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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 (Borisjiuk 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 (Wassner 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 ($30\ \mu mol\ m^{-2}\ s^{-1}$ 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),

Table 1. Composition of culture medium in each step of the experiments.

Germination	Induction	Formation
6 g/l agar	6 g/l agar	6 g/l agar
58.67 mM sucrose (or 0 mM)	88 mM sucrose (glucose at 44, 88, or 166.24 mM; fructose at 88 mM)	88 mM sucrose (or sucrose at 46.58 mM, or fructose at 88 mM)
MS salts 0.5×	MS salts 0.3×	MS salts 0.3×
	0.5 g/l *myo*-inositol	0.5 g/l *myo*-inositol
	0.4 mg/l thiamine	0.4 mg/l thiamine
	10 mg/l IBA (or 0 mg/l)	1 g/l activated charcoal

Treatments in brackets refer to changes from the standard protocol tested in this study.

devoid of auxin, which is inhibitory to root growth (De Klerk et al. 1997). Starting at the induction phase, flasks were kept in the dark at 25 ± 2 °C. Treatments that included light in the root formation step are specified in the text. All reagents were analytical grade and the media were prepared with distilled water, followed by autoclaving at 121 °C and 0.11 MPa for 20 min; pH was set with NaOH and HCl to 5.8 prior to autoclaving.

Effects of irradiance and sucrose availability to donor-plants

Glass jars with 2 month-old plants grown in presence or absence of sucrose were exposed to 0, 30 or 60 μmol m^{-2} s^{-1} of PAR (Photosynthetically Active Radiation) for 1 month, before excision of microcuttings. Cuttings from plants in each combination of light and sugar conditions were rooted with or without exogenous IBA.

Use of different carbohydrates as carbon source in the rooting of cuttings

To analyze if carbohydrates have any regulatory function in the rooting of the *Eucalyptus* species, microcuttings were excised from 3 months-old mother-plants grown with sucrose and rooted in induction/formation media with the following combinations of sugars (equimolar concentrations: 88 mM), respectively: sucrose/sucrose (control), glucose/glucose, sucrose/glucose, glucose/sucrose. The same combinations and concentrations were also examined with fructose/sucrose in *E. globulus* (the species most responsive to carbohydrates) cuttings to verify if the response was the same to another hexose. All induction media had exogenous IBA (10 mg/l) in their composition.

In a second set of experiments carried out with *E. globulus*, a gradient of carbohydrates concentrations was tested in relation to the rooting response in six combinations: induction with 44, 88 or 166.24 mM glucose and formation with 46.58 or 88 mM sucrose.

In a third set of assays, the rooting response of *E. globulus* cuttings to the carbohydrate compositions sucrose/sucrose and glucose/sucrose (all at 88 mM) in the induction/formation phase were examined in the absence of auxin, with 1 or 10 mg/l of IBA. In addition, the same carbohydrate exposure sequences were carried out in the presence of 3 mg/l of kinetin, a well known rooting inhibitor, in the induction step both in the absence of auxin and with 10 mg/l IBA.

For the second and third set of experiments, as well as for the fructose assays, modifications in relation to the initial set of experiments were done to maximize explant availability. Seedlings used as cutting donors had approximately 4 months of age and cuttings were made from several segments of each seedling shoot, not only from the apical portion. The presence or absence of the apical meristem in the tip portion of seedlings was shown not to significantly affect the rooting response of cuttings derived therefrom exposed to exogenous auxin (Fogaça and Fett-Neto, unpublished). However, in order to avoid topophysis effects, cuttings from different parts of seedlings were evenly distributed among treatments.

Measurements

Parameters analyzed were percent rooting, mean number of roots per rooted cutting (root number)

and mean length of longest root (root length), which were measured 20 days after transferring to formation medium.

To calculate mean rooting time, cuttings were evaluated every 2 days in a binary fashion (rooted or non rooted). Rooting criterion was the presence of at least one visible whitish polar cylindrical structure of approximately 2 mm in length.

