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PRELIMINARY REPORT ON TISSUE CULTURE OF *ARAUCARIA ANGUSTIFOLIA*

NOTA PRELIMINAR SOBRE A CULTURA DE TECIDOS DE *ARAUCARIA ANGUSTIFOLIA*

Walter Handro[1] *e Claudio Martins Ferreira*[2]

SUMMARY - Stem segments from dark grown *Araucaria angustifolia* seedlings were cultured on synthetic nutrient medium supplied with kinetin plus auxins (NAA, IAA, IBA, or 2,4-D). Several kinds of calluses were produced, especially in conditions of high auxin (2mg/l) and low kinetin (0.1mg/l). High kinetin and low auxin promoted the development of the lateral buds. These buds can be isolated and remain alive on basal medium lacking hormones for 6 months, without rooting.

RESUMO - Segmentos de caule de plântulas de *Araucaria angustifolia* crescidas no escuro foram cultivados em meio sintético com adição de cinetina e auxina (ANA, AIA, AIB ou 2,4-D). Foram produzidos vários tipos de calos, especialmente em condições de alta concentração de auxina (2mg/l) e baixa de cinetina (0,1mg/l). Alta cinetina e baixa auxina promoveram o desenvolvimento das gemas laterais. Estas gemas podem ser isoladas e permanecem vivas no meio básico sem hormônios por 6 meses, sem enraizar.

INTRODUCTION

Tissue and cell culture seem to be a good alternative method to obtain rapid multiplication and improvement of tree species. Recently, Winton (1978) listed about 60 tree species which have been regenerated from tissue cultures, ca. 20% being Gymnosperms. This group of plants has long been cultured *in vitro* (see Brown & Sommer 1975) but studies on the genus *Araucaria* are restricted to those reported by De Fossard and Haines (1977). This paper reports some preliminary results obtained on tissue cultures of *Araucaria angustifolia* (Bert.) O. Ktze., the Brazilian pine.

MATERIAL AND METHODS

De-coated seeds were germinated in the dark at 27°C, giving rise (after 30d) to etiolated seedlings, with long internodes (Figure 1). Stems were then cut off, sterilized in 5% calcium hipochlorite (10 min) and rinsed in sterile water. Sections 0.5 mm long were then prepared, the leaves cut at the base, and

(1) Plant Tissue Culture Laboratory. Dept. of Botany, Institute of Biosciences, University of São Paulo. C P 11461 - 05421 São Paulo, Brazil.
(2) Undergraduate student.

the explants placed into the culture tubes. Two kinds of explants were prepared: from internodes, without lateral buds ; and from node regions with one or more lateral buds.

The basal medium comprised the macronutrients of Knop (see Gautheret 1959) or Murashige and Skoog (1962); micronutrients , FeEDTA, growth factors and vitamins following Nitsch (1969), 2% sucrose, 50mg/l arginine, 20mg/l glutamine, 20mg/l urea and 150 mg/l citric acid. The last four substances were added to the previously autoclaved medium by filtration through Millipore membranes. The pH was adjusted to 5.5. To the basal medium , growth regulators were added in several combinations. Two experiments were performed, differing in the macronutrients (Murashige and Skoog, or Knop), each experiment with several combinations of kinetin (0.1, 0.5, 2.0mg/l) plus IAA, IBA, NAA or 2,4-D (0.1, 0.5, 2.0mg/l). The media were gelled with 0.7% agar.

The cultures (12 per treatment) were kept under photoperiod of 16 h provided by fluorescent and incandescent lamps (about 3000 lux), or maintained in the dark, at $27 \pm 2^{o}C$.

RESULTS AND DISCUSSION

The schematic aspects and the summary of responses of cultures are shown in table 1. Depending on the treatment, after 10-30 days the explants have produced several kinds of calluses with peculiar size, location, colour or consistency. The more vigorous calluses were produced under low kinetin (0.1mg/l) combined with high auxin (2.0mg/l). NAA and 2,4-D were the most effective in callus production, even combined with high kinetin. However, the more remarkable result was the development of the lateral buds under treatments with high kinetin (2mg/l) plus low auxin (0.1mg/l), especially 2,4-D and IBA. This occurred in the basal medium with Knop macronutrients. If the explants after bud development are kept in the original medium, lateral branches grow slowly reaching about 0.5 mm. When transferred to growth regulator-free medium, the branches continue to grow. Typical cultures of some treatments are shown in figures 2-5.

Both calluses and branches originating from primary cultures were isolated to be subcultured or rooted. Few calluses are able to survive after subculturing. Isolated branches were able to survive up 6 months in medium without growth regulators, but no roots were formed. Attempts are being made in view of culturing callus tissues in liquid medium, and of producing large amounts of lateral branches to assay different concentrations of growth regulators for rooting purposes.

The preliminary results reported here seem to be encouraging in view of the possibility in obtaining high rates of plant multiplication with minimum risks of genetic variation , or in view of using tissue culture for genetic manipulations . On the other hand, physiological and structural studies on the development of the lateral buds are also of interest.

