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Adventitious shoots from nodule cluster cultures of *Vriesea reitzii*: an endemic and endangered bromeliad from atlantic forest

Broto adventícios a partir de culturas nodulares de *Vriesea reitzii*: uma bromélia endêmica e em extinção da Mata Atlântica

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- NOTE -

ABSTRACT

The Atlantic Forest is a biome with megadiversity and a number of bromeliads take part of it. This is the case of *Vriesea reitzii*, an endemic bromeliad threatened with extinction. Tissue culture techniques are valuable tools for the mass propagation of bromeliads, thus reducing pressure in the natural habitat. The aim of the present work was to establish an *in vitro* protocol based on the induction and proliferation of nodule cluster cultures of this species. Plantlets maintained in MS liquid culture medium plus NAA (2µM) and BAP (4µM) had the basal regions of leaves excised and then inoculated in gelled with agar (7g L⁻¹) MS culture medium plus with Dicamba (2.5; 5; 10; 20 e 30µM) and Kin (2µM) or free of plant growth regulators. Nodule cluster cultures arose from the basal region of explants. The subculture to MS liquid medium plus GA (10µM) and in MS liquid medium free of plant growth regulators resulted in a high proliferation rate. The mean regenerative rate was 39 plantlets/0.03g of nodule culture. Plantlets were acclimatized in a mix substrate of 1:1 (v:v) of carbonized rice coat and Turfa Fertil[®] mineral supplement.

Key words: bromeliads, microshoots, *in vitro* regeneration, conservation, acclimatization.

RESUMO

A floresta tropical atlântica é um bioma de alta diversidade, rico em espécies endêmicas de bromélias, entre elas *Vriesea reitzii*, que se encontra ameaçada de extinção. Técnicas de cultura de tecidos possibilitam a propagação massal de bromélias, reduzindo a pressão de coleta na natureza. O presente trabalho teve como objetivo desenvolver um protocolo regenerativo baseado na indução e no desenvolvimento de culturas nodulares desta espécie. Plantas multiplicadas em meio MS líquido com ANA (2µM) e BAP (4µM) tiveram suas folhas excisadas e inoculadas em meio MS

geleificado com ágar (7g L⁻¹) e suplementado com Dicamba (2,5; 5; 10; 20 e 30µM) e Kin (2µM) ou isento de fitorreguladores. A indução de culturas nodulares foi observada na região basal dos explantes. Estas culturas foram subcultivadas em meio de cultura MS líquido suplementado com GA (10µM) e subsequentemente para meio MS líquido isento de fitorreguladores, resultando em altas taxas de proliferação de culturas nodulares que originaram brotos adventícios e microbrotos. A taxa média de regeneração foi de 39 brotos/0,03g de culturas nodulares. As mudas foram aclimatizadas com sucesso em substrato composto por fertilizante organomineral Turfa Fertil[®] e casca de arroz carbonizada na proporção de 1:1 (v:v).

Palavras-chave: bromélias, microbrotos, regeneração *in vitro*, conservação, aclimatização.

The Brazilian Atlantic biome contains high genetic diversity and endemic species. Bromeliads are important components of this biome and among them *V. reitzii*, an endemic and endangered bromeliad. This species occurs in regions with altitudes from 750m to 1200m in the states of Santa Catarina, Paraná and Rio Grande do Sul (REITZ, 1983), and reveals excellent potential as ornamental (Figure 1a). The ecosystem in which this bromeliad is distributed, the Araucarian Mixed Forest, was severely devastated during the last century.

