The influence of radiation quality on the *in vitro* rooting and nutrient concentrations of peach rootstock

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Abstract

The effect of radiation quality (350 - 740 nm) and darkness (D) on *in vitro* rooting, and chemical composition of the peach rootstock GF 677 was studied. Shoot explants were exposed for four weeks to cool white (control) (W), red (R), blue (B), green (G) or yellow (Y) radiation from fluorescent tubes. Some of the explants were kept in D during the rooting stage and others were maintained only for the first 2- or 4-d under R, B, G, Y or D, and subsequently were transferred to W. W was the most effective radiation source for adventitious root formation of GF 677 explants. Rooting was inhibited in those plants that remained in continuous D, and R reduced root growth in all treatments. The 2- or 4-d exposure to D, Y or B followed by W helped adventitious root development similarly as did W. G significantly increased Fe concentration in roots.

Additional key words: hybrid, micropropagation, monochromatic radiation, nutrients, *Prunus.*

Introduction

The most broadly used peach rootstock is the hybrid GF 677 (*Prunus amygdalus* × *P. persica*), as it is suitable for soils of low fertility, high calcium carbonate content and is employed to face the peach replant problem (Monticelli *et al*. 2000). Since GF 677 is propagated commercially by tissue culture, much research has been conducted to improve the multiplication rate and quality of the *in vitro* rooted plantlets (Dimassi-Theriou 1989, Molassiotis *et al*. 2003). Apart from GF-677, attepts are made to micropropagate new hybrids of this group of rootstocks (Fotopoulos and Sotiropoulos 2004)

The growth and development of plants is dependent on photosynthetically active radiation (PAR) important for photosynthesis, photomorphogenesis and phototropism. One of the properties of PAR that influences *in vitro* morphogenesis and photosynthesis is the wavelength or spectral quality (Hoenecke *et al*. 1992, Saebo *et al*. 1995). The PAR source generally used for *in vitro* culture is fluorescent lamps and research has shown that when the proper lamps are chosen, then rooting can be stimulated. Chée and Pool (1989) enhanced rooting of *Vitis* explants in red radiation (R) and Baraldi *et al*. (1988) obtained rooting of GF 655/2 in R without auxin. However, green - yellow (G-Y; 550 nm) and blue radiation (B) have been found to inhibit adventitious root formation by destroying auxin (Fuernkranz *et al*. 1990, George 1996). Many authors have reported the promotory effect of a short period of darkness on the *in vitro* rooting. Hence, keeping cultures of *Prunus cerasifera* (Hammerschlag 1982) and almond/peach hybrid rootstock Titan × Nemaguard (Channuntapipat *et al*. 2003) in the dark (D), prior to incubation in white (W) radiation, increased the rooting of explants. Furthermore, Shin *et al*. (2003/4) working with R found that radiation quality affected biomass accumulation.

Although previous results have confirmed morphological and physiological effects of radiation quality, responses vary according to plant species. There are, however, only a few reports on the effects of radiation quality on growth and development of *in vitro* cultured plantlets (Reid 1990, Herrington and McPherson 1993). The rational of light requirement for root formation has not been studied. Therefore, we explored whether rooting and nutrient uptake of GF 677 explants in *in vitro* culture can be induced under different radiation stimuli.

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Abbreviations: B - blue radiation; D - darkness; G - green radiation; IBA - indole-3-butyric acid; MS - Murashige and Skoog (1962) culture medium; R - red radiation; W - white radiation; Y - yellow radiation.

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Materials and methods

Shoot tips (1.5 - 2.5 cm) of the rootstock GF 677 were obtained from previous subcultures and transferred to MS nutrient medium (Murashige and Skoog 1962). All media contained 4.90 µM IBA, 30 g dm^{-3} sucrose and 6 g dm^{-3} agar. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121 °C, for 20 min. Cultures were incubated at 22 ± 2 °C, with a 16-h photoperiod, under different *Philips* fluorescent tubes, while some explants were kept in the dark. The approximate wavelengths and the peak wavelength were: W (350 - 740 and 580 nm), D (>740 and 1400 nm), R (640 - 740 and 680 nm), B (425 - 490 and 460 nm), G (490 - 560 and 520 nm) and Y (560 - 585 and 580 nm), respectively. The explants of some treatments were incubated only for the first 2 or 4 d under the previously mentioned monochromatic radiation or in the dark and subsequently were transferred to W. Photon flux density was 90 µmol m^{-2} s^{-1}. After four weeks, the experiment terminated and the explants were washed in distilled water and the roots were cut, counted and weighed in order to assess root growth. All samples (shoots and roots) were dried at 75 °C for 48 h, weighed, ground to a fine powder and ashed at 550 °C. Subsequently, the samples were analysed for P by the phospho-vanado-molybdate method, and for K, Ca, Mg, Fe, Mn and Zn by atomic absorption spectroscopy. Each treatment included ten replications. The experimental layout was completely randomised and the experiment was repeated twice. The reported data are the means of two experiments. Means were compared using Duncan's multiple range test.

