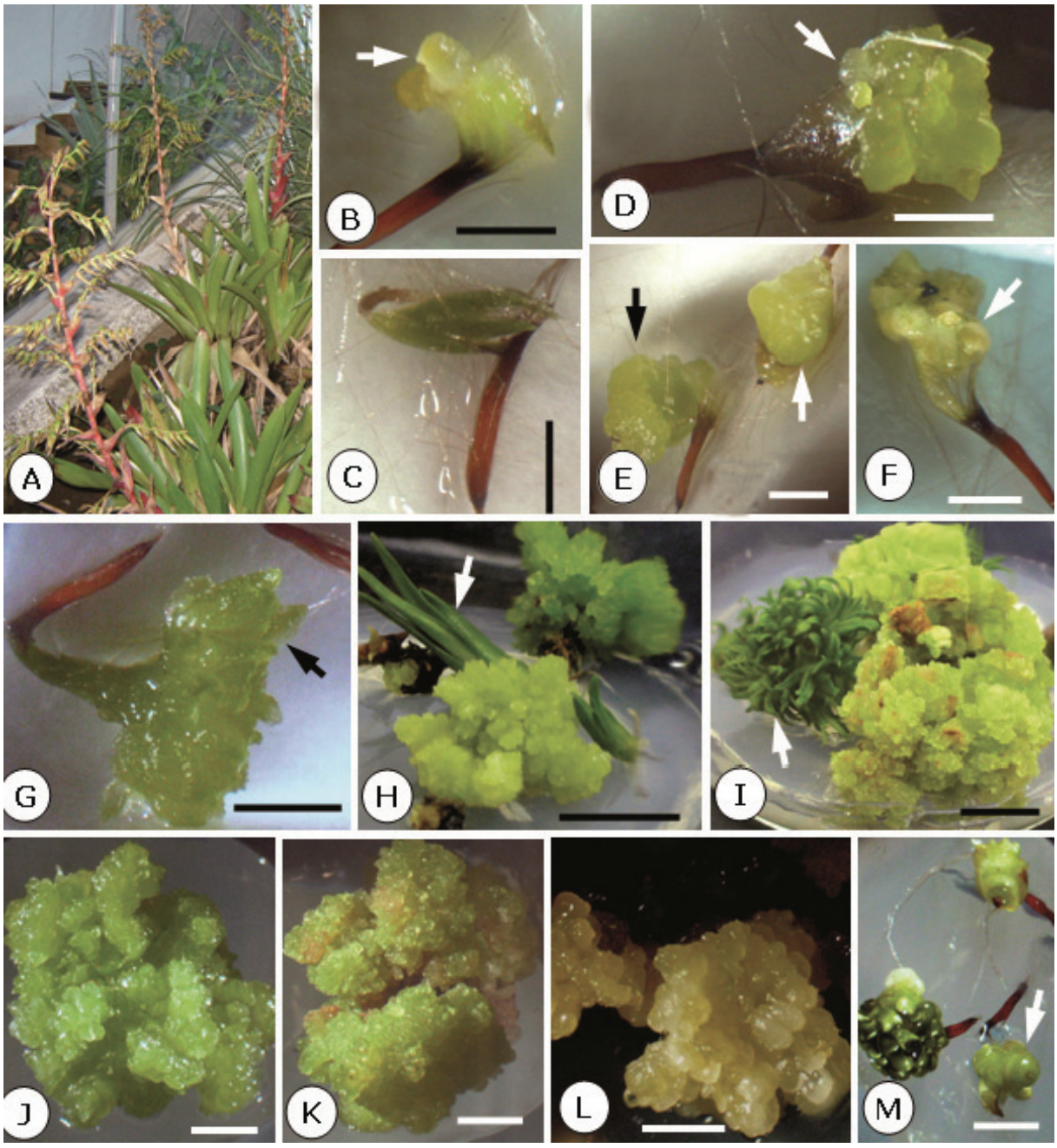

Figure 1. Induction and morphological features of *V. reitzii* in *in vitro* cultures obtained from seeds of A) Matrix plant with mature capsules; B) Induction of cultures after two weeks; C) Seed germination in a MSB medium culture; D-G) start of proliferation of nodular cultures (arrow) after 6 weeks: D) In MSB supplemented with 4 μM of NAA; E-F) In MSB with 0.1 μM of TDZ: E) Induction of compact nodular cultures and F) Detail of nodule formation; G) Green nodular cultures in MSB supplemented with 4 μM of BAP; H) Nodular cultures in gelled MSB supplemented with 4 μM of NAA. Note germination of seeds (arrow) and I) Granular NC in MSB medium with 4 μM of BAP and induction of multiple shoots (arrow); J-L) Maintenance of nodular cultures in gelled medium, originated from: J) Green cultures in MSB supplemented with 4 μM of NAA; K) yellowish to green cultures in MSB supplemented with 4μM of BAP; L) Regeneration of yellowish nodular cultures on MSB; M) Slow growing nodular cultures with compact texture low proliferation (arrow) cultures on MSB supplemented with 0.1 μM of TDZ. Bar: B-G and J-M=3 mm and H and I bar=1 cm.

The use of 340 mL glass bottles with gelled medium triggered the proliferation and induction of nodular cultures and high germination rates, even in the presence of PGR, after 17 weeks in the culture (Figure 1H, I - arrows). These cultures showed granular texture with green color when grown in the presence of 4 μM of NAA (Figure 1H) and yellowish green color associated with the development of multiple microshoots when grown on culture medium supplemented with 4 μM of BAP (Figure 1I).

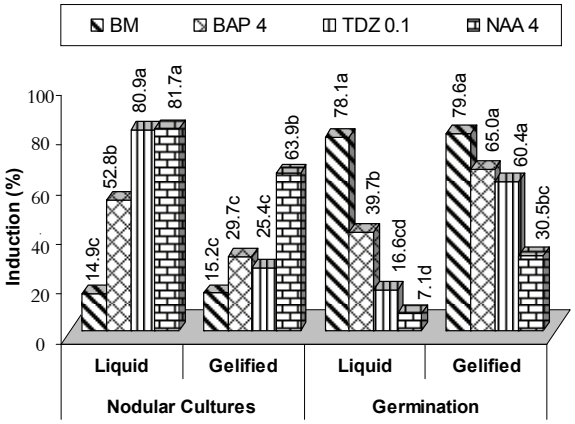