The *in vitro* morphogenesis in bromeliads from different explant sources has been associated with organogenesis in *Vriesea fosteriana* (MERCIER & KERBAUY, 1992). In *Dyckia distachya* *in vitro* culture

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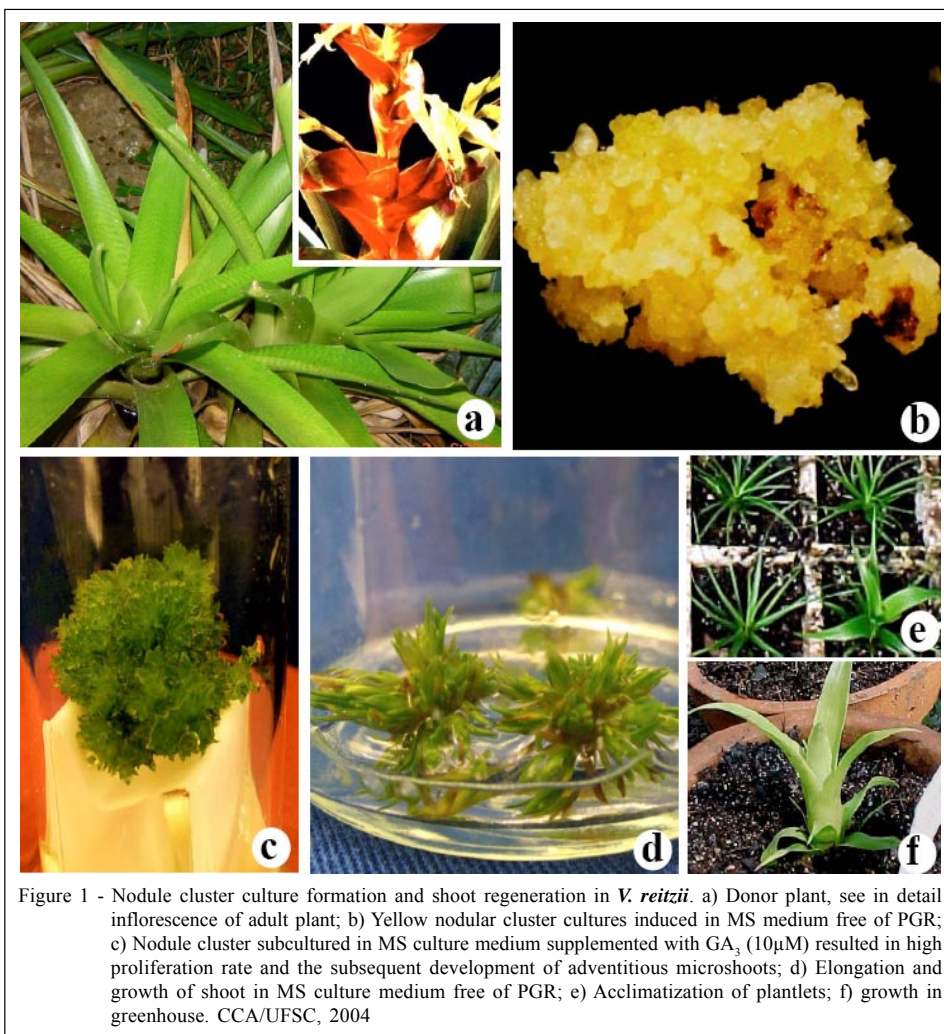


Figure 1 - Nodule cluster culture formation and shoot regeneration in *V. reitzii*. a) Donor plant, see in detail inflorescence of adult plant; b) Yellow nodular cluster cultures induced in MS medium free of PGR; c) Nodule cluster subcultured in MS culture medium supplemented with GA_3 ($10\mu M$) resulted in high proliferation rate and the subsequent development of adventitious microshoots; d) Elongation and growth of shoot in MS culture medium free of PGR; e) Acclimatization of plantlets; f) growth in greenhouse. CCA/UFSC, 2004

allowed the mass regeneration for conservation purposes (POMPELLI & GUERRA, 2005). In *Vriesea friburgensis* microbuds were induced from plantlets (ALVES & GUERRA, 2001). Microbuds were also observed in *V. reitzii* (RECH FILHO et al., 2005). ALVES et al. (2006) showed that the leaf basal region of *V. reitzii* was competent for the long-term regeneration and multiplication of nodule cluster cultures induced in MS culture medium supplemented with 2,4-D - 2,4-dichlorophenoxyacetic acid ($20\mu M$).

