SHOOT REGENERATION FROM ROOT EXPLANTS OF *Adenocalymma nodosum* (SILVA MANSO) L.G.LOHMANN

REGENERAÇÃO DE BROTAÇÕES A PARTIR DE EXPLANTES RADICULARES DE *Adenocalymma nodosum* (SILVA MANSO) L.G.LOHMANN

JANAÍNA FERNANDA DE OLIVEIRA[1], VESPASIANO BORGES DE PAIVA NETO[2], MÔNICA CRISTINA REZENDE ZUFFO[3], MARIA RITA MARQUES[4], JOSIMARA NOLASCO RONDON[4], SEBASTIÃO FERREIRA LIMA[2]

[1]Graduanda em Agronomia - Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do Sul - Cx. P. 112 - 79560-000 - Chapadão do Sul, MS - jfernanda_oli@hotmail.com
[2]Doutor em Agronomia - Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do Sul - Cx. P. 112 - 79560-000 - Chapadão do Sul, MS - vespasiano@nin.ufms.br, sebastiaofl.*lima*@gmail.com
[3]Bacharel em Agronomia - Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do Sul - Cx. P. 112 - 79560-000 - Chapadão do Sul, MS - mczuffo@nin.ufms.br
[4]Doutora - Universidade Federal de Mato Grosso do Sul, Departamento de Morfofisiologia - Cidade Universitária, Cx. P. 549 - 79070-900 - Campo Grande, MS. - mrmarx@nin.ufms.br, josimararondon@yahoo.com.br

ABSTRACT

A reproductible *in vitro* shoot regeneration system in *Adenocalymma nodosum* (Silva Manso) L.G.Lohmann using root explants was obtained in this investigation. Shoots were induced *in vitro* directly from root explants of 50-d-old seedlings on Murashige and Skoog (MS) medium through adventitious shoot bud regeneration. The ability of root explants to produce shoot buds occur without plant growth regulators addition. Shoot buds elongated into healthy shoots in the same induction medium. Results reforce hyphotesis that this species probably have an increased endogenous cytokinins biosynthesis.

Index terms: cytokinin, brazilian savanna, direct organogenesis, *in vitro*

RESUMO

Um sistema de regeneração *in vitro* de fácil reprodutibilidade para *Adenocalymma nodosum* (Silva Manso) L.G.Lohmann, utilizando explantes de raiz, é apresentado na presente pesquisa. Brotações adventícias foram originadas diretamente de explantes radiculares obtidos de plântulas com 50 dias de idade e cultivados em meio MS. A capacidade dos explantes para produzir gemas ocorreu sem adição de reguladores de crescimento vegetal. As brotações alongaram e geraram ramos saudáveis no mesmo meio de indução. Os resultados obtidos reforçam a hipótese de que esta espécie tem, provavelmente, uma elevada capacidade para biossíntese de citocininas.

Termos para indexação: citocininas, cerrado, organogênese direta, in vitro

INTRODUCTION

Adenocalymma nodosum (Silva Manso) L.G. Lohmann (Bignoniaceae) popularly named "carobinha do campo" is a perennial shrub mainly found in the the Brazilian Savanna, locally known as Cerrado biome, with up to 1.70 m high, large and yellow flowers, and compound leaves (SILVA, 1998). Until recently this species was botanically known as *Memora nodosa* (Manso) Miers. However, Alcantara & Lohmann (2010) reclassified and renamed it to *Adenocalymma nodosum*. In recent years, researches have been carried out with *A. nodosum* in respect to essential oil composition and action (TRESVENZOL et al., 2005; TRESVENZOL et al., 2009; TRESVENZOL et al., 2010). Siqueira (1988) describes the popular use of the stem and leaf infusions in the treatment of external wounds and ulcers. Silva (1998) reports the use of root tea for intestinal pain. Guarim Neto & Morais (2003) listed *A. nodosum* as a medicinal plant and pharmacognostical studies conducted by Tresvenzol et al. (2005) demonstrated the presence of essential oil, flavonoids, carbohydrates and traces of heterosides saponins in leaves of *A. nodosum*. However, personal observations showed a special regeneration capacity of this species from root fragments, meanly after a drastic pruning. This characteristic makes it an agressive plant in pasture areas with mechanical weed control.

