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Short Communication / Nota Científica
Propagation of *Dyckia vicentensis*, an endemic bromeliad of the Pampa biome, Brazil

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Abstract
Dyckia vicentensis is an endemic species of the southwestern region of Rio Grande do Sul (RS, Brazil), which presents high ornamental and economic value. Thus, the aim of this study was to test *in vitro* and *ex vitro* conditions for its propagation. For *in vitro* germination, disinfested seeds were inoculated on Murashige and Skoog medium with different salt concentrations and containing or not active charcoal. The *ex vitro* emergence of the seeds was evaluated using different compositions of substrates. Results showed that the propagation of *D. vicentensis* could be successfully performed *in vitro* on medium with 50% salt concentration or *ex vitro* using commercial substrate. The seedlings showed good adaptation during acclimatization in a greenhouse, although the *in vitro* germinated plants presented higher survival rate, number of leaves, and biomass in relation to those grown in *ex vitro* substrate. This is the first study carried out on the propagation of *D. vicentensis*, which may be used to subsidize its propagation and conservation.
Key words: Bromeliaceae, germination, *in vitro* culture, ornamental plant, substrates.

Resumo
Dyckia vicentensis, uma espécie endêmica do sudoeste do Rio Grande do Sul (RS, Brazil), possui alto valor ornamental e econômico. Assim, o objetivo deste estudo foi testar condições *in vitro* ou *ex vitro* para a sua propagação. Para a germinação *in vitro*, as sementes foram desinfestadas e inoculadas em meio de Murashige e Skoog (MS) com diferentes concentrações de sais minerais e contendo ou não carvão ativo. A emergência de plântulas, em condições *ex vitro*, foi avaliada em diferentes composições de substratos. Os resultados mostraram que a propagação de D. vicentensis pode ser realizada em condições *in vitro*, utilizando meio de cultura com 50% da concentração de sais do meio MS, e também em condições *ex vitro*, em substrato comercial. Em geral, as plântulas mostraram uma boa adaptação durante a aclimatização em casa de vegetação, embora aquelas germinadas *in vitro* tenham apresentado maior taxa de sobrevivência, maior numero de folhas e biomassa em relação às plântulas oriundas da germinação em substrato. Este estudo é pioneiro para *D. vicentensis* e poderá fornecer subsídios para propagação e conservação dessa espécie.
Palavras-chave: Bromeliaceae, germinação, cultivo *in vitro*, planta ornamental, substratos.

The Bromeliaceae family is known for its recent adaptive radiation (Zanella *et al.* 2012). It likely originated in the Andean region and subsequently spread throughout the millennia, reaching tropical forests about 200,000 years ago. Currently, this family is widely distributed in the American continent. In Brazil, the bromeliads include 43 genera and 1,341 species (Forzza *et al.* 2014; Alves *et al.* 2015), which represent almost 40% of the diversity of the family.

Bromeliads have high ornamental and economic value (Zanella *et al.* 2012) due to their lush shapes and colors. In addition to their easy maintenance and adaptability, they can be used for

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interior decoration and landscaping. According to SEBRAE (2015), the production and marketing of ornamental plants (including bromeliads) is one of the most dynamic and promising segments of the Brazilian agribusiness. In this reality, the state of Rio Grande do Sul (RS) is the second most important region of Brazilian floriculture.

The Bromeliaceae family is also characterized by high ecological value and degree of endemism since 1,172 species are endemic to Brazil (Alves *et al*. 2015). However, this family is the second plant group in absolute number of endangered species (202 spp.) (Rio Grande do Sul 2014; Alves *et al*. 2015). The main threats to bromeliads are the loss of habitats due to the degradation of native forests, since most have epiphytic habit, and the predatory removal of individuals in nature (Santos *et al*. 2005) for commercialization. Although this practice is illegal in Brazil, it still occurs in some regions (Negrelle *et al.* 2012; Negrelle & Anacleto 2012).

These data highlight the urgent need for studies aiming at developing strategies for the cultivation and conservation of the existing germplasm, especially those endemic and/or endangered species. In addition, the production and use of native plants with ornamental potential can contribute to the valorization and conservation of regional biodiversity. It is an economically and ecologically viable option and contributes to the conservation of natural ecosystems (Santos *et al.* 2005; Stumpf *et al.* 2009). However, a great number of species of native flora are little studied, hindering the use of management techniques and their sustainable use.