The present research describes the establishment of an *in vitro* regenerative protocol based on nodule cluster cultures aiming at the mass propagation and conservation of *V. reitzii*. Basal leaves were excised from shoots cultured *in vitro* in MS culture medium (MURASHIGE & SKOOG, 1962) plus $2\mu M$ of naphthaleneacetic acid (NAA) and $4\mu M$ 6-

benzylaminopurine (BAP). For the induction phase explants were inoculated in Petri dishes containing 15mL of MS culture medium gelled with agar ($7g L^{-1}$). The basal MS culture medium free of plant growth regulators (PGR) was used as control and this same basal medium was supplemented with Dicamba - 3,6-dichloroanistic acid (2.5; 5; 10; 20 and $30\mu M$) and Kinetin (Kin, $2\mu M$) composing the different treatments. The experimental design was completely randomized blocks with three replicates. Each experimental unit was constituted of four Petri dishes containing five explants (60 explants per treatment). Nodule cluster cultures (0.03g fresh weight) were subcultured to test tubes over paper filter bridges. Three different culture media were tested: 1) MS free of PGR; 2) MS plus NAA ($2\mu M$) and BAP ($4\mu M$), and; 3) MS plus giberellic acid (GA_3 , $10\mu M$). Microshoots derived from the previous phase

were subcultured in 300mL glass flasks containing 20mL of liquid MS culture medium free of PGR. The plantlets were subcultured for more 60 days in MS culture medium PGR free. Plantlets longer than 2.5cm were acclimatized in trays with 72 cells (120cm³) containing a substrate of 1:1 (v:v) of carbonized rice coat and Turfa Fertil® mineral supplement (N-4, P₂O₅-14, K₂O-8).

The resulting *in vitro* morphogenetic route is summarized in the Figure 2. MS medium PGR-free promoted the induction of nodule cultures (Figure 1b) with high proliferation (Table 1). Nodule cultures resulting from MS culture medium supplemented with Dicamba and Kin showed low proliferation, most of them with necrosis, mainly in response to high levels of Dicamba (Table 1). This PGR is commonly employed in agriculture as herbicide. However, when employed in low levels in the tissue culture of monocotyledons it shows remarkable morphogenic effects (GEORGE, 1993).

Nodule cultures subcultured in MS liquid medium with GA₃ (10µM) showed proliferation of microshoots (Figure 1c). When these microshoot clusters were subcultured to glass flasks (300ml) containing liquid MS culture medium free of PGR a high proliferation rate and the subsequent development and elongation of multiple adventitious shoots were observed (Figure 1d). Each 0.03g of the nodule cluster culture yielded a regeneration rate of 39 shoots (Data not shown). Plantlets 2.5cm long were transferred to trays filled with the substrate described previously. The survival rate of acclimatization was 100% (Figure 1e).

The *in vitro* morphogenesis in bromeliads shows specific features. In *V. fosteriana* (MERCIER & KERBAUY, 1992) these structures were denominated cell masses. In *D. distachia*, a threatened endemic bromeliad of South Brazil, a regenerative protocol was successfully developed for the *in vitro* conservation and mass propagation (POMPELLI & GUERRA, 2005).

