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BRIEF COMMUNICATION

Effects of short light-dark regimes on *in vitro* shoot rooting of some fruit tree rootstocks

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Abstract

Experiments were carried out to evaluate the effects of 4/2 light-dark cycles (4 h of light followed by 2 h of dark) on the rooting responses of shoots cultivated *in vitro* of the fruit tree rootstocks GF 677 (peach × almond hybrid), Mr.S. 2/5 (*Prunus cerasifera*), MM 106 (apple Nothern Spy × Paradise M1) and BA 29 (*Cydonia oblonga*). Under this light regime rooting percentage of GF 677, Mr.S. 2/5 and MM 106 shoots reached 100 % as in the control treatment (16/8), while in BA 29 shoots, short light-dark cycles increased rooting response by about 65 %. Moreover, the shoots of all rootstocks submitted to the 4/2 cycle showed an appreciable increase in root number and length, and an earlier root emergence of about 4 - 5 d compared to the 16/8 cycle. Finally, rooting percentage of BA 29 shoots submitted to the 4/2 light regime and treated with 0.2 mg dm^{-3} indolebutyric acid (IBA), was equal to that reported with 0.4 mg dm^{-3} IBA under the 16/8 regime, indicating that the former light regime also amplified the rhizogenic effect of auxin.

Additional key words: *Cydonia*, *Malus*, micropropagation, photoperiod, *Prunus*, rootstocks.

Photoperiod is one of the factors that characterise the environmental conditions of cultures in micropropagation. Many research studies (Economou and Reed 1987) have shown that its variation can influence the growth of the cultures to a greater or lesser degree depending on the genotype: some species benefit from longer photoperiods, others from shorter photoperiods and still others appear to be indifferent. Photoperiod has also shown to influence plant growth during acclimatization stage (Vaillant *et al*. 2005).

Research carried out in our laboratory has shown that *in vitro* cultures of some fruit tree rootstocks submitted to light-dark periods shorter than 16/8, by keeping the quantity of light radiation applied to the culture unchanged, performed more pronounced growth responses (Morini *et al*. 1990). Thus it was observed that a 4/2 regime (4 h light followed by 2 h dark) was more efficient than 16/8, in terms of fresh and dry masses of the culture, new axillary shoots produced and shoot leaf area (Morini *et al*. 1990). This effect was also observed in the presence of a low sucrose concentration in the culture medium (Morini *et al*. 1992). The higher growth potential of the cultures subjected to this light-dark regime seemed to be associated with a reduction in apical dominance that induced a culture growth pattern characterised by the production of a higher number of secondary and tertiary axillary shoots (Morini *et al*. 1991).

As regards the effect of the 4/2 light regime on adventitious rooting of *in vitro* cultured shoots, information is rather scarce. It is only known that root length in shoots of two clonal rootstocks treated with this light regime was significantly higher than with 16/8 treatment (Morini *et al*. 1990). This finding would suggest a positive role of 4/2 light regime on rhizogenesis as well but deeper knowledge is still necessary to better evaluate rooting performance under this photoperiod. Purpose of this work was to investigate in more detail the effects of the 4/2 light regime on *in vitro* rooting of shoots of different fruit tree rootstocks.

Shoots from mother cultures already established to *in vitro* conditions of the following fruit tree clonal rootstocks were used: GF 677 (peach × almond hybrid), Mr.S. 2/5 (*Prunus cerasifera*), MM 106 (apple Northern Spy × Paradise M1) and BA 29 (*Cydonia oblonga*). Proliferation medium of the mother cultures was made up of mineral components of Murashige and Skoog (1962;

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Abbreviations: BA - 6-benzyladenine; GA_3 - gibberellic acid; IBA - indole-3-butyric acid; MS - Murashige and Skoog.
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MS) enriched with 0.4 mg dm^{-3} thiamine-HCl, 100 mg dm^{-3} myo-inositol, 30 mg dm^{-3} FeNaEDTA, 30 g dm^{-3} sucrose, 0.1 mg dm^{-3} gibberellic acid (GA_3) and 0.05 mg dm^{-3} indole-3-butyric acid (IBA); 6-benzyl-adenine (BA) was added at 0.4 mg dm^{-3} for Mr.S. 2/5, at 0.6 mg dm^{-3} for GF 677, and at 3 mg dm^{-3} for MM 106 and BA 29. The pH of all growth media was adjusted to 5.2. For MM 106 the growth medium was also enriched with phloroglucinol at the concentration of 160 mg dm^{-3}. In the growth chamber irradiance was 50 ± 5 µmol m^{-2} s^{-1} (fluorescent tubes *Philips TLD 18/33*), 16-h photoperiod, and temperature 24 ± 1 °C. Before collecting the shoots for rooting, the cultures were submitted to a 10-d period of growth on medium containing the same components mentioned above but with BA reduced at 0.1 mg dm^{-3} for all the species. On this culture medium, the shoots elongated and acquired greater vigour, well-differentiated internodes and larger leaves dark green in colour.

