In vitro propagation of *Cordia verbenacea* L. (Boraginaceae)

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ABSTRACT: *In vitro* propagation of *Cordia verbenacea* L. (Boraginaceae). Clonal propagation by tissue culture is useful to regenerate large populations of plants with similar characteristics. There are no previous reports of micropropagation of Cordia verbenacea. In this study, in vitro techniques were applied multiply this important Brazilian medicinal plant. Apical and nodal segments were cultured on Murashige and Skoog solid (0,6%) medium (MS) supplemented with 0.1, 1.0, 5.0 and 10.0 µM kinetin and 0.01µM naphthaleneacetic acid (NAA). Segments apical yielded more propagules than nodal segments. The number and length of propagules increased with in kinetin levels at 5 µM kinetin, decrease with more concentration. The treatment containing 5 µM kinetin and 0.01µM NAA yielded 2.7 propagules per explant. Propagules rooted on MS medium without growth regulators. Finally, 90-95% of the micropropagated plants survived when transferred to greenhouse conditions.

Key words: nodal segment, medicinal plant, Cordia, micropropagation

INTRODUCTION

Brazil has a great number of medicinal plants species distributed along its territory. These plants have been used in pharmacology and popular medicine since the early days of civilization. Unfortunately, many of the species are threatened or endangered, mainly due to habitat destruction and predation.

Cordia verbenacea (Boraginaceae), an important medicinal plant, grows over a large area in Brazil, particularly in the coastal region. It has very flexible branches, showning leaves verrucose and aromatic typically, inflorescence is a spike with white aromatic flowers, and the fruit is red at maturity (Silva Junior *et al*., 1995). The common name of this native Brazilian bush is "Erva baleeira". Extracts from leaves of this plant contain analgesic and antiinflammatory agents for the treatment of arthritis, rheumatism, and spinal column ache. Artemetine is the principal active ingredients (Sertié *et al*., 1990).

During the last few years, in vitro culture techniques have been developed into a successful and rapid mean of asexually propagating a number of plant species. Clonally propagating by tissue culture is highly desirable to regenerate sufficient populations of plants with similar characteristics, decreasing or eliminate the possibility of anomaly what occurring with others methods (Bajaj et al., 1988). Also, plant tissue culture is useful for conservation and rapid propagation of rare and endangered medicinal plants. Regeneration through micropropagation has been obtained in many medicinal species (Anad *et al*., 1998; Augustine & D'Souza, 1997; Johnson *et al*., 1997; Lameira *et al*., 1994; Oliveira *et al*., 1995; Mantel *et al*., 1978).

There are no previous reports on micropropagation of any species of *Cordia*. So, the objective of this investigation was to develop efficient systems for the in vitro propagation of this medicinal specie by apical and nodal segment proliferation.

MATERIAL AND METHOD

Apical and nodal segments of *Cordia verbenacea* approximates 30 to 40 mm long were harvested from three year old greenhouse grown plants. In order to prevent the oxidation of phenolic compounds initially released from the material, the explants were washed in tap water for 30 minutes. The explants were surface-sterilized with 70% (vol/vol) ethanol for 30 sec., followed by 0.6% (vol/vol) sodium hypochlorite for 10 minutes and then rinsed four times with sterile distilled water. The explants with 5 mm long were placed on MS medium (Murashige & Skoog, 1962) supplemented with 0.1, 1.0, 5.0 and 10.0 µM kinetin and 0.01µM naphthaleneacetic acid (NAA).

Culture media were adjusted to pH 5.7-5.8 before adding 0.6% (wt/vol) agar, and they were sterilized by autoclaving at 121º C for 20 min.

All cultures were maintained under a photoperiod regime of 16 h light, provided by Philips cool white fluorescent lamps (25 $\mu mol.m^2.s^1$) for 4 weeks at 26 ± 1º C. Each treatment was applied to 20 explants which were incubated vertically in 25×150 mm culture tube containing 20 ml of medium, and capped with a plastic cap. The data were submitted to analysis of variance and regression test and mean separation was tested using Duncan's test. The significance level was fixed at $P < 0.01$. The propagules

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were rooted on MS medium without growth regulators after 4 weeks. Plantlets rooted were transferred to greenhouse conditions to acclimatization under nebulization intermittente water misting applied automatically.