Statistical analyses

For percent rooting a Welch Analysis of Variance was applied, complemented by Dunnett-C test. This test was chosen because of lack of normality of this parameter, a pre-requisite of the classic Analysis of Variance (ANOVA).

Two-way analysis of variance followed by Duncan test was used for root density and root length. Data were transformed with square root or log when necessary to achieve normal distribution. Single effects are cited only when there are no interactive effects among the factors.

Mean rooting time, their standard errors and confidence intervals were calculated based on the concept of mean time of germination (Labouriau and Osborn 1984; Fett-Neto et al. 2001). Significance level ($p \leq 0.05$) was the same for all experiments.

Experiments with *E. saligna* and *E. globulus* were performed separately. The number of samples varied among the experiments; 20 replicates per treatment were used for experiments with donor plants and 30 replicates per treatment, for the sugar source experiments. All experiments were independently carried out at least twice, with similar results.

Results

No microcutting, in any condition, rooted before transference to formation medium. All samples, with the exception of occasional contaminated vials, were computed for percent rooting calculation; however, non-rooted cuttings were not considered for the other measurements. There was no prominent callus formation in any cutting. Some roots emerged from clusters on leaves, but these were not considered in the analyses. Generally, *E. saligna* cuttings developed more roots than *E. globulus*; however, the latter developed longer roots. Only individual effects are shown in the graphics, because interactions among the factors were frequent. Single effects are specified in the text, when present. There was no significant difference among treatments applied to donor plants within each species for mean rooting time and longest root length.

Sucrose and irradiance as pre-treatments

In *E. globulus*, no rooting was observed in microcuttings taken from plantlets exposed to a 4-week dark pre-treatment. The presence of sugar in the donor plant medium (germination medium) yielded plantlets with diverse morphology compared to plantlets without sucrose. The former had longer and darker leaves with more trichomes and a robust stem. Sugar removal from the donor plant medium caused a trend toward higher rooting percentage in the cuttings derived from them, particularly in the absence of auxin in the induction step, as revealed by a comparison between "aa" vs. "pa" treatments and "ap" vs. "pp" treatments within each irradiance level (Figure 1a). Auxin promoted root density (Figure 1b). At 60 μmol m^{-2} s^{-1} there was a trend toward root density increase with sugar removal in *E. globulus*, particularly in the presence, but also in the absence of auxin in the root induction medium, which was not observed at 30 μmol m^{-2} s^{-1}; at this irradiance, sugar removal caused a slight reduction in root density in the presence of auxin (Figure 1b). Taking together treatments with the same mother plant carbohydrate condition at both irradiance levels, a clear beneficial effect of sugar removal on percent rooting can be observed.

In *E. saligna*, some rooting was observed with dark pre-treatment as long as sucrose was present in the donor plant medium, and percent rooting was not affected by carbohydrate availability to mother plants (Figure 2a). Root density increased with sugar as pre-treatment at 30 μmol m^{-2} s^{-1} and a trend for increase was also observed at 60 μmol m^{-2} s^{-1} (Figure 2b, compare as done for Figure 1a). Shorter dark exposure times for donor plants were also tested with both species, but none was advantageous for rooting (data not shown).