For each clone, 90 shoots uniform in vigour of about 4 - 5 nodes were selected. These were divided into two groups of 45, each of which was distributed, in groups of 15, in three 500 cm^3 vessels containing 120 cm^3 of rooting medium. This medium contained the same components as the shoot proliferation medium except that BA and GA_3 were omitted and IBA added at 0.4 mg dm^{-3}. The two groups of shoots were then placed in two separate growth chambers where the photoperiod was 16/8 and 4/2, respectively, while temperature and irradiance were the same as used for the mother cultures.

On quince BA 29, the effect of light regime was studied also in relation to the presence of IBA at the concentration of 0.0 and 0.2 mg dm^{-3} applying the same methodology as that utilized for the 0.4 mg dm^{-3} IBA treatment.

In all the experiments the period of rooting was fixed at 35 d, at the end of which rooting percentage and number and length of the roots produced were determined. Data were subjected to analysis of variance (*ANOVA*). Rooting percentages were adapted to variance analysis by transformation into the corre-sponding angular values.

Shoots of MM 106, Mr.S. 2/5 and GF 677 submitted to 4/2 light-dark cycles rooted at 100 % as in the control treatment (16/8), while those of BA 29 took more advantage of the 4/2 cycle and rooted more extensively; in this case rooting percentage of shoots treated with IBA at 0.4 mg dm^{-3} was greater than the control by about 65 % (Fig. 1*A*). Furthermore, with BA 29 shoots the auxin rhizogenic effect appeared to be amplified by the 4/2 cycle. Indeed, rooting response obtained with the latter light cycle and IBA at 0.2 mg dm^{-3} was equal to that obtained with IBA at 0.4 mg dm^{-3} in treatment 16/8 (Fig. 1*A*). It should also be noted that with the 4/2 light regime, even in the absence of auxin treatment, rhizogenesis occurred, although to a small extent, whereas no root was produced under 16/8. The number and the length of roots produced on BA 29 shoots at varying IBA concentrations were positively influenced by 4/2 cycles as well (Fig. 1*B*,*C*), and showed trends very similar to those observed for rooting percentage.

Positive effects of 4/2 cycles were also observed on the rooting response of MM 106, Mr.S. 2/5 and GF 677; they were represented by a higher number and length of neo-formed roots that in most of the cases exhibited statistically significant differences compared to the 16/8 treatment (Fig. 2*A*,*B*). The greater root length on shoots submitted to the 4/2 light regime may be the result of an earlier triggering of rhizogenic process by which root emergence occurred 4 - 5 d earlier compared to the 16/8 control (data not shown). Although no specific measurements were taken, the leaf area also appeared distinctly greater in the shoots rooted under the 4/2 light regime, confirming the results obtained in a previous work (Morini *et al*. 1990).

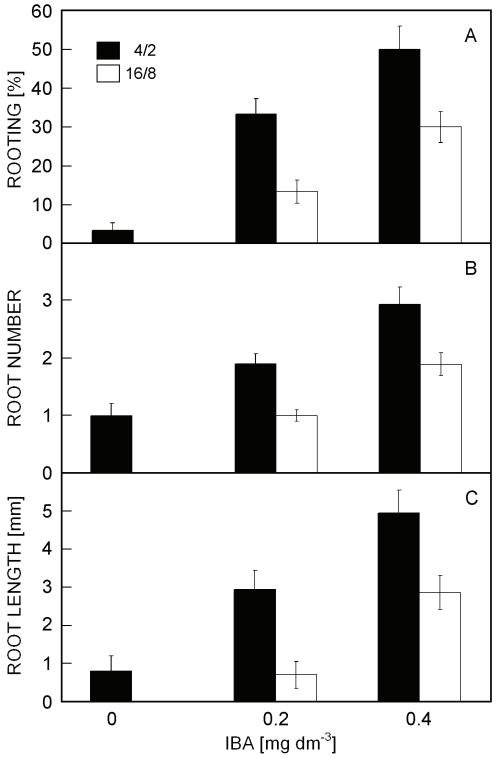

Fig. 1. Effect of 4/2 light-dark cycle on *in vitro* rooting percentage (*A*), root number (*B*) and length (*C*) of quince BA 29 shoots treated with different concentrations of IBA. Means ± SE, $n = 3$.

