Full Length Research Paper

Micropropagation and *in vitro* conservation of *Jacaranda decurrens* Cham.

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Accepted 19 January, 2012

Several pharmacological activities have been attributed to *Jacaranda decurrens* a medicinal plant species native to Brazilian Cerrado regions. Its root extracts have been used as anti-inflammatory, antiseptic and tonic agent and the infusion of its leaves has been efficacious in healing external ulcers. In this work, it was aimed at the development of protocols for *J. decurrens* micropropagation and *in vitro* conservation in germplasm bank. Asepsis of seeds and nodal segments was effective achieving 97 and 84% disinfestation, respectively and multiplication ratio ranged 6.98. Axillary buds used as explants, regardless of their position on the stem, produced vigorous *in vitro* plantlets. Multiplication rate of explants cultured in temporary immersion system RITA® was superior (9.61) compared to the proportion obtained in semi-solid culture medium. Optimized protocol for the *in vitro* conservation of *J. decurrens* in germplasm bank promoted 56.67% survival. Moreover, enhanced root formation (96.7%) was achieved.

Key words: *Jacaranda decurrens*, Bignoniaceae, micropropagation, conservation.

INTRODUCTION

Jacaranda decurrens, popularly known as “carobinha” is endemic to the Brazilian Cerrado but it is subjected to genetic erosion by indiscriminate collection and frequent deforestation of natural occurrence areas. Root extracts from that Bignoniaceae medicinal species are used as anti-inflammatory, antiseptic and tonic agent and the infusion of its leaves has been effective to heal external ulcers. The triterpene ursolic acid isolated from *J. decurrens* aerial parts (Varanda et al., 1992) is considered anti-inflammatory agent, HIV-1 protease inhibitor and also antineoplastic drug (Baglin et al., 2003; Kassi et al., 2009; Slamenová et al., 2006; Xavier et al., 2009).

Moreover, *J. decurrens* plays an important ecological role by fixing atmospheric carbon and it is considered a promising species to be used in phytomediation

environmental programs for pollution clearing (Pereira, 2007). Due to their medicinal importance, several Bignoniaceae species have been micropropagated, e.g. *Anemopaegma arvense* (Pereira et al., 2003), *Stereospermum personatum* (Shukla et al., 2009); *Tabebuia serratifolia* (Nery et al., 2008).

Cerrado endemic plants like *J. decurrens* represent great economic potential for pharmaceutical, biofuel, lumber and food industry, but the lack of studies on the agronomic aspects of most species obstructs sustainable use. Data presented on this paper describe an efficient methodology for the micropropagation and *in vitro* conservation of *J. decurrens*.

MATERIALS AND METHODS

Plant material

J. decurrens plants and seeds were collected in Uberaba-MG, Brazil and identified by Dr. Inês Cordeiro from the São Paulo

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Table 1. Effect of different cytokinins on the *in vitro* development of *J. decurrens* explants.

Plant growth regulators	Shoot proliferation (%)	Number of shoots/bud	Number of buds/stem	Shoot height (cm)	Callus production (%)
TDZ (mg L⁻¹)					
0	100.00 ^a	1.30 ^b	3.33 ^a	1.33 ^a	-
0.1	91.46 ^b	1.40 ^b	3.80 ^a	1.20 ^a	100.00 ^a
1.0	97.76 ^a	1.56 ^b	2.76 ^a	0.86 ^c	100.00 ^a
3.0	80.00 ^b	1.56 ^b	3.30 ^a	1.16 ^b	82.20 ^a
5.0	86.66 ^b	1.80 ^a	3.03 ^a	0.73 ^c	95.53 ^a
BAP (mg L⁻¹)					
0	95.56 ^a	1.33 ^c	2.50 ^b	1.10 ^b	-
0.1	97.76 ^a	1.66 ^b	2.73 ^b	0.93 ^b	8.90 ^c
1.0	97.76 ^a	1.96 ^b	3.56 ^a	1.26 ^a	24.46 ^b
3.0	80.00 ^a	2.60 ^a	2.16 ^b	0.43 ^c	15.56 ^c
5.0	81.76 ^a	2.03 ^b	2.23 ^b	0.36 ^c	40.00 ^a
Kinetin (mg L⁻¹)					
0	100.00 ^a	1.45 ^b	3.30 ^a	0.70 ^a	-
0.1	100.00 ^a	1.67 ^b	2.53 ^a	0.50 ^a	-
1.0	76.67 ^a	1.27 ^b	2.47 ^a	0.97 ^a	-
3.0	86.67 ^a	1.53 ^b	3.10 ^a	0.70 ^a	-
5.0	90.00 ^a	1.90 ^a	2.50 ^a	0.60 ^a	-

Means followed by the same letters do not significantly differ (Duncan test, 5% significance).

