Plant Tissue Cult. & Biotech. **18**(2): 151-156, 2008 (December) **PTC&B**

In vitro Propagation of *Albizia guachapele*, *Cedrela odorata*, *Platymiscium pinnatum* and *Guaiacum sanctum*

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Key words: *In vitro,* Propagation, *Albizia guachapele*, *Cedrela odorata*, *Platymiscium pinnatum, Guaiacum sanctum*

Abstract

In Costa Rica *Albizia guachapele, Cedrela odorata, Platymiscium pinnatum* and *Guaiacum sanctum* are important plant species in both economic and ecological terms and their wood is precious and reported to be highly resistant material. This research has evaluated the *in vitro* micropropagation as a technology focused to conserve these species. Findings include percentage of germination of seeds and contamination, induction of buds, rooting and growth of micro-cuttings of these four species.

Introduction

Some decades ago, the national timber industry was more inclined towards the extraction of fine-wood or highly decay-resistant wood species. Due to the continued diminution in the number of such species, others of equal or less marketing value used to fill the gap, which caused a selective harvesting (the best specimens of each species were extracted). This diminution brings in an alarming situation today, as a consequence of the dramatic population growth in many species. Threatened species are vulnerable and susceptible to factors that may influence their extinction in various ways including a population reduction, habitat destruction or any other environmental disturbance (Jiménez 1999).

Currently, there is an interest for establishing plantations of native species. However, the lack of studies on this field restricts the technological development to a small number of species and prevents their large scale production. For recalcitrant seed species, other production alternatives should be considered; including the use of *in vitro* techniques that would contribute to obtain material, for the *in vitro* conservation of material improved in any characteristic of

agronomic importance, and the massive propagation of clones. However, this can only be achieved if the *in vitro* regeneration systems are obtained.

The *in vitro* propagation of adult forest trees is experiencing many difficulties in the various steps of the process, including, in particular, extremely high contamination levels in the first explants, and high emission of polyphenols and tannins that impede the explant development and frequently cause necrosis, vitrification and a deficient rooting capacity. It has been demonstrated that young tissues have a better response in the culture. For this reason, and since there is no genetic improvement program for the *Albizia guachapele, Cedrela odorata, Paltymiscium parviflorum* and *Guaiacum sanctum*, it seemed necessary to initiate studies with young material from such species.

Materials and Methods

Four hundred seeds each of *Cedrela odorata, Albizia guachapele* and *Paltymiscium parviflorum* were collected from the tree, washed and disinfected with commercial chlorine at 100% (3% a.i.) for 25 minutes. The sterilized seeds were again washed four times in sterile distilled water in a laminar flow cabinet. Before the disinfection, the top of each seed was removed. The *Guaiacum sanctum* seeds were also separated in groups to plant some of them with the fruit coat, scarified and not scarified, and treated with 100% concentrated sulfuric acid. The seeds of *Paltymiscium parviflorum* were washed under tap water for 30 minutes; then ringed in a water-soap solution for ten minutes; immersed in a 10% antimicrobial solution for five minutes; and finally, immersed in 100% commercial chlorine (3.5% a.i.) for five minutes. All seeds were inoculated in an agar and water medium. The that germinated seedlings were divided in nodes. These nodes were placed in MS at different concentrations of cytokinin depending on the species, Kn and TDZ, and evaluated every eight days, for 30 days. After reproducing the material, apical buds of 1.5 - 2.5 cm length (depending on the species) were separated and inoculated in culture media under different concentrations of IBA.

For species that did not exhibit a good rooting, trials were performed for the induction of somatic embryos from zygotic embryos through various combinations of growth regulators, including BAP and NAA. All cultures were kept in the growth chamber at 27ºC and 16 hour photoperiod.

Results and Discussion

The germination of *Cedrela odorata* seeds was cent per cent. The microcuttings from the apical buds of the seedlings showed a somewhat slow *in vitro* growth, and a good leaf area development. Moreover, the material was contaminated by

a bacterium, reducing the number of explants available for trials. The species had a good response to growth regulators in the culture media. No significant differences were found ($p > 0.05$) among the BAP concentrations; however, an average of 2.1 axillary buds per explant and a better appearance of the buds (Table 1) was obtained with 0.3 mg/l BAP. The rooting trial reported a higher percentage of rooted stems in a 1 mg/l IBA and, although the average number of roots per stem was higher with the 2 mg/l IBA (Núñez and Mesén 1999). The root distribution was better in the 1 mg/l IBA, and presented the higher average number of roots per stem (Table 2).

Table 1. Effect of the three BAP concentrations in the induction of buds from *Cedrela odorata*.

BAP (mg/l)	0.1	0.3	0.5	0.7	1.0	1.5
Average number of buds per explant	1.1	2.1	2.0	2.3	1.6	1.2

Table 2. Effect of the three IBA concentrations on the rooting of *Cedrela odorata* stems.