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TABLE 1 - Treatments and morphogenetic responses of stem segments

AUXINS	KNOP macronutrients						MURASHIGE and SKOOG macronutrients											
	kinetin (mg/l)						kinetin (mg/l)											
	0.1		0.5		2.0		0.1			0.5			2.0					
	light		light		light		light		dark	light		dark	light			dark		
IAA	0.1	white or green callus	0.5	brownish callus	2.0	swollen bud	0.1	light greenish callus	greenish callus	0.5	greenish callus	light greenish callus	2.0	green callus		brownish, friable callus		
2,4-D	0.1	greenish callus	0.5	green callus	2.0	green callus	0.1	light greenish callus	light greenish callus	0.5	greenish callus	white, friable callus	2.0	bud, or green callus		bud, or white callus		
IBA	0.1	greenish callus	0.5	greenish callus	2.0	no response	0.1	light greenish callus	whitish callus	0.5	greenish callus	greenish callus	2.0	bud		greenish, compact callus		
NAA	0.1	greenish callus	0.5	greenish callus	2.0	greenish callus	0.1	greenish callus	whitish callus	0.5	light greenish callus	light greenish callus	2.0	green, compact callus		whitish, friable callus		

Aspects of cultures	brownishment in the explant	brownishment in the medium	lateral bud developed	callus in the lateral bud	callus with regular surface	callus with irregular surface

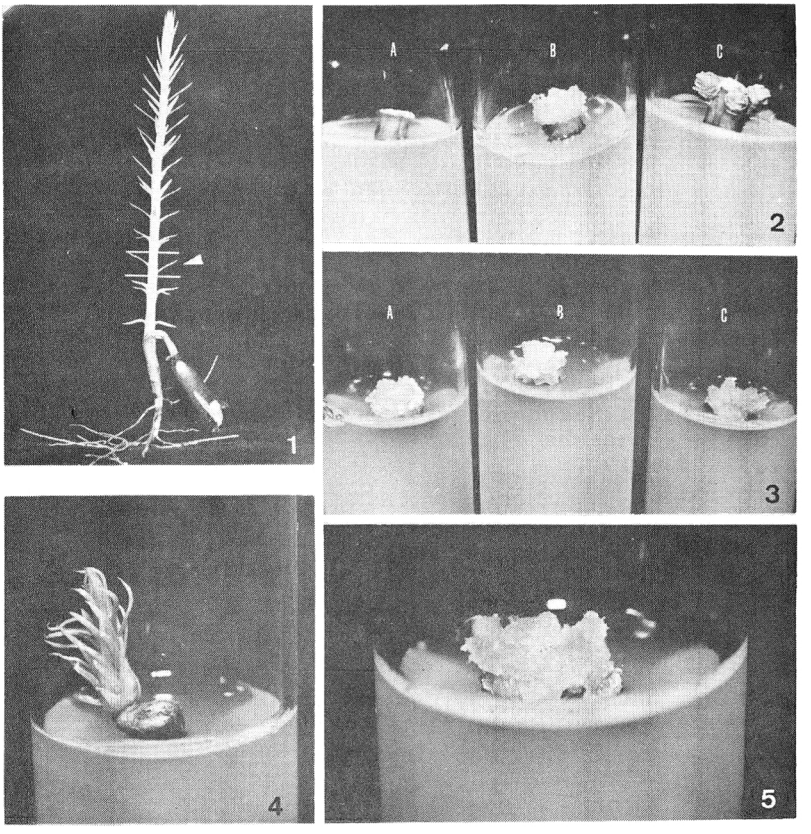

Fig. 1 - 30d old seedling used as source of explants. Fig. 2 - 30d old cultures, on media with Knop macronutrients. A - basal medium; B - kinetin (2.0mg/l) + NAA (0.1mg/l) (dark); C - kinetin (2.0mg/l) + 2,4-D (0.1mg/l) (light). Fig. 3 - 10d old cultures (light), on media with Knop macronutrients. A - kinetin (0.1mg/l) + IAA (2.0mg/l); B - kinetin (0.1mg/l) + 2,4-D (2.0 mg/l); C - kinetin (0.5 mg/l) + IAA (0.5mg/l). Fig. 4 - 50d old culture, previously cultured on medium as in fig. 2,C for 30d, then transferred to basal medium lacking growth regulators. Fig. 5 - Callus tissue cultured on medium as in figure 3, C.

Fig. 1 - Plântula de 30 dias, fonte dos explantes. Fig. 2 - Culturas de 30 dias, em meio com macronutrientes de Knop. A - meio básico; B - cinetina (2,0mg/l) + ANA (0,1mg/l) (escuro); C - cinetina (2,0mg/l) + 2,4-D (0,1mg/l) (luz). Fig. 3 - Culturas de 10 dias (luz) em meio com macronutrientes de Knop. A - cinetina (0,1mg/l) + AIA (2,0mg/l); B - cinetina (0,1mg/l) + 2,4-D (2,0mg/l); C - cinetina (0,5mg/l) + AIA (0,5mg/l). Fig. 4 - Cultura de 50 dias previamente cultivada em meio como na figura 2, C, por trinta dias, e então transferida para meio básico sem reguladores de crescimento. Fig. 5 - Tecido de calo cultivado em meio como na figura 3,C.