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Micropropagation of *Araucaria columnaris* Hook.

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Introduction

The genus *Araucaria* comprises several magnificent evergreen forest tree species, including *Araucaria columnaris* Hook., which has a high ornamental value as well. Conventional propagation by seeds is slow and inadequate for producing large uniform progenies. Cuttings are difficult to root and they produce plagiotropic plants except when taken from apical shoots. Successful micropropagation of this species has not been reported. The aim of this study was to use explants from normally growing mature trees for *in vitro* propagation of *A. columnaris.*

Materials and Methods

Tips (3–5 mm) of secondary and tertiary lateral branches, taken from 14 yr old trees, were sterilized in mercuric chloride (0.05%) for 1 min and cultured on Murashige and Skoog's (MS) medium (Murashige and Skoog, 1962), containing different concentrations of various growth regulators and/or other chemicals. The cultures were kept at 25°C under cool white fluorescent light (1.5 klux), from 40 W tubes, for 16 h each day. At least 20 replications were used for each treatment.

Results

The explants, initially cultured on MS medium supplemented with kinetin (5–12 μM) and naphthalene acetic acid (1–3 μM), were established on the medium containing only kinetin (10 μM). The establishment of cultures was maximum (40–50%) when the explants were collected in April and November, whereas it was low (10–30%) during other months of the year. The development of axillary buds was obtained by subculturing on a medium containing 6-benzylaminopurine (BAP, 12.5 μM), indole butyric acid (IBA, 1 μM) and 3% sucrose.

Explants taken in January, prechilled for 1 mo at 5°C and cultured on MS medium (containing 10 μM kinetin and 1 μM IBA)

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Table I. Shoot growth and axillary bud development of *A. columnaris in vitro*.

Treatment	*Axillary bud development*	*Shoot growth (cm)*
MS + 12.5 μM BAP + 1 μM IBA + 3% sucrose	2–3 minute buds visible	1.03 ± 0.08
MS + 10 μM kinetin + 1 μM IBA + 2% sucrose	none	0.73 ± 0.10
Explants prechilled for 1 mo, MS + 10 μM kinetin + 1 μM IBA + 2% sucrose	3–4 axillary branches, up to 1 cm long	1.44 ± 0.16
MS + 10 μM kinetin + 1.3 μM thiourea + 2% sucrose	2–3 axillary branches, up to 1.6 cm long	1.99 ± 0.22
MS + 10 μM kinetin + 1 μM IBA + 2% sucrose + 0.3% charcoal	2–4 axillary branches, up to 0.7 cm long	1.16 ± 0.16

Values represent means of 20 explants ± 1 SD, observed after 6 wk.

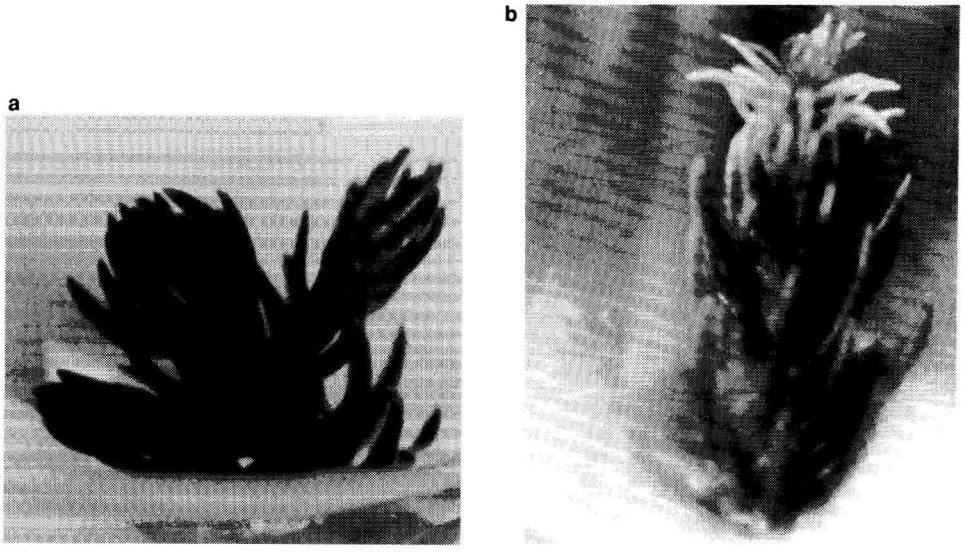

Fig. 1. *In vitro* growth of *A. columnaris* on MS medium containing thiourea. **a.** 1.6 cm axillary branches in 6 wk. **b.** After subculturing, 3 cm shoot in 12 wk.

produced almost twice the shoot growth rate as compared to the ones not chilled, giving a shoot length of 1.5 cm in 6 wk (Table I).

Subculturing or direct planting on a medium containing kinetin (10 μM) and thiourea (1.3 μM) enhanced the shoot length and production of axillary branches

(Fig. 1). The latter were separated and used for multiplication. Addition of 0.3% activated charcoal to the medium also indicated a faster rate of growth (Table I).

Discussion and Conclusion

Micropropagation of most of the conifers has not been successful. Out of 6 or so reports on *in vitro* culture of about 12 species of *Araucaria* (Haines and de Fossard, 1977; Maene & Debergh, 1987), only Burrows (1983) used coppice shoots from mature trees. Others have utilized orthotropic shoots from juvenile plants. Recently, Handro (1986) reported the development of axillary shoot *in vitro,* starting from branches of a 20 yr old tree of *A. angustifolia.* The only report on *A. columnaris* is a preliminary study using seedling stem segments (Burrows, 1983). In our study, tips of very small branchlets appearing naturally on plagiotropic branches of 14 yr old trees were used. In nature, these branchlets hardly show any growth. In culture, however, these shoots appeared to show normal orthotropic growth. In *Sequoia,* Boulay (1979) also obtained orthotropic behavior of *in vitro* shoots derived from stump sprouts of mature trees. Explants from mature trees of some other conifers have also been used (Bonga, 1981; Gupta, 1987).

The present studies also show that season and prechilling of explants effect subsequent response in culture. Thiourea seems to promote axillary branches and shoot growth, as observed by Maene and Debergh (1987).

Araucaria is a hard-to-root material in culture, and limited success in rooting has been reported only for one species *viz., A. cunninghamii* (Haines and de Fossard, 1977; Burrows, 1983). Nevertheless, the production of shoot growth and axillary branches obtained in culture in the present study is an important step in micropropagation. Attempts to induce *in vitro* rooting are in progress.

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