The genus *Dyckia* Schult. & Schult. f. (subfam. Pitcairnioideae) comprises about 164 species (Gouda *et al.* 2012) spread across the southern and southeastern regions of South America (Benzing 2000). In Rio Grande do Sul (southern Brazil), this genus is considered to have the highest diversity, with 29 species (Strehl 2008; Büneker *et al.* 2013) and several endemic species (Hmeljevski *et al.* 2007; Büneker *et al.* 2015; Gomes-da-Silva *et al*. 2017), among which is *D. vicentensis* Strehl.

D. vicentensis is endemic to the Pampa biome, occurring especially in rocky outcrops in the southwestern region of RS. The name of the species is a tribute to the municipality São Vicente do Sul (RS, Brazil), where it was collected for the first time (Strehl 2008). The great ornamental potential of this species is that its inflorescences are composed of showy flowers, with coloration varying from yellow to red (Fig. 1a,b) and with potential to be grown in parks or gardens.

The Pampa biome, which is restricted to the southern half of RS, contains a high level of genetic diversity and several wild ornamental species (Stumpf *et al*. 2009). However, several factors have contributed to the drastic reduction of this biome, such as the expansion of areas for agriculture (especially for soybean cultivation), the indiscriminate use of agrochemicals, and the presence of exotic species. Thus, *D. vicentensis* specie is distributed in endangered biome, is endemic or have a relict distribution, therefore threatening the survival of many members of this family. Today, this specie is on the endangered category of the official list of Brazilian plant species threatened with extinction (Rio Grande do Sul 2014).

The propagation of the other species of the genus *Dyckia* is done using seeds (Pompelli & Guerra 2004; Vieira *et al.* 2007; Aoyama *et al.* 2012). However, to this date there are no reports on the physiology of germination aimed at the propagation of *D. vicentensis* plants, which is fundamental knowledge to subsidize their conservation. Thus, this study focused on evaluating the viability of the propagation of *D. vicentensis* using seeds. For this, the culture media for *in vitro* germination and different substrates in the emergency of seedlings were tested.

Seed-containing capsules were collected from natural populations in the municipality of Alegrete (RS, Brazil) and cool-stored under refrigeration at 4 ± 2 ºC according to Pompelli & Guerra (2004). For disinfestation, the seeds were immersed in 70% (v/v) ethanol solution for 3 min followed by 30 min immersion with agitation in 1.0% sodium hypochlorite (v/v) plus two drops of commercial detergent per 100 mL solution. The seeds were then rinsed five times in sterile and distilled water. These disinfected seeds were used for *in vitro* germination and emergence assay in different substrates.

For *in vitro* germination, the disinfected seeds were inoculated into glass culture tubes containing 2 mL of the solidified culture Murashige & Skoog (MS; Murashige & Skoog 1962) with 50 or 100% of salt concentrations and supplemented or not with activated charcoal (1.0 g L^{-1}). All media were added with 100 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose, and 7 g L^{-1} agar. The pH was adjusted to 5.8 using HCl or NaOH (1N) prior to autoclaving at 121 °C and 1.15 kg cm^{-2} for 20 min.

