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Ex situ conservation of *Dyckia distachya*: an endangered bromeliad from South Brazil

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ABSTRACT *- The Brazilian Atlantic Forest biome contains a high level of genetic diversity. Historically, several factors have contributed to the drastic reduction of its cover, today restricted to less than 7%. Large forest areas have been cut down in the states of Santa Catarina and Paraná to make way for hydroelectric power plant dams and many plant species are threatened to various degrees, especially the endemic. This is the case of the* Dyckia distachya *bromeliad, native to the rocky banks of the River Uruguay that is threatened with extinction because of the construction of a dam for the Itá Hydroelectric power plant. The present study describes the plant's most relevant characteristics and the results of* ex situ *and* in vitro *conservation. Plants and seeds were colleted and established* ex situ *in the bromeliad germplasm bank. Seeds were germinated in the laboratory and the resulting plants conserved* in vitro *using the growth reduction technique. Both methodologies were considered effective for germplasm conservation.*

Key words: Atlantic forest, bromeliads, germplasm conservation, tissue culture.

INTRODUCTION

The devastation of the Atlantic forest, power plant constructions, and sharp endemism have restricted the *Dyckia distachya* bromeliad to a few remaining populations on the rocky banks of the Uruguay River in the west of Santa Catarina (SC) State and the south of Paraná (PR) State, and in restricted areas in Paraguay and Argentina. This species is commonly mistaken for *D. brevifolia* from which it differs mainly in the greater number of leaves, the more attractive rosette, and a more robust and ramified inflorescence (Reitz 1983, Strehl 1994). *Dyckia distachya* is on the official list of Brazilian plant species threatened with extinction (Ibama 1992).

Tissue culture techniques have been used for the germplasm conservation of a wide range of plant species (Martin et al. 1998). In Brazil, the use of these techniques for bromeliads has allowed the mass micropropagation of *Vriesea, Tillandsia, Guzmania, Neoregelia,* and other species to supply the landscape gardening market, preventing extractivism (Mercier and Kerbauy 1995).

Bromeliad history and decline

Bromeliads are typical plants of the American continent and probably originated in the Andean region. The species spread out throughout the millennia and reached the tropical forest about 200,000 years ago. In this taxonomic group, 2700 species were described, all native to the American continent except for a single species, *Pitcairnia feliciana*, which is found in West Africa (Benzing 2000). Both terrestrial and epiphyte bromeliad are found even in the most desert and arid regions of the world (Rundel and Dillon 1998).

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Bromeliaceae is subdivided into three sub-families: Bromelioideae, Tillandsioideae and Pitcairnioideae. The first sub-family is composed of 536 species (Rundel and Dillon 1998). They are predominantly epiphytes and seed dispersal occurs through animals (Fischer and Araujo 1995). The subfamily Tillandsioideae consists of 810 species (Rundel and Dillon 1998). *Tillandsia, Vriesea,* and *Guzmania* are the most common genera for commercial and ornamental purposes. Their seeds are wind-dispersed (Fischer and Araujo 1995). Pitcairnioideae comprise 750 species (Rundel and Dillon 1998) and include the genera *Puya, Dyckia,* and *Pitcairnia* (Mercier and Kerbauy 1997). This subfamily is considered the most primitive (Medina and Troughton 1974, Galetto and Bernardello 1992), but recent molecular studies have suggested that Tillandsoideae are the basal derived species (Rundel and Dillon 1998).

There are three dispersal centers of the family, one in the Andean region stretching to Mexico and the Antilles, another on the Guiana Plateau, and a third in Brazil (Smith and Downs 1977) (Figure1). It is estimated that about 40% of the species dwell in Brazil, many of which are endemic and concentrated mainly in the Atlantic Forest biome (Leme and Marigo 1993).

The bromeliads, being mostly epiphytes, depend on the forest conservation. The devastation process of the Atlantic Forest biome is the main cause of the genetic erosion of the bromeliad species. Only 18.06% of this forest is maintained in form of dispersed fragments in its associated ecosystems (Fundação SOS Mata Atlântica 2001).

