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MICROPROPAGATION OF *NIDULARIUM INNOCENTII* LEM. AND *NIDULARIUM PROCERUM* LINDM (BROMELIACEAE)

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Abstract

Nidularium procerum and *Nidularium innocentii* belong to the family Bromeliaceae and have ornamental and medicinal characteristics. A procedure for the micropropagation for these bromeliads is described. Seedling explants were cultured on MS media (Murashige and Skoog, 1962), liquid and solid supplemented with 0, 2, 4 and 8µM BAP (6-benzylaminopurine) and 2µM NAA (naphthalene acetic acid). Leaf explants were cultured on MS media, liquid and solid supplemented with 0, 0.67, 1.35, 2.7 and 5.4µM NAA (naphthalene acetic acid) and 0.55µM BAP (6-benzylaminopurine). Isolated shoots were cultured on MS double-phase supplemented with 0, 4.1 and 8.2µM GA_3 (gibberellic acid). Rooted plants were acclimatized in conventional and hydroponics system. *In vitro* multiplication is more suitable for seedlings than leaf explants. We recommend MS liquid or solid medium supplemented with 4µM BAP for *Nidularium procerum* and 8µM BAP for *Nidularium innocentii*, both media supplemented with 2µM NAA for *In vitro* multiplication. *In vitro* elongation and rooting can be promoted efficiently on medium free of plant regulators growth. Rooted plants can be satisfactorily acclimatized in Plantmax™ HT substrate in the greenhouse with intermittent nebulization.

Introduction

Nidularium procerum Lindm., and *Nidularium innocentii* Lem., belong to the family Bromeliaceae and have ornamental and medicinal characteristics. Leaves of *N. procerum* has potent analgesic (Amendoeira *et al*., 2005a) and anti-inflammatory activities (Amendoeira *et al*., 2005b), this species presents also potent anti-eosinophil activity (i.e. Anti-allergic properties) (Vieira-de-Abreu *et al*., 2005). *N. innocentii* is used in popular medicine as anti-inflammatory, analgesic, anti-helmintic and diuretic (Chedier *et al*., 2000). *N. procerum* and *N. innocentii* are threatened and facing extinction due to predatory collect of its habitats. Micropropagation can supply enough plantlets for research, conservation, medicinal and ornamental purposes.

Micropropagation of bromeliads is already established for many species. However, *Nidularium* genus had few reports. *In vitro* growth of *Nidularium fulgens* was evaluated on MS and Knudson media (Paiva *et al*., 2006) and a protocol for *In vitro* propagation of *Nidularium fulgens* was established (Paiva *et al*., 2009). Several explants can be used for *In vitro* multiplication of bromeliads, such as seedlings (Droste *et al*., 2005; Silva *et al*., 2008; Paiva *et al*., 2009; Silva *et al*., 2009), nodal buds from crowns of young fruits (Khan *et al*., 2004) and leaves (Mercier & Kerbauy, 1992; Rech Filho *et al*., 2009; Silva *et al*., 2009). This paper describes a protocol for micropropagation of *N. innocentii* and *N. procerum* through shoot and leaf explants.

Materials and Methods

Culture establishment: Two species of bromeliads, *Nidularium procerum* Lindm., and *Nidularium innocentii* Lem. were *In vitro* established. Disinfection consisted of seeds immersion in 70% ethanol during one minute, followed by immersion in commercial bleach (1% NaOCl) for 20 min, and rinsed three times with distilled sterilized water. The germination medium was MS (Murashige & Skoog, 1962), with 30g.L^{-1} sucrose and solidified with 6g.L^{-1} agar.

***In vitro* multiplication:** In order to induce *In vitro* multiplication, a three-way (2x2x4) ANOVA (analysis of variance) experiment was carry out, factor A consisted of 2 species (*N. procerum* and *N. innocentii*), factor B consisted of two consistency of medium (solid and liquid) and factor C consisted of BAP (6-benzylaminopurine) levels which were 0, 2, 4 or 8µM. Basal medium was MS with 30g.L^{-1} sucrose and 2µM NAA (naphthalene acetic acid). The solid media experiment was solidified with 6 g.L^{-1} agar and liquid media experiment was shaking at 75 RPM. Shoot number per explant and shoot percentage were evaluated after 120 days of *In vitro* culture.