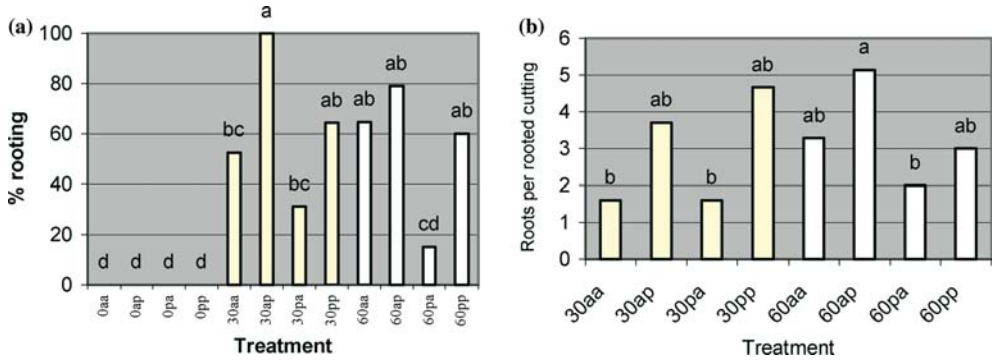

Figure 1. Effect of light and sucrose pre-treatments of donor plants of *Eucalyptus globulus* on the adventitious rooting of cuttings in presence or absence of exogenous auxin. Legend code: a – absent, p – present; number: light intensity provided to donor plants in μmol m^{-2} s^{-1}; first letter: sucrose available to donor plant; second letter: exogenous IBA in the induction phase. (a) percent rooting; (b) root density. Columns sharing a letter are not significantly different by a Duncan test or Dunnett-C ($p \leq 0.05$).

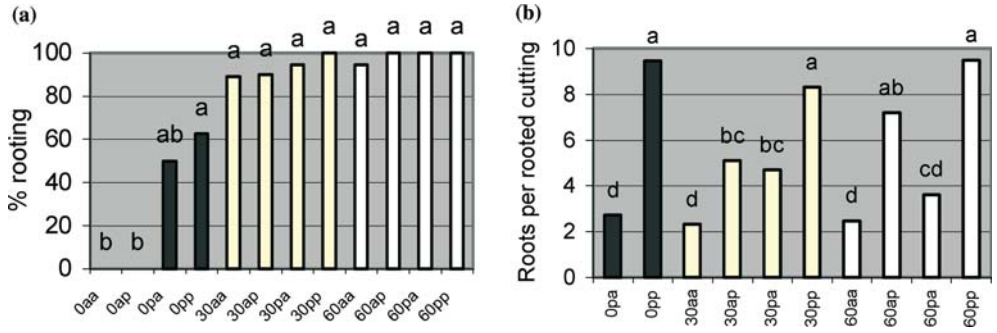

Figure 2. Effects of light and sucrose pre-treatments on donor plants of *Eucalyptus saligna* on the adventitious rooting of cuttings in presence or absence of exogenous auxin. Legend code: as in Figure 1.

Effects of different carbohydrates on cuttings

In both species, application of glucose during the induction phase had a clear beneficial effect on root elongation, compared to the control with continuous sucrose and the other sugar combinations. In *E. globulus*, although the difference was not statistically significant compared to continuous glucose, roots were approximately 40% longer in the glucose in induction step followed by sucrose in the formation step treatment (Figure 3c). When compared to the continuous sucrose control, root length was approximately two-fold higher in the glucose followed by sucrose treatment. Besides root length, root density was also significantly improved with glucose in induction (2.5-fold over the sucrose control) (Figure 3b). Mean rooting time was also significantly reduced for *E. globulus* when glucose was used in the induction phase followed by sucrose in the formation step (Figure 3d). Continuous glucose was the second best treatment for the rooting parameters considered, although in most cases it had a performance equivalent to the control with continuous sucrose and to sucrose in induction phase, followed by glucose in the formation step (Figure 3). Root length was also approximately doubled in *E. saligna* with glucose exposure during the induction phase compared to the continuous sucrose control, whereas other parameters were not affected (Figure 4).

Because *E. globulus* rooting was more significantly improved by the glucose/sucrose combination during the root induction and formation phases respectively, additional experiments were carried out to verify the existence of a dose response between carbohydrates and rooting. In these experiments the overall rooting response was