Results

After four weeks in the culture room, 100 % rooting of GF 677 was obtained in all the treatments, except for the explants maintained continuously in the dark or kept for 2 d under R or B and then transferred to W (Table 1).

The explants that were kept in the dark during the rooting stage, formed the least number of roots (2.6) and after 7 - 10 d their leaves turned yellow and shoot tips died (Table 1). In the other treatments, the average number of roots ranged from 5.3 to 5.8. The average root length was the greatest in the control (W) (5.2 cm) treatment, while the shortest roots were formed in the darkness and under the R (Table 1). Compared to the W, root fresh mass was reduced in the Y treatment (Table 1). Concerning the root dry mass in the W treatment, this was greater than that of the dark, R or B treatments (Table 1). The control explants (W) had greater fresh and

Table 1. Effects of radiation quality and darkness on *in vitro* rooting and growth of roots and shoots of GF 677 explants. Explants were continuously (28 d) exposed to white or monochromatic radiation or darkness or exposed only for 2 or 4 first days to monochromatic radiation or darkness and subsequently transfer to white radiation. Values with different letters in the same column and in the same treatment differ significantly for $P \leq 0.05$ (Duncan's multiple range test).

Treatment		Rooting [%]	Root number	Root length [cm]	Root fresh mass [g]	Root dry mass [g]	Lateral roots [%]	Shoot fresh mass [g]	Shoot dry mass [g]
28 d	W	100 b	5.60 b	5.19 c	0.139 c	0.014 c	40 c	0.181 b	0.026 b
	D	90 a	2.60 a	0.70 a	0.025 a	0.003 a	0 a	0.074 a	0.012 a
	R	100 b	5.30 b	0.92 a	0.037 a	0.007 ab	10 a	0.079 a	0.013 a
	B	100 b	5.80 b	2.64 b	0.045 ab	0.007 ab	10 a	0.141 ab	0.018 ab
	G	100 b	5.30 b	2.54 b	0.066 ab	0.010 abc	20 b	0.109 a	0.015 a
	Y	100 b	5.80 b	2.84 b	0.096 bc	0.013 bc	40 c	0.190 b	0.025 b
2 + 26 d	W	100 b	5.60 b	5.19 b	0.139 b	0.014 b	40 c	0.181 b	0.026 b
	D	100 b	6.40 b	4.84 ab	0.098 ab	0.012 ab	10 a	0.180 b	0.026 b
	R	90 a	2.90 a	2.74 a	0.038 a	0.005 a	20 b	0.093 a	0.015 a
	B	90 a	4.90 ab	4.46 ab	0.088 ab	0.011 ab	70 d	0.147 ab	0.022 ab
	G	100 b	4.10 ab	2.75 a	0.047 a	0.006 a	0 a	0.110 ab	0.018 ab
	Y	100 b	5.10 ab	4.01 ab	0.071 a	0.008 ab	20 b	0.153 ab	0.021 ab
4 + 24 d	W	100 a	5.60 b	5.19 b	0.139 c	0.014 c	40 c	0.181 ab	0.026 b
	D	100 a	6.00 b	2.85 a	0.037 a	0.005 ab	30 b	0.157 ab	0.020 ab
	R	100 a	3.30 a	2.21 a	0.033 a	0.004 a	0 a	0.113 a	0.015 a
	B	100 a	3.80 a	3.79 ab	0.045 ab	0.005 ab	30 b	0.147 ab	0.018 ab
	G	100 a	3.30 a	2.54 a	0.029 a	0.003 a	10 a	0.105 a	0.015 a
	Y	100 a	6.00 b	4.15 ab	0.085 b	0.009 b	40 c	0.211 b	0.023 ab

Table 2. Effects of radiation quality and darkness on concentrations of nutrient elements in roots and microshoots of GF 677 explants. Explants were continuously (28 d) exposed to white or monochromatic radiation or darkness or exposed only for 2 or 4 first days to monochromatic radiation or darkness and subsequently transfer to white radiation. Values with different letters in the same column and in the same treatment differ significantly for $P \leq 0.05$ (Duncan's multiple range test).

