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ORIGINAL PAPER

Pre and post-severance effects of light quality on carbohydrate dynamics and microcutting adventitious rooting of two *Eucalyptus* species of contrasting recalcitrance

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Abstract Adventitious rooting is a complex developmental response affected by genetic and environmental factors. Radiation quality effects on adventitious rooting depend on characteristics such as species, growth stage, irradiance, spectral quality, and time of exposure. *Eucalyptus* is an essential genus for the paper industry, and high yield plantations depend on adventitious rooting of selected genotypes. This work addressed two hypotheses: (1) radiation quality equally affects adventitious rooting in *Eucalyptus* species of different recalcitrance; (2) adventitious rooting outcome depends on both donor plant and cutting radiation quality treatments. To that end, the easy-to-root *Eucalyptus grandis* and the recalcitrant *Eucalyptus globulus* were evaluated. The effect of white, blue, red and far-red radiation enrichment on microcuttings and donor plants of both species was evaluated in relation to rooting. There was no effect of radiation quality on adventitious rooting of *E. grandis* or when radiation treatments were applied to *E. globulus* microcuttings. In contrast, donor plants of *E. globulus*, grown in medium devoid of sucrose and exposed to far-red radiation, yielded microcuttings showing higher rooting percentage, even in the absence of exogenous auxin in the rooting medium. Sucrose in donor plant medium abolished the positive effect of far-red radiation. An increase in endogenous soluble sugars and starch contents in basal microcuttings was associated with far-red radiation treatment of donor plants. These results underline the importance of appropriate carbohydrate partitioning in donor plants for adventitious rooting of cuttings and provide a basis for understanding and overcoming rooting recalcitrance in *E. globulus* clones.

Keywords Adventitious rooting · Carbohydrates · Donor plant · *Eucalyptus* · Radiation quality

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Introduction

Eucalyptus is one of the most widely cultivated tree genera in the world due to its high adaptation ability to different environments (Eldridge et al. 1993). Most plantations are established to provide pulp for paper (Turnbull 1999). Brazil is one of the largest producers of eucalypt pulp and its plantations are based on the vegetative propagation of selected elite genotypes with high productivity (Mora and Garcia 2000; Schwambach et al. 2008).

Adventitious rooting is an essential step in the vegetative propagation of trees and may be divided in two main phases: (1) induction, corresponding to the molecular and biochemical events prior to any visible morphological changes, and (2) formation, comprising cell divisions involved in root meristem organization and radical primordia establishment, followed by root elongation and emergence (De Klerk et al. 1999; Fett-Neto et al. 2001; De Klerk 2002). This complex developmental process can be affected by internal and

external factors including radiation, temperature, hormones and sugars (Ruedell et al. 2009).

Radiation is a fundamental regulatory signal in plant development. Plants have evolved complex methods of sensing quality, quantity, direction and duration of radiation, and interpreting these signals to produce the appropriate physiological and developmental response (Möller et al. 2002; Montgomery and Lagarias 2002). Radiation quality can have major impacts on plant development, as exemplified by leaf morphology changes in the medicinal plant *Alternathera brasiliana* grown in vitro under different radiation environments (Macedo et al. 2011). Although previous results have described morphological and physiological effects of radiation on rooting, responses vary according to plant species, quantity or quality of radiation and if the exposure was done in donor plants or during the rooting process (Hansen et al. 1978; Chée and Pool 1989; Fuernkranz et al. 1990; Saebo et al. 1995; Baraldi et al. 1988; Fett-Neto et al. 2001; Antonopoulou et al. 2004; Corrêa et al. 2005). Some studies in *Eucalyptus* show that radiation could affect rooting in different ways. Low irradiances seem to favor rooting of stem cuttings in *Eucalyptus globulus* in greenhouse assays (Wilson 1998), and the combination of exposure of microcuttings to IBA (indole butyric acid), followed by culture in darkness, also had positive effects (Fett-Neto et al. 2001). In contrast, exposure of donor plants of *E. globulus* to dark periods was detrimental to rooting (Corrêa et al. 2005). Different radiation intensities supplied to donor plants of *E. globulus* and *Eucalyptus saligna* had limited influence on rooting of microcuttings derived therefrom (Corrêa et al. 2005), whereas high irradiance induced longer roots, but decreased percent rooting when applied to microcuttings of *E. saligna* (Fogaça and Fett-Neto 2005). Despite studies analyzing radiation quantity in adventitious root formation of *Eucalyptus*, radiation quality effects were poorly investigated for this genus and other tree species.

Carbohydrates are key mobile providers of both energy and carbon used to synthesize all other essential constituents needed for adventitious rooting (Druege 2009). Carbohydrates can also act as signaling molecules and regulate many developmental processes in plants, including the formation of roots (Roland and Sheen 2005; Corrêa et al. 2005; Roland et al. 2006; Altamura and Falasca 2009). The presence of sugar in the culture medium was determinant for root development in apple and *Arabidopsis* (Pawlicki and Welander 1995; Takahashi et al. 2003; Calamar and De Klerk 2002). This was especially observed in the first 48 h for apple, when a dose–response curve with auxin, the main phytohormone involved in root induction, showed an interaction with carbohydrates (Calamar and De Klerk 2002). In addition, efficient utilization and partioning of carbohydrates, both of which can be modulated by radiation (Rapaka et al. 2005), can be crucial factors for adventitious rooting in cuttings (Haissig 1984; Friend et al. 1994; Druege 2009).