According to George (1993) and Taiz & Zeiger (2004) roots are the more important organs of cytokinins biosynthesis in plants. So, we hypothesized that it has probably an increased genetic capacity to cytokinins

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biosynthesis that allows enhanced regeneration ability from underground tissues. Additionally, a very similar plant of the same genus called *Adenocalymma peregrinum* (Miers) L.G.Lohmann (cited as *Memora peregrina*) has the same agressive behavior in field and its strategy is focused in nitrogen metabolism (MARCHETTI, 2006; DUTRA, 2007). So, initially we have two interests to obtain a protocol to *in vitro* cultivation of *A. nodosum*: first, to check the capacity of this species to produce endogenous cytokinins; and second, to investigate aspects of the *in vitro* nitrogen metabolism.

MATERIAL AND METHODS

The work was carried out at the LabTec (Biotechnology Laboratory) at *Universidade Federal de Mato Grosso do Sul, Campus de Chapadão do sul*, located in Chapadão do Sul, Mato Grosso do Sul state, Brazil.

Winged seeds of *A. nodosum* were collected from adult field-grown plants in a cerrado area at Chapadão do Sul, Mato Grosso do Sul State, Brazil. They were manually selected, taking healthy seeds. To facilitate germination process and to reduce contamination, seeds were pre-sterilized by immersion in hypochlorite solution (3 % active chlorine) for five min followed by manual coat removal. After that, embryos were dipped into an ethanol solution 70 % (v/v) for 1 min, and afterwards in a sodium hypochlorite solution (1 % active chlorine) for 5 min, and rinsed 5 fold with sterile distilled water. Following surface-sterilization process, embryos were germinated in glass flasks (250 mL) containing 30 mL of medium with MS-based salts (MURASHIGE & SKOOG, 1962), supplemented with MS vitamins, 58.4 mM sucrose, 100 mg L^{-1} myo-inositol, 5 g L^{-1} activated charcoal pH 5.6 ± 0.1, and solidified with 2.8 g L^{-1} agar. Cultures were maintained under a 16 h photoperiod, irradiance of 36 mmol m^{-2} s^{-1} provided by two fluorescent tubes (Luz do Dia Especial, 20 W, Osram, Brazil), and temperature of 27 ± 2 °C in a growth room. After fifty days in germination culture medium, root seedlings were cut (15 mm approximately) and used to perform regeneration experiment. Root explants were placed in culture flasks (250 mL) containing 40 mL of MS-medium with total or half strenght salts, and supplemented as germination medium except to activated charcoal. Culture glass flasks were maintaned in growth room as described before. After forty days in culture, of the number of shoots per explant and number of elongated shoots (> 0,3 mm) data were collected. The regeneration experiment was performed with fourteen flasks with at least four root segments each, using a completely randomized design. Data were submitted to analysis of variance (ANOVA) and statistical averages comparations were made through Tukey's test at 5% significance level using the statistical program Sisvar (FERREIRA, 2002).

RESULTS AND DISCUSSION

Seed germination initiated 20 days after embryo innoculation, and fungi contamination rate was 10% (data not shown). Fifty days after innoculation, seedlings have large root height and almost complete shoot absence (Figure 1A). This could be an interesting characteristic of adapted species to cerrado conditions, once that water disponibility in this biome soils could be a critical factor. So seedlings invest in root system trying to ensure their survival. The cerrado biome is often characterized as an "inverted forest" (RODIN, 2004; ABDALA et al., 1998), in which the underground biomass (root system) is greater than the air biomass. This development pattern can be observed in other savannas plants around the world, and the uptake of water and nutrients are the main explanation (CAIRNS et al., 1997).