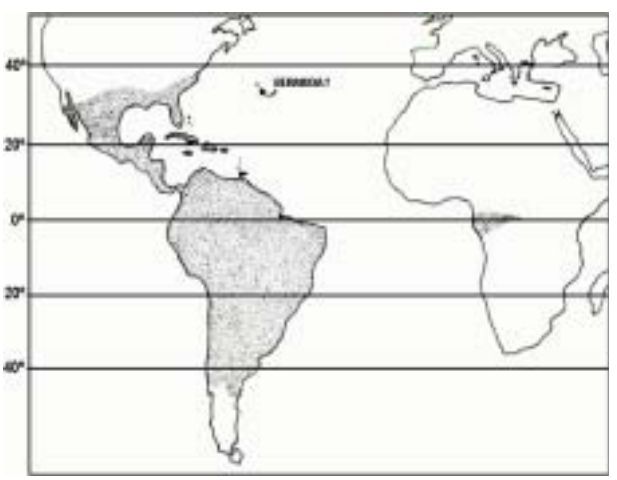

Figure 1. Bromeliaceae distribution around the world. Note the endemism of the American region (Reproduced from Benzing 2000)

As if the vast devastation of the Atlantic Forest in Brazil and SC State (Figure 2) were not enough, the bromeliads were also identified as hosts of the *Anopheles* mosquitoes in the middle of the last century. Thus, millions of bromeliads were pulled up and/or burnt with the justification of eradicating the flies that reproduced there. In Florianópolis, the capital of SC State alone, 15,981,431 bromeliads were collected and destroyed between 1944 and 1950, which was associated to a drop in the city's malaria cases. The total value of the plants eradicated in SC alone was estimated at 319,900,314 units. This was the price paid to control malaria in several cities in southern Brazil (Geocities 2001).

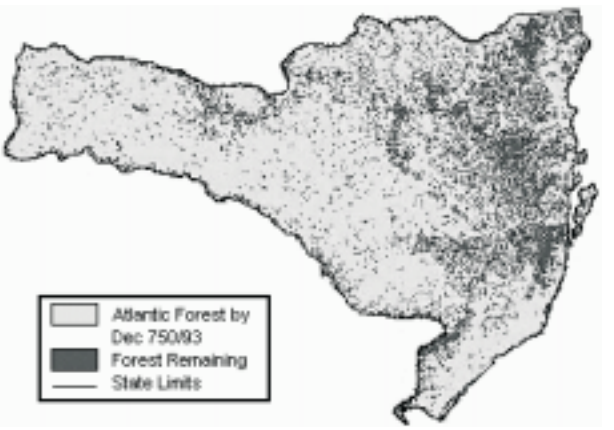

Figure 2. Forest remnants in the Brazilian State of Santa Catarina

Distribution of the bromeliad genera in south Brazil and in Santa Catarina

The most frequent genera in SC are *Vriesea, Dyckia, Tillandsia, Bromelia, Aechmea,* and *Billbergia* with a wide dispersal from Rio Grande do Sul (RS) to Rio de Janeiro (RJ) and Minas Gerais (MG) States. *Catopsis, Nidularium, Wittockia,* and *Canistrum* species are also present predominantly in the east of this region. *Pitcairnia* and *Ananas* are distributed in the northern coastal region and Itajaí valley in SC and the coastal region of PR (Reitz 1983). The genera *Hohembergia* and *Neoregelia* are also present in this region (Figure 3).

Dyckia distachya – a brief description

The *Dyckia* genus consists of 120 species, all terrestrials, spread across the south and southeast of South America (Benzing 2000). The plants have small rosettes with fleshy triangular leaves, forming compact sets under the rocks. Some species are adapted to extreme environmental variations caused by alternating droughts and floods, with stand drought in the dry period and submergence when the rivers flood.

Figure 3. Geographic distribution of the genera in the State of Santa Catarina, Brazil

Dyckia distachya is highly endemic, probably because of the restricted seed dispersion by wind (Benzing and Stiles 1998). It is a rupestris and rheophyte flowering plant, 70 to 130 cm in height, with many leaves in a beautiful rosette. The 12-10 cm long leaves are rigid, concave-channeled with many claw-shaped, sharply pointed up-turned thorns on the edges, inflorescence with side exit, not very twisted, 70 to 130 cm tall, generally forming a terminal panicle of 2-8 branches, yellow-orange flowers, very similar to *D. brevifolia*. It is characteristic and exclusive to the islands or along the rocky banks of the Uruguay River and the Paraná River rapids in the south of PR and west of SC.