Auxin has a central role in determining rooting capacity (De Klerk et al. 1999), and radiation conditions affect auxin metabolism and tissue sensitivity (Reid et al. 1991). Polar auxin transport can be modulated by flavonoids through direct and indirect interactions with cellular transport and regulatory mechanisms (Murphy et al. 2000; Brown et al. 2001; Peer and Murphy 2007) and the biosynthesis of these compounds is also dependent on environmental conditions, such as radiation and temperature (Quattrocchio et al. 2006). An interaction between glucose and auxin signaling controlling primary and lateral root growth and development was shown in *Arabidopsis* (Mishra et al. 2009). Since radiation can affect the content of carbohydrates, auxins and their interactions, the manipulation of this factor represents a potential means of improving adventitious rooting of cuttings.

To address whether radiation quality can significantly regulate rooting in eucalypts, the effects of environmental enrichment with different radiation qualities on donor plants from which cuttings were obtained and during cutting adventitious rooting of two commercially relevant *Eucalyptus* species were analyzed. Two hypotheses were tested in this work: (1) radiation quality equally affects adventitious rooting in *Eucalyptus* species of different recalcitrance; (2) adventitious rooting outcome depends on both donor plant and cutting radiation quality treatments. The easy-to-root *Eucalyptus grandis* is one of the most common eucalypts planted in Brazil (Canettieri et al. 2007), and the difficult-to-root *E. globulus* (Le Roux and Van Staden 1991; Serrano et al. 1996) has characteristics of interest for the pulp industry in southern Brazil, such as relative frost resistance and low lignin content, facilitating cellulose extraction (Chiang 2002; Bison et al. 2007). Interactions between radiation quality and sucrose supplied to donor plants and the internal content of soluble carbohydrates, starch, and flavonoids were also analyzed during the rooting process.

Materials and methods

Plant material

Seeds of *E. grandis* and *E. globulus* (kindly provided by Fibria, former Aracruz Celulose, Guaíba, RS, Brazil) were surface sterilized in 70 % (v/v) ethanol (1 min) and 1.5 % (v/v) NaOCl (15 min) with constant stirring, followed by four washes in sterile distilled water. About fifteen seeds were planted on 300 ml glass jars (capped with a double layer of aluminum foil or transparent plastic film in radiation experiments) containing 60 ml of germination medium with or without sucrose (Table 1) and kept at 25 ± 2 °C and 16 h

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

Germination	Root induction	Root formation
6 g/l agar	6 g/l agar	6 g/l agar
58.67 mM sucrose (or 0 mM)	88 mM sucrose	88 mM sucrose
MS[a] salts 0.5X	MS[a] salts 0.3X	MS[a] salts 0.3X
	100 mg/l myo-inositol	100 mg/l myo-inositol
	0.4 mg/l thiamine. HCl	0.4 mg/l thiamine. HCl
	10 mg/l IBA (or 0 mg/l)	1 g/l activated charcoal

[a] Murashige and Skoog (1962)

photoperiod (45 μmol m^2/s). After 3.5 and 4 months for *E. globulus* and *E. grandis*, respectively, microcuttings (about 3-cm-long tip cuttings, containing the meristematic apex) were excised from seedlings and used for in vitro rooting experiments. In donor plant assays, pre-treatments were applied during the last month of seedling growth.

Culture conditions

Rooting experiments were carried out according to a two-step basal sequential medium protocol (Fett-Neto et al. 2001). Microcuttings were placed in an induction medium (Table 1) for 4 days and then transferred to formation medium (Table 1) for 20 days. Auxin concentration was defined in previous experiments after tests with various amounts of the phytohormone in culture media (Fett-Neto et al. 2001). Culture flasks were 20 ml vials with 6 ml medium covered with a double layer of aluminum foil or plastic film (in the radiation quality experiments) with 2 microcuttings per vial. All reagents were analytical grade and media were prepared with distilled water. Media were sterilized by autoclaving at 121 °C and 0.15 MPa for 20 min.

Experiments with radiation quality enrichments on microcuttings (post-severance)

Microcuttings excised from seedlings of *E. grandis* and *E. globulus* growing in germination medium with sucrose were exposed to white (control), blue, red or far-red radiation enrichment during the rooting period. Changes in spectral quality were provided by filtering the output of the white fluorescent tubes through double cellophane sheets (Lin and Yang 1999; Héraut-Bron et al. 2001). The transmittance spectra of the filters (Fig. 1) were measured and recorded in a Spectramax automated spectrophotometer (Molecular Devices, USA). Filters were replaced every 5 days to avoid minor changes in spectral quality of the transmitted radiation due to possible color fading. Microcuttings were harvested for biochemical analyses after 20 days in root formation medium. In the case of *E. globulus*, harvests and biochemical analyses were also done after 5 and 10 days in root formation medium. Microcuttings were kept in a growth room at 27 ± 2 °C and a 16 h photoperiod (45 μmol m^2/s). Radiation intensity was normalized for all treatments by adjusting the height of the shelves with the flasks closer or farther from the radiation source, as required, based on the readings of a portable radiometer (Li-Cor LI-250A, USA). Each experiment had 20 replicate explants per treatment and was independently repeated at least twice with similar results.