Herbarium at the Institute of Botany, SP. A voucher specimen (HPMU-SP 570) is deposited in the Herbarium of Medicinal Plants at the University of Ribeirão Preto, (UNAERP).

Plant and seed disinfection

J. decurrens seeds were submerged in 1% Benomyl solution for 1 h, dipped in 70% alcohol for one minute and immersed in 0.5% calcium hypochlorite during 30 min. Disinfested seeds were washed thrice in distilled water, autoclaved and inoculated in glass test tubes containing WP medium.

J. decurrens plants were transplanted into 15 L pots containing soil/sand mixture (1:1) and kept in green house. After removal of all leaves the stems were sprayed with 1% (m/v) Benomyl and 10 mg/ml⁻¹ of each of the following antibiotics: gentamicin, cefotaxime, chloramphenicol and kanamycin. Fresh nodal segments developed after treatments, were excised, surface disinfested with Tween[®], rinsed in running water, immersed in 1% Benomil under orbital agitation (100 rpm) for 8 h, dipped in 70% alcohol for 1 min and immersed in 0.5% calcium hypochlorite for 30 min. Following asepsis, explants were inoculated in test tubes containing MS medium and cultured for 60 days with one subculture to fresh MS medium at the 30th day of culture. Explant contamination rates, proportion of seed germination, axillary bud formation and plant survival were scored.

Plant proliferation and influence of bud position on its development

Nodal segments from *in vitro* germinated plantlets were inoculated on MS medium supplemented with 30 g/L⁻¹ sucrose and different

concentrations of BAP (benzylaminopurine), Kinetin and TDZ (thidiazuran) (Table 1).

After selecting the most appropriate type and concentration of cytokinin for *J. decurrens in vitro* multiplication, nodal segments were inoculated on, MS (Murashige and Skoog, 1962), B5 (Gamborg et al., 1968) and WP (Lloyd and Mccown, 1981) culture media in their original concentrations or diluted two or four times (Table 2) to determine the best culture medium for *J. decurrens* multiplication. Every tested medium was supplemented with 1.0 mg/L⁻¹ BAP and 30.0 g/L⁻¹ sucrose.

Buds sprouted in different positions on the stem (Table 3) were excised and inoculated on MS culture medium supplemented with 1.0 mg/L⁻¹ BAP and 30.0 g/L⁻¹ of sucrose. Percentage of explants with shoots, number of shoots per bud and per stem, shoot height and callus proliferation were recorded.

Effects of co-cultivation *in vitro*

J. decurrens plantlets cultured in glass flasks (5 x 8.5 cm) containing 20 ml of WP semi-solid medium supplemented with 1.0 mg/ml⁻¹ BAP were co-cultured with either 1 or 5 explants. Concurrently, 50 plantlets were placed into Erlenmeyer flasks (500 ml) under orbital agitation (100 rpm) and flasks for temporary immersion (RITA[®]) (Figure 1) containing 200 ml of WP liquid culture medium. In RITA[®] explants were immersed for 15 min with immersion cycle every 4 h cycle.

The temporary immersion system (TIS) also called RITA[®] (Teisson et al., 1995) is a type of bioreactor where plant tissues are immersed into the two (superior and inferior) compartment flask, one connected to the other by a tube. The low compartment holds the culture medium and the explants are placed in the upper

Table 2. Effects of different salt dilutions of culture media (WP, MS and B5) on the *in vitro* development of *J. decurrens* explants.