RESULT AND DISCUSSION

The effects of kinetin concentrations on propagules multiplication are presented in Table 1. It was observed that explant type and kinetin concentration had a significant (P = 0.01) influence on the number of propagules produced per explant as well as on the length of regenerated propagules.

TABLE 1. Effect of kinetin and 0.01mM NAA on number and length of apical and nodal segments in *Cordia verbenacea.*

		Means square	
Source	Df	Propagules number	Length (mm)
Explant	1	12.8*	348.6**
Kinetin	3	3.5**	177.8**
Explant × Kinetin	3	0.4ns	16.0ns
Error	72	0.2	6.5
Total	79		
SV (%)		18.105	29.8

Ns, not significant
** significant at 1%; df, Degree of freedom; F, F-ratio.

The number and length of propagules increased with increase in kinetin levels at 5 µM, decreasing with more concentration (Figures 1A and 1B). The maximum number (2.7) and length (12.6 mm) propagules per segment were obtained on a medium with 5 µM kinetin, whereas the minimum number (1.7) and length (5.5 mm) propagules per segment were formed on a medium with 0.1 µM kinetin. The apical segment yielded in mean more number (2.6) and length (10.5 mm) propagules than nodal segment (Table 2).

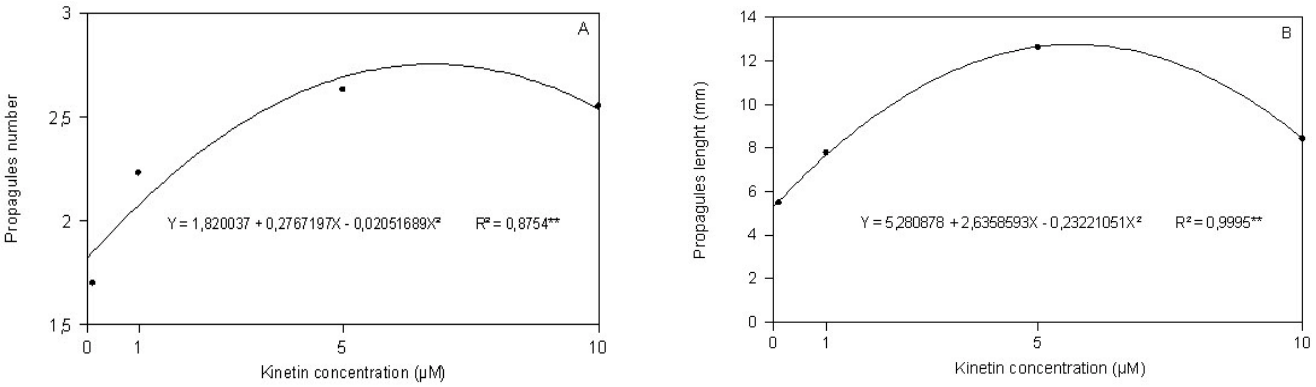

FIGURE 1. Effect of kinetin on propagules number (A) and length (B) of *Cordia* verbenacea

TABLE 2. Influence of type explant on number and length of propagules produced per apical and nodal segments in *Cordia verbenacea*.

Segment	Propagules number mean*	Propagules length mean (mm)*
Apical	2.6a	10.5a
Nodal	1.9b	6.6b

Values within columns with different letters indicating significant differences according to the Duncan's test at the level of 1%.

The benefitc effect of cytokinin obtained in this paper was also observed by Lameira et al., (1994) when the efficiency of shoot proliferation rate and plantlet formation of *Cephaelis ipecacuanha* was obtained after three subcultures in vitro.

The type of explant, apical segment versus nodal segment has been reported as a significant source of variation in vitro shoot proliferation. In *Morus nigra* L. (Yadav et al., 1990), in *Myrtus communis* L. (Parra & Amo-Marco, 1998), shoot proliferation was influenced mainly by stock origin, with higher responses from the adult material than from the seedling material. Therefore, was no found effect of explant type on shoot multiplication in either adult or seedling material.