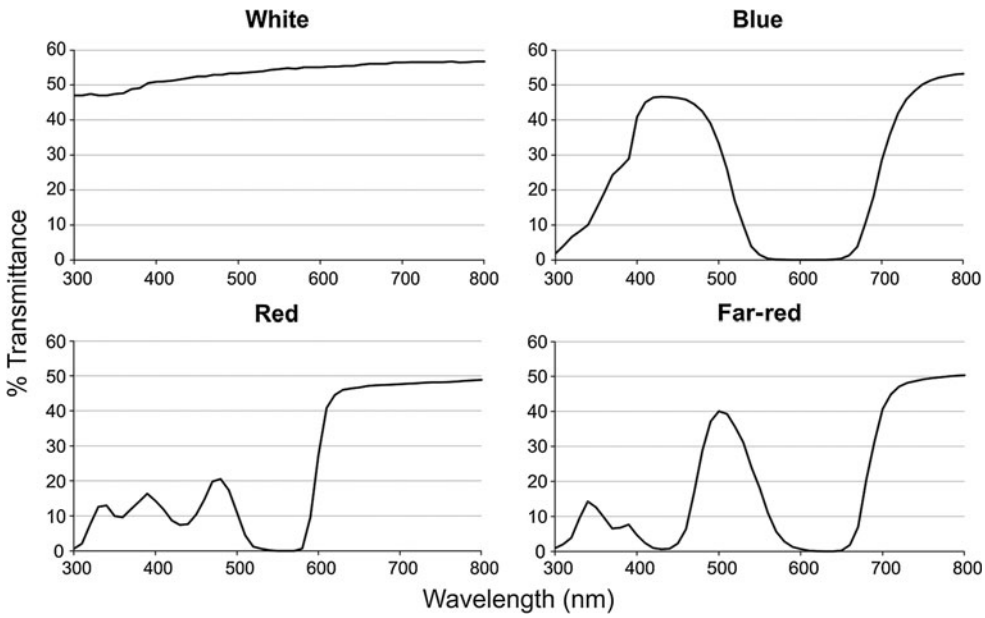

Fig. 1 Transmittance spectra of filters used to enrich for different colors

Experiments with radiation quality enrichments on donor plants (pre-severance)

Seedlings of *E. grandis* and *E. globulus* growing on germination medium without sucrose (and also with sucrose for *E. globulus*) under white radiation for 3 and 2.5 months respectively, were exposed to white (control), blue, red or far-red radiation enrichment for 1 additional month. Radiation intensity for all treatments was 45 µmol m^2/s and sources and filters were as described above (post-severance experiments). Donor plants were harvested for biochemical analyses upon the completion of the additional 1 month period of radiation enrichment treatments. Microcuttings obtained were used in rooting experiments with or without auxin in the induction medium and kept in a growth room at 25 ± 2 °C and 16 h photoperiod (45 µmol m^2/s) during the experiment. Each experiment had 20 explants per treatment and was independently repeated three times. Samples of shoots from donor plants without sucrose before and after radiation treatments, and shoots and roots of microcuttings obtained during the rooting formation were frozen and analyzed for the contents of total soluble sugars, starch and flavonoids (the last one only for *E. globulus*).

Total soluble sugars content

The extraction and quantification of soluble sugars was done according to Dubois et al. (1956), with minor modifications. Frozen samples of about 10 mg of fresh weight were homogenized in liquid nitrogen, extracted with 1 ml 80 % (v/v) ethanol and incubated in a water bath at 75 °C for 15 min. The extracts were centrifuged at 10,000*g* for 15 min and the supernatant was recovered. The pellets were re-extracted with 500 µl 80 % (v/v) ethanol. For quantification, 500 µl of the sample, diluted in the same proportion of 80 % ethanol, were mixed with 5 ml of concentrated sulfuric acid and 1 ml of freshly prepared 5 % (w/v) phenol. After agitation, solutions were maintained for 20 min at room temperature (25 ± 3 °C). The absorbance at 490 nm was measured in a Spectramax automated spectrophotometer (Molecular Devices, USA). The standard curve was established with D-glucose.

Starch content

The pellet obtained from the soluble sugars extraction was used for starch extraction as described by McCready et al. (1950), with minor modifications. The pellets were homogenized with 250 µl of distilled water and 320 µl of 52 % (v/v) perchloric acid, submitted to sonication in a water bath for 15 min and centrifuged at 10,000*g* for 15 min. The supernatant was collected and the pellet was re-extracted. For quantification, 500 µl of the extract reacted with 500 µl of freshly prepared anthrone reagent (10 mg anthrone + 5 ml 95 % (v/v) sulfuric acid); the resulting solution was mixed and kept in a boiling water bath for 10 min. After cooling, the absorbance at 630 nm was determined in a Spectramax automated spectrophotometer (Molecular Devices, USA). The standard curve was established with D-glucose in perchloric acid. The glucose content was multiplied by 0.90 to estimate the amount of starch. This procedure allows for the molecular weight of one molecule of water—18 g/mol—to be subtracted from the weight of each molecule of glucose—180 g/mol, thereby accounting for the removal of water during the covalent bonding of glucose molecules to form starch (Hall 2003).

