Carbohydrates as regulatory factors on the rooting of *Eucalyptus saligna* Smith and *Eucalyptus globulus* Labill

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Abstract

Comparisons between related species with different rooting capacities can provide insights into the mechanisms controlling adventitious root development. The availability of carbohydrates is often considered exclusively as an energetic requirement to drive root development; the major regulatory role in the process is often attributed to phytohormones, particularly auxin. The roles of light quantity (irradiance) and carbohydrate supply available to young aseptic donor-plants on the adventitious rooting response of *Eucalyptus globulus* (rooting recalcitrant) and *Eucalyptus saligna* (easy-to-root) were examined. The effects of the type of carbohydrate supply (sucrose or glucose) on the rooting response of cuttings was also evaluated. Light intensity supplied to mother-plants (30 or 60 µmol m⁻² s⁻¹) had limited influence on the rooting response of both species, whereas dark periods were detrimental, particularly for *E. globulus*. In *E. globulus*, rooting was promoted by the absence of sucrose in donor-plant media. Presence of sucrose in donor plant medium promoted root number but did not affect rooting percentage of *E. saligna*. A positive effect of glucose on cutting rhizogenesis was found if this hexose was supplied during the root induction phase, followed by sucrose in the root formation step, especially for *E. globulus*. The same effect was not seen with fructose. The beneficial effect of glucose in the induction phase on root number was also evident under suboptimal auxin concentrations.

Introduction

Adventitious rooting is an essential step in the vegetative propagation of trees, in order to multiply selected genotypes. This developmental process may be divided in two main phases: (1) induction, corresponding to the molecular and biochemical events prior to any visible morphological change, and (2) formation, comprising cell divisions involved in root meristem organization and radical primordia establishment, followed by root elongation and emergence (Fett-Neto et al. 2001). These last events are sometimes treated as a third phase named root expression.

Auxins seem to be the main class of phytohormones involved in adventitious rooting (De Klerk et al. 1999). Other biotic or abiotic factors often modulate auxin metabolism, transport and perception. In spite of the central role of auxins, the importance of a number of other rooting factors,
such as carbohydrates, nutrition and light, cannot be underestimated (Kevers et al. 1997; Bennett et al. 2003).

Light is a major environmental factor in the life of a plant. It is not only the energy source for photosynthesis, but also a fundamental regulatory factor in development. Carbohydrates are necessary as metabolic “building blocks” and energy source for plant tissues. The availability of carbohydrates is often considered exclusively as an energetic requirement and carbon skeleton source to drive root development. However, it has been shown that sugars can have an important regulatory role, repressing the transcription of photosynthetic genes (Sheen 1990) and interacting with abscisic acid and ethylene signaling (León and Sheen 2003). Besides, the ratio between glucose and sucrose concentrations have influence on morphogenesis, affecting cell division rates (Borisjuk et al. 1998). Effects of carbohydrates concentrations and types on rooting of apple have been reported (Pawlicki and Welander 1995; Calamar and De Klerk 2002).

The physiological status of the donor-plant is of considerable importance, especially in ex vitro assays. The influence of donor-plant age (Wilson 1999), position of the cutting in the donor-plant (Wasnner and Ravetta 2000) and shading/etiolating treatments (Benz and Midmore 1996; Wilson 1998) have been analyzed in relation to adventitious rooting. The maintenance of cuttings in the dark during the first days of the rooting treatment increased the efficiency of the process for some woody species (McClelland et al. 1990). Rooting of *Eucalyptus grandis* ex vitro has been shown to be stimulated by low red : far red irradiance in donor plants and rooting success was associated with low pre-severance starch and water-soluble sugar concentrations, and a greater total water-soluble carbohydrate content per cutting (Hoad and Leakey 1996). Many of these factors, such as shading and light quality, seem to affect at least in part some aspect of phytohormone metabolism and transport (Morelli and Ruberti 2002). More investigations are required to examine the effect of carbohydrate status of donor-plants on the rooting of cuttings.

In the present work, the effects of carbohydrate supply to donor plants and carbohydrate supply and composition available to cuttings derived therefrom have been examined in relation to the adventitious rooting response of *Eucalyptus saligna* Smith, an easy-to-root species, and the recalcitrant *Eucalyptus globulus* Labill. Both species are of interest to the cellulose pulp and paper industry in southern Brazil. Interaction among analyzed factors (carbohydrates, irradiance) and exogenous IBA (indol-butyric acid) was also considered. In addition to help establishing more efficient rooting protocols for the propagation of recalcitrant clones for industrial use, this work also aimed at characterizing the relative importance and interactions between carbohydrate source and availability with the amount of irradiance for rooting *in vitro*-grown eucalypts with different recalcitrance degrees.

**Material and methods**

**Plant material**

Seeds of *E. globulus* and *E. saligna* were kindly provided by Teotônio F de Assis (Aracruz Celulose, Guaíba, RS, Brazil). They were washed in distilled water, surface sterilized in 70% (v/v) ethanol for 1 min and 2.5% (v/v) NaClO (with a few drops of neutral detergent) for 15 min with constant stirring, followed by four washes in sterile distilled water. About 16 seeds were sown in 250 ml glass jars containing 40 ml MS (Murashige and Skoog 1962) germination medium (Table 1); jars were capped with aluminum foil and kept at 25 ± 2 °C and 16 h photoperiod (30 μmol m⁻² s⁻¹ of photosynthetically active radiation). After germination, seedlings were grown for 3 months (when needed, pre-treatment was applied during the last month). Explants used in the rooting experiments were approximately 3 cm long epicotyl segments, containing the meristematic apex. All inoculations were performed aseptically in a laminar flow hood.

**Culture conditions**

Culture flasks were 20 ml vials with 6 ml medium (2 cuttings per flask), covered with a double layer of aluminum foil. Microcuttings were placed on an induction medium (Table 1) for 4 days; they were then transferred to formation medium (Table 1),