In this paper, apical segment showed better proliferation, this results probably demonstrate what occurred a best favorable response of the cytokinin in the growth apical region than in growth axillary region. Similar results were obtained by Wysokinska (1993) when the shoot proliferation rate in the micropropagation of *Penstemon serrulatus* from shoot tip and nodal segments were compared. The shoot formation rate from shoot tips were eight times more than nodal segment. However, in *Morus nigra*, Yadav *et al.* (1998), reported that both multiplication and elongation were higher with nodal explants than with shoot tips.

Barna & Wakhlu (1995), when developing a new micropropagation method for chickpea (*Cicer arientimum* L.), obtained different results. The number of propagules formed per nodal segment from a modified single node culture method, were four times more efficient than shoot tip from axilary shoot proliferation.

Propagules rooting occurred on MS medium without growth regulators after 32 days of initial culture. The acclimatization of plantlet was achieved with 90-95% success when transferred to greenhouse conditions.

The benefict effect of cytokinin on propagules number and length was observed only at 5 µM concentration in this paper. After this level the effect was inhibitory to two evaluated variables. In all experiments apical segment were more efficient than nodal segment yielding more number and length propagules than nodal segment.

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REFERENCE

ANAD, A.; RAO, C.S.; LATHA, R. et al. Micropropagation of *Uraria Picta*, a medicinal plant, through axillary bud culture and callus regeneration. **In vitro Cell Development Biology,** v.34, p.136-140, 1998.

Augustine, A.C.; D'Souza, L. Regeneration of an anticarcinogenic herb, Curaligo orchioides (Gaertn.). **In vitro Cell Development Biology,** v.33, p. 111-113, 1997.

Bajaj, Y.P.S.; Furmanowa, M.; Olszowska, O. Biotechnology of the micropropagation of medicinal and aromatic plants**.** In: Bajaj, Y.P.S., ed. **Biotechnology in agriculture and forest: medicinal and aromatic plants**. 4. New York: Springer Verlag, 1988. p.60-103.

Barna, K.S.; Wakhlu, A.K. Modified single node culture method – a new micropropagation method for chickpea. **In Vitro Cell Development Biology,** v.31, p.150-152, 1995.

Johnson, T.S.; Narayan, S.B. and Narayana, D.B.A. Rapid in vitro propagation of Saussurea lappa, an endangered medicinal plant through multiple shoot cultures. **In vitro Cell Devopment Biology**, v.33, p. 128-130, 1997.

Lameira, O.A.; Costa, M.P.da.; Pinto, J.E.B.P. The efficiency of shoot and plantlet formation of Cephaelis ipecacuanha after three subcultures in vitro. Ciência Rural. v.24, p.523-526, 1994.

Mantel, S.H.; Haque, S.Q.; Whitehall, A.P. Clonal multiplication of Dioscorea alata and D. rotundata Poiryams by tissue culture. **Journal Horticulture Science**, v.53, p.95-98, 1978.

Murashige, T.; Skoog, F. A revised medium for rapid growth and biossays with tobacco tissue cultures. **Physiology Plantarum**, v.15, p.473-497, 1962.

Oliveira, S.A. de; Machado, M.F.P.S.; Prioli, A.J. et al. In vitro propagation of Cereus peruvianus Mill. (Cactaceae). **In vitro Cell Development Biology**, v.31, p.150-152, 1995.

Parra, R. and Amo-Marco, J.B. Factors affecting in vitro shoot proliferation of Myrtus communis L. A comparison of adult and seedling material. **In vitro Cell Development Biology**, v.34, p.104-107, 1998.

Sertié, J.A.A.; Basile, A.C.; Panizza, S. et al. Anti-inflammatory activity and sub-acute toxicity of artemetin. **Planta Medica,** v.56, p.36-40, 1990.

Silva Junior, A.A.; Vizzoto, V.J.; Giorgi, E. et al. **Plantas medicinais caracterização e cultivo**. Florianópolis: EPAGRI; 1995. 71p. Boletim Técnico, 68.

Wysokinska, H. Micropropagation of Penstemon serrulatus and iridoid formation in regenerated plants. **Plant Cell Tissue Organ Culture**, v.33, p.181-186, 1993.

Yadav, U.; Lal, M.; Jaiswal, V.S. Micropropagation of Morus nigra L. from shoot tip and nodal Explants of mature trees. **Science Horticulture**, v.44, p. 61-67, 1998.