This species is under serious threat of extinction mainly because the river beds and banks of its primary dispersion area have recently been dammed for the construction of hydroelectric power plants at Itaipú (PR), Itá, and Machadinho (SC) (Reitz 1983).

Dykia distachya Hassler micropropagation and conservation

The conservation of genetic resources of the tropical biomes is a very important subject. While many species are at risk of extinction in the temperate regions, several tropical species have disappeared with alarming speed. The protection of species threatened with extinction is therefore a priority issue. The conservation and management of the biodiversity even in protected areas in the tropics consists of complex challenges that require basic knowledge of species distribution and abundance, their mutual interactions, reproductive biology, and the genetic structure of their populations (Park et al. 1998).

The *in vitro* conservation techniques can be valuable methods for conserving plant genetic resources (Harding et al. 1997). It has been postulated that the *in vitro* maintenance of cultures is expensive; nevertheless this technique holds a great potential for application (Withers and Williams 1999). Vulnerability, as the main disadvantage of germplasm collections maintained under field conditions should also be considered. Plants are exposed to pathogen attack, climatic variations, and can be lost by faults in identification or human errors. The possibility of *in vitro* conservation is therefore attractive for economic as well as practical reasons. Even considering these aspects, Withers and Williams (1999) emphasize that the *ex situ* conservation is the most efficient form of plant germplasm conservation. *In situ* conservation prioritizes habitat protection, thus allowing the continuous evolution of species and the maintenance of the intra and inter-specific diversities of populations, individuals, and genes.

The dynamics of tropical forest destruction have led to serious alterations in the ecosystems that compose the respective biomes due in particular to the high fragmentation of the habitats and to genetic erosion (Heringer and Montenegro 2000), making the establishment of germplasm conservation programs necessary and urgent. The present study assessed the potential of applying *ex situ* and *in vitro* conservation methods to *Dyckia distachya*.

MATERIAL AND METHODS

Plant collection

Fifteen *Dyckia distachya* populations were identified in an approximately 500 meter long area on the Uruguay River banks, in the Chapecó region of west SC before the dam of the Itá hydroelectric power plant was closed in January 2000. A *Dyckia distachya* population was assessed at approximately every 30 meters and three or four plants removed, packed in cardboard boxes, and transported by road (600 km) to the bromeliad germplasm collection at the Department of Plant Sciences at the Santa Catarina Federal University, Florianopólis, Brazil. All plants were planted in plastic or clay pots containing a mixture of xaxim (fibers of *Dycksonia sellowiana*):vermiculite:sand (2:2:1) and placed on 1.3 m high tables, under screened conditions with 40 % luminosity reduction.

Germplasm collection

To prepare the material *in vitro*, seed-containing capsules were collected from the aforementioned populations and cool-stored under refrigeration at 4 ± 2 ºC. The seeds were immersed in a 70 % ethanol solution during 3 min. and then in commercial bleach (5 % NaOCl) for 30 min. in an aseptic chamber, followed by five rinses in sterilized water. Seeds were then inoculated in Petri dishes containing 20 ml KC culture medium (Knudson 1946) supplemented with Morel vitamins (Morel and Wetmore 1951), sucrose (30 g L^{-1}), and gelled with agar (10g L^{-1}). The pH was adjusted to 4.7 by adding HCl (0.5

N) before being autoclaved for 15 min. at 1.5 atm. Cultures were maintained in a growth room at 25 ± 3 ºC, 60 ± 5% relative humidity, and 60 mmol m^{-2} s^{-1} light photons provided by cool white lamps during 16 hours of light regime.

After 30 days, the germinated plantlets were transferred to test tubes (100 x 15 mm) containing 10 mL KC medium, pH adjusted to 4.5, and solidified agar (10g L^{-1}) or placed in groups of 10 plantlets in 500 ml glass flasks containing 30 ml of liquid KC medium. Cultures were kept under the conditions above-described.

RESULTS AND DISCUSSION

Ex vitro conservation

Dyckia distachya seeds are dispersed by wind forming dense clumps. Many of these clumps derive from the development of the shoots itself or from the germination of seeds that drop near the mother-plant (Figure 4).