A highly reproducible *in vitro* shoot regeneration system in *A. nodosum* using root explants was developed in this investigation (Figures 1B and 1C). Shoots were induced *in vitro* directly from root explants through adventitious shoot bud regeneration, despite having only 1.1 shoots per explant (Table 1), representing a low number of regenerated shoots. Root explants have been proven to be regenerative for *in vitro* propagation of a limited number of species, so use of root as explants source is not an usual practice. According to Son & Hall

(1990), the root explant source offers obvious advantages (easy of manipulation, availability, less oxidation after excision etc.) in comparison with other organ cultures. However it has been used with success in a small number of plant species, for example, *Populus alba* L. × *Populus grandidentata Michx.* (SON & HALL, 1990), *Albizia jublibrissin* Durazzo (SANKHOLA et al., 1995), *Populus tremula* L. (VINOCUR et al., 2000), *Solanum melongena* L. (FLANKLIN et al., 2004), *Aralia elata* (Miq.) *Seem.* (KARIM et al., 2007); *Lilium* (KAPOOR et al., 2008; KUMAR et al., 2008), Rosa hybrida (KIM et al., 2009) and *Allium schoenoprasum* L. (ZDRAVKOVIÆ-KORAÆ et al., 2010). Use of plant growth regulators to obtained shoot regeneration from root explants are reported by above cited authors and by Kapoor et al. (2008) that obtained shoot regeneration in lily hybrids root explants only in NAA and BA supplemented medium and by Franklin et al. (2004) that obtained indirect shoot induction in Eggplant (*Solanum melongena* L.) in the medium containing 0.45 µM thidiazuron and 13.3 µM 6-benzyladenine. These authors are supported by Karim et al. (2007) that demonstrated the ability of *Aralia elata* root explants to produce shoot buds depended on the supplementation of plant growth regulators.

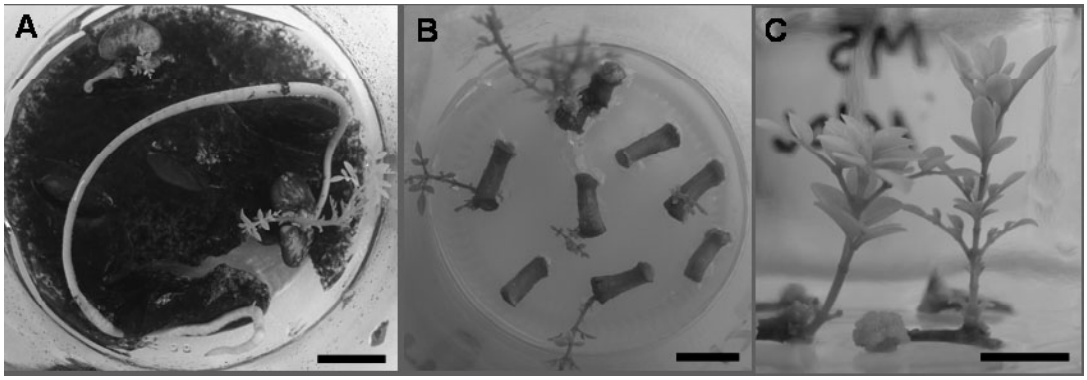

FIGURE 1 – Regeneration process of *Adenocalymma nodosum in vitro* from root explants. A – Germination process showing large root height. B, C – Direct organogenesis in root explants. Bars = 1 cm.

TABLE 1 – Morphogenic response of the *Adenocalymma nodosum* root explants.

MS salts	Shoot regeneration (%)	Elongated shoots/explant	Average lenght of elongated shoots (cm)
Total strength	75 a	1.3 a	3.2 a
Half strength	89 a	1.1 a	3.6 a

Means in a column with different letters are significantly different ($P \leq 0.05$, Tukey's test)

CONCLUSIONS

Initial and easy protocol for *in vitro* propagation of *Adenocalymma nodosum* (Silva Manso) L.G.Lohmann from *in vitro* root explants in a free-growth-regulator-medium was shown.

The obtained sucessful supports our hypothesis that this species produces a large quantity of cytokinins. So, here we saw a repeating of this species pattern response found in the field natural conditions when it's under wounding conditions.

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