Flavonoid content

The flavonoid content was determined by the aluminum chloride spectrophotometric method reported by Zhishen et al. (1999) with some modifications. Approximately 25 mg of frozen plant tissues were ground in liquid nitrogen, extracted in 1.25 ml EtOH 95 % and submitted to sonication in a water bath for 30 min in the dark. All of the following procedures were carried out under dim indirect light. The extracts were centrifuged at 12,000*g* for 10 min and the supernatant was collected. For quantification, 250 µl of extract was added to 1 ml of H_2O and 750 µl of $NaNO_2$ 5 % (w/v), mixed and then kept at 25 °C for 5 min. Next, 750 µl of $AlCl_3$ 1 % was added, mixed and then incubated at 25 °C for 6 min. Then, 500 µl of NaOH and 600 µl of H_2O were added and mixed. Reading was done at 510 nm in spectrophotometer. The standard curve was established with quercetin (Sigma, USA).

Plant measurements and statistical analyses

The morphological parameters analyzed were percent rooting, mean number of roots per rooted microcutting (root number), mean length of longest root (root length) and mean rooting time (as described by Fett-Neto et al. 2001). Data were measured 20 days after transferring to formation medium, except for the mean rooting time observations which were noted every 2 days after transferring to formation medium. Analyses of variance (ANOVA) followed by Duncan test when appropriate ($P \leq 0.05$) were performed for morphological parameters. Descriptive statistics (mean and standard error) were calculated and Student t tests were performed for the biochemical analyses. The Student t test ($P \leq 0.05$, unless noted otherwise) compared donor plant contents of carbohydrates of each radiation condition against that of white radiation control; shoot and root contents of carbohydrates and flavonoids within each radiation condition against that of white radiation control in microcuttings; shoot or root

content of carbohydrates and flavonoids of each radiation condition against that of white radiation control; and donor plants and microcuttings carbohydrate contents between *E. grandis* and *E. globulus*.

Results

Experiments with radiation quality enrichments on microcuttings (post-severance)

The different radiation treatments during the rooting period had no significant effect on adventitious rooting morphological parameters in microcuttings in both species (Fig. 2). The only differences observed in *E. globulus* were due to the presence or absence of auxin in the induction phase; microcuttings exposed to IBA had a higher percentage of rooting (Fig. 2b) and root number per cutting (overall mean ± SD: IBA = 5.67 ± 0.35; control = 1.58 ± 0.34) than the control without auxin.

Experiments with radiation quality enrichments on donor plants (pre-severance)

There was no effect of radiation quality enrichment on morphological parameters analyzed when treatments were applied to donor plants of *E. grandis* (Fig. 3a). Only a slight improvement in percent rooting of microcuttings was observed on the pre-severance experiments when compared with post-severance experiments, but without statistical significance (Figs. 2a, 3a).

Far-red radiation enrichment and absence of sucrose in donor plants had a strong positive effect on percent rooting of *E. globulus* microcuttings; microcuttings rooted without auxin in the induction medium derived from donor plants grown without sucrose under far-red enriched environment yielded 51 % of rooting (Fig. 3b). The combination of these two factors increased more than two-fold the ability of microcuttings to develop roots when compared with microcuttings derived from control treatment, which resulted in about 20 % rooting (Fig. 3b). The presence of exogenous sucrose in donor plant medium abolished this stimulatory response even after the far-red enrichment period (Fig. 3b). For all of the other morphological parameters evaluated, radiation treatment and/or exogenous sucrose availability had no significant effect. Moreover, blue and red radiation treatments on donor plants had no effect on rooting of microcuttings when compared with the control in white radiation (data not show). As previously reported (Fett-Neto et al. 2001), rooting percentage, root number and root length in the presence of IBA was higher for all radiation treatments in *E. globulus*, representing an auxin effect (Fig. 3b).

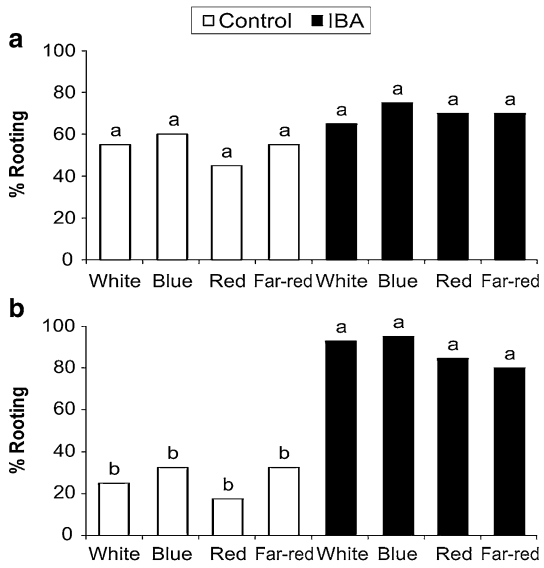

Fig. 2 Percent rooting of microcuttings of *E. grandis* (**a**) and *E. globulus* (**b**) submitted to treatments with different radiation quality enrichments with (*dark bars*) and without (*white bars*) transient exposure to auxin during the root induction step. Data taken after 20 days on root formation medium. Values with different letters are significantly different according to a Duncan test ($P \leq 0.05$)

