Anatomical events during rooting and effects of IBA on adventitious roots of in vitro microcutting from *Eucalyptus pellita*

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1. Introduction

*Eucalyptus* spp. originated from Australia have been extensively planted as exotics for commercial reforestation in many parts of the world. Today, they constitute the majority of the world’s exotic hardwood trees and one of the major sources of biomass. Among the *Eucalyptus* species, *E. pellita* is a very attractive species because of its fast-growing, superior wood quality and wide adaptability to a variety of environments.

In the present study, we studied the anatomical events leading to root induction and development in *E. pellita*.

2. Materials and methods

Shoots cultures maintained on DKW medium and subcultured every 4 weeks. Shoots from 6–7 cm long, were cutted for rooting. Their basal ends (0.5–1.0 cm) were dipped in talc paste containing 100 ppm IBA and the shoots were then transferred to plastic box containing artificial soil (vermiculrite). All cuttings were grown under cool-white fluorescent lamps providing a PPFD of 30 μmol m⁻² s⁻¹ during a 16 h photoperiod 25°C.

Batches of ten stem pieces were collected from cuttings 0–12 d during root induction. Each piece was fixed for histological observation of the basal 0.5–0.7 cm of the stem.

The samples fixed
- Histochemical method (Yeung, 1999)
  - Fixation: Fixative solution containing 1.5 % glutaraldehyde and 1.6 % paraformaldehyde buffered with 0.05 M phosphate buffer, pH 6.8 (for 24–48 h)
  - Dehydration and embedding: Alcohol series, Technovit 7100 (kluzer, Germany)
  - Section: 3 μm in thickness (Leica 2040, Autocut rotary microtome)
  - Histochemical staining
    - Total carbohydrates: PAS (Periodic acid – Schiff’s reaction)
    - Counter staining: Toluidine Blue O

3. Results

The anatomical observation showed that the process of adventitious root initiation and development proceeded as follows: On the 1st day of cuttings, shoot section showed a typical tissue organization without any cell divisions. After 3 days of cuttings, the number of dividing cells increased markedly, and meristemoids were evident between primary phloem and vascular
cambium region. On the 6 days, the meristemoids developed outwards showing increased cell volume and numerous cell division. On the 9 days, meristematic tissue progressively formed from dedifferentiated cells and polarized cells were visualized giving rise to typical pointed shape of root primordium. On the 12 days two or more adventitious roots emerged from the surface of the basal part of shoots.