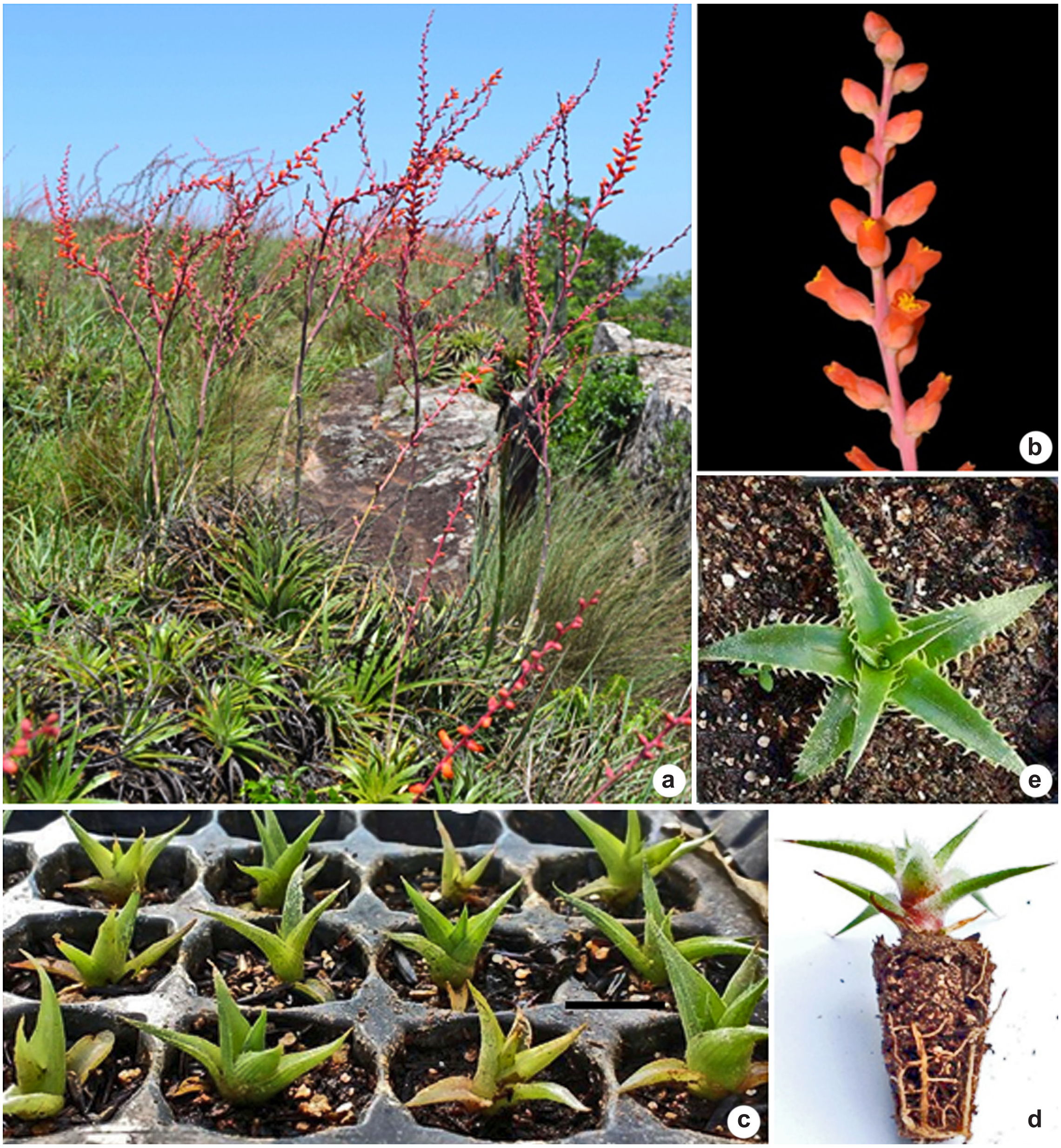

Figure 1 – Plants of *Dyckia vicentensis* in nature (a) and detail of inflorescence (b); plantlet rustication in a greenhouse after 60 days of planting (c); general aspect of plants after 180 days of planting (d); rusticated plantlets transferred to strays containing commercial substrates (e).(Bars: c = 1.0 cm; d = 2.3 cm; e = 3.0 cm)

After inoculation (one seed per flask), the seeds were incubated in a growth room at 25±2°C under a 16/8 h light/dark cycle with 35 µmol m^{-2} s^{-1} h of irradiance provided by 40 W cool-white fluorescent tubes. The percentage of germination and seedlings production were assessed every 3 days until the stabilization of results. The criterion used for germination was radicle protrusion (1.0 mm).

To evaluate the best condition for seedling emergency, five compositions of substrates were tested: i) commercial substrate (Carolina Soil II; Santa Cruz do Sul, RS, Brazil); ii) commercial substrate + bark of pine + charcoal + granite fragments (3:1:1:1, v/v/v); iii) commercial substrate + granite fragments (1:1, v/v); iv) bark of pine + charcoal + granite fragments (1:1:1, v/v/v) and v) granite fragments. All substrates were autoclaved for 30 min for disinfection. After this, the amount of 500 cm^3 of each substrate was placed inside plastic containers (1000 cm^3) with transparent covers and moistened with distilled and autoclaved water before sowing. Substrates were chosen considering *D. vicentensis* has rupicolous habitat (Strehl 2008).

After sowing, the material was kept in the growth room at 25±2 °C under a 16/8 h light/dark cycle with 35 µmol $m^{-2} s^{-1}$ h of irradiance provided by 40 W cool-white fluorescent tubes. Irrigation was done every three days with distilled and autoclaved water using a sprinkler. Evaluations were carried out every three days depending on the percentage of seedling emergence until results stabilized.

After 65 days, seedlings from *in vitro* germination and *ex vitro* emergency in different substrates were transferred to plastic trays (52 × 26 × 4 cm) containing Carolina Soil II substrate and kept in a greenhouse for rustication. Irrigation was done every three days with distilled and autoclaved water using a sprinkler. The survival rate (%) of all plants was evaluated after 60 days of planting. After 180 days, plants (n = 10) originated from *in vitro* germination and *ex vitro* emergency were evaluated for the number of leaves per plant and dry biomass (DW; g $plant^{-1}$). After this phase, all plants were transferred to larger trays (36 × 22 × 7 cm) containing the same substrate and maintained in a greenhouse for growth and development.