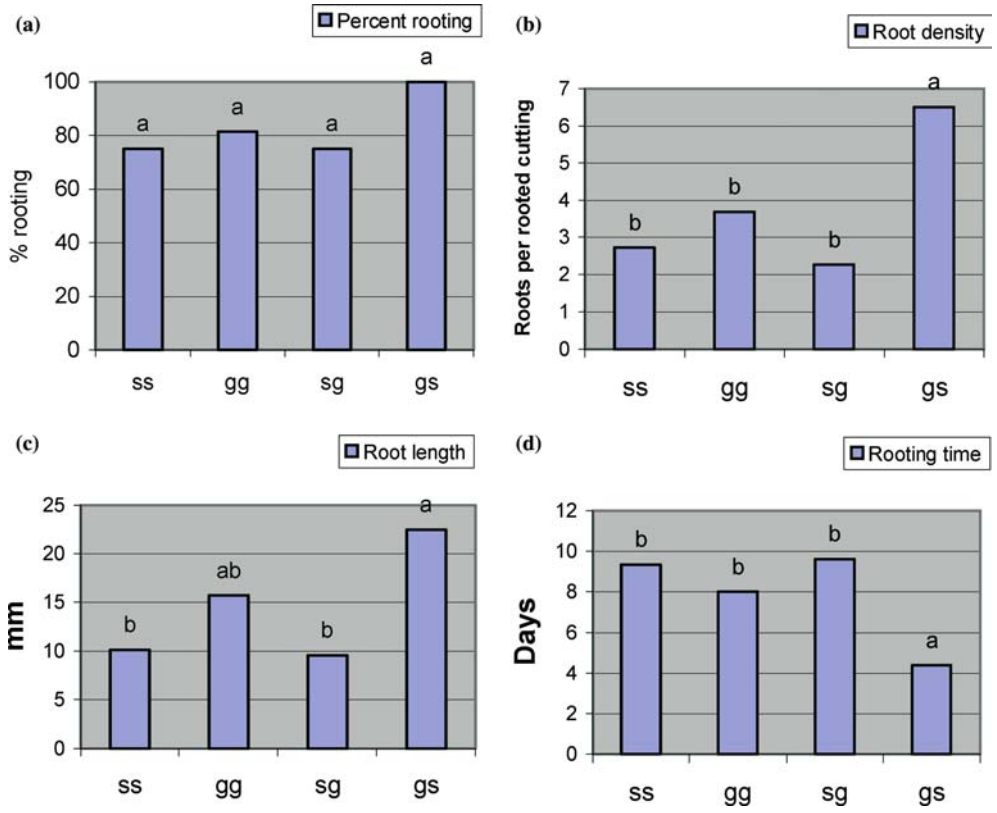

Figure 3. Effects of carbohydrate sources available at the rooting phases on the rooting response of cuttings of *E. globulus*. ss = sucrose on both phases, gg = glucose on both phases, sg = sucrose in induction and glucose in formation, gs = glucose in induction and sucrose in formation. (a) percent rooting; (b) root density; (c) mean length of longest root; (d) mean rooting time. Columns sharing a letter are not significantly different by a Duncan test or Dunnett-C ($p \leq 0.05$).

lower than in the previous ones, possibly due to the older seedlings used as cutting donor and to the inclusion of non-tip segments for cutting preparation (Figure 5). Increase in rooting percentage as glucose concentration increased from 44 to 166.24 mM was not significant (Figure 5a). Root density per cutting did not show a clear response (Figure 5b), whereas root length increase in cuttings grown in 88 mM sucrose during root formation phase compared to those cultivated at 46.58 mM was not significant (Figure 5c). The lack of significance may be due to the small sample size. Significant differences could be noted by analysing the single effect of glucose (non-interactive effect by ANOVA test) on percent rooting (Figure 5d), which showed a significant difference between 44 and 166.24 mM, but not between the latter and 88 mM. The single effect of sucrose on root length (Figure 5e) was significantly higher at 88 mM compared to 46.58 mM.