Treatment			P [%]	K [%]	Ca [%]	Mg [%]	Fe [μg g^{-1}]	Zn [μg g^{-1}]	Mn [μg g^{-1}]
Roots	28 d	W	0.47a	3.98c	0.34a	0.11ab	821ab	206a	91ab
		D	1.21b	0.44a	1.38b	0.12b	1217b	250b	83a
		R	0.59ab	0.78a	0.34a	0.05a	847ab	199a	74a
		B	0.96b	2.22b	0.43a	0.12b	1242b	237ab	98ab
		G	0.54ab	2.06b	0.30a	0.09ab	1085ab	239ab	112b
		Y	0.46a	2.32b	0.24a	0.08ab	536a	183a	115b
	2 + 26 d	W	0.47a	3.98b	0.34a	0.11ab	821a	206a	91ab
		D	0.53a	2.25a	0.27a	0.09a	741a	196a	73a
		R	0.52a	2.23a	0.49a	0.17b	1628b	270b	95ab
		B	0.55a	2.79ab	0.37a	0.11ab	1106ab	201a	106b
		G	0.46a	2.05a	0.49a	0.14ab	3679c	212a	103ab
		Y	0.50a	3.02ab	0.33a	0.12ab	1468ab	254b	75a
	4 + 24 d	W	0.47a	3.98a	0.34b	0.11ab	821a	206a	91a
		D	0.54ab	2.92ab	0.50ab	0.14b	1583b	282ab	128b
		R	0.91b	3.18ab	0.68b	0.09a	2620c	320b	100a
		B	0.54ab	1.79a	0.48ab	0.13b	1476b	304ab	101a
		G	0.57ab	3.25ab	0.61ab	0.09a	3491d	291ab	91a
		Y	0.56ab	2.23a	0.34a	0.09a	1168ab	212a	124b
Shoot	28 d	W	0.44a	2.21b	0.33ab	0.11ab	425b	107a	113b
		D	0.60ab	1.49a	0.36b	0.11ab	350ab	135ab	66a
		R	0.65b	2.20b	0.34ab	0.12b	322ab	159b	70a
		B	0.43a	2.22b	0.31ab	0.11ab	441b	107a	105b
		G	0.48a	2.34b	0.31ab	0.11ab	462b	118a	85ab
		Y	0.42a	2.21b	0.25a	0.09a	179a	101a	94ab
	2 + 26 d	W	0.44a	2.21a	0.33ab	0.11b	425a	107a	113ab
		D	0.81b	2.25a	0.25a	0.08a	639ab	121ab	99ab
		R	0.50a	2.22a	0.30ab	0.09ab	992b	151b	91a
		B	0.40a	2.54a	0.34b	0.11b	568ab	107a	125b
		G	0.57ab	2.32b	0.30ab	0.12b	968b	135ab	103ab
		Y	0.48a	2.41a	0.33b	0.11b	744ab	124ab	113b
	4 + 24 d	W	0.44a	2.21a	0.33ab	0.11a	425a	107a	113ab
		D	0.42a	2.30a	0.30a	0.10a	507ab	111a	102ab
		R	0.44a	2.34a	0.31ab	0.10a	629ab	111a	95a
		B	0.45a	2.06a	0.29a	0.10a	656ab	126ab	89a
		G	0.59b	2.32a	0.35b	0.11a	807b	131b	114b
		Y	0.45a	2.40a	0.33ab	0.11a	521a	116ab	108ab

dry mass than those in the darkness or in R. 40 % of the explants growing *in vitro* under W or Y formed lateral roots.

Among the nutrient elements, the P concentration of roots increased with the dark and the B treatment almost twice above the control (Table 2). Potassium concentration in roots was the greatest in the W treatment (Table 2). Those roots, which formed in the darkness, had the greatest Ca concentration, while Mg concentration was constant, irrespective of the radiation (Table 2). Among the microelements, Fe and Mn concentrations of roots were constant in all the treatments, while Zn concentration increased only when the roots were developed in the darkness (Table 2).

In the shoots, P concentration was greater in the R treatment compared to W (Table 2). Potassium concentration was the least in the D treatment and Ca and Mg concentrations were constant in all the treatments (Table 2). Y reduced the concentration of Fe in shoots (Table 2). The Zn concentration had the greatest value in the R treatment (Table 2). The concentration of Mn decreased with the D and the R treatments (Table 2).

After 2-d exposure to monochromatic radiation or D and subsequent transfer to W the least number of roots (2.9) was recorded under R radiation (Table 1), while the smallest length of roots (2.74 cm) was measured under R and G (Table 1). The fresh mass of roots was reduced under R, G and Y, while the dry mass declined only under R and G (Table 1). The explants of GF 677 grown under R had the smallest fresh and dry masses. 70 % of the explants maintained for 2 d under B and then transferred to W produced lateral roots (Table 1).