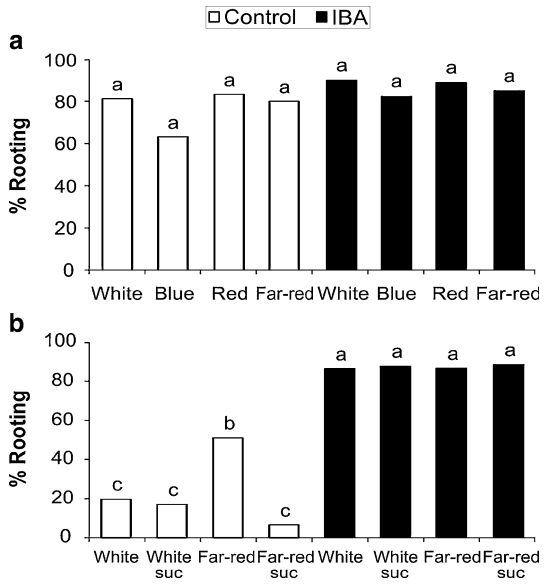

Fig. 3 Percent rooting of microcuttings of *E. grandis* (**a**) and *E. globulus* (**b**) excised from donor plants submitted to treatments with different radiation quality enrichments with (*dark bars*) and without (*white bars*) transient exposure to auxin during the root induction step. For *E. globulus* donor plants, presence or absence of sucrose in the culture medium was tested in presence or absence of far-red enrichment. Data was taken after 20 days on root formation medium. Values with different letters are significantly different according to a Duncan test ($P \leq 0.05$)

To better understand the improved adventitious rooting in microcuttings of *E. globulus* derived from donor plants exposed to far-red radiation enrichment, the content of

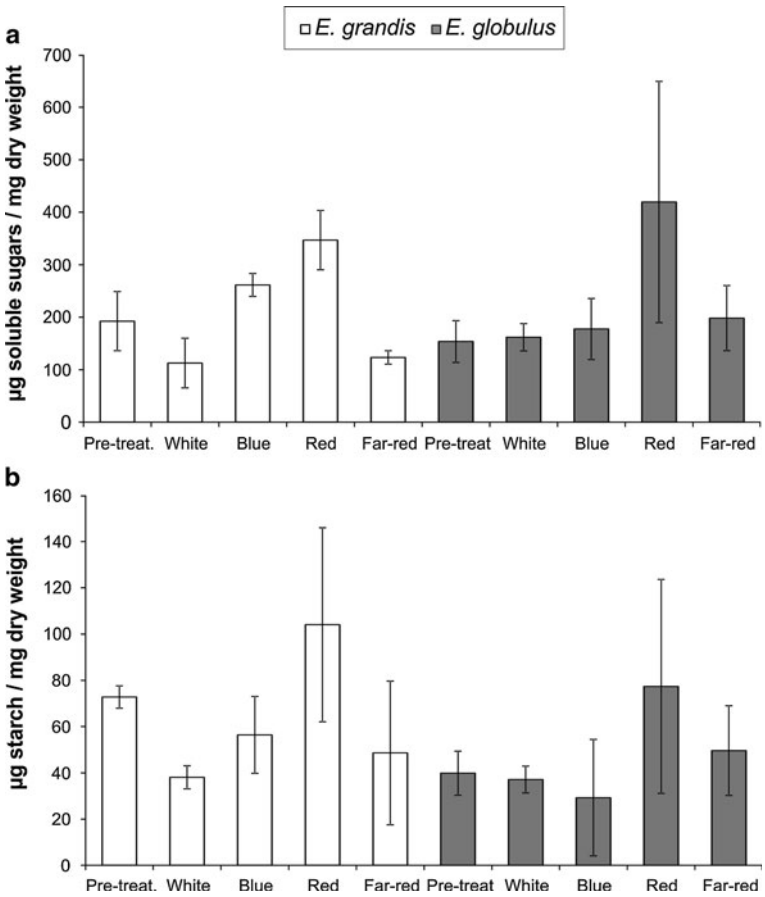

Fig. 4 Quantification of total soluble sugars (**a**) and starch (**b**) in donor plants of *E. grandis* (*white bars*) and *E. globulus* (*grey bars*), exposed to different radiation treatments during 1 month and without sucrose in the germination medium. Pre-treat. refers to seedlings before radiation treatment. Top vertical lines indicate standard error of the means. No significant differences were detected between color and white radiation control

soluble sugars and starch for both species was determined. The flavonoid content of the only species affected by radiation treatment, *E. globulus*, was also evaluated. These biochemical analyses were done in donor plants grown in medium without sucrose and exposed to radiation treatments, as well as in microcuttings derived thereof and cultured in absence of auxin. Donor plants had more soluble sugars than starch for all radiation treatments in both species (Fig. 4). The total content of soluble sugars and starch in donor plants exposed to far-red radiation enrichment was not significantly different from that of plants before radiation treatments (pre-treat), or from those exposed to the other treatments in both species. A trend for higher contents of carbohydrates and starch in donor plants exposed to red light is apparent, but not significant (Fig. 4). Microcuttings of *E. globulus* had more soluble sugars and starch than microcuttings of *E. grandis*, a difference that was not seen in donor plants (Figs. 4, 5).

