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BRIEF ARTICLE

In vitro organogenesis in *Albizia guachapele*, *Cedrella odorata* and *Swietenia macrophylla* (Fabaceae, Meliaceae)

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Abstract: Regeneration of adventitious buds was achieved from hypocotyl explants of *Albizia guachapele* (Guayaquil) and *Cedrella odorata* (Spanish cedar), and from epicotyl explants from *Swietenia macrophylla* (Honduran Mahogany). Seeds were obtained from CATIE's Latin American Forest Seed Bank and germinated under aseptic conditions. Four explants were cultured in each Petri dish on half strength modified Murashige and Skoog basal medium, and five concentrations of BA (benzyladenine) were studied; *A. guachapele* and *S. macrophylla* responded positively to the presence of BA in the culture medium. Otherwise, *Cedrella odorata* required media supplemented with citokinin and auxin combinations to induce adventitious buds.

Key words: *Cedrella odorata, Albizia guachapele, Swietenia macrophylla*, adventitious bud induction.

During the last years, Central America has been affected by an increasing deforestation. At present, the deforestation rhythm reaches 370 000 to 400 000 hectares. One can calculate that at this rate, in year 2000 only will remain small forested areas in highlands, in regions far from the Atlantic coast and in protected areas such as National Parks (Funes 1992). As a consequence a population decrease occurred, as will as a genetic erosion that does not guaranty the *in situ* survival of many species (Jiménez Madrigal 1993). Therefore there is an urgency to look for new alternatives that would allow us to multiply and maintain the genetic pool of those endangered species.

There has been considerable progress made in regeneration plantlets of tropical hardwood trees through tissue culture (Monteuuis & Bon 1996, Etienne *et al.* 1997, Valverde Cerdas *et al.* 1997). More recently Maruyama *et al.* (1997), developed the protocols for the germplasm conservation of *Cedrella odorata*, *Jacaranda mimosaefolia* D. Don, and *Guazuma crinita* at temperature conditions above freezing by alginate encapsulation of shoot tips in the production of artificial seeds.

Guayaquil, Spanish cedar and Honduran Mahogany are important timber yielding trees and their economical and ecological importance warrants the application of tissue culture techniques for their clonal propagation and improvement.

The present study was conducted to induce adventitious bud of three tropical forest species: *Albizia guachapele* (Guayaquil), *Cedrella*

odorata (Spanish Cedar) and *Swietenia macrophylla* (Honduran Mahogany).

MATERIALS AND METHODS

Albizia guachapele, *Cedrella odorata* and *Swietenia macrophylla* seeds stored at CATIE´s Latin American Forest Seed Bank under a temperature of 5 °C, were used for this research. The seeds were washed with water and soap and kept under running water for 30 min. They were surface sterilized with 70 percent alcohol for three s and immersed for 20 min in a 5.5 percent commercial sodium hypochlorite solution and then washed with sterile distilled water.

The seeds were germinated in the dark, under aseptic conditions in an agar and water-based medium. Once they all germinated -*A. guachepele* after 5 days, *C. odorata* and *S. macrophylla* after about 10 to 15 days-, their hypocotyls were divided into 1.5 cm long sections and cultured in a medium containing: half-strength Murashige and Skoog (1962) inorganic salts, 2 $mg.l^{-1}$ nicotinic acid, 2 $mg.l^{-1}$ pyridoxine-HCl, 10 $mg.l^{-1}$ thyamine-HCl, 4 $mg.l^{-1}$ glycine, 200 $mg.l^{-1}$ myoinositol, 3% sucrose, and solidified with 7 $g.l^{-1}$ agar (Agar-agar, Sigma). In the case of *S. macrophylla*, the epicotyl used as an explant.

All media were adjusted to pH 5.8 before autoclaving and dispensed in Petri dishes (150x15 mm). Cultures were incubated at 27± 1 °C under 16 photoperiods at 45 $Em^{-2}s^{-1}$ intensity and 87 ± 3% relative humidity.

Explants were distributed in an irrestricted design, with five benzyladenine concentrations: 8.8, 17.7, 26.6, 35.5 and 44.4 µM. Another assay was designed for *C. odorata* where benzyladenine (BA) and naphthaleneacetic acid (ANA) were combined in a 5x4 factorial design with concentrations of 4.4, 8.8, 13.3, 17.7, and 22.2 µM BA; 0, 2.6, 5.3 and 10.7 µM ANA.