Culture media*	Shoot proliferation (%)	Number of shoots/bud	Number of buds/stem	Shoot height (cm)	Callus production (%)
WP	100.00 ^a	1.67 ^a	3.17 ^a	0.50 ^a	-
WP/2	100.00 ^a	1.90 ^a	2.37 ^b	0.50 ^a	-
WP/4	83.33 ^a	1.80 ^a	1.70 ^c	0.33 ^a	-
MS	100.00 ^a	1.84 ^a	2.60 ^a	0.40 ^b	100.00 ^a
MS/2	90.00 ^a	1.73 ^a	3.27 ^a	0.80 ^a	100.00 ^a
MS/4	80.00 ^b	1.40 ^a	3.03 ^a	0.57 ^b	100.00 ^a
B5	60.00 ^b	1.73 ^b	2.23 ^a	0.37 ^a	-
B5/2	83.33 ^a	1.97 ^a	1.77 ^a	0.23 ^a	-
B5/4	73.33 ^b	1.53 ^b	1.87 ^a	0.27 ^a	-

Means followed by the same letters do not significantly differ (Duncan test, 5% significance). * media + 1.0 mg L⁻¹ de AP.

Table 3. Influence of the different positions of buds on the stem, on the *in vitro* development of *J. decurrens* explants.

Bud position on the stem	Shoot proliferation (%)	Number of shoots/bud	Number of buds/stem	Shoot height (cm)	Callus production (%)
1	86.67 ^a	1.70 ^a	2.50 ^a	0.50 ^a	-
2	96.67 ^a	2.10 ^a	2.37 ^a	0.47 ^a	-
3	93.33 ^a	2.03 ^a	2.33 ^a	0.47 ^a	-
4	100.00 ^a	1.90 ^a	2.63 ^a	0.37 ^a	-
5	96.67 ^a	1.97 ^a	2.47 ^a	0.47 ^a	-
6	96.67 ^a	1.83 ^a	3.10 ^a	0.50 ^a	-

Means followed by the same letters do not significantly differ (Duncan test, 5% significance).

compartment. Injection of compressed air in the lower compartment allows the flow of the liquid media from one vessel to the other. When the full medium volume reaches the upper compartment, the air forces the liquid medium up and bath the culture. The air flow oxygenates and agitates the medium producing air bubbles. The air is liberated through a filter fitted orifice in the cover of the upper compartment. When the flow is turned off, pressure drops, and the medium returns to the bottom of the vessel until the next cycle starts. After 90 days of culture, the number of shoots per bud, buds per stem, shoot height, callus proliferation and explant survival were evaluated.

Conservation in the germplasm bank

Nodal segments (1 cm long), without leaves, were excised from micropropagated plantlets maintained *in vitro*. Explants were inoculated on MS, MS/2 and MS/4 media supplemented with sucrose, sorbitol, manitol, calcium pantothenate and spermidine as shown in Table 4 and cultured at 18°C, under germplasm bank conditions, for 6 months.

In vitro rooting

Plantlets (3 cm long) with two axillary buds, were inoculated in glass test tubes (8.5 x 2.5 cm) containing different combinations of WP or

MS culture media supplemented with Indole-3-Butyric Acid (IBA), naphthalene acetic acid (NAA), sorbitol, dextrose and/or spermidine (Table 5).

General experimental conditions

Flasks (8.5 cm high x 2.5 cm or 8.5 cm high x 5.0 cm) were filled with 5.0 ml or 15 ml of culture medium, respectively and covered with plastic closures.

The growing room was kept at 25 ± 2°C, 60 ± 5% relative humidity and 16 h photoperiod with an intensity of 2.0 × 10⁷ µE.cm⁻².s⁻² provided by two fluorescent white cold bulbs (GE, 85W). The conditions of the germplasm bank room were kept at approximately 18 ± 2°C, 60 ± 5% relative humidity and 16 h photoperiod with an intensity of 2.0 × 10⁷ µE.cm⁻².s⁻² provided by two fluorescent white cold bulbs (GE, 85W).

Acclimatization

J. decurrens in vitro rooted plantlets were transplanted into styrofoam boxes containing Plantmax[®] and maintained inside a glass greenhouse.

Each plantlet was covered with a glass flask (8.5 x 5 cm) to avoid excessive transpiration. Plantlet survival was evaluated after 95 days of culture.

Figure 1. RITA® Flask used in the temporary immersion system.

Table 4. Effect of different *in vitro* culture systems on the development of *J. decurrens* explants.

Type of flasks/number of explants inoculated	Number of shoots/bud	Number of buds/stem	Shoot height (cm)	Callus production (%)	Explant survival (%)
Test tubes/1	1.97 ^a	3.77 ^a	1.03 ^b	85.48 ^a	58.10 ^c
Glass flask/5	1.37 ^a	3.73 ^a	1.63 ^b	100.00 ^a	79.35 ^b
Erlenmeyer/50	-	-	-	-	-
RITA/50	1.77 ^a	5.43 ^b	1.93 ^a	100.0 ^a	100.00 ^a

Means followed by the same letters do not significantly differ (Duncan test, 5% significance).