The carbohydrate distribution between the rooting zone (basal portion of the stem) and shoots (root/shoot ratio of carbohydrates) was analyzed in microcuttings. The content of soluble sugars and starch in rooting zone and shoots of *E. grandis* microcuttings were similar for all treatments and control (Fig. 5a, b). On the other hand, the root zone of microcuttings derived from *E. globulus* donor plants exposed to far-red radiation had consistently more soluble sugars and starch than its shoots (Fig. 5a, b). Microcuttings derived from far-red enriched donor plants had high root/shoot ratio of carbohydrates, a feature not observed for the other radiation treatments (Fig. 5c, d). The same pattern of carbohydrate distribution was observed analyzing its content in a time course during the formation phase of adventitious rooting in microcuttings, spanning 5, 10 and 20 days after transfer to root formation medium. The higher root/shoot ratio of soluble sugars and starch in microcuttings derived from far-red treated donor plants started to become evident after 5 days. There was no clear difference between shoot and root content of soluble sugars or starch in microcuttings derived from white, red or blue radiation exposed donor plants.

There was no difference in the content of flavonoids in donor plants of *E. globulus* when comparing the radiation treatments (overall average ± SD of flavonoid content in donor plants = 19.67 ± 1.53 µg/mg dw). On the fifth day of the formation phase, the content of flavonoids in the root zone of microcuttings in all radiation treatments tended to be higher than in shoots, but without significant difference (overall average ± SD of flavonoid content in microcuttings: basal stem: 19.25 ± 2.22 µg/mg dw; shoot: 28.5 ± 7.23 µg/mg dw).