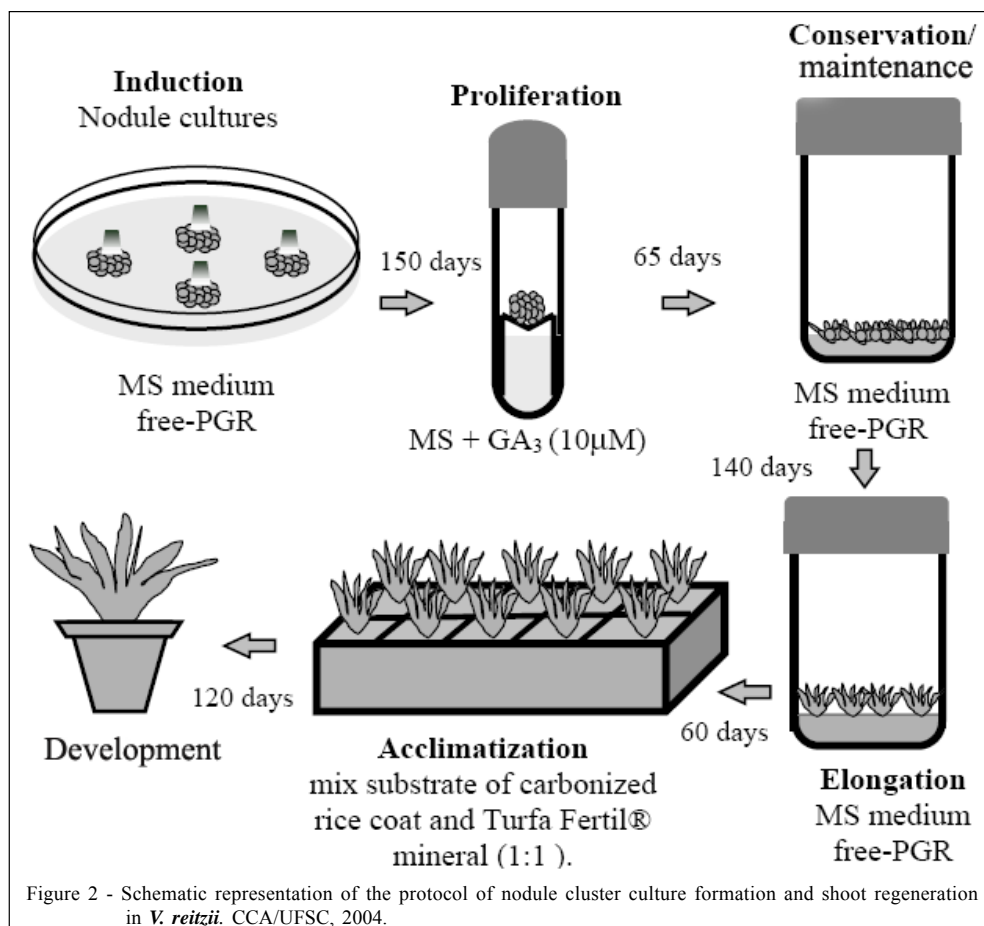


Table 1 - Morphogenetic responses of leaves *V. reitzii* cultured in MS culture medium free of PGR or supplemented with Dicamba and Kin, after 150 days in culture. CCA/UFSC, 2004.

CULTURE MEDIUM	SCORE
MS PGR free	+++
MS + Dicamba (2,5µM) + Kin (2µM)	++
MS + Dicamba (5µM) + Kin (2µM)	++
MS + Dicamba (10µM) + Kin (2µM)	++
MS + Dicamba (20µM) + Kin (2µM)	+
MS + Dicamba (30µM) + Kin (2µM)	+

(+++): friable, granular and uniform nodular cluster cultures; (++) compact callus, less uniform and presence of necrosis in the limit of 50%; (+) compact callus with necrosis in a rate higher than 50 %.

Microbuds were regenerated from plantlets of *V. friburgensis* var. *paludosa* germinated *in vitro* in MS medium supplemented with NAA and BAP (ALVES & GUERRA, 2001).

An advantage of nodule cultures as it was observed in bromeliads (ALVES et al., 2006) is the high regeneration rate. In the present research the resulting mean regenerative rate (39 shots/0.03g callus) is considerably higher than those observed in other bromeliads. Previously it was showed the induction and multiplication of shoots in *V. reitzii* from protuberances (RECH FILHO et al., 2005). Also in this same species it was demonstrated the competence for long-term regeneration and multiplication of nodule clusters (ALVES et al., 2006).

In conclusion the results of the present work show the induction of *V. reitzii* nodule cultures from the basal region of leaves inoculated in MS culture

medium free of PGR. The transference of these cultures to MS medium with GA₃ or free of PGR promoted the multiplication of adventitious shoots, and the development of plantlets. Plantlets higher than 2.5cm were acclimatized. These results open new possibilities for the bromeliad mass propagation and conservation.

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