It is unknown the physiological level to which the 4/2 light regime operates. We presume two factors, one related to photosynthesis and one to photomorphogenesis, are involved. As concern photosynthetic activity, the *in vitro* cultures are hampered by the rapid depletion of CO_2 in the culture vessels due to reduced gas exchanges with the outside (Kozai 1991). Low irradiance is another limiting factor for photosynthesis but it has been ascertained that CO_2 assimilation occurs even at values of about 40 - 50 µmol m^{-2} s^{-1} (Morini *et al*. 1993), which is that commonly used in micropropagation laboratories. Thus, under 16-h photoperiod the concentration of CO_2 in

the culture vessel reduces rapidly, following assimilation by the cultures during the first hours of light, and then fluctuates around the compensation point in the remaining period until the lights are turned off (Morini *et al*. 1993). With the application of a 4/2 light-dark regime, the cultures appeared able to photosynthesize longer during the day, as consequence of the more frequent production of CO_2 by dark respiration that never reached the compensation point (Morini *et al*. 1993). Since in the 4/2 cycle the quantity of CO_2 assimilated during the light period was practically equivalent to that emitted during the dark respiration (Morini *et al*. 1993), it is suggested that the energetic contribution of photosynthesis was irrelevant to culture growth, but the longer periods of photosynthetic activity monitored under the 4/2 cycle, compared to 16/8, encouraged more efficient growth processes in the cultures.

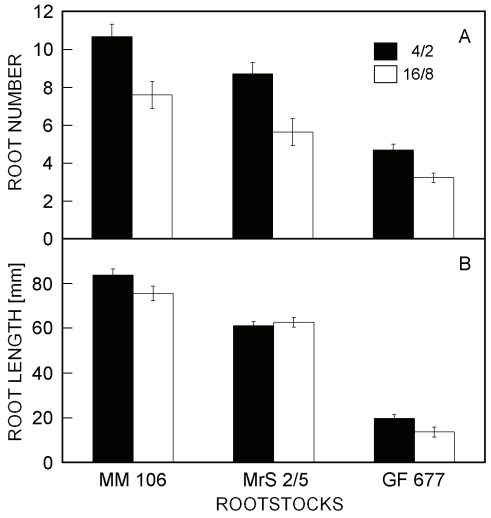

Fig. 2. Effect of 4/2 light-dark cycle on number (*A*) and length (*B*) of roots produced by shoots of the clonal rootstocks MM 106 (apple), Mr.S. 2/5 (plum) and GF 677 (peach × almond), cultivated *in vitro*. Means ± SE, $n = 3$.

A further positive effect of the 4/2 light regime on photosynthetic activity of *in vitro* cultures might be the smaller accumulation of starch and soluble sugars in the chloroplasts. As observed *in vivo* (Azcon-Bieto 1983, Flore and Lakso 1989), the production of these compounds might be so high to inhibit the regular activity of the chloroplast, particularly if their production is greater than their transport to outside the chloroplast. Unpublished data from our laboratory have shown that with 4/2 light regime, starch granules accumulated in the chloroplasts were of smaller size compared to those observed under 16/8 light regime. This result might be related to the shorter light periods of this photoperiod and/or to a greater transport of sugars to the outside of the chloroplast as consequence of the higher number of axillary meristems (Morini *et al*. 1990), or growing roots which could represent sinks potentially effective in determining a high energy requirement. Thus the presence of smaller starch granules might enable the chloroplasts to be less subjected to possible damages to the structure (Carmi and Shomer 1979, Cave *et al*. 1981, Sasek *et al*. 1985) in favour of their activity.

Another factor that might be related to the positive effect of the 4/2 light regime on shoot rooting is represented by a more effective photoreceptor activation. Phytochrome is one of the photomorphogenic photoreceptors involved in different plant growth mechanisms (Mancinelli 1994) amongst which there are the control of apical dominance (Phillips 1975, Tucker 1976, Muleo and Thomas 1997), the outgrowth of axillary buds and the rooting of shoots in micropropagation (Morini and Muleo 2003). The physiologically active form of phytochrome has a peak under red light, which represents a high fraction of the emission spectrum of most of the fluorescent tubes emitting white light, used in micropropagation laboratories. The active form of phytochrome is subjected to destruction during the dark period, and the destroyed amount may differ in function of the length of the light-dark cycles and the quality of the light applied (Mancinelli 1994). On the basis of this knowledge, the results obtained with the 4/2 cycle could possibly be further explained by the shorter length of the dark periods or in the greater frequency of light stimuli, with respect to the 16/8 regime, thanks to which the active form of the phytochrome would be less prone to being destroyed.

In conclusion, although the physiological effects are not demonstrated, the 4/2 light regime seem therefore to be more successful than 16/8 in *in vitro* culture growth. This result could lead one to presume that other cycles apart from the 4/2, might also be able to exert a greater positive effect on shoot rooting. Information available on growth responses of cultures subjected to different light-dark cycles like, for example, 8/4 and 2/1 (Morini *et al*. 1990), would seem to confirm, however, that the hours of light and dark in the ratio of 4/2 is, at the moment, the best photoperiod. Also on *in vitro* cultures of different peach genotypes, the same light-dark cycle, in the presence of an optimal concentration of cytokinin, influenced positively the shoot growth potential (Zimmerman and Scorza 1994). From a practical point of view, the 4/2 light regime prospects the possibility of increasing the efficiency of micropropagation in terms of a higher shoot proliferation rate (Morini *et al*. 1990) and a greater and faster rooting of shoots.

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