Figure 2. Induction of nodular culture[1] and germination[2] from seeds of *V. reitzii* grown in different medium culture: 1) MSB; 2) MSB + BAP (4 µM); 3) MSB with TDZ (0.1 µM) and 4) MSB with NAA (4 µM), combined with the liquid medium culture, on paper filter bridge or gelled, after 6 weeks in culture. *Mean of three replications. Means followed by different letters indicate rates that differ for SNK test (5%). [1]CV (%) = 17.6; [2]CV (%) = 18.3.

The highest percentage of bud induction and the highest number of buds/explants in *T. eizii* occurred in response to culture medium supplemented with BAP plus NAA (PICKENS et al., 2006). The use of TDZ plus NAA also induced the formation of callus from the leaf explants of *A. bromelifolia* (ARANDA-PERES; RODRIGUEZ, 2006). In *Guzmania "Hilda"*, the regeneration of adventitious buds into plantlets was obtained on culture medium supplemented with NAA plus TDZ (HUANG et al., 2011b). The induction of nodular cultures in *B. zebrina* started from nodal segments and resulted in high regenerative frequency in response to the basal medium supplemented with TDZ (DAL VESCO et al., 2011).

Maintenance of NC

The different morphologic characteristics in the cultures were also related to their original medium. Thus, the cultures originated from MSB, with NAA (4 μM), maintained the formation of agglomerates of friable organogenic nodules with incipient differentiation and greenish-yellowish translucence (Figure 1J). Cultures originated from MSB, supplemented with BAP (4 μM), kept the friable and granular texture (Figure 1K). However, when originated from MSB and sub-cultivated on medium supplemented with NAA plus 2-iP (2 μM each), the proliferation of a new NC was registered, with friable texture, translucent yellow coloration, resembling embryogenic cultures (Figure 1L). The sub-culture every 15 weeks of the cultured to gelled MSB supplemented with NAA plus 2-iP (2 μM each) resulted in the repetitive proliferation for over 2.5 years in culture, as seen in Figure 1J. However, the regeneration of shoots was reported when compared with PGR-free MSB.