The root induction/formation carbohydrate sequence suc/suc vs. glc/suc was analyzed at three concentrations of exogenous IBA (0, 1 and 10 mg/l) for *E. globulus*. A trend toward higher rooting percentages and higher root density per rooted cutting was observed for all IBA concentrations when cuttings were cultured under the glc/suc sequence compared to suc/suc (data not shown). Root number per rooted cutting most significant increase was observed in the absence of exogenous IBA. On the other hand, root length showed a trend toward reduction as IBA concentration in the induction phase increased, a situation observed in both carbohydrate exposure sequences. In all carbohydrate regimens, the exposure of cuttings to kinetin at 3 mg/l during the induction step with or without 10 mg/l IBA severely inhibited rooting (maximum rooting reached only 12%).

The use of fructose instead of glucose in the combination with sucrose during the *E. globulus*

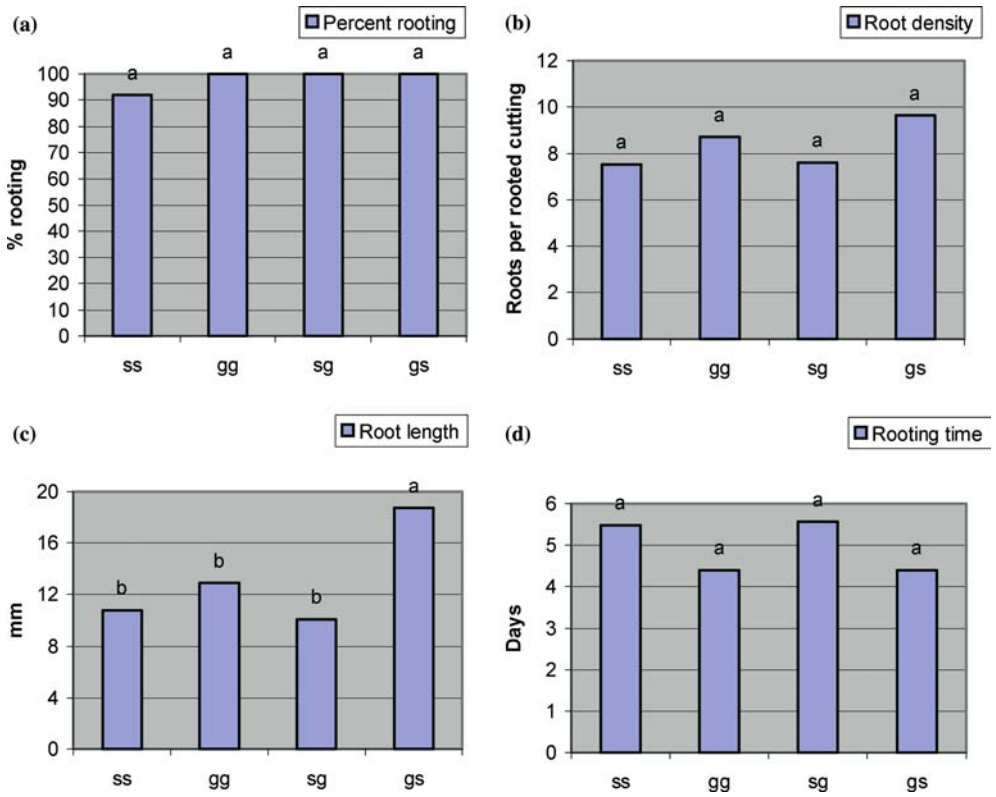

Figure 4. Effects of carbohydrate sources available at the rooting phases on the rooting response of cuttings of *E. saligna*. Legend: as in Figure 3.

root induction/formation steps at equimolar amounts (88 mM) did not result in any significant rooting response compared to the suc/suc control. However, roots tended to be longer when fructose was used as carbon source during the formation phase. Problems with agar solidification were observed in fructose containing media, although the pH was at values equivalent to the sucrose based media at the end of the experiment (approximately 5).