Among the macroelements in roots, the concentrations of P, Ca and Mg were constant in all treatments (Table 2), while that of K decreased with D, R and G (Table 2). From the microelements in roots, Fe concentration in G was almost four times greater than that of W (Table 2). R and Y increased the concentration of Zn, while spectral radiations and the darkness similarly affected the concentration of Mn (Table 2).

Concerning shoots, P concentration increased with the dark treatment above that in the control, K and Ca concentrations were constant in all the treatments, while Mg concentration decreased in D (Table 2). Fe concentration was two times greater than that in the control with the R and the G treatment (Table 2). R also increased the concentration of Zn, while that of Mn was constant in all the treatments (Table 2).

After 4-d exposure to monochromatic radiation or D and subsequently transfer to W the smallest average number of roots was obtained with R, B or G treatments (Table 1). When the explants were maintained in the dark or under Y for the first 4 d, the number of developed roots was equal to that under W (Table 1). The smallest length of roots was found in explants treated with D, R or G radiation (Table 1). The greatest root fresh and dry mass was observed with W (Table 1). The dry shoot mass declined under R or G radiations. 40 % of the explants kept under Y for the first 4 d and subsequently transferred to W formed lateral roots (Table 1).

The P and Ca concentrations of roots in the R treatment were greater than those under W (Table 2), while the concentration of K was affected negatively by B and Y (Table 2). The Mg concentration of roots was independent of spectral quality of irradiation or darkness (Table 2). G seemed to enhance Fe uptake and its concentration was four times greater than that in W (Table 2). Furthermore, Zn concentration increased only in the R treatment. Darkness and Y resulted in the greatest Mn concentration (Table 2).

G promoted P transport to shoots and its concentration was greater than that in W treatment (Table 2). Radiation quality and darkness did not influence the concentrations of K, Ca and Mg in shoots (Table 2). G increased the concentrations of Fe and Zn (Table 2), while the concentration of Mn was constant in all the treatments (Table 2).

Discussion

Visible radiation plays an important role in the *in vitro* morphogenesis. It has been found that some species, including peach, seem to form roots in *in vitro* only in the light (Rugini *et al*. 1988). However, in other species, visible radiation exerts inhibitory effects and keeping the cultures in the dark for a short period enhances root initiation (Hammerschlag 1982, Channuntapipat *et al*. 2003).

Exposure of GF 677 cultures to W irradiation during the whole rooting stage had the most stimulating effect on all the rooting parameters. This could be explained on the basis that W consists of all the wavelengths necessary for photosynthesis and other physiological processes, while continuous D or exposure to R inhibited rooting and the growth of shoots. The negative effect of R on rooting could be explained via its effect on phytochrome, as it alters the endogenous hormonal level (IAA/GAs < 1) of plants in favour of rooting inhibition (Fletcher and Zalik 1964, Reid *et al*. 1968, Tucker and Mansfield 1973).

When the *in vitro* cultures of GF 677 were maintained only for the first 2 or 4 d under monochromatic radiation or in D, W was again the most effective on the rooting of explants. Except for W radiation, the initial maintenance of the explants in the darkness or under Y or B, followed by W, promoted rooting. The favourable effect of a short period of D on rooting has been reported also for other plant species of *Prunus* (Hammerschlag 1982, Channuntapipat *et al*. 2003). Radiation exclusion protected auxin from photodegradation (Bassuk and Maynard 1987) and reduced the activity of peroxidase, which is an enzyme contributing to auxin degradation (Druart *et al*. 1982). The promotory effect of Y to rooting has also been reported by Fuernkranz *et al*. (1990) for *Pyrus serotina* and by Dimassi-Theriou *et al*. (1999) for *Actinidia chinensis*. The positive effect of B on the rooting of GF 677 is contradictory with the data reported for other plants (Fuernkranz *et al*. 1990).

The question of the effect of spectral quality of radiation on *in vitro* rooting through the nutrient uptake was not answered. The increase of Fe was considerable, reaching toxic concentrations when the explants were exposed for 2- or 4-d to R or G and then transferred to W. Under R, which had the most unfavourable consequences on the adventitious root formation of GF 677, the concentrations of P and Zn were greater than those of the control treatment, while the concentration of Mn was lower. If those elements are involved in the inhibition of rooting under R, then further experiments are necessary in order to elucidate this point.

In conclusion, exposure of *in vitro* cultures of the peach rootstock GF 677 to W during the rooting stage had the best results for rooting. Continuous D, or monochromatic radiation could not promote greater rooting than W. Nevertheless, the 2-d or 4-d exposure to D, Y or B followed by W could help root initiation and root growth, similarly with continuous W.

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