Shoot regeneration from leaves: Leaves were removed with tongs and cut at the half; two pieces were cut, a proximal and distal part from the leaf base. In order to induce *In vitro* multiplication from leaf explants, two experiments were carried out, a using proximal part of leaves and other using distal part of leaves. These experiments were organized in a three-way ANOVA (2x2x5) and the factor A consisted of 2 species (*N. procerum* and *N. innocentii*), factor B consisted of two consistency of medium (solid and liquid) and factor C consisted of NAA levels which were 0, 0.67, 1.35, 2.7 or 5.4µM. Basal medium was MS with 30g.L^{-1} sucrose and 0.55µM BAP. Solid media were solidified with 7g.L^{-1} agar and liquid media were shaking at 75 RPM. Flasks were covered with polypropylene lids. Number and percentage of shoots, and number and percentage of roots were evaluated after 60 days of *In vitro* culture.

***In vitro* elongation and rooting:** Shoots (2cm height) from clusters cultured *In vitro* were used as explants. Double-phase medium consisted of the same medium for solid and liquid phase with some differences. Basal medium was MS with 30g.L^{-1} sucrose. For the solid phase was used 30mL of medium solidified with 7g.L^{-1} agar and 4mL of medium for the liquid phase. This experiment were organized in a two-way ANOVA (2x3) and the factor A consisted of both the species (*N. procerum* and *N. innocentii*) and factor B consisted of GA_3 (gibberellic acid) levels which were 0, 4.1 and 8.2 µM. Solid media phase supplemented with GA_3 had this regulator growth autoclavated and liquid media phase supplemented with GA_3 had this regulator growth microfiltered (0.22µm) and placed in media after autoclaving. Root number, rooting percentage, height of the aerial part (cm), leaf number, lateral shoot number, fresh weight (g) and lateral shoot percentage were evaluated after 50 days of *In vitro* culture.

Acclimatization: Plantlets from elongation media (0, 4.1 and 8.2µM GA_3) was removed from flasks and their roots were washed with tap water (faucet). Two acclimatization system was tested, a conventional and hydroponics system. Conventional acclimatization consisted on the culture of the plantlets in substrate Plantmax^{TM} HT in the greenhouse with intermittent nebulization during 15 days. Hydroponics acclimatization consisted on the culture of the plantlets an alveolated tray with thick sand as a substrate (≥1mm). This tray stayed on a nutritive solution composed with half strength MS medium salts (macro and micronutrients) (Fig. 1d). Myo-inositol and vitamins were not added. This solution stayed inside a basin and pH was adjusted to 5.8 each 5 days, and solution level was adjusted to one liter with distilled water. Hydroponic solution was oxygenated with aid the air compressor (1.5 L.min^{-1}), which resulted in 202 L.day^{-1}. Basin was covered with transparent plastic perforate (5 cm^2 each hole) during 10 days of hydroponic culture, and these plantlets were cultured more five days without covered, the period for hydroponic culture was 15 days. Hydroponic culture remained at a growth room with a temperature at 25±2^oC and 16 h of photoperiod, under light intensity of 30 µM m^{-2} s^{-1} obtained by white fluorescent lamps. At the end of 15 days of acclimatization, plantlets were cultured in a substrate plantmax^{TM} HT in the greenhouse with manual irrigation.

This experiment was organized in a three-way ANOVA (2x2x3) and the factor A consisted of 2 acclimatization systems (conventional and hydroponics), factor B consisted of 2 species (*N. procerum* and *N. innocentii*) and factor C consisted of *In vitro* elongation and rooting media which plantlets were produced, these media were supplemented with GA_3 (gibberellic acid) levels which were 0, 4.1 and 8.2µM. Survival percentage was evaluated after 15 days of culture in acclimatization systems and 15 days of culture in the greenhouse without intermittent nebulization.

Culture conditions and statistical analysis: All media had the pH adjusted to 5.8 and were autoclaved at 1 atm and 121ºC for 20 min. The cultures were kept at 25 ± 2º C under white fluorescent light (28µM m^{-2} s^{-1}) with a 16 h photoperiod. The experimental design was completely randomized in a factorial arrangement with five replicates of five explants. The data was submitted in a normality analysis for the Bartlett´s method and, followed by analysis of variance (ANOVA) followed by Duncan´s test, both at a $p<0.01$ and 0.05. Variables from counting were transformed to $\sqrt{x+0.5}$ and variables from percentage were transformed to arcsin $\sqrt{x/100}$. All statistical analyses were done following the procedures of the software GENES (Cruz, 2001).

Results and Discussion

***In vitro* multiplication from seedlings:** For shoot number per explant there were statistical differences for factors, type of species (*N. procerum* and *N. innocentii*), BAP levels (0, 2, 4 and 8µM) and also for interaction between these factors. *N. procerum* presented higher multiplication rate than *N. innocentii* (Table 1). Solid and liquid media did not show statistical differences and there were not interaction between consistency of medium and BAP levels. The best BAP level for *N. procerum* were 4µM which obtained 14.9 shoot per explant and for *N. innocentii* was 8µM which resulted 2.8 shoot per explant (Table 1). In *Nidularium fulgens*, the best result was 5.75 shoot per explant using 4.44µM BAP and 0.54µM NAA (Paiva *et al*., 2009).