Discussion

The overall percent rooting of *E. globulus* cuttings used in the first series of experiments was relatively higher than previously reported (Fett-Neto et al. 2001), a fact that is probably related to the different origin of the seeds, because all other aspects of the culture and experimental conditions were exactly the same. In fact, cuttings derived from the same batch of seedlings with 3.5 or 4 months of age displayed much lower rooting. Therefore, seedlings derived from the batch of seeds used at least in the first part of the present experiments required more time to fully lose adventitious rooting capacity. Nonetheless, the relative rooting recalcitrance of *E. globulus* compared to *E. saligna* is evident by all rooting parameters.

Some general patterns can be observed throughout the experiments. First, auxin application promoted rhizogenesis in all situations, raising the percent rooting and root density, mainly in *E. globulus*; this is in agreement with previous reports (Kevers et al. 1997; De Klerk et al. 1999; Ludwig-Müller 2000; Fett-Neto et al. 2001). Auxin application partially reversed the negative effects of keeping mother-plants for 4 weeks in the dark, suggesting that the inhibition of the shikimate pathway operation and indole biosynthesis by this condition brought auxin content down to inadequate amounts (Kevers et al. 1997).

The effect of poor rooting of cuttings after dark pre-treatment of seedlings may have reflected the lower vitality associated with that condition,

