

Initiation of Callus Tissue of *Abies concolor* (White Fir) by Tissue Culture Techniques

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Abstract

Callus tissue of *Abies concolor* (Gord. and Glend.) Lindl. was initiated from inocula prepared from terminal stem tissue on a modified Winton medium containing basis salts, organic nutrients, growth regulators and a stem extract of *Abies concolor*. Under controlled environmental conditions, the callus has grown and has been maintained through subsequent subculture for ten months.

Introduction

Culturing of gymnosperm tissues under aseptic conditions has been difficult to achieve. Approximately 27 gymnosperm species have been successfully cultured, however (1-13). Among these 27 is grand fir (*Abies grandis*) the only representative of the genus *Abies* to have been successfully cultured to date (6).

We now report the initiation of callus tissue from stem tissue of mature *Abies concolor* (Gord. and Glend.) Lindl. trees.

Materials and Methods

The inocula used in this study were obtained from branches (which were less than 3 m above the ground level) of mature *Abies concolor* trees. Terminal stem sections approximately 10 cm long and consisting of the current year's growth were used; before taking, each stem section was sprayed with 70% ethyl alcohol. Cuts were made with sterile scalpels. After making the cut, the basal end of each stem section was sprayed with 70% ethyl alcohol and placed in a separate, unused, plastic bag and the opening of the bag was fastened securely.

In the laboratory, one stem section at a time was removed from its plastic bag and immersed in a shallow container of 70% ethyl alcohol. While immersed in the alcohol, all the needles were removed with a scalpel at their junction with the stem. Each de-needled stem section was transferred to a dry, sterilized, 500 ml Ehrlenmeyer flask equipped with a screw cap. After the stem sections were de-needled and placed in the flask, 250 ml of fresh, household bleach (5.25% sodium hypochlorite) were added, along with 2 drops of Tween 20 (polyoxyethylene sorbitan monolaurate), a wetting agent used as a surfactant. The flask was agitated frequently during the 10-minute interval that the stem sections were sterilized. The bleach was decanted, and the stem sections were rinsed 3 times with sterile, distilled water. The sterilized portions of stems were cut into sections approximately 8-10 mm in length. The end sections were discarded, while the others were inserted, basal end up, in 25 ml of an agar nutrient medium in 20 x 100 mm petri dishes.

Results and Conclusions

Callus was successfully initiated and maintained on a modification of Winton's medium for triploid quaking aspen (14-17). Winton's medium was modified by the addition of gibberellic acid, indoleacetic acid, naphthaleneacetic acid, ascorbic acid, L-glutamine, myostatin (a fungicide), and a stem extract of *Abies concolor*. On this undefined medium (Table 1), cell proliferation occurred within 14 days while the plates were being maintained in the dark in an incubator at $27.5 \pm 2^\circ$ C under satur-

TABLE 1. Undefined medium for initiating and maintaining *Abies concolor* callus.

Constituents	Conc mg/l	Constituents	Conc mg/l
MgSO ₄ ·7H ₂ O	764.0	pyridoxine	0.1
Na ₂ SO ₄	425.0	Fe-EDTA	5.5
Ca(NO ₃) ₂	170.0	2,4D	0.5
KNO ₃	425.0	kinetin	1.0
KCl	140.0	sucrose	20000.0
NaH ₂ PO ₄ ·H ₂ O	34.0	bacto-agar	8000.0
myo-inositol	100.0	gibberellic acid	0.5
MnSO ₄	9.0	indoleacetic acid	1.0
ZnSO ₄ ·7H ₂ O	3.2	naphthaleneacetic acid	1.0
H ₃ BO ₃	3.2	ascorbic acid	0.1
KI	1.6	L-glutamine	250.0
nicotinic acid	0.5	myostatin	2.5 ¹
thiamin	0.1	<i>Abies concolor</i> stem extract	85.0 ^{1,2,3}

¹ml/l

²The stem extract was prepared by adding 150 g of terminal stem sections of the current year's growth of *Abies concolor* to 50 ml of distilled water; pulverizing for 15 min at high speed in a Waring Blender; and suction filtering and collecting the liquid extract.

³pH of medium was adjusted to 5.7 with 0.1N NH₄OH.

ated humidity conditions. Figure 1 shows the small mound of callus which formed on representative inocula after 42 days of growth. At 42 days the callus tissue was transferred to fresh medium where it continued to grow and, thereafter, was subcultured every 21 days. Figure 2 shows a representative mound of callus tissue after 90 days. Cells on the surface of tissue this age are still near white, but basal cells are reddish-brown. The pigmentation in the older cells did not appear to hinder the proliferation of *Abies concolor* callus since the callus has been subcultured and maintained for 10 months. Harvey and Grasham (6) also reported pigmentation

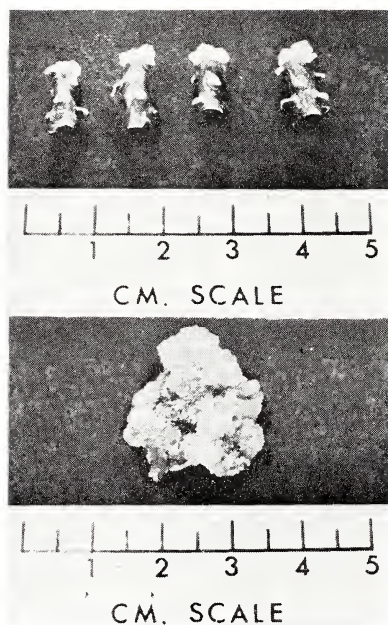


FIGURE 1. *Abies concolor* callus at 42 days (top).

FIGURE 2. *Abies concolor* callus at 90 days. (bottom)

in the callus of *Abies grandis*, but it lost its vigor by the end of 90 days. Further study with the established *Abies concolor* callus is now in progress; parameters being investigated are growth rate, minimal nutrient medium, effects of varying photoperiod, and callus differentiation and maturation.

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