Moreover, cultures originated from medium with TDZ (0.1 μM) and sub-cultivated either in PGRs-free MSB or in medium supplemented with NAA plus 2-iP showed low proliferative capacity and more compact texture (Figure 1M). Induced from the basal leaf region of *V. reitzii*, NC was multiplied and maintained in PGR-free gelled medium (DAL VESCO; GUERRA, 2010). Long term NC in *B. zebrina* was maintained on BM gelled medium supplemented with TDZ plus 2-iP and sub-cultured every 17–18 weeks during 2.5 years. However, the regeneration of shoots was reported when they were cultured in liquid medium (DAL VESCO et al., 2011).

Regeneration of microshoots

Cultures grown in MSB medium supplemented with IAA (4 µM) resulted in a high average number of microshoots (1,468 shoots g^{-1} of NC) after nine weeks (Table 1). Intense proliferation was registered during this period and the regeneration of microshoots occurred in a repetitive manner (Figure 3A). The supplementation of the culture medium with IAA and GA_3 (4 µM each) caused the elongation of the microshoots, allowing their individualization (Figure 3B).

Table 1. Regenerative efficiency* in relation to initial and final fresh mass (g) from nodular cultures of *Vriesea reitzii* and the estimated number of microshoots g^{-1} of NC** compared to the number of microshoots produced, in response to the medium MSB culture, supplemented or not with different PGRs, after nine weeks of growth.

Growth regulators (µM)	Fresh mass (g)		Regenerative efficiency*	Microshoots produced	Estimated number of micro shoots g^{-1}**
	Initial	Final			
IAA (4)	0.27	1.55	4.8 A (±0.47)	391 A (±49)	1,468 A (±202)
GA_3 (4)	0.28	1.32	3.8 A (±0.48)	318 B (±42)	1,150 B (±170)
IAA (4) + GA_3 (4)	0.27	1.24	3.6 A (±0.71)	292 B (±70)	1,084 B (±226)
NAA (1) + BAP (2)	0.27	1.27	3.7 A (±0.58)	266 B (±24)	993 B (±111)
MS	0.27	1.29	3.8 A (±0.19)	265 B (±28)	988 B (±131)
Mean	0.27	1.33	4.0 (±0.49)	305 (±43)	1,137 (±168)
CV (%)			14.2	14.7	14.4

Mean of four replications. Means followed by different letters in the column indicate rates that differ from SNK test (5%). Mean (± standard deviation).*Regenerative Efficiency = (Final mass - initial mass)/initial mass; **Estimation of micro shoots g^{-1} = Number of microshoots produced/initial mass (g). CV (%), coefficient of variation.