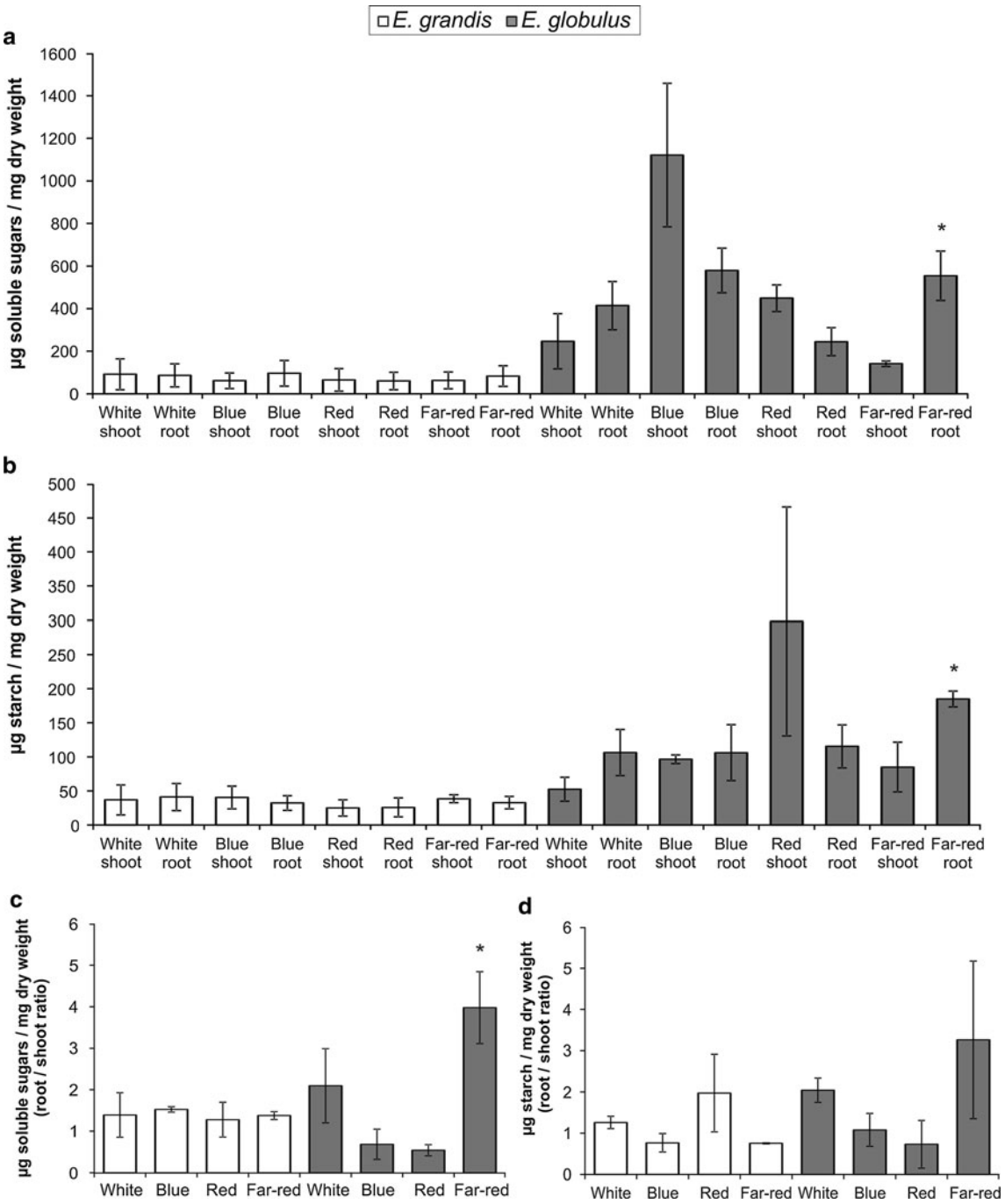

Fig. 5 Quantification of total soluble sugars (**a**) and starch (**b**) and the corresponding root/shoot ratios (**c, d**) in *E. grandis* (*white bars*) and *E. globulus* (*grey bars*) microcuttings excised from donor plants exposed to different radiation treatments during 1 month and without sucrose in the germination medium, after 20 days in the formation medium and without auxin exposure in the induction step. *Top vertical lines* indicate standard error of the means. The *asterisk* indicates significant difference between root and shoot content (**a, b**) and between white radiation and far-red enrichment treatment (**c**). The significance levels for Student t test were $P = 0.012$ (**a**), $P = 0.033$ (**b**) and $P = 0.138$ (**c**)

Discussion

The results of the experiments allowed us to refute the first hypothesis, since radiation quality affected adventitious rooting only in the recalcitrant species *E. globulus* with no significant effects on the easy-to-root *E. grandis*. The second hypothesis was also refuted because the outcome of adventitious rooting was only affected by donor plant radiation quality treatments. The physiological status of the donor plants is the result of interactions between genotype

and environmental factors (Moe and Andersen 1988) and is of considerable importance in the rooting process. The results obtained in this work indicated that adventitious rooting of *E. globulus* was more influenced by exposing the donor plants to radiation treatments than the cuttings during the rooting process *per se* (Figs. 2b, 3b). These data support the important role of the physiological status of donor plants from which cuttings are excised and emphasize the importance of controlling donor plants growth conditions. Other studies with radiation quality also reported that radiation treatments could be more effective when applied to donor plants. Different radiation quality treatments on donor plants yielded better results in subsequent adventitious rooting of *Betula pendula* cuttings (Saebo et al. 1995).

The rooting of *E. globulus* increased 255 % by combining absence of sucrose with far-red radiation enrichment in donor plants, even without auxin in the rooting induction medium (Fig. 3b). This result could be correlated with the higher content of total soluble sugars and starch in the rooting zone when compared with the shoots in microcuttings derived from far-red exposed donor plants, treatment which resulted in high root/shoot ratio of carbohydrates in these microcuttings (Fig. 5). None of the other radiation treatments promoted rooting or carbohydrate accumulation in the new root formation area (Fig. 5). The presence of exogenous sucrose in medium of donor plants exposed to far-red radiation enrichment probably disrupted the conditions to establish this balance of carbohydrate distribution along the microcuttings derived from them, resulting in reduced rooting capacity, similar to that seen in the control (Fig. 3b). These results further support the view that relationships between rooting ability and the availability of stored and/or actual supplies of carbohydrates are important for clonal propagation (Hoad and Leakey 1996).

The developing roots establish a new sink that competes for assimilates with the shoot meristems, and how the cutting manages these resources is crucial for adventitious root formation and subsequent restoration of the whole plant condition (Eliasson 1971; Druege 2009). The content of soluble sugars in shoots of microcuttings derived from *E. globulus* donor plants exposed to far-red radiation was diminished when compared to donor plants in other radiation treatments (Fig. 5a, c). In this particular condition, most resources were allocated to the stem base to form new roots. An adequate carbon balance between cutting base and shoot could result in improved adventitious root development, as observed in microcuttings derived from donor plants exposed to far-red treatment. A detailed analysis of physiological events taking place during adventitious rooting of *Petunia* cuttings provided evidence of the importance of an early increase in carbohydrate sink strength in the root forming region (Ahkami et al. 2009). The importance of establishing a strong carbon sink during adventitious rooting was also shown in carnation cuttings (Agulló-Antón et al. 2011). In *Petunia* cuttings, it has also been shown that dark pre-treatment enhanced carbohydrate availability at the cutting base during rooting under radiation, strongly promoting adventitious root development (Klopotek et al. 2010). The results of the present study suggest that the higher capacity to accumulate carbohydrates in cutting bases induced by far-red treatment of donor plants makes more energy and carbon available for new root formation, therefore contributing to overcoming *E. globulus* rooting recalcitrance.

