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EFFECT OF MEDIUM DARKENING ON IN VITRO ROOTING OF GF 677 AND KIWI EXPLANTS

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Abstract

The effect of darkening of the nutrient medium on *in vitro* rooting of GF 677 rootstock (*Prunus amygdalus* x *P. persica*) and kiwi (*Actinidia chinensis*, cv. Hayward) explants was studied. Two different dyes, Brilliant Black MN-SIGMA (BB) (50, 100 and 200 mg L^{-1}) and active charcoal (AC) (200 and 500 mg L^{-1}) were added in the Murashige and Skoog medium. Moreover, the rooting medium of the control treatment was darkened by covering the outside of the test tubes with black electrical tape. In both plant species, adding 50 mg L^{-1} BB in the nutrient medium had the same positive results as did the black electrical tape, enhancing rooting percentage, root number and growth. On the other hand, AC did not promote *in vitro* rooting of the explants. Finally, increasing the concentration of BB it reduced the amount of total chlorophyll in the leaves of kiwi explants.

Keywords: active charcoal, darkening, kiwi, micropropagation, peach

INTRODUCTION

In vitro rooting is a crucial stage of the micropropagation of woody plant species and depends on many endogenous and exogenous factors (George, 1996). Illumination of the rooting medium can either promote (Antonopoulou et al., 2004) or inhibit (Hammerschlag, 1982) *in vitro* rooting of different plant species. However, keeping the whole *in vitro* cultures in the dark during *in vitro* rooting can reduce the survival of the rooted plantlets during acclimatization (von Arnold and Erikson, 1984). It is reported that darkening only the base of the explants can replace the initial dark period for a successful *in vitro* rooting (Antonopoulou, 2009). Rugini et al. (1993) enhanced *in vitro* rooting of olive explants by painting black the base of the culture vessels and by covering the surface of the medium with black sterile polycarbonate granules. Total or partial darkening of the rooting medium can also be achieved by adding activated charcoal (AC) (Adak et al., 2010). Mencuccini (2003) reported that dying the nutrient medium with BB it increased *in vitro* rooting of olive explants. BB is listed as Food Black 1 and is largely used in food-dye

mixtures (Mencuccini, 2003). The aim of the present work was the study of the influence of darkening the nutrient medium by different ways on *in vitro* rooting of peach rootstock GF 677 and kiwi microcuttings.

MATERIALS AND METHODS

Apical shoot tips (1.5-2.5 cm) of GF 677 rootstock (*Prunus amygdalus* x *P. persica*) and kiwi (*Actinidia chinensis*, cv. Hayward) from previous subcultures were used as explants in 25x100 mm test tubes containing 10 mL basal MS (Murashige and Skoog, 1962) medium supplemented with 2 mg L^{-1} indole-3-butyric acid (IBA), 3% sucrose and 0.6% agar. The rooting media were darkened by adding separately two different dyes, Brilliant Black MN-SIGMA (BB) (50, 100 and 200 mg L^{-1}) and active charcoal (AC) (200 and 500 mg L^{-1}). Moreover, the rooting medium of the control treatment (CNT) was darkened by covering the outside of the test tubes with black electrical tape and by covering the surface of the medium with sterile perlite. Medium pH was adjusted to 5.8 before autoclaving at 121°C for 20 min. Cultures were maintained at 22±2°C under cool white fluorescent light (Phillips, 45 μmol $m^{-2}s^{-1}$), with a 16h photoperiod. Rooting percentage, root number, root length and weight were recorded after 4 weeks. Chlorophyll (chl) content of the leaves was extracted with ethanol (96%) after incubation in a water bath (78°C) and its amount was calculated according to Wintermans and Mots (1965) and expressed on a fresh mass (FM) basis. The experiment was repeated twice. The results of both experiments were calculated together and the means were compared using the Duncan multiple range test ($P \leq 0.05$).

RESULTS

Brilliant Black and the dark control treatment resulted in 90-100% rooting in both plant species, while AC had an inhibitory effect, especially on GF 677 microcuttings (Fig. 1a). Kiwi and GF 677 produced more roots when the medium was darkened by the black tape or BB (Fig. 1b). The lowest root numbers were recorded in the presence of AC (Fig. 1b). On the other hand, regarding GF 677 explants, CNT, 50 mg L^{-1} BB and AC promoted root elongation (Fig. 1c). The highest fresh and dry root weight of GF 677 plantlets was recorded at the CNT, while of kiwi plantlets at the CNT and at 50 mg L^{-1} BB (Fig. 1d-e). Leaves of all plantlets did not present differences at their chl content (Fig. 1f). However, increasing the concentration of BB it reduced the amount of total chl in kiwi microshoots (Fig. 1f).