The collected plants adapted well to the *ex vitro* germplasm collection conditions. There have been three flowering cycles since the arrival of the plants, with shoot emission comparable to the field behavior of the species (Figure 5 and 6). The formation of capsules with fertile seeds has also been observed, indicating adequate acclimatization of the collected plants to the new environment. Thus this method is recommended for *Dyckia distachya* conservation. According to Luther (1994), *ex vitro* germplasm conservation should be encouraged since it represents a form of escape from extinction threats to plant species caused by catastrophes, both natural and human, as for example, dam building and/or hydroelectric power plant constructions.

According to Benzing (2000), *ex situ* conservation is probably more promising for endangered Bromeliaceae, but only if priorities change. According to this author, about 200 notable collections of bromeliads exist in the USA of which perhaps two dozen are also well-documented. It should be stressed that the Brazilian legislation enforces the need of establishing safeguards for the collection and conservation of endangered plant species mainly when activities or projects offer risks to the biodiversity as in the case of the construction of hydroelectric power plants.

Figure 5. *Dyckia distachya* plants recovered from the Uruguay River bed kept in the bromeliad germplasm collection at the Santa Catarina Federal University in Florianópolis, Brazil

In vitro conservation

The extrusion of the first leaves was observed three days after inoculating the seeds, and the seedling development was completed after 30 days. The plantlets were then replicated individually (Figure 7-A). At the initial stage, the transference intervals between the subcultures and fresh

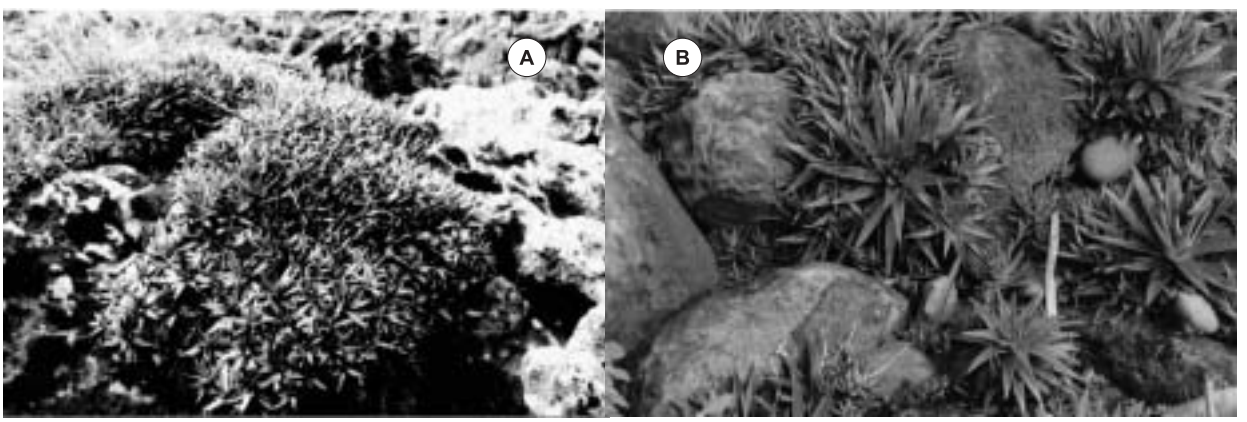

Figure 4. *Dyckia distachya* population in the Uruguay river bed in the low water period, before closing the dam of the Itá Hydroelectric power plant in the region of Chapecó, west of Santa Catarina, in southern Brazil

Figure 6. A general view of the bromeliad collection (on the left) and a flowering *Dyckia distachya* plant kept in the germplasm collection (on the right), at the Santa Catarina Federal University in Florianópolis, Brazil

culture media lasted 90 days. Cultures could be maintained in test tubes for over a year without subculturing. After this period a basal shoot was selected, isolated, and re-inoculated in test tubes for *in vitro* conservation purposes.

The *in vitro* germplasm collection currently consists of 50 test tubes containing one plantlet each, and 15 flasks (500 ml) containing 10 plantlets each, totaling more than 250 genotypes collected in the region of natural occurrence. They are derived from seeds and not from somatic tissues that would generate a clonal population. This is an important aspect because the main proposal of any germplasm collection is the conservation of the genetic variability that represents the target species. The cultures are kept under conditions that are easily manipulated and available at any time for mass multiplication, population reintroduction in the regions of origin, and germplasm exchange.