Hoad and Leakey (1996) also described a positive effect of exposing donor plants to higher far-red:red radiation ratios on adventitious root formation in *E. grandis*. In this study, under such radiation conditions, better rooting response was associated with pre-severance lower starch and hexose content in shoots (no measurement was done for the rooting zone of cuttings). This correlation was not apparent in the present study with both *Eucalyptus* species; content of soluble sugars and starch in shoots of far-red treated donor plants was not significantly different from those of white radiation grown control plants (Fig. 4). This difference could be due to experimental conditions, such as *ex vitro* and in vitro environments and age of donor plants and cuttings. The rooting ability of *Triplochiton scleroxylon* was also improved by exposure to far-red enriched radiation (Leakey and Storeton-West 1992; Newton et al. 1996). On the other hand, high far-red and blue radiation yielded better rooting responses for *Terminalia spinosa* and *Betula pendula* (Newton et al. 1996; Saebo et al. 1995). In leaf explants of *Morinda citrifolia* grown for 5 weeks in medium with auxin and sucrose, far-red radiation resulted in the lowest root induction (Baque et al. 2010). Although at least some of these distinct results may reflect differences in experimental conditions, they also confirm that the effects of radiation quality on rooting may vary considerably between species (Moe and Andersen 1988; Antonopoulou et al. 2004).

For all the morphological parameters analyzed, radiation quality only had an effect on percent of rooting in recalcitrant *E. globulus* and no effect in the easy-to-root *E. grandis* (Fig. 3). This suggests that radiation quality may have a stronger effect on rooting capacity of recalcitrant species. Auxin can improve rooting in both easy-to-root *E. saligna* and hard-to-root *E. globulus* (Fett-Neto et al. 2001) and the presence of this phytohormone in the induction medium caused higher rooting percentage in all radiation treatments; however, most large scale propagation operations do not use auxin to root cuttings of eucalypt hybrids in clonal gardens. Far-red enrichment treatments in donor plants, as described herein, could be evaluated as a possible strategy to further improve adventitious rooting of "elite" *E. globulus* hybrids that display rooting limitations.

Radiation intensity applied to donor plants and microcuttings during our experiments (45 μmol m^2/s) is adequate for *Eucalyptus* grown in vitro (Fett-Neto et al. 2001; Corrêa et al. 2005; Schwambach et al. 2005). In large scale greenhouse conditions for commercial production of *Eucalyptus*, plants are usually exposed to higher radiation intensity, in a range from 80 to 200 μmol m^2/s, for example (Schwambach et al. 2008). Therefore, caution is required in trying to extrapolate results obtained in vitro to ex vitro conditions.

Polar auxin transport is affected by radiation (Jensen et al. 1998) and an increasing body of evidence suggests that there are important interactions between phytochrome, radiation signaling and auxin transport (Morelli and Ruberti 2002). Tyburski and Tretyn (2004) established a positive correlation between radiation, auxin transport and adventitious rooting in tomato. However, this does not appear to occur to a significant extent in *E. globulus* under the experimental conditions used. Flavonoids can modulate auxin transport (Murphy et al. 2000; Brown et al. 2001; Peer and Murphy 2007), so the content of these compounds may interfere with allocation of auxin. Since far red treatment of donor plants promoted the rooting of microcuttings of *E. globulus*, the content of flavonoids was examined only in this species. No significant difference in flavonoid content was observed between plants exposed to different radiation quality treatments in the present work. In addition, endogenous concentration of free indole-3 acetic acid quantification by liquid chromatography did not differ between pooled samples of *E. globulus* plants treated with white or far-red radiation exposure (data not shown). In continuous far-red enriched environments, such as the ones used in the corresponding treatments of this experiment, PhyA is expected to be the predominant form of phytochrome mediating plant responses to radiation (Franklin and Quail 2010). PhyA has been proposed to affect auxin sensitivity presumably through phosphorylation and modulating stability and/or activity of Aux/IAA proteins, which are known to often act as repressors of auxin response factor (ARF)-regulated gene expression (Stowe-Evans et al. 2001). Hence, an auxin-mediated response to radiation cannot be ruled out.

Overall, data point to a key role of the rooting zone carbohydrate accumulation in cuttings derived from far-red treated donor plants. The higher proportion of carbohydrate allocation to the cutting base versus shoot may serve not only to provide energy and carbon skeletons for root differentiation in a larger number of cuttings, but also play a regulatory role in interaction with phytohormones (Corrêa et al. 2005). Microarray investigations have recently shown that glucose can affect several aspects of auxin metabolism, transport and action relevant for primary and lateral root development of *Arabidopsis*, involving both transcriptional and non-transcriptional processes, such as protein stability/degradation (Mishra et al. 2009).

Conclusion

Radiation quality can be modulated in a manner to improve adventitious rooting of the recalcitrant species *E. globulus*. The evidence indicates that the improvement in adventitious rooting capacity in microcuttings of *E. globulus* derived from donor plants exposed to far-red radiation in an autotrophic system (without sucrose in the culture medium) was probably associated with an adequate balance between the content of endogenous sugars in shoots and basal rooting zone, with a higher content in the latter. Ongoing studies analyzing the expression profile of genes involved in carbohydrate metabolism and auxin biosynthesis and transport in *Eucalyptus* may shed further light on the mechanisms underlying this response.

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