Table 1. Shoot number per explant of *Nidularium innocentii* and *Nidularium procerum* on liquid and solid media supplemented with different BAP (6-Benzylaminopurine) levels after 120 days of culture. All media was supplemented with 2 µM naphthalene acetic acid (NAA).

	N. procerum				*N. innocentii*		
BAP (µM)	**Liquid**	**Solid**	**Mean**		**Liquid**	**Solid**	**Mean**
0	4.3	5.2	4.7 Ac[1]		2.5	2.4	2.4 Bab
2	15.1	12.9	14 Ab		2.4	2.1	2.2 Bb
4	13.9	15.9	14.9 Aa		2.7	2.4	2.5 Bab
8	16.9	11.7	14.3 Ab		3.3	2.3	2.8 Ba
CV (%)			16.6				

[1]Means within a column followed by the same lower case letter and means within a line followed by the same capital letter for each parameter are not different at $p<0.05$ by Duncan's Test

Similar results for *In vitro* multiplication rate of *N. innocentii* was found in *Vriesea scalaris* which reached 2 shoot per explant, however instead of BAP, it was used KIN (6-furfurylaminopurine or kinetin) (Silva *et al*., 2009). It is probable that other cytokinins can improve rate proliferation in *N. innocentii*, as in *Dyckia maritima* which KIN was more efficient than BAP (Silva *et al*., 2008). Nevertheless, different species has different rate multiplication, what is a genetic characteristic of the species.

Synergic effect between BAP and NAA have been demonstrated to several bromeliad species for adventitious shoot proliferation, as demonstrated in *V. fosteriana* cultivated in KC medium and supplemented with 8.9µM BAP and 2.7µM NAA which produced ca. 22 explants per seedling (Mercier & Kerbauy, 1992), in *Nidularium fulgens*, the best result was 5.75 shoot per explant using 4.44µM BAP and 0.54µM NAA (Paiva *et al*., 2009), in *Vriesea gigantea* and *V. philippocoburgii* were used 8.9µM BAP and 2.7µM NAA (Droste *et al*., 2005) and in *Vriesea reitzii*, the highest rate of shoot multiplication was with 2-4µM BAP and 1-2µM NAA (Rech-Filho *et al*., 2005).

For shoot percentage statistical differences were observed for type of species only. *N. procerum* was superior to *N. innocentii* for this variable. However, there were not significance for BAP levels, consistency of media and interaction. *N. procerum* vary from 92 to 100% and *N. innocentii* from 55 to 80% for shoot percentage (Table 2). These results can be due to juvenility explant; finally it was used shoot as explants on the contrary seeds. When bromeliad seeds are used as explants, it results in 100% shoot formation (Pompelli & Guerra, 2005) and when seedlings (shoot from seeds) are used it results in minor shoot formation rate (Silva *et al*., 2008).

There was no hyperhydricity occurrence in these species (*N. innocentii* and *N. procerum*); when cultivated in the liquid media, whereas the most common cause of hyperhydricity is the liquid culture (Scheidt *et al*., 2011). Moreover, we did not observe hyperhydricity in these species in stationary cultures (Data not shown). Similar results were found in *Melaleuca alternifolia* cultured in stationary liquid media (Oliveira *et al*., 2010). This capacity to tolerate the stationary culture is very important to reduce production costs, whereas the use of agar and electric energy to move shakers is not necessary.

Table 2. Shoot percentage of *Nidularium innocentii* and *Nidularium procerum* on liquid and solid media supplemented with different BAP (6-Benzylaminopurine) levels after 120 days of culture. All media was supplemented with 2µM naphthalene acetic acid (NAA).

	N. procerum			*N. innocentii*	
BAP (µM)	**Liquid**	**Solid**		**Liquid**	**Solid**
0	93.3[1]	96		55	60
2	100	96		60	55
4	95	100		76.6	61
8	100	92		80	65
CV (%)			17,7		

[1]No statistical differences among treatments

***In vitro* multiplication from leaf explants:** Leaf explants presented different morphogenic answers, distal parts from the leaf base does not show visual effects and the most part died, instead of proximal parts from the leaf base formed roots and shoots in some NAA levels, these results were observed for both species. Similar results were found in *Vriesea scalaris* which shoots were formed at the base from leaves (Silva *et al*., 2009). Another similar result with morphogenic capacities of proximal part from the leaf base was found in *Cryptanthus*, which calli were formed at the base from leaf explant (Koh & Davies, 1998). Calli were not observed in leaf explants cultivated with NAA.

