Tissue, cell culture and micropropagation of *Mandevilla velutina*, a natural source of a bradykinin antagonist

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**ABSTRACT**

Leaf, stem and root explants of *Mandevilla velutina* were cultured in vitro and produced vigorous callus in LS basal medium containing one auxin (2,4-D or NAA) plus BAP. Cali can be subcultured indefinitely with vigorous growth. Subculture of calli to NAA (1.0 mg/l) plus BAP (5.0 mg/l) caused profuse regeneration of shoots. Isolated shoots were rooted in basal medium plus NAA (5.0 mg/l) or IBA (8.0 mg/l). Rapidly growing cell suspensions can be easily obtained from friable callus cultured in liquid medium.

**INTRODUCTION**

*Mandevilla velutina* Mart. ex Stadelm (Apocynaceae) is a perennial herb possessing an aerial portion and an underground organ (xylopodium) (Fig. 1 and 2), and occurs chiefly in the Brazilian savanna-like vegetation named "Cerrado" (see Eiten 1982). Extracts of the xylopodium are used in folk medicine for treatment of venomous snake as an anti-inflammatory agent. Recently, a selective antagonism to bradykinin action was demonstrated in crude extracts of *Mandevilla xylopodium* (Calixto et al. 1985, Calixto & Yunes 1986). Tissue and cell culture techniques should be useful in this species for fundamental and applied studies such as micropropagation, genetic selection and production of active principles in vitro. This work reports the basic procedures for the establishment of tissue and cell cultures and plant regeneration of *M. velutina*.

**MATERIAL AND METHODS**

Plants of *Mandevilla velutina* were obtained from the Cerrado vegetation of the State of São Paulo (Brazil), and cultivated in the Department of Botany - Institute of Biosciences, University of São Paulo. Young leaves and stem segments were surface sterilized by immersion in sodium hypochlorite for 10 min, then washed in sterile water. Explants were prepared as leaf discs (0.5 cm in diameter) and stem cylinders (0.2 cm long), and washed 10 min in sterile water before inoculation, to eliminate latex exudation. In the case of xylopodium tissues, the organ was washed, peeled and the surface sterilized with 80% ethanol, and the inner tissues removed with the aid of a corkborer, to obtain cylindrical explants (0.5 cm in diameter and 2.0 cm long).

The culture medium was composed of LS micro- and macronutrients (Linsmaier & Skoog 1965), vitamins (Nitsch 1969), 3% sucrose, several combinations of growth regulators, and gelled with 0.8% agar. The pH was adjusted to 5.5 before autoclaving. Cultures were kept in the light (16 h/day, 10 W.m⁻²) or in the dark, at 26±1°C.

Suspension cultures were started from highly friable callus subcultured in liquid medium with BAP (0.5 mg/l) and 2,4-D (1.0 mg/l), kept in agitation (orbital shaker, 104 rpm), in the light (18 h/day, 5 W.m⁻²) at 26±1°C.

**RESULTS**

Callus induction - A variety of explants (leaf and xylopodium pieces, stem segments) were able to develop callus in medium with several combinations of NAA or 2,4-D (0.1–1.0 mg/l) plus BAP (0.1–1.0 mg/l). Explants from leaves and xylopodium gave the best results (90% of cultures formed callus); young and adult leaves showed similar behavior. No response was obtained when auxin or cytokinin were applied alone. The best callus induction was in medium with NAA (1.0 mg/l) plus BAP (1.0 mg/l). The primary callus can be subcultured showing vigorous growth.
growth in most of the treatments tried; also in this case the use of auxin together with cytokinin is necessary. For growth of the sub cultured callus 2,4-D was more effective than NAA, and the latter more effective than IAA. In general, high auxin/cytokinin ratio produces friable callus, whereas the contrary produces more compact callus. CA accumulation enhances growth and friability. Callus growth is quite similar in light and dark, differing only by the green colour in light.

Differences in growth potential and texture were observed in calli derived from leaf and xylopodium tissues (Fig. 4), the former showing a growth rate three times higher, and the latter higher friability. These characteristics remained stable after 18 months in culture.

Figure 1 - Young aerial portion of M. velutina plant emerging from xylopodium. Figure 2 - Xylopodium. Figure 3 - Multiple shoots developed in callus culture, in medium with 0.1 mg/l NAA + 5.0 mg/l BAP. Figure 4 - 26th callus subculture from xylopodium (left) and leaf tissue (right), in medium with 0.5 mg/l 2,4-D + 1.0 mg/l BAP, in light. Figure 5 - Suspension culture showing cell aggregates. Figure 6 - Aspects of thickening in the basal portion of isolated shoots, after 150 days in medium for rooting. Figure 7 - Plants regenerated in vitro, after extra-vitrum rooting (120 days in rooting conditions).