IBA (mg/l)	1.0	2.0	3.0
Percentage of rooted stems	70	65	50
Average number of roots per stem	3.8	6.5	4.2

Cent per cent seeds of *Albizia guachapele* germinated; however, this species did not present a good *in vitro* performance : leaf development was poor and growth was very slow. In the three types of cytokinin evaluated, shoots tended to be small, under developed, did not develop in some cases, and had a slow growth. Dropping of leaf was observed, and the explant recovery was slow. Significant differences ($p < 0.05$) were observed in BAP versus Kn and TDZ. However, BAP produced higher number of buds per node in average (Table 3), a low effect of the treatment was observed on the primodia in the presence of Kn. Huetterman and Preece (1993) reported that TDZ is one of the most active cytokinins in tissue culture for recalcitrant woody plants and that low concentrations (< 1 mg/l) may result in a important axillary proliferation, compared to other cytokinins; however, TDZ may inhibit shoot elongation (George 1993). In the case of *Albizia guachapele*, the TDZ effect was similar to Kn.

The stem did not show any formation of roots; only callus was formed at the base of the stem.

The *in vitro* germination of immature seeds of *Paltymiscium parviflorum* was 100%. A vigorous growth of seedlings was observed, but the microcuttings grew very slowly, and went through a frequent defoliation period (Table 4). Nevertheless, the material recovered easily and continued its normal growth. In

any way, this limited the availability of vegetative material for the subsequent *in vitro* culutres.

Table 3. Response of the *Albizia guachapele* nodes at different concentrations of cytokinins.

	Kn (mg/l)			BAP (mg/l)			TDZ (mg/l)		
Variable	0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5
Av. No. buds	2.3	2.5	2.1	2.3	3.3	2.6	3.0	2.5	2.1

Table 4. Percentage of growth of *Paltymiscium parviflorum* microcuttings in MS at different concentrations of cytokinin.

Variable	25	50	75	100
Growth	1.7	1.8	1.6	1.5

The somatic embryogenesis trials with *Paltymiscium parviflorum* explants provided any other results than the formation of a non-embryogenic callus.

The results of the *Guaiacum sanctum* seed treatments are presented in Table 5. Removing the apical portion of the seed and disinfecting it in concentrated sulfuric acid did neither contribute to increase the clean material obtained nor improved the germination percentage. Removing the apical portion of the seed help to improve the germination percentage, but it is not comparable with the cent per cent germination obtained from the embryo extraction. The mature seeds of *Guaiacum sanctum* showed the same *in vitro* behavior of the radicle emergence, without development of the aerial portion i.e. seeds did not germinate completely; in such cases, the embryo extraction was necessary for the embryo development. It was also necessary to supplement the culture medium with GA_3 or BAP 0.5 mg/l, to activate the germination of some embryos, and to add an antibacterial agent to reduce contamination. This treatment can be omitted if the apical portion of the seed is removed at the beginning. The *Guaiacum sanctum* immature embryos showed cent per cent *in vitro* germination. This facilitated the somatic embryogenesis. The *Guaiacum sanctum* species exhibited a very slow growth, and the explants tended to become old, limiting the multiplication of the species as demonstrated in the values of the average number of buds obtained from Kn and TDZ concentrations (Table 6). However, the possibility of increasing the cytokinin concentrations in the culture media is not discarded.

For this reason, somatic embryogenesis was tried as a technique for more efficient reproduction of the species. However, the somatic embryogenesis from *Guaiacum sanctum* zygotic embryos was inconsistent and scarce. The higher average number of somatic embryos obtained was 3.0 per explant (Table 7), and

the embryo development is very slow and most of the embryos stay in a permanent globular stage. Valdez (2002) mentioned that the *in vitro* tissue shows differences in response according to the endogenous number of receptor hormones and proteins in the plants, which vary in genotype, age, portion of the plant and phenological stage. Therefore, Valdez (2002) conclude on the necessity of conducting studies to identify the type and dosage of growth regulators for each type of plant or species, as a model for their application.

Table 5. *In vitro* germination of *Guaiacum sanctum* seeds.

Treatment	Germination (%)	Contamination (%)
Seeds with apical portion removed	29	10
Seeds not extracted from the coat	5.7	47
Seeds scarified and treated with sulfuric acid	25	75
Seeds not scarified and treated with sulfuric acid	5.5	2.5

Table 6. Response of *Guaiacum sanctum* microcuttings of *in vitro* seedlings germinated in Kn and TDZ.

	Kn (mg/l)		TDZ (mg/l)	
	0.1	0.3	0.1	0.3
Average number buds	0.6	0.4	0.1	0.2

Table 7. Average number of somatic embryos in *Guaiacum sanctum* embryo explants.

Variable	BA + NAA (mg/l)			
	2.5 + 0.5	2.0 + 1.0	3.0 + 0.5	3.0 + 1.0
Average number of somatic embryos per explant	1.2	3.0*	0.3	1.1

* Significant differences at 5% of significance.

The results obtained from the *Albizia guachapele*, *Guaiacum sanctum* and *Platymiscium pinnatum* species demonstrate their recalcitrance *in vitro* and the necessity of conducting more studies to establish an efficient *in vitro* regeneration system for these species.

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