DISCUSSION

Although photosynthesis provides the carbohydrates necessary for root induction and growth, keeping the whole explants or just their base in the dark may enhance *in vitro* rooting (George, 1996). Rugini et al. (1993) achieved *in vitro* rooting of olive explants by painting black the base of the culture vessels. In the present study, the base of the test tubes was covered with black electrical tape and the surface of the medium with sterile perlite. This treatment promoted *in vitro* rooting of GF 677 and kiwi explants. Similar results were found for *Rosa hybrida* (Khosh-Khui and Sink, 1982) and olive explants (Antonopoulou, 2009). Plant

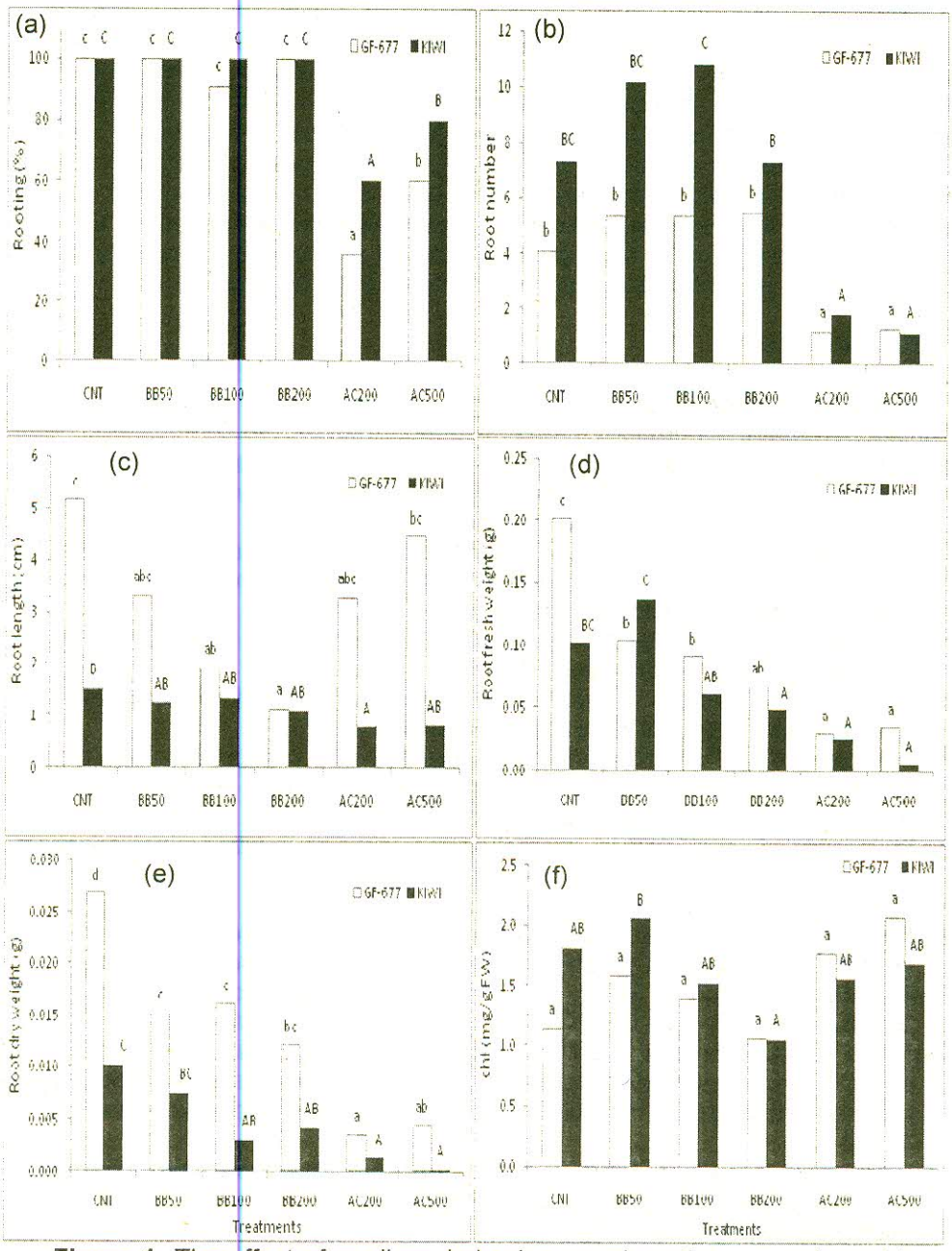

Figure 1. The effect of medium darkening on: a) rooting percentage, b) root number, c) root length, d) root fresh weight, e) root dry weight and f) chlorophyll concentration of GF 677 and kiwi explants. (CNT-black electrical tape, BB-Brilliant Black 50, 100, 200 mg/L, AC- activated charcoal 200 and 500 mg/L)

(Values with different letters are significantly different at $P \leq 0.05$)

tissues grown in the absence of light are less differentiated, are more sensitive to auxins, contain more rooting synergistic compounds and auxin degradation is slower (George, 1996). However, since it was not a practical method, the black tape was replaced by darkening totally or partially the nutrient medium with two different dyes. In both plant species, adding 50 mg L^{-1} BB in the nutrient medium had the same positive results as did the black tape. Similarly, Mencuccini (2003) promoted *in vitro* rooting of olive explants by using BB. On the other hand, AC did not promote *in vitro* rooting, although it promoted root elongation in GF 677 explants. Activated charcoal can inhibit (Montoliu et al., 2010) or increase (Adak et al., 2010) *in vitro* adventitious rooting depending on the plant species.

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