Statistical analysis

All experiments were carried out with $n = 10$ samples and each treatment was performed in triplicate. The experimental design was completely randomized and the Duncan's Multiple Range Test ($P < 0.05$) was used to compare the treatment means.

RESULTS AND DISCUSSION

Asepsis was considered efficient since only 3.0% (± 0.89) of seeds were contaminated, mainly by fungi. Germination reached 53% (± 8.6), starting 45 days after inoculation, but after 82 days of culture, it could not be noticed. The contamination (by fungi and bacteria) of nodal segments of source plants kept in the green house, ranged $4.0 \pm 0.2\%$ and $3.0 \pm 0.1\%$, respectively and plant survival after treatments reached $84.0 \pm 5.1\%$. Disinfestation of *J. decurrens* explants was highly successful considering that Moraes et al. (2007) reported that due to the high number of endophytic microorganisms present in adult plants collected in the

field the micropropagation of endemic plants from the Cerrado in general starts with the germination of seeds. Addition of 3.0 mg L^{-1} BAP and 5.0 mg L^{-1} TDZ to MS medium, induced number of shoots per bud and number of buds per stem. MS medium supplemented with 1.0 mg L^{-1} BAP promoted shoot proliferation (1.96 shoots per bud and 3.56 buds per stem) and elongation (1.26 cm) and callus incidence was low (24.46%) compared to explants cultured with TDZ. Kinetin did not induce callus formation, but shoot height was inferior compared to explants cultured on media supplemented with TDZ and BAP.

During the first thirty days of culture, explants cultured on MS medium supplemented or not with plant growth regulators, did not show developmental differences, suggesting that *J. decurrens* plant tissues contained endogenous reserves of growth regulators. However, after 40 days of culture explants on MS medium devoid of plant growth regulators stopped growing and died in the next 30 days. Those data indicate that explants may be inoculated on MS culture medium depleted of growth regulators, but after 30 days, they should be subcultured

Table 5. Development of plantlets cultured with growth-retarding agents and maintained in germplasm bank.

Treatments	Shoot proliferation (%)	Number of buds/stem	Number of buds/stem	Shoot height (cm)	Root formation (%)	Explant survival (%)
MS/4 + 2% sucrose	46.7±1.5	1.0±0.0	2.4±0.1	0.6±0.6	-	90.0±10.0
MS + 2% sucrose	15.0±7.1	1.0±0.0	1.3±0.4	0.5±0.0	-	26.7±5.8
MS/2 + 2% sucrose	43.3±25.2	1.0±0.05	2.4±0.2	0.7±0.3	-	60.0±26.5
MS + 2% sucrose + 4% sorbitol	23.3±5.8	1.0±0.0	2.9±0.7	1.3±0.2	-	-
MS/2 + 2% sucrose + 4% sorbitol	66.7±15.3	1.4±0.4	2.2±0.6	0.4±0.2	-	-
MS + 2% sucrose + 4% sorbitol + 2.0 mg L ⁻¹ Ca pantothenate	56.7±25.2	1.5±0.3	2.0±0.4	0.3±0.05	-	-
MS/2 + 2% sucrose + 4% sorbitol + 2.0 mg L ⁻¹ Ca pantothenate	60.0±20.0	1.3±0.0	3.1±0.8	0.6±0.2	-	-
MS + 2% sucrose + 3% manitol + 2.0 mg L ⁻¹ Ca pantothenate	36.7±11.5	1.1±0.1	2.0±0.9	0.6±0.3	-	-
MS/2 + 2% sucrose + 3% manitol + 2.0 mg L ⁻¹ Ca pantothenate	66.7±25.2	1.2±0.3	0.6±0.8	0.7±0.2	-	-
MS + 2% sucrose + 2% sorbitol + 2% dextrose + 2 mg L ⁻¹ spermidine	56.7±5.8	1.5±0.1	2.7±0.8	0.4±0.1	-	-
MS/2 + 2% sucrose + 2% sorbitol + 2% dextrose + 2.0 mg L ⁻¹ spermidine	96.7±5.8	1.2±0.3	3.5±0.7	1.0±0.2	36.7±5.8	33.3±5.8
MS/2 + 2% sucrose + 2% sorbitol + 2% dextrose + 4 mg L ⁻¹ spermidine	100.0±0.0	1.3±0.2	1.7±0.1	0.3±0.05	-	10.0±0.0
MS/2 + 2% sucrose + 4% sorbitol + 2% dextrose + 2 mg L ⁻¹ spermidine	100.0±0.0	1.4±0.2	1.2±0.1	0.7±0.8	-	30.0±26.5
WP/2 + 2% sucrose + 2% sorbitol + 2% dextrose + 2 mg L ⁻¹ spermidine	100.0±0.0	1.1±0.32	3.0±0.95	1.8±0.25	25.0±7.1	80.0±0.0
WP/2 + 2% sucrose + 2% sorbitol + 2% dextrose + 4 mg L ⁻¹ spermidine	100.0±0.0	1.07±0.06	3.57±0.80	1.24±0.43	36.7±20.8	63.3±32.1
WP/2 + 2% sucrose + 2% sorbitol + 2% dextrose + 6 mg L ⁻¹ spermidine	100.0±0.0	1.1±0.11	3.17±0.23	1.42±0.28	23.3±11.6	70.0±20.0