The experimental design was completely randomized. Four replicates and each replicate in *in vitro* germination and *ex vitro* emergency consisted of 25 seeds (100 seeds per treatment). All data were subjected to analysis of variance (ANOVA; $P \leq 0.05$) followed by Tukey's multiple range test.

Regardless of the treatment, the start of *in vitro* germination occurred four days after inoculation of seedsand stabilization occurred at 13 days. These results indicate that *D. vicentensis* presents viable seeds without dormancy and with high *in vitro* germinative potential, which seems to be a characteristic of this genus. For example, in *D. distachya*, Pompelli & Guerra (2004) observed the extrusion of the first leaves three days after seed inoculation and seedling development after 30 days.

Both salt concentrations and activated charcoal influenced *D. vicentensis* seed germination and seedling development (Tab. 1). The culture media with 50% of salt concentration and with 100% of salts plus activated charcoal were the most efficient treatments favoring seed germination and seedling development after 13 days *in vitro* (Tab. 1). In other bromeliads, such as *Vriesea recurvata*, the reduction of MS salt concentrations and the active charcoal (5 g L^{-1}) were beneficial for *in vitro* germination, development, and acclimatization of the plants (Sasamori *et al*. 2016). However, in other species of *Dyckia*, such as *D. distachya* (Pompelli & Guerra 2005) and *D. maritima* (Silva *et al.* 2008), the MS medium with 100% salt concentration was used for *in vitro* germination. This suggests that the requirement of nutrients for germination of seedling development is species-dependent.

The seedlings (*i.e.*, genotypes) of *D. vicentensis* obtained in this study were derived from seeds representing the genetic variability in the populations of the collection region, as recommended by Pompelli & Guerra (2005). Thus, *in vitro* germination was adequate for this species, since it favored the germination of a large number of plants using a small space and in a short period. In addition, aseptic seedlings have been produced, which may be used for other *in vitro* techniques (Mercier & Kerbauy 1997) such as clonal propagation of genotypes with ornamental potential.

Table 1 – Effect of salt concentration of MS culture and activated charcoal on the percentage of germination and seedling of *Dyckia vicentensis,* after 13 days *in vitro*.

Salt concentration of MS medium (%)	Activated charcoal (g L^{-1})	Germination (%)*	Seedling (%)*
50	-	91.6 a	82.6 a
	1	69.8 b	65.8 b
100	-	51.5 c	48.1 c
	1	87.6 a	82.5 a

*Mean values followed by the same lower-case letters in each column are not significantly different by Tukey's multiple range test at $P \leq 0.05$. Each value represents mean calculated from four replicates of 25 seeds by treatment (n = 100).

Moreover, substrate has a great impact on the germination process and development of seedlings because factors such as texture, airing, and water retention capacity can vary among different substrates (Estevan *et al*. 2010). In *D. vicentensis*, seedlings were visualized after 13 days of planting the seeds in all substrates, and the stabilization of the number of seedlings occurred only after 35 days of planting. In this period, the highest percentage of emergency (100% plantlets) was observed with the use of commercial substrate (Tab. 2).

The use of a suitable substrate is essential for the production of high quality seedlings, although most of the study was carried out with epiphytic bromeliads (Ferreira *et al*. 2007) and not in relation to bromeliads that occur exclusively in rocky outcrops, such as *D. vicentensis*. Seedling emergence is favored in substrates with greater water retention, such as the commercial substrate used in this study, whose water retention capacity is 55%. In addition, it has a slightly acidic pH (around 5.5), which is a characteristic of the soils (Reinert *et al*. 2007) where it occurs naturally.

Other advantages of the use of commercial substrates are the fact that they have a suitable composition and are free of pathogens, avoiding possible sanitary problems as emphasized by Kämpf (2000). Similarly, in other bromeliads such as *Neoregelia cruenta* (R. Graham) L.B. Smith, which is an epiphyte of large trees, although it also occurs on the ground, the commercial substrate showed the best results for height, leaf number, and aerial biomass of plants (Ferreira *et al*. 2007). On the other hand, more drained substrates containing sphagnum, coconut fiber, or washed river sand were more favorable for seed germination of *Dyckia pectinata* Smith & Reitz (Estevan *et al*. 2010).

