An embryogenic cell suspension culture of *Picea glauca* (White spruce)

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**ABSTRACT**

A cell suspension culture of *Picea glauca* (White spruce) which continuously produces somatic embryos has been established. Embryogenic callus derived from cultured zygotically embryonic cells was used to initiate the culture. Numerous embryos at various early stages of development were recognized; they exhibited a meristematic embryonic region and suspensor consisting of elongate, vacuolated cells. The culture also contained clumps of meristematic cells and large irregular-shaped cells. The culture could be readily re-established on solid medium.

**INTRODUCTION**

Embryogenesis has been achieved in tissue cultures of a number of angiosperms, both dicotyledons and monocotyledons (see review by Ammirato 1983). Success with gymnosperms has been more limited. Recently embryogenic callus cultures have been established from cultured zygotic embryos (e.g. Hakman et al. 1985, Hakman and von Arnold 1985, von Arnold and Hakman 1986, Hakman and Fowke 1986, Gupta and Durzan 1986) and female gametophyte tissues (Nagmani and Bonga 1985) of conifers. These callus cultures contain multiple embryos at different stages of development. Under certain conditions the embryos develop into rooted plantlets. Further refinement of this technology should provide a valuable method to propagate selected conifers and provide an excellent source of experimental material for studies of embryo growth and differentiation as well as for future genetic manipulation of conifers.

This paper describes a method for the establishment of a liquid culture which has continuously produced embryogenic embryos of *Picea glauca*.

**MATERIALS AND METHODS**

Embryogenic callus of *Picea glauca* (Moench) Voss. (White spruce) were produced as previously described (Hakman and Fowke 1986). Briefly, seed cones were collected on the 19th of August 1985 at Christopher Lake, Prince Albert, Saskatchewan. Immature embryos possessing tiny cotyledons were isolated from surface sterilized seeds and cultured on solid LP medium (von Arnold and Eriksson 1981) containing 3% sucrose, 2,4-dichlorophenoxyacetic acid (10^-5 M) and N6-benzyladenine (5x10^-6 M). Cultures were incubated in the dark at 25°C with subculture carried out monthly to fresh medium. Small pieces (about 400 mg f. wt.) of two month old embryogenic callus were transferred to liquid LP medium of the same composition as used for callus cultures. Initially the cultures were grown in 10 ml medium in 50 ml Erlenmeyer flasks on a gyratory shaker (about 100 rpm) and fresh medium was replenished weekly. Once sustained growth was established (after 3-4 weeks), subculturing of approximately 5 ml aliquots was carried out biweekly to 25 ml medium in 250 ml Erlenmeyer flasks with agitation increased to 150 rpm. The cell suspension cultures were maintained in continuous light (1.614 x 10^-4 W cm^-2; Westinghouse 'Cool White' fluorescent lamps) at 28°C. After 3 months of sustained growth samples from the liquid cultures were plated on solid LP medium (as above).

Samples were removed from the liquid cultures, examined and photographed using bright field and Nomarski optics in a Zeiss Universal microscope. Callus cultures re-established from the cell suspension cultures were photographed with a Zeiss Tessoar macro photographic unit.

Samples were also fixed, embedded and sectioned for light microscope observation according to methods previously published (Fowke 1984). In brief, they were fixed sequentially with 1% and 3% glutaraldehyde (in 0.025 M sodium phosphate buffer, pH 6.8) for 1 and 2 h, respectively, at room temperature. Following a brief wash with buffer they were postfixed in 1% OsO4 in the same buffer overnight on ice. The samples were slowly dehydrated in ethanol to propylene oxide at 0°C, infiltrated with resin at room temperature and finally flat embedded in Araldite by baking for 48 h at 60°C. Embryos and cell clusters were sectioned at approximately 1 μm, stained with toluidine blue (1% in 1% borax) and photographed using bright field optics in a Zeiss Universal microscope.

**RESULTS AND DISCUSSION**

Cell suspension cultures derived from embryogenic callus have been maintained for a period of 8 months. Although superficially the cultures resemble "normal" suspension cultured cells, a close examination reveals that the culture is much more complex. The most interesting feature of the culture is that it contains numerous somatic embryos of different developmental stages at any given time (Figs. 1-5). The somatic embryos are essentially the same as those found in embryogenic callus cultures of conifers (e.g. *Picea abies* - Hakman et al. 1985, *Picea glauca* and *Picea mariana* - Hakman and Fowke 1986, *Larix decidua* - Nagmani and Bonga 1985, *Pinus lambertiana* - Gupta and Durzan 1986). They consist of a terminal embryonic...
region consisting of meristematic cells and a suspensor region consisting of large elongated highly vacuolated cells. Structures similar to those illustrated in figures 1 and 2 were commonly observed in the liquid cultures and likely represent very early stages in the development of somatic embryos. The meristematic embryonic region seems to be initiated by an asymmetric division of particular vacuolated cells in the culture. The structure in figure 3 is clearly recognizable as a young somatic embryo. As the embryos increase in size, the most striking change is an increase in the length and diameter of the suspensor region resulting from a proliferation of the vacuolated cells (Figs. 4, 5). These cells seem to arise directly from the embryonic region and expand rapidly into the suspensor. The suspensor is usually larger and not as precisely organized as in zygotic embryos. This type of development appears to be characteristic of somatic embryos of conifers produced in tissue culture (e.g. Hakman and Fowke 1986).

Somatic embryogenesis has previously been reported for cell suspensions of *Picea glauca*, *Pseudotsuga menziesii* (Durzan 1982) and *Picea abies* (Hakman et al. 1985). While the embryos described by Durzan are unusual in that they do not possess an elongate suspensor region characteristic of conifer embryos. In fact the embryoids described by Durzan are similar to those reported in this paper, the embryoids described by Durzan are unusual in that they do not possess an elongate suspensor region characteristic of conifer embryos. In fact the embryoids superficially resemble angiosperm embryos. It will be interesting to see whether such embryos are capable of developing into normal plantlets.

The embryogenic cell suspension cultures contain different types of cells in addition to the somatic embryos. The most common are large highly vacuolated