There were statistical differences ($p<0.01$) for factors (type of species, consistency of the medium and NAA levels) and for all interactions in the variables shoot number, shoot percentage, root number and root percentage. However, exceptions were there for factor consistency of the medium and for the interaction among species and consistency of medium in the shoot percentage that had no statistical differences.

Shoot percentage in leaf explants using NAA was low; the best NAA level was 1.35µM for *N. procerum* in the liquid medium (40% shoot) (Fig. 1a) and 2.7µM for *N. innocentii* in the solid medium (20% shoot) (Table 3). For *N. procerum* the most suitable NAA level (1.35µM) in the liquid medium produced 2.1 shoots per explant, and the best NAA level (2.7µM) in the solid medium produced 4 shoots per explant. For *N. innocentii*, the most suitable NAA level (5.4µM) in the liquid medium produced 1.3 shoots per explant, and the best NAA level (2.7 µM) in the solid medium produced 2 shoots per explant (Table 4).

Comparing these data obtained with leaf explants with those obtained with seedlings (Tables 1 and 2), it is probable that seedlings have more competence for multiplication rate; of course that plant growth regulators used for these explants were different. Nevertheless, similar results were found in *Vriesea fosteriana*, which frequency of regeneration in leaf explants and shoot number formed per explant were lower than in seedling explants (Mercier & Kerbauy, 1992).

Leaf explants (whole leaves) of *Vriesea scalaris* cultivated on MS medium supplemented with 0.4 µM BAP, 0.5 µM NAA and 0.06 $g.L^{-1}$ NH_4NO_3 produced ca. 8 shoots per explant after 60 days of culture (Silva *et al*., 2009). In *Neoregelia cruenta* was observed direct shoot formation from whole leaves (at the base) cultivated on MS medium supplemented with 22 µM BAP and 2.5 µM NAA, which produced 50.3 shoots per explant after eight months of culture (Carneiro *et al*., 1999).