Table 2 – Effect of different substrates on the percentage of seedling emergency of *Dyckia vicentensis* after 35 days of planting the seeds.

Substrates (v/v)	Seedlings (%)*
Commercial substrate (1)	100 a
Commercial substrate + bark of pine + charcoal + granite fragments (3:1:1:1)	83 b
Commercial substrate + granite fragments (1:1)	56 c
Bark of pine + charcoal + granite fragments (1:1:1)	57 c
Granite fragments (1)	37 d

*Mean values followed by the same lower-case letters in each column are not significantly different by Tukey's multiple range test at $P \leq 0.05$. Each value represents mean calculated from four replicates of 25 seeds by treatment (n = 100).

Thus, these results indicate that although *D. vicentensis* occurs naturally in rocky and highly drained soils, seed germination seems to depend on a greater availability of water. It is likely that adequate water conditions for seed germination do not always occur in nature due to the climatic changes observed in the Pampa biome. Consequently, the establishment of new populations of *D. vicentensis* is reduced. In fact, according to Pompelli (2006), high germinative potential observed in this genus, unlike what happens in nature where it finds other barriers, is probably because of the physical nature that decreases germination. This has also been observed in other bromeliads, which have high percentages of *in vitro* germination, although the reverse occurs in nature (Mercier & Kerbauy 1995).

In general, the seedlings derived from *in vitro* germination or in substrates showed good adaptation to environmental conditions in the greenhouse (Fig. 1c), with 96% and 82.3% survival rate ($P \leq 0.05$) after 60 days of planting, respectively. In addition, the conditions used in this study favored the rooting of seedlings (Fig. 1d), which probably influenced positively the higher survival rate of the plants in a greenhouse. Notably, unlike the epiphytic bromeliads, in the genus *Dyckia*, the root system is well developed and functional (Pita & Menezes 2002), hence the importance of the appropriate substrate for its development.

After 180 days in a greenhouse, the plants from the *in vitro* culture presented a larger number of leaves and higher DW (mean of 7.9 leaves and 200 mg $plant^{-1}$) in relation to those germinated in *ex vitro* conditions (mean of 6.4 leaves and 76 mg $plant^{-1}$) ($P \leq 0.05$). After this phase, plants were transferred to larger trays (Fig. 1e) and then destined for *ex situ* conservation in a conserved area in the municipality of São Vicente do Sul (RS, Brazil).

Several studies have shown the advantages of *in vitro* cultivation of other species of *Dyckia* (Pompelli & Guerra 2005; Silva *et al.* 2008). Moreover, in a recent study carried out by Sasamori *et al.* (2016) with *Vriesea incurvata* (Bromeliaceae), the *in vitro* germinated plantlets also showed high survival rate during acclimatization. In this study, it was also found that the reduction of all macronutrients (25%) or of the nitrogen salts (25%) was beneficial for both plantlets development and survival during acclimatization (97%). In *D. vicentensis*, the plants derived from *in vitro* germination presented higher survival rate and superior biomass in relation to those germinated in substrates, which may be justified by the higher supply of minerals, carbohydrates, and vitamins present *in vitro*.

Due to the high degree of threat of extinction of the native bromeliads, studies related to their biology, propagation, and management together with the creation of areas of environmental protection for the *in situ* conservation should be priority actions. According to Barroso *et al.* (2007), the cultivation of bromeliads is an emergency solution, since it protects part of the genetic structure of the populations of the anthropic impacts in their natural environment, which ensures the survival of the species and its subsequent reintroduction into nature. In general, the cultivation of native ornamental plants can constitute a strategy of valorization and conservation of biodiversity, in addition to being an excellent alternative of income for small farmers.

In conclusion, the results of the present study showed that *in vitro* or *ex vitro* germination are useful tools for *D. vicentensis* propagation, an endangered bromeliad species of the Pampa biome. Plant propagation can be successfully performed *in vitro* using MS medium with 50% salt concentration or, alternatively, *ex vitro* using commercial substrate. However, *in vitro* germination presents advantages over the use of commercial substrates, such as higher speed and uniformity during germination, the production of aseptic and vigorous seedlings with higher number of leaves and biomass, together with greater survival rate of the plants in a greenhouse.

In this study, the seedlings produced (about 300 plants) are genotypes collected in a region of natural occurrence, which could be used to subsidize the conservation of this species by maintaining the genetic variability of natural populations. In addition, the plants produced can be also used for clonal propagation programs, ecophysiological and/or genetic studies, plant production and reintroduction into the wild, as well as genetic breeding.

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