Endangered bromeliad could be inexpensively preserved *in vitro* at low temperature but at this point not without risk. Cryopreservation is still experimental and few taxa have been tested after long-term storage (Benzing 2000, Hamilton 1992). However, recent advances in the general procedures and methodologies are honing these techniques for the conservation of endangered plant species (Gagliardi 2003)

In short, the results obtained here showed the potential use of *ex situ* and *in vitro* techniques for *D. dystachia* conservation, an endangered bromeliad species from the Atlantic Forest biome.

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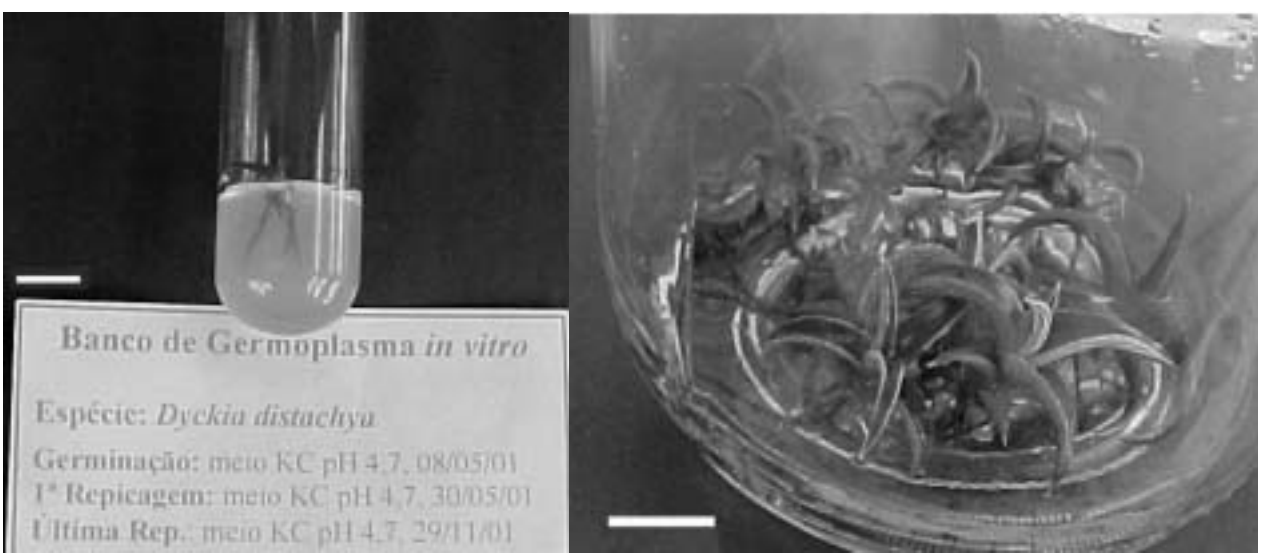

Figure 7. *Dyckia distachya* conservation in test tubes containing agar-solidified KC medium (A), and in groups of 10 plants in flasks with liquid medium (B) – bar = 10 mm

Conservação *ex situ* de *Dyckia distachya*: uma bromélia do sul do Brasil ameaçada de extinção

RESUMO - *O bioma da Mata Atlântica brasileira contém elevados níveis de diversidade genética. Historicamente diversos fatores contribuíram para drástica redução da sua cobertura, restrita a menos de 7% da sua área original. Grandes áreas, principalmente nos Estados de Santa Catarina e Paraná, foram desmatadas para dar lugar a usinas hidroelétricas, com risco para diversas espécies de plantas, especialmente as endêmicas. Este é ocaso da* Dyckia distachya, *uma bromélia nativa dos costões rochosos do Rio Uruguai, a qual apresenta-se ameaçada de extinção devido à construção da Usina Hidroelétrica de Itá. O presente estudo descreve suas características mais relevantes e os resultados da conservação* ex situ *e* in vitro. *Plantas e sementes foram coletadas e dispostas no banco de germoplasma de bromélias. Sementes foram germinadas em laboratório e as plantas resultantes foram conservadas* in vitro, *utilizando-se as técnicas de redução do crescimento. Ambas as metodologias foram consideradas eficientes para a conservação.*

Palavras-chave: Mata Atlântica, bromélias, conservação de germoplasma, cultura de tecidos.

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