Table 6. Effect of different auxins, osmotic agents and polyamine spermidine on rooting of *J. decurrens*.

Treatments	Rooted plants (%)	Plants with callus
WP +30.0 g L ⁻¹ sucrose +0.1 mg L ⁻¹ IBA	53.3±5.8	-
WP +30.0 g L ⁻¹ sucrose +0.5 mg L ⁻¹ IBA	60.0±20.0	26.7±5.8
WP +30.0 g L ⁻¹ sucrose +3.0 mg L ⁻¹ IBA	66.7±11.6	70.0±20.0
WP +30.0 g L ⁻¹ sucrose +0.1 mg L ⁻¹ NAA	15.0±7.1	60.0±30.0
WP +30.0 g L ⁻¹ sucrose +0.5 mg L ⁻¹ NAA	53.3±15.3	56.7±15.3
WP +30.0 g L ⁻¹ sucrose +3.0 mg L ⁻¹ NAA	53.3±15.3	50.0±10.0
WP +3% sucrose +2 mg L ⁻¹ spermidine	26.7±5.3	-
MS +3% sucrose +2 mg L ⁻¹ spermidine	16.7±5.8	-
WP +2% sucrose +2% sorbitol +2% dextrose +2.0 mg L ⁻¹ spermidine	25.0±7.1	20.0±14.1
MS +2% sucrose +2% sorbitol +2% dextrose +2.0 mg L ⁻¹ spermidine	26.3±15.3	23.3±15.3

Table 6. Contd.

MS+2%sucrose+2%sorbitol+2%dextrose+2.0 mg L ⁻¹ spermidine+0.5 mg L ⁻¹ IBA	96.7±5.8	50.0±20.0
MS/2+2%sucrose+2%sorbitol+2%manitol+2.0 mg L ⁻¹ spermidine	0.0±0.0	0.0±0.0
MS/2+2%sucrose+2%sorbitol+2%manitol+2. mg L ⁻¹ spermidine+0.5 mg L ⁻¹ IBA	3.33±1.53	8.33±1.53

to WP medium supplemented with phyto regulators. *J. decurrens* explants cultured on MS/2 medium developed well but showed callus formation, what may represent an inconvenience for rooting. Explants cultured on B5 medium showed inferior growth (Table 2). WP culture medium in its original concentration was considered the most appropriate for *J. decurrens in vitro* development, because it did not induce callus formation, promoted shoot proliferation (100%) and enhanced number of buds per stem (3.17).

Studies with different species have shown that concentrations of micro and macro nutrients added to the culture medium directly interfere in the development of *in vitro* plantlet cultures. Micropropagation of woody species, in general, present better propagation and rooting rates when cultured in WP than in more concentrated culture media as MS (Dutra et al., 2009; Ferreira and Pasqual, 2008; Mantovani et al., 2001; Thorpe et al., 1991).

J. decurrens buds excised from different positions on the stem showed similar shoot regeneration ability (Table 3) and thus may be considered potentially useful for multiplication processes. In general, however, the bud position influences *in vitro* propagation and plantlet survival (El Morsy and Millet, 1996). Rose axillary buds excised from the central part of stems, developed more rapidly than apical buds (Bressan et al., 1982). Similar results were reported by Malosso et al. (2008) with *Acmella oleracea*.