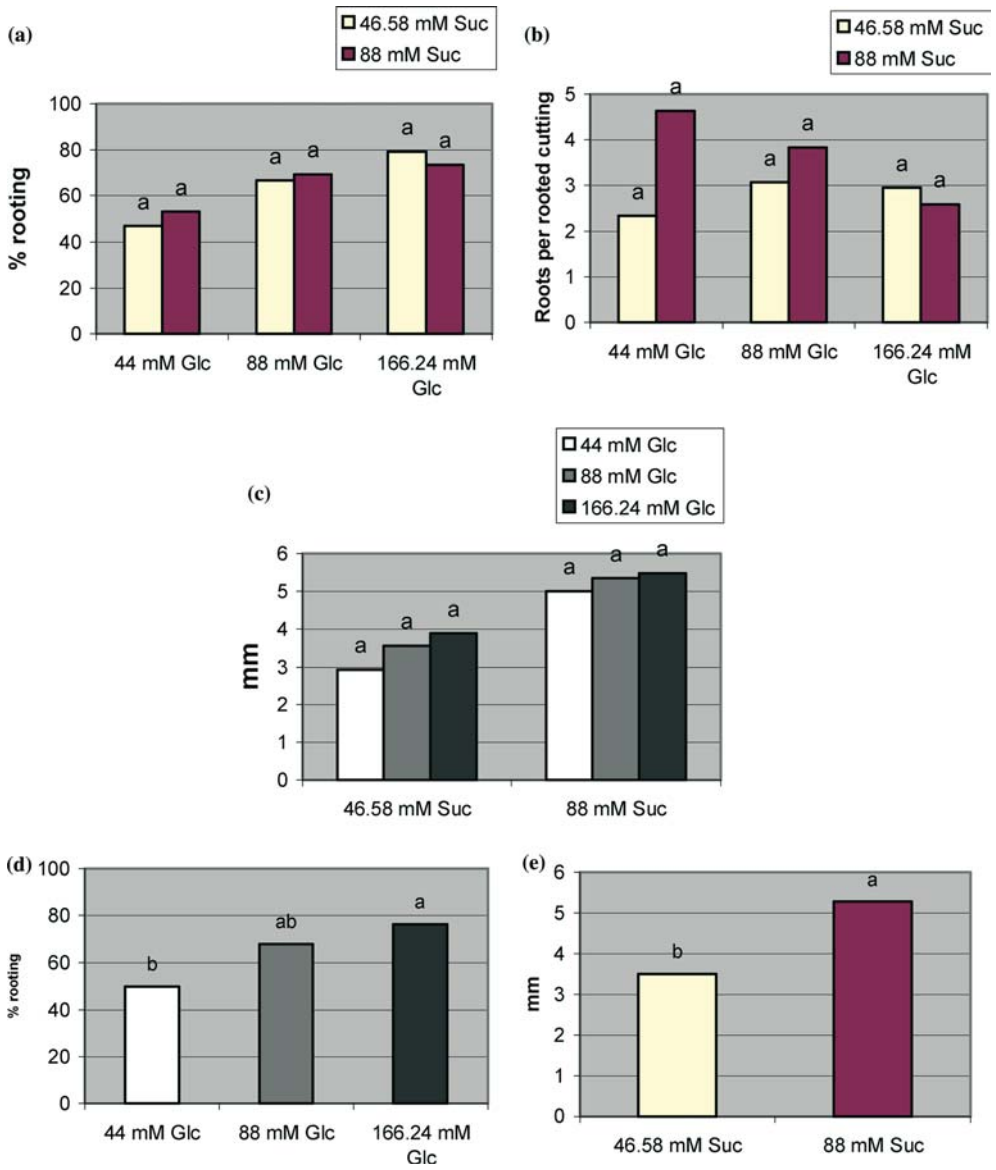

Figure 5. Effect of carbohydrate doses on the rooting response of cuttings of *E. globulus*. Glucose concentrations refer to the rooting induction phase and sucrose, to formation phase. (a) percent rooting, individual effects; (b) root density, individual effects; (c) mean length of longest root, individual effects; (d) percent rooting, glucose's single effect; (e) root length, sucrose's single effect. Columns sharinge a letter are not significantly different by Analyses of Variance followed by a Duncan test ($p \leq 0.05$).

characterizing an indirect effect of light as a rooting factor. However, the maintenance of the low rooting response with sucrose supplementation in the dark, the lack of beneficial effects of shorter dark periods (for example, 1 week) to the rooting response, the fact that seedlings survived the dark treatment and the species-related differences observed at the two irradiance levels used suggest that light treatments in mother plants actually can play a regulatory role in the rooting of *Eucalyptus* species.

Sucrose supplementation was more positive when plantlets were kept for longer periods in the dark. Because of photosynthate depletion, the importance of sucrose as a carbon source was probably magnified. However, when examining

cuttings derived from plantlets grown under light treatments, it can be seen that percent rooting in *E. globulus* was favored by lack of sucrose in donor-plant culture media. Sucrose can inhibit the expression of several photosynthetic genes and photoautotrophic metabolism (Sheen 1990). The phenotypical differences observed between *E. globulus* plantlets grown in the presence and absence of sucrose suggest a delay in the development of mother-plants in the latter case; this would imply in a longer ''juvenile'' state, more responsive to rooting treatments (Ford et al. 2002).

The analysis of experiments involving modification of irradiance levels indicated only a moderate effect of light treatments on donor plants in subsequent rooting responses. Increase in photosynthesis, light dependent metabolic pathways (*e.g.* shikimate derivatives), water, nutrient and auxin uptake and endogenous transport could be responsible for differences in rooting response among the irradiance level treatments.

The basal concentration of sucrose in the control medium of the present experiments was 3% (w/v), chosen on the basis of preliminary assays. Sucrose concentrations in the range of 2–3% have been shown to be beneficial for the rooting of *Eucalyptus sideroxylon* using a one-step medium protocol, avoiding excess callus formation and detrimental effects on explants (Cheng et al. 1992). Beneficial effects of exogenous glucose in rooting media were reported for apple (Moncousin et al. 1992), although rooting performance was not significantly improved compared to equimolar amounts of sucrose. Application of glucose instead of sucrose in induction phase and maintenance of sucrose in the formation step promoted root elongation in both species and root density in *E. globulus*; reduced mean rooting time in *E. globulus* was also observed.

Considering that equimolar quantities (and not equal weights) of both sugars were used in the treatments, the better performance of rooting with the use of sucrose in the formation phase could be explained by the larger carbon skeleton provided by this carbohydrate, resulting in higher availability of biosynthetic building blocks. This observation is further supported by the better response of root length with 88 mM sucrose in the formation step instead of 46.58 mM.