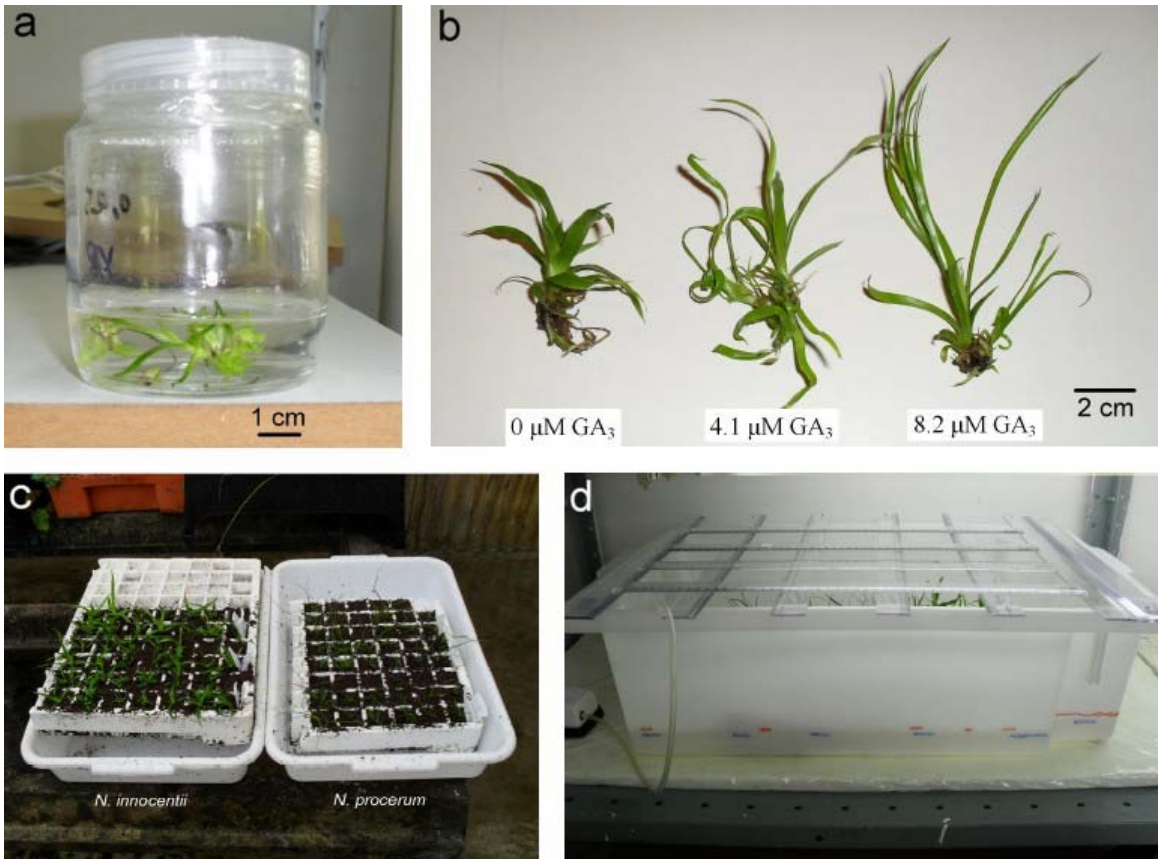

Fig. 1. (a) Multiple shoot formed from base at the leaf of *Nidularium procerum* cultivated on liquid medium supplemented with 1.35 µM NAA and 0.55 µM BAP,(b) *Nidularium innocentii* cultivated at the different levels of gibberellic acid on double-phase medium, (c) *Nidularium procerum* and *Nidularium innocentii* acclimatized on Plantmax^{TM} HT substrate and (d) Hydroponics system for plant acclimatization.

Table 3. Shoot percentage of *Nidularium procerum* and *Nidularium innocentii* from leaf explants (proximal part from leaf base) in solid and liquid media with different naphthalene acetic acid (NAA) levels after 60 days of *In vitro* culture. All media was supplemented with 0.55µM 6-benzylaminopurine (BAP).

	N. procerum			*N. innocentii*	
NAA (µM)	**Liquid**	**Solid**		**Liquid**	**Solid**
0.00	0 Ab[1]	12 Aa		0 Aa	0 Ab
0.67	0 Ab	0 Aa		0 Aa	0 Ab
1.35	40 Aa	4 Ba		0 Ba	0 Bb
2.70	0 Bb	8 ABa		0 Ba	20 Aa
5.40	0 Ab	4 Aa		10 Aa	0 Ab
CV (%)			69.1		

[1] Means within a column followed by the same lower case letter and means within a line followed by the same capital letter for each parameter are not different at p<0.05 by Duncan's Test

Table 4. Shoot number of *Nidularium procerum* and *Nidularium innocentii* from leaf explants (proximal piece) in solid and liquid media with different naphthalene acetic acid (NAA) levels. All media was supplemented with 0.55 µM 6-benzylaminopurine (BAP).

	N. procerum			*N. innocentii*	
NAA (µM)	**Liquid**	**Solid**		**Liquid**	**Solid**
0.00	0 Bb	2.0 Ab		0 Bb	0 Bb
0.67	0 Ab	0 Ad		0 Ab	0 Ab
1.35	2.1 Aa	1.3 Bc		0 Cb	0 Cb
2.70	0 Cb	4.0 Aa		0 Cb	2.0 Ba
5.40	0 Bb	1.1 Ac		1.3 Aa	0 Bb
CV (%)			17.3		

[1]Means within a column followed by the same lower case letter and means within a line followed by the same capital letter for each parameter are not different at p<0.05 by Duncan's Test

Roots were formed at the leaf base. Rooting in *N. procerum* occurred with 1.35 µM NAA in solid medium only, resulting in one root per explant and 4% of rooting. On the contrary, *N. innocentii* had higher rooting rate in liquid medium, the most suitable result was with 5.4 µM NAA (70%), and with 0.67, 1.35, 2.7 µM NAA (40%). Root number varied from 7 to 10.5 roots per explant in *N. innocentii* (Data not shown). Similar results were found in *Vriesea fosteriana*, which shoots were rooted with 1.1 µM NAA and after 60 days of culture every shoot formed 3-4 roots (Mercier & Kerbauy, 1992).

***In vitro* elongation and rooting:** There were no statistical differences in the factors, species and GA_3 levels and there was no interaction among these factors for root percentage and leaf number variables. Factor type of species was significant for fresh mass ($p<0.01$) and for percentage of lateral shoots ($p<0.05$), nevertheless the factor GA_3 levels and interaction among factors type of species and GA_3 levels was not significant. There were no statistical differences for height of the aerial part among species and there was not interaction, although GA_3 levels were significant ($p<0.01$) (Fig. 1b). For the variable number of lateral shoots there were no statistical differences for factors type of species and GA_3 levels, however, this interaction was significant ($p<0.05$). The variable root number showed statistical differences for factors type of species and GA_3 levels ($p<0.01$), however, their interaction was not significant (Table 5).

Table 5. Summary of two-way ANOVA (analysis of variance). Height of the aerial part (AP cm), number of lateral shoots (BL), percentage of lateral shoots (BL %), root number per explant (NR), percentage of rooting (E %), fresh mass (MF g), leaf number per explant (NF) observed in shoots of *Nidularium innocentii* and *Nidularium procerum* after 50 days of *In vitro* culture on double-phase media with different levels of gibberellic acid (0, 4.1 and 8.2µM GA_3).