Response was evaluated at the end of the experiment and recorded as percentage of explants with buds, average number of buds per explants and total number of buds.

The statistics used was variance analysis. The data requiring conversion were transformed as $x=\sqrt{x+0.5}$. For *A. guachepele* and *S. macrophylla* variables, CATMOD procedure for X^2 (Grizzle *et al.* 1969) was used. Each experimental unit consisted of a Petri dish containing four explants and forty repetitions.

RESULTS

A. guachepele, hypocotyls and *S. macrophylla* epicotyls responded to the presence of BA in the culture medium by forming adventitious bud capable of developing. That was observed between 30 and 40 days in Guayaquil and between 20 and 40 days in Honduran mahogany.

For *A. guachapele*, the percentage of explants with buds, the total number of buds and the average number of buds per explant, showed significant differences ($X^2=10.7$, $p=0.02$) between the BA concentrations. The highest percentage of explants with buds (20%) and the highest total number of buds (14.5) was obtained with 17.7 µM BA. The highest average number of buds per explant (2.5 buds) were obtained with 35.5 µM BA (Table 1). At this concentration a better bud development was observed.

In the case of *S. macrophylla*, the largest amount of total buds was obtained at 44.4 µM BA. The average percentage of explants with buds ranged around 15% and the differences between treatments were not statistically different at the 5% level. Lineal tendency ($X^2=9.7$, $p=0.05$) was observed for the total number of buds and the average number of buds per explant which increased with the BA concentration (Table 2).

TABLE 1

Effect of five BA concentrations on bud induction in the hypocotyl explants of Albizia guachapele

BA[1] concentration (μM)	Percent with buds	Total No. of buds	Mean no. buds per explant
8.8	4.4	2.7	1.5
17.7	20*	14.5*	1.5
26.6	2.2	1.8	1.0
35.5	8.8	9.2	2.5*
44.4	11.0	5.0	1.2

1 Explants were cultured on half-strength MS. Data were collected from 20 replicates after six weeks for all parameters. *Significant at P = 0.05

TABLE 2

Effect of five BA concentrations on bud induction in the epicotyl explant of Swietenia macrophylla

BA[1] concentration (μM)	Percent with buds	Total No. of buds	Mean no. buds per explant
8.8	10	2	1.0
17.7	10	4	1.5
26.6	15	6	2.0
35.5	15	6	2.0
44.4	15	8	2.6*

1 Explants were cultured on half-strength MS. Data were collected from 20 replicates after 6 weeks for all parameters. *Significant at P = 0.05

C. odorata was the only species which did not respond organogenically to the BA presence. The explants were organogenic only when BA was combined with an auxin such as NAA, which indicates that this species required the presence of an auxin into the culture medium. The highest total number of adventitious buds (5 buds) was obtained with 17.7 μM BA and 5.3 μM NAA, and there were not significant differences between treatments. These buds were visible after 40 to 45 days of culture.

DISCUSSION

The results obtained in this study indicate the potential *in vitro* propagation of the three tropical forest species investigated. However, their in vitro requirements need to be further explored to establish the pattern for production of large number of propagules.

Media supplemented with BA was effective in inducing organogenesis in epicotyl explants of *S. macrophylla* and hypocotyl explants of *A. guachapele*. Adding Broadleaved Tree Medium benzyladenine also induced multiple shoot formation in *S. macrophylla* (Maruyama *et al.* 1989). Lee and Rao (1988), obtained adventitious shoots from the friable callus only when the nodal segments from seedlings were cultured on benzyladenine media. BA is a cytokinin known for strongly stimulating bud formation. It also stimulates the explant metabolism (George and Sherrington 1984). Finally, BA has been reported in several instances as the cytokinin of choice for adventitious budding in both gymnosperms and angiosperms. It is usually the only phytohormone required (Thorpe *et al.* 1991). *Cedrella odorata* required the addition of an auxin in order to express a morphogenic response. Otherwise, Maruyama *et al.* (1989), obtained multiple shoot formation in *Cedrella odorata* with addition of benzyladenine to Woody Plant Medium.

All the studied species showed the same bud formation pattern: a direct organogenesis without callus formation.

This work describes for the first time the adventitious bud induction in *Albizia guachapele*, a commercially valuable species of Costa Rica.

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