Glucose is an important signaling molecule for abscisic acid and ethylene and indirectly for auxin, due to interactions of these phytohormones (León and Sheen 2003); however, this may not be the main effect of this carbohydrate, because the glucose effects were also seen in cuttings that had exogenous IBA supply (which is a strongly active auxin for root induction) in an adequate concentration for these species rooting (Fett-Neto et al. 2001). Nonetheless, an effect of glucose on auxin synthesis/response cannot be ruled out. The antagonistic relationship between glucose and ethylene signaling could have helped root development (De Klerk et al. 1999; Sheen et al. 1999); however, the importance of ethylene in eucalypt adventitious rooting was not supported by experiments using an inhibitor of ethylene action in the induction phase (Fogaça and Fett-Neto, 2004, unpublished results).

Borisjuk et al. (1998) found a positive effect of high hexose to sucrose ratio in cell division of *Vicia faba* L. cotyledons. The presence of glucose in the induction phase may cause more cells to be recruited for root induction (*via* dedifferentiation and mitosis), improving the rooting response. This fact may also explain the effectiveness of glucose in the induction step in promoting root development even in the absence or at suboptimal exogenous auxin concentrations, compared to cuttings supplied with sucrose throughout the rooting process under the same auxin conditions. The stimulatory rooting effect of glucose availability during the induction phase, however, was obliterated by citokinin present in the same phase of the process, a period in which this type of phytohormone is particularly inhibitory to rooting (DeKlerk et al. 1999). Positive effects of glucose on the rooting of leaf disks of apple have been related to faster and effective metabolization of this carbohydrate compared to sucrose (Pawlicki and Welander 1995). The dose-dependence of the rooting percent response with different glucose concentrations in the induction step may reflect this readily available carbon demand. The more pronounced rooting response of *Eucalyptus globulus* to the presence of glucose in the induction step compared to *E. saligna* may reflect specific differences related to the rooting recalcitrance of the former species, perhaps based on differences related to sugar metabolism and sensing or interactions between sugar and phytohormone

signaling networks. As carbohydrates can be involved in many complex intracellular events, they may also influence other components of the auxin-induced adventitious rooting cascade, such as secondary messengers for the process, *e.g.* nitric oxide (NO), cGMP, Mitogen-Activated Protein Kinases (MAPKs) (Pagnussat et al. 2004).

The fact that fructose was unable to replace glucose in the stimulation of *E. globulus* rooting indicates that the response is not universal to all hexoses. As fructose is one of the breakdown products of sucrose, it is an obvious candidate to be evaluated. The problem of agar softening by fructose had already been described for other cultures and cannot be ruled out as factor in the rooting response; other possibility for the lack of stimulatory effect of fructose could be the phytotoxicity of furfural formed from fructose after medium autoclaving (Moncousin et al. 1992). Apple stem discs exposed to fructose formed less roots than discs treated with sucrose or glucose, regardless of concentration, under continuous exposure (Pawlicki and Welander 1995). Nevertheless, it will be interesting to examine combinations of glucose and fructose in a different experimental system, using liquid medium and filter-sterilized carbohydrates.

In conclusion, this study showed that mother-plant sugar availability can affect the adventitious rooting response of *Eucalyptus*. In the rooting recalcitrant *E. globulus*, absence of exogenous sugar and presence of irradiance in donor plants promoted adventitious rooting, whereas in the easy-to-root *E. saligna* exogenous sugar for donor plants was beneficial and irradiance level to these plants had no major effect on subsequent rooting. Different kinds of carbon sources can modulate rooting ability of eucalypt microcuttings, and this effect is strongly dependent of the rooting phase. A sequence of glucose in the induction phase and sucrose in the formation phase was beneficial for root number per rooted cutting, root length and rooting speed of *E. globulus*; the same condition was also able to promote root length in *E. saligna*. The positive effects of glucose in the induction phase seem to show a dose-response in the rooting of *E. globulus*; such effects are also evident in the absence of exogenous auxin or at suboptimal concentrations, but apparently are not maintained by replacing glucose with fructose.

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