	Mean square							
Source	**d.f.**	**AP cm**	**BL**	**BL %**	**NR**	**E %**	**MF g**	**NF**
A[1]	1	1.260 ^{ns}	0.05270 ^{ns}	2452.2 **	7.7516 *	94.09 ^{ns}	0.738 *	0.2897 ^{ns}
B[2]	2	17.566 *	0.2194 ^{ns}	814.54 ^{ns}	0.5289 *	23.52 ^{ns}	0.035 ^{ns}	0.8385 ^{ns}
A x B	2	2.951 ^{ns}	0.7231 **	960.18 ^{ns}	0.1282 ^{ns}	23.52 ^{ns}	0.024 ^{ns}	0.8403 ^{ns}
Residual	24	0.9829	0.1505	319.50	0.0747	47.046	0.0143	0.3628
CV(%)		17.1	24.1	28.8	10.9	7.7	40.0	13.2

[1]Species (*Nidularium innocentii* and *Nidularium procerum*), 2 GA_3 levels (0, 4.1 and 8.2µM), * $p<0.01$, ^{ns}Not significant

Root number was favored with absence of GA_3. Similar result was found in *Vriesea scalaris* which culture free of plant growth regulators promoted best results (Silva *et al*., 2009). *N. innocentii* formed more roots than *N. procerum*, 8.5 and 3.4 roots per shoot, respectively. Root percentage varied from 96 to 100% for both species and GA_3 levels (Table 6). The most suitable level of GA_3 was 8.2µM for variable height of the aerial part, resulting in 7.1cm against 4.4cm at absence of GA_3. Similar result was observed in *Dyckia maritima* cultivated on MS medium, the most suitable level of GA_3 for height of the aerial part of multiple shoots was 7.13µM, and also observed that increases of GA_3 levels decreases number of lateral shoots (Silva *et al*., 2004).

Number of leaves varied from 15.7 to 25.7 leaves per shoot. *N. procerum* presented more percentage of lateral shoots than *N. innocentii*, 80 and 61.3%, respectively. Number of lateral shoots was higher at 8.2µM GA_3 in *N. innocentti* and higher at the absence of GA_3 in *N. procerum*, 3.4 shoots per explant in both species. *N. innocentii* showed higher fresh mass than *N. procerum*, 0.455 and 0.142g, respectively, although fresh mass do not show statistical difference for GA_3 levels, results of the fresh mass found in shoots cultivated with GA_3 was lower than shoots cultivated in the absence of GA_3 (Table 6). Similar result was found in *Vriesea friburgensis* cultivated on MS medium, the increase of GA_3 levels resulted in decrease of the fresh mass after 60 days of culture (Alves & Guerra, 2001).

Acclimatization: There were no statistical differences after 15 days of acclimatization for factors, type of species, GA_3 levels and type of acclimatization, the survival rate varied from 91.6 to 100% (Data not shown). However, after 30 days of acclimatization, there were statistical differences for factors, type of species and type of acclimatization method and for interaction triple. *N. procerum* cultivated on hydroponics presented lower survival rate than *N. innocentii*, although these species were not statistical different when cultivated on conventional acclimatization. Survival rate was lower using hydroponics acclimatization than conventional and the GA_3 levels do not had statistical differences (Table 7). The most suitable result for acclimatization of micropropagated plants of *N. procerum* and *N. innocentii* was acquired with conventional acclimatization (Table 7 and Fig. 1c). Similar result was found in micropropagated plants of *Nidularium fulgens* cultivated in greenhouse irrigated by a nebulization system during 60 days, which survival rate was 100% (Paiva *et al*., 2009).

Micropropagated plants of *Dyckia maritima* were acclimatized using hydroponics, 80% of survival rate was reached when plants were cultivated on coconut fiber, and 60% when cultivated on worm humus and a mixture of coconut fiber: worm humus (1:1 v/v) (Silva *et al*., 2007). It is probable that our results can be more elevated using other substrates for hydroponics acclimatization. Although, acclimatization using hydroponics is efficient for the hydrophilic plants (Nhut *et al*., 2004). Nevertheless, acclimatization using hydroponics is an alternative for laboratories that do not have greenhouse with intermittent nebulization.

Table 6. Characteristics observed in *N. innocentii* and *N. procerum* cultivated on double-phase media supplemented with different levels of gibberellic acid (GA_3) after 50 days of *In vitro* culture.