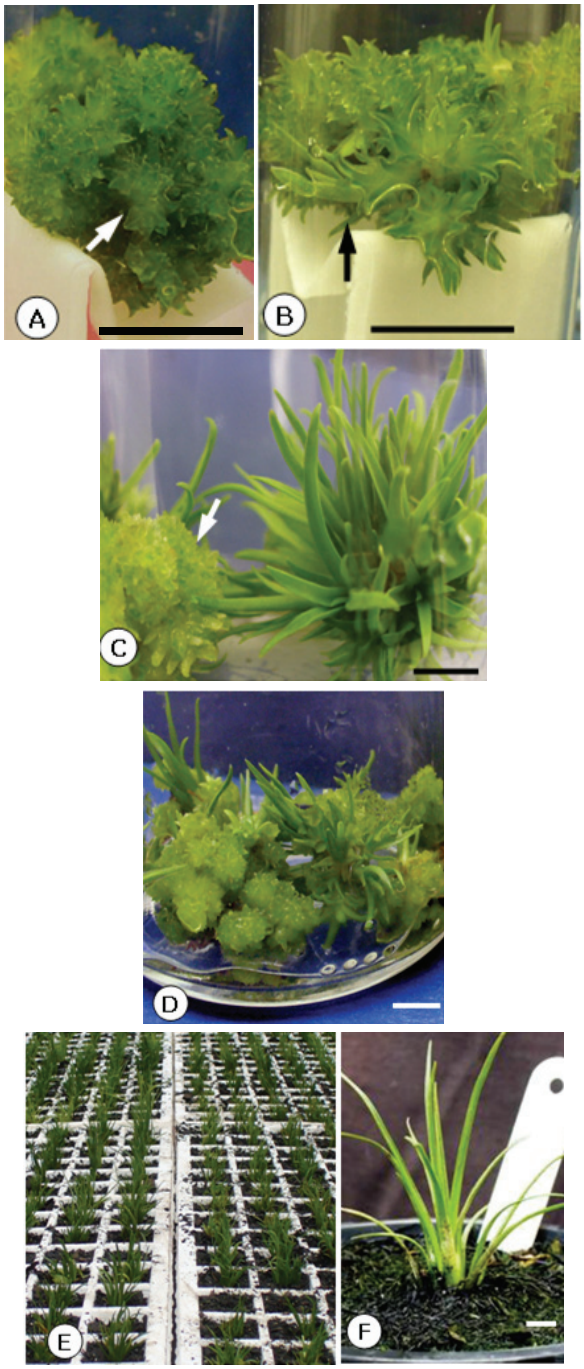

Figure 3. Regeneration and elongation of microshoots from nodular culture (NC) of *Vriesea reitzii*, A) Microshoots in MSB medium supplemented with 4 μM of IAA after 9 weeks in culture; B) Elongation and individualization of microshoots in MSB medium supplemented with 4 μM of IAA plus GA_3; C) Elongation of microshoots and regeneration of NC in MSB liquid medium supplemented with GA_3; D) Proliferation and NC elongation of microshoots in a medium culture supplemented with IAA plus GA_3; E) Acclimatization of shoots, and F) Plantlet development after 3 months. Bar = 1 cm.

Alves et al. (2006) reported a regenerative rate of 60 shoots g^{-1} of NC of *V. reitzii* originated from the leaf basal region cultivated in MS medium supplemented with BAP, Kin and 2-iP. Rech Filho et al. (2005) obtained a higher increase of fresh mass and a larger number of regenerated shoots with this same species in response to NAA and BAP. Dal Vesco and Guerra (2010) reported a high regenerative efficiency (12.4-fold) and an estimated number of 5,329 g^{-1} of NC of microshoots in culture medium with 2-iP and NAA. In *V. splendens*, TDZ supplementation to the medium MS culture resulted in a greater production of microshoots (GUERRA; DAL VESCO, 2010).

Elongation of shoots

The use of 10 µM of GA_3, combined or not with the four IAA tested levels, caused a synchronic elongation of microshoots after 20 weeks of growth (Figure 3C, Figure 4). In this case, the quadratic model of the regression analysis was the best to describe the evolution of the average number of elongated shoots per gram of NC in response to the combination of IAA GA_3 and rates of r^2 and T-value ($p < 0.05$). These reliable indices indicate the quadratic trajectories that, in the case of biological systems, are considered high rates of r^2 occurring between 0.5 and 0.9 (COMPTON, 1994). It may be inferred from the derivation of regression models proposed (Figure 4) that the maximum number of elongated shoots (67.3 shoots g^{-1} of NC) may be obtained in response to the use of 10 µM of GA_3 combined with 2 µM IAA.

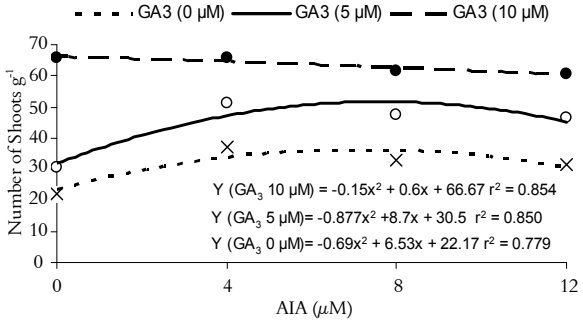

Figure 4. Evolution of the average number of elongated microshoots per gram of NC *V. reitzii* to MSB culture medium supplemented with IAA (0; 4; 8 and 12 µM) and GA_3 (0; 5 and 10 µM) after 20 weeks of culture. *Mean of three replications.