Moreover, bud position probably influenced rooting of micro sticks cultivated *in vitro* or not (Nicoloso et al., 2001; Malosso et al., 2008; Silva et al., 2009).

Explants (50) co-cultivated in Erlenmeyer flasks containing liquid culture media presented oxidation and total necrosis after 20 days of culture, though flasks were kept under continuous agitation (100 rpm). Woody cerrado vegetation like *J. decurrens* usually presents xeromorphic characteristics and do not resist to constant hydric stress. Quite the opposite, explants co-cultured on semisolid media or in liquid media using RITA® system showed enhanced shoot proliferation rates if compared to explants cultured isolated in semisolid media and 100% survival (Table 4). That is consistent with field-grown *J. decurrens* that always present dense growth and adaptation in cohabiting with several individuals of the same species.

The temporary immersion system promoted explant development and survival rates reached 100% while survival of plantlets co-cultured (either 1 or 5 per flask) on semi-solid medium, ranged 58.1 and 76.3%, respectively. If compared to the other systems tested, the RITA® system enhanced shoot height (1.93 cm) and induced proliferation (number of shoots per bud x number of buds per stem = 9.61). There are two great advantages in using RITA® system, the first is the elimination of Phytigel™ and consequently reduction of costs (79%) and decrease of contamination risks. Explants cultured in either

systems evaluated, RITA® or, individually and co-cultured in semi-solid medium, remained green and vigorous for 90 days in the same culture medium. That demonstrates the low nutritional demands by *J. decurrens*, what is a common characteristic of endemic plants from Cerrado. More recently, several studies with different species have shown the superiority of the temporary immersion system compared to conventional *in vitro* cultures on semisolid culture media, exhibiting higher multiplication rates as for the ones obtained with *J. decurrens* (Escalona et al., 2003; Mordocco et al., 2009; Pisowotzki et al., 2008; Rodrigues et al., 2006).

Sorbitol, calcium pantothenate and manitol did not protect *J. decurrens* explants against cold stress. On the other hand, explants cultured on MS/2 presented higher survival ratio, indicating that the salt stress promotes explants mortality. The most adequate culture medium for the *in vitro* conservation of *J. decurrens* plantlets in germplasm bank was WP/2 supplemented with 20.0 g/L⁻¹ sucrose, 2% sorbitol, 2% dextrose, 2.0 mg/L⁻¹ spermidine and 4.0 g/L⁻¹ Phytigel™, for presenting better survival indexes and for promoting shoot and bud proliferation (Table 5). The reduced concentration of salts and the addition of spermidine protected plantlet tissues under low temperatures in the germplasm bank (18°C). Polyamines in the culture media or synthesized in the plant tissue, protect explants and/or plantlets against environmental low temperatures (Capell et al., 2004) or osmotic

stress besides playing important role in plant development and growth and in the regulation of nucleic acid metabolism (Galston and Kaur-Sawhney, 1990; Maki et al., 1991).

Plantlets cultured on MS supplemented with 20 mg/L⁻¹ sucrose, 2% sorbitol, 2% dextrose, 2.0 mg/L⁻¹ spermidine and 0.5 mg/L⁻¹ IBA and kept in the green house at 25°C rooted better (96.7 ± 5.8%) in comparison with the other culture media evaluated in the rooting experiments (Table 6). *J. decurrens* *in vitro* rooting was dependent on exogenous auxins, and the association of spermidine and IBA enhanced rooting. Intense mitotic activity seems to be indispensable for root formation *in vitro* and this activity is mainly promoted by auxins and chemical interactions with other substances as polyamines, which promote root development (Naija et al., 2009; Sankhla and Upa dhyaya, 1987).

Obtained results indicate that the temporary immersion culture system is the most appropriate for large scale production of *J. decurrens* plantlets. Micropropagated plants may be used in reforestation programs of Cerrado areas where the species is depleted. The *in vitro* conservation of *J. decurrens* in germplasm bank will allow the establishment of sustained cultures for biomass production with application in the phyto-pharmaceutical industry.

ACKNOWLEDGEMENTS

The authors thank FAPESP - Fundação de Amparo à Pesquisa do Estado de São Paulo (Proc. n.º 2010/18132-2); CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and UNAERP - Universidade de Ribeirão Preto for financial support.

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