	Root number				Rooting (%)		
GA_3	Species				Species		
(µM)	*N. innocentii*	*N. procerum*	Mean		*N. innocentii*	*N. procerum*	Mean
0.0	10.8	4.1	7.4 a[1]		100[2]	96	98
4.1	7.6	3.6	5.6 b		100	100	100
8.2	7.3	2.7	5.0 b		100	96	98
Mean	8.5 a	3.4 b			100	97.3	
	Heigth of the aerial part (cm)				**Lateral shoots (%)**		
GA_3	Species				Species		
(µM)	*N. innocentii*	*N. procerum*	Mean		*N. innocentii*	*N. procerum*	Mean
0.0	5.2	3.7	4.4 c		64	100	82
4.1	5.4	6.1	5.7 b		48	72	60
8.2	7.3	6.9	7.1 a		72	68	70
Mean	5.9	5.5			61.3 b	80 a	
	Number of lateral shoots				**Fresh mass (g)**		
GA_3	Species				Species		
(µM)	*N. innocentii*	*N. procerum*	Mean		*N. innocentii*	*N. procerum*	Mean
0.0	1.9 Bb	3.4 Aa	2.6		0.5677	0.1528	0.3602
4.1	1.2 Bc	2.1 Ab	1.6		0.3485	0.1331	0.2408
8.2	3.4 Aa	1.4 Bc	2.4		0.4513	0.1401	0.2957
Mean	2.2	2.3			0.4558 a	0.1420 b	

[1]Means within a column followed by the same lower case letter and means within a line followed by the same capital letter for each parameter are not different at $p<0.05$ by Duncan's Test

[2] Means without letters do not have statistical differences

Table 7. Survival percentage of micropropagated plants of *Nidularium procerum* and *Nidularium innocentii* on conventional and hydroponics acclimatization after 30 days of *ex vitro* culture.

GA_3	Conventional			Hydroponics	
(µM)	*N. procerum*	*N. innocentii*		*N. procerum*	*N. innocentii*
0.0	100 aA[1]	100 aA		50 aC	83.3 aB
4.1	91.6 aA	100 aA		66.6 aC	83.3 aB
8.2	91.6 aA	100 aA		58.3 aC	83.3 aB
CV(%)			6.7		

[1] Means within a column followed by the same lower case letter and means within a line followed by the same capital letter for each parameter are not different at $p<0.05$ by Duncan's Test

Conclusions

In vitro multiplication is more suitable for seedlings than leaf explants. We recommend MS liquid or solid medium supplemented with 4µM BAP for *Nidularium procerum* and 8µM BAP for *Nidularium innocentii*, both media supplemented with 2µM NAA for *In vitro* multiplication. *In vitro* elongation and rooting can be promoted efficiently on medium free of plant regulators growth. Rooted plants can be satisfactorily acclimatized in Plantmax™ HT substrate in the greenhouse with intermittent nebulization.

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References

Alves, G.M. and M.P. Guerra. 2001. Micropropagation for mass propagation and conservation of *Vriesea friburgensis* var. paludosa from microbuds. *Journal of the Bromeliad Society*, 51: 202-212.

Amendoeira, F.C., V.S. Frutuoso, C. Zanon, L.M. Chedier, M.R. Figueiredo, M.A.C. Kaplan, C.B. Melo, P.T. Bozza and H.C. Castro-Faria-Neto. 2005b. Anti-inflammatory Activity in the Aqueous Crude Extract of the Leaves of *Nidularium procerum*: A Bromeliaceae from the Brazilian Coastal Rain Forest. *Biol. Pharm. Bull*., 28: 1010-1015.

Amendoeira, F.C., V.S. Frutuoso, L.M. Chedier, A.T. Pearman, M.R. Figueiredo, M.A. Kaplan, S.M. Prescott and H.C. Castro-Faria-Neto. 2005a. Antinociceptive effect of *Nidularium procerum*: a Bromeliaceae from the Brazilian coastal rain forest. *Phytomedicine*, 12: 78-87.

Carneiro, L.A., R.F.G. Araújo, G.J.M. Brito, M.H.P.B. Fonseca, A. Costa, O.J. Crocomo and E. Mansur. 1999. *In vitro* regeneration from leaf explants of *Neoregelia cruenta* (R. Graham) L.B. Smith, an endemic bromeliad from eastern Brazil. *Plant Cell Tiss. Organ Cult*., 55: 79-83.

Chedier, L.M., M.R. Figueiredo and M.A.C. Kaplan. 2000. Chemical and biological investigations on *Nidularium innocentii* Lemaire. *An. Acad. Bras. Ciênc*., [online] 72: 295-295.

Cruz, C.D. 2001. *Programa genes: versão Windows; aplicativo computacional em genética e estatística*. Viçosa, Universidade Federal de Viçosa.

Droste, A., A.M. Silva, A.V. Matos and J.W. Almeida. 2005. *In vitro* culture of *Vriesea gigantea* and *Vriesea philippocoburgii*: Two vulnerable bromeliads native to southern Brazil. *Braz. Arch. Biol. Technol*., 48: 717-722.