The sub-culture in 340 mL glass bottles resulted in an intense proliferation of NC, albeit associated with a low elongation rate of microshoots (Figure 3D). It has also been reported that the use of 4 µM IAA or 10 µM of GA_3 resulted in a higher and significant ($p < 0.001$) average number of shoots per gram of inoculated culture (51.5 and 63.5 shoots g^{-1}, respectively) when compared to the different doses of IAA and GA_3 (Table 2). However, no significant

Table 2. Regenerative efficiency and average number of microshoots g^{-1} nodular culture (NC) of *Vriesea reitzii* and average length of shoots (cm) from NC in response to the culture medium supplemented with IAA and MSB GA_3 after 20 weeks in culture.

Growth regulators	Fresh mass (g)		Regenerative efficiency*	Number of micro shoots g^{-1} of NC	Height (cm)
IAA (μM)	Initial	Final			
0	2.09	10.36	3.96A (±0.39)	39.4B (±23)	0.3A (±0.07)
4	2.08	10.81	4.20A (±0.46)	51.5A (±14)	0.4A (±0.16)
8	2.12	10.82	4.10A (±0.20)	47.3AB (±14)	0.5A (±0.08)
12	2.11	10.43	3.94A (±0.14)	46.4AB (±15)	0.3A (±0.07)
Mean	2.10	10.60	4.05 (±0.29)	46.1 (±17)	0.4 (±0.09)
GA_3 (μM)					
0	2.12	10.48	3.94A (±0.14)	31.0C (±6)	0.3A (±0.09)
5	2.08	10.72	4.15A (±0.10)	44.0B (±9)	0.5A (±0.15)
10	2.10	10.61	4.06A (±0.29)	63.5A (±3)	0.4A (±0.08)
Mean	2.1 (±0.02)	10.6 (±0.33)	4.05 (±0.18)	46.1 (±6)	0.4 (±0.11)
CV (%)			8.6%	[1]11.0%	35.9%

Mean of three replications. Means followed by different letters in the column indicate rates that differ from SNK test (5%). Mean (± standard deviation); *Regenerative Efficiency = (final weight - initial weight) / initial weight; [1]Data transformed in $(x+0.5)^{½}$. CV (%) = coefficient of variation.

differences were observed in response to different concentrations of IAA and GA_3 tested to the average height of microshoots and regenerative efficiency of cultures (Table 2). In this context, the elongation to take place of synchronized micro shoots of *V. splendens* hybrid took 2 - 3 sub-cultures on medium MS with GA_3 (GUERRA; DAL VESCO 2010). Likewise, two successive sub-cultures in MS medium culture with GA_3 were required to promote elongation of microshoots *V. reitzii* (RECH FILHO et al., 2005). The elongation of shoots in the same species occurred with the alternated cultivation between GA_3 and PGRs-free MSB (RECH FILHO et al., 2009).

In the case of several species of bromeliads, microshoots sub-cultivated on MS medium supplementation with GA_3 are suitable for shoot elongation (GUERRA; DAL VESCO, 2010; DAL VESCO et al., 2011). An increased height of shoots has also been observed in *Nidularium innocentii* and *N. procerum* when cultivated on MS medium with GA_3 (SILVA et al., 2012). However, increase of GA_3 level above 10 μM may reduce the elongation of shoots (DAL VESCO; GUERRA, 2010).

Acclimatization

Elongated shoots with a height over 3.0 cm resulted in more than 95% survival rate, after 30 days from the transference to the trays (Figure 3E). Transplant of seedlings acclimatized to vases of 350 mL resulted in the full development of plants after three months (Figure 3F). *V. reitzii* shoots longer than 2 cm were suitable for a successful acclimatization, with a survival rate higher than 90% (ALVES et al., 2006; DAL VESCO; GUERRA, 2010; RECH FILHO et al., 2005; RECH FILHO et al., 2009).

Conclusion

The regenerative model based on induction and development of NC in *V. reitzii* is an *in vitro* system of high-efficiency for the micro-propagation for the bromeliad. Induced NC on MSB medium with NAA (4 μM) and sub-cultivated on MSB medium with NAA (2 μM) plus 2-iP (2 μM) showed granular texture and high proliferation rate. The sub-culture of NCs on MSB medium with IAA (4 μM) resulted in a higher number of microshoots. The synchronic elongation of microshoots was achieved on liquid MSB medium with GA_3 (10 μM) and shoots bigger than 3.0 cm, with more than 95% of *ex vitro* survival rate.

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