Khan, S., A. Nasib and B.A. Saeed. 2004. Employment of *İn vitro* technology for large scale multiplication of pineapples (*Ananas comosos*). *Pak. J. Bot*., 36: 611-615.

Koh, Y.C. and F.T.J. Davies. 1997. Micropropagation of *Cryptanthus* with leaf explants with attached intercalary meristems excised from greenhouse stock plants. *Sci. Hortic*., 70: 301-307.

Mercier, H., G.B. and Kerbauy. 1992. *In vitro* multiplication of *Vriesea fosteriana*. *Plant Cell Tiss. Organ Cult*., 30: 247-249.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*., 15: 473-497.

Nhut, D.T., N.T.D. Huong and D. Van-Khiem. 2004. Direct microtuber formation and enhanced growth in the acclimatization of *In vitro* plantlets of taro (*Colocasia esculenta* spp.) using hydroponics. *Sci. Hortic*., 101: 207-212.

Oliveira, Y., F. Pinto, A.L.L. Silva, I. Guedes, L.A. Biasi and M. Quoirin. 2010. An efficient protocol for micropropagation of *Melaleuca alternifolia* Cheel. *In Vitro Cell Dev. Biol. Plant.*, 46(2): 192-197.

Paiva, P.D.O., V. Coelho-Naves, L. Ferreira-Dutra, R. Paiva and M. Pasqual. 2009. *In vitro* propagation of *Nidularium fulgens* lem. INCI 34: 593-596.

Paiva, P.D.O., V.C. Naves, R. Paiva and M. Pasqual. 2006. Avaliação de diferentes formulações de sais minerais para a micropropagação de *Nidularium fulgens* Lem.. *Plant Cell Culture and Micropropagation*, 2: 9-14.

Pompelli, M.F. and M.P. Guerra. 2005. Micropropagation enables the mass propagation and conservation of *Dyckia distachya* Hassler. *Crop Breed Appl. Biotechnol*., 5: 117-124.

Rech Filho, A., L.L. Dal Vesco and M.P. Guerra. 2009. Adventitious shoots from nodule cluster cultures of *Vriesea reitzii*: an endemic and endangered bromeliad from atlantic forest. *Ciênc. Rural*., 39: 909-912.

Rech Filho, A., L.L. Dal Vesco, R.O. Nodari, R.W. Lischka, C.V. Müller and M.P. Guerra. 2005. Tissue culture for the conservation and mass propagation of *Vriesea reitzii* Leme and Costa, a bromeliad threatened of extinction from the Brazilian Atlantic Forest. *Biodivers and Conserv*., 14: 1799-1808.

Scheidt, G.N., A.L.L. Silva, Y. Oliveira, J.L. Costa, L.A. Biasi and C.R. Soccol. 2011. *In vitro* growth of *Melaleuca alternifolia* Cheel in bioreactor of immersion by bubbles. *Pak. J. Bot*., 43: 2937-2939.

Silva, A.L.L., E.T.H. Franco, D.A. Bisognin, E.B. Dornelles and J.M. Walter. 2004. Ensaios sobre o alongamento de brotos laterais de *Dyckia maritima* Baker dispostos em agrupamentos - Bromeliaceae. *Caderno de Pesquisa Série Biologia*, 16: 37-46.

Silva, A.L.L., E.T.H. Franco, E.B. Dornelles and J.P.A. Gesing. 2008. Micropropagation of *Dyckia maritima* Baker-Bromeliaceae. *Iheringia Série Botânica*, 63: 135-138.

Silva, A.L.L., E.T.H. Franco, E.B. Dornelles, C.L. Reichert Bortoli and M. Quoirin. 2009. *In vitro* multiplication of *Vriesia scalaris* E. Morrem (Bromeliaceae). *Iheringia Série Botânica*, 64: 151-156.

Silva, A.L.L., E.T.H. Franco, M.A. Horbach, D.A. Bisognin and M. Quoirin. 2007. Acclimatization of *Dyckia maritima* Baker using hydroponics (Bromeliaceae). *Caderno de Pesquisa Série Biologia*, 19: 16-23.

Vieira-de-Abreu, A., F.C. Amendoeira, G.S. Gomes, C. Zanon, L.M. Chedier, M.R. Figueiredo, M.A. Kaplan, V.S. Frutuoso, H.C. Castro-Faria-Neto, P.F. Weller, C. Bandeira-Melo and P.T. Bozza. 2005. Anti-allergic properties of the bromeliaceae *Nidularium procerum*: inhibition of eosinophil activation and influx. *Int. Immunopharmacol*., 5: 1966-1974.

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