Agronomy Research **3**(1), 3–8, 2005

In vitro rooting of PR 204/84 rootstock (*Prunus persica* x *P. amygdalus*) as influenced by mineral concentration of the culture medium and exposure to darkness for a period

S. Fotopoulos and T.E. Sotiropoulos*

N.AG.RE.F., Pomology Institute, P.O. Box 122, 59200 Naoussa, Greece
*Corresponding author; e-mail: thosotir@alfanet.gr

Abstract. Reduction of the mineral concentration of MS medium to half the normal value increased the rooting percentage of PR 204/84 explants when IBA concentration was 2.5 μM, and mean root number when IBA concentrations were 2.5 and 5 μM. Root elongation was stimulated at all IBA levels on both full and half strength media. By increasing IBA concentration from 0 to 10 μM, an increase in the mean root number per shoot was observed in both media (full and half strength). The mean length of roots was not significantly affected by IBA and mineral concentration of the culture media. In a second experiment, after 12 days of culture of shoots in a dark room followed by 12 days in standard growth room conditions, rooting percentage of shoots increased in comparison to shoots grown for 24 days in standard growth room conditions with IBA concentrations 1 and 2.5 μM.

Key words: adventitious roots, indole-3-butyric acid, irradiance, *Prunus* micropropagation, rhizogenesis

INTRODUCTION

The PR 204/84 rootstock is a hybrid between peach and almond and was selected in the Pomology Institute of Greece. It is an alternative rootstock to GF-677 (peach x almond). It adapts well in low fertility soils, arid or semi arid regions, as well as in calcareous soils and replant sites. Furthermore, PR 204/84 has greater resistance to *Phytopthora*, *Verticillium dahliae* and *Agrobacterium tumefaciens* than GF-677 (Tsipouridis, 2003). The mineral concentration of the culture medium affects rooting characteristics, and some researchers have proposed its reduction to half normal strength for rooting improvement (Dimassi-Theriou & Economou, 1993). Keeping micropropagated shoots in darkness for a determined period during the rooting phase is generally favourable to rooting of *Prunus* and *Malus* shoots (Moncousin, 1988; George, 1996). The objectives of the present research were to study: a) the effect of mineral concentration (full and half strength) and IBA concentration of the MS medium, and b) the effect of light conditions of the growth chamber and IBA concentration of the medium on the rooting response of the PR 204/84 shoots cultured *in vitro*.

MATERIALS AND METHODS

The explants employed were shoots of the PR 204/84 peach rootstock (*Prunus persica* x *P. amygdalus*) of about 25 mm in length, preserved from previous *in vitro* cultures and maintained in the growth room. Each explant was transferred and grown in a 15x100 mm glass test tube containing 10 ml of the MS culture medium (Murashige & Skoog, 1962). Two experiments were conducted. In the first one, the effect of inorganic salts concentration of a MS medium (full strength, half strength except iron in order to avoid symptoms of chlorosis) supplemented with IBA at four concentrations (0, 2.5, 5, 10 μM) on the rooting performance of the PR 204/84 rootstock was investigated. A total of 8 treatments were employed (2 concentrations of salts of MS medium x 4 IBA concentrations) with 20 replications (tubes) per treatment. The tubes were closed with aluminum foil and maintained in the growth room at standard conditions (22 ± 1°C and 16-h photoperiod with irradiance of 45 μmol m^{-2} s^{-1} and wavelength of 400–700 nm). The experiment lasted 36 days. In the second experiment, shoots were placed in a full strength MS medium containing five concentrations of IBA (0, 1, 2.5, 5, 10 μM), transferred in a dark room for 12 days and then transferred for 12 days to standard growth room conditions as described previously. In another treatment (control), shoots were grown for 24 days in standard growth room conditions. In both experiments, the pH of the media was adjusted to 5.8 before autoclaving at 121°C for 15 min. This pH value was adopted by Murashige & Skoog (1962) protocol but still needs to be optimised for this plant material. At the termination of both experiments, the percentage of rooting was recorded. Furthermore, the mean root length, the mean number of roots per shoot and the fresh and dry mass of roots was measured. The experiments were conducted twice, and the reported data are the means of the two experiments. The statistical design employed was the randomised complete block one. Differences between means were evaluated using LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

1. First experiment. After 36 days in culture, excellent rooting of shoots (100%) occurred on MS media (full strength) containing 5 and 10 μM IBA and MS media (half strength) containing 2.5, 5 and 10 μM IBA (Table 1). On the contrary, shoots were not rooted on the media without auxin (control). By increasing IBA concentration from 0 to 10 μM, an increase in the mean root number per shoot was observed in both media (full and half strength). The highest mean root number per shoot was obtained in the MS media (full strength) containing 10 μM IBA, although it was not significantly different than that on the MS media (half strength) plus 5 or 10 μM IBA (Table 1). The mean length of roots was not significantly affected by IBA and mineral concentration of the culture medium. By increasing the IBA concentration of both media types, the fresh and dry mass of roots increased, too. The promotory effect of mineral concentration of the culture medium on rooting can be attributed to the participation of inorganic ions in processes regulating hormonal balance (Amzallag et al., 1992). Satisfactory rooting can take place on full strength culture media but it is very common practice to transfer shoots to be rooted from high strength media to less concentrated solutions. This practice is used for herbaceous plants as well as for woody ornamentals,

fruit trees or forestry species (Moncousin, 1988; George, 1996). The favourable effect of a diluted mineral solution on rooting can be explained by the reduction of nitrogen concentration (Driver & Suttle, 1987). Dimassi-Theriou (1995) reported that reducing mineral concentration of the MS medium to half the normal value increased rooting percentage and stimulated root elongation of the GF 677 rootstock *in vitro*. Ruzic et al. (1984) have proposed the use of MS medium at half of normal strength for rooting improvement of the GF 677 rootstock shoots. Fouad et al. (1997) reported that a half strength medium supplemented with different concentrations of auxin resulted in high rooting percentages of the *Prunus* rootstocks Nemaguard and Meet-Ghamre. Moncousin (1988) suggested that the dilution of salt concentration to half prepared the plants in the tubes for better adaptation to the acclimatisation medium, while the growth of shoots and leaf size increased. The results of our experiment with PR 204/84 suggested that reducing mineral concentration of MS medium to half of the normal value increased rooting percentage when IBA concentration was 2.5 μM and the mean root number with IBA concentration was 2.5 and 5 μM. Root elongation was stimulated at all IBA levels on both full and half strength media. Common IBA concentrations giving satisfactory rooting responses of various *Prunus* cultures *in vitro* range from 2.5 to 7 μM (Dimassi-Theriou & Economou, 1993; Dimassi-Theriou, 1998).

2. Second experiment. After 12 days of culture in a dark room followed by 12 days in standard conditions, and the rooting percentage of shoots increased in comparison to the control when IBA concentrations were 1 and 2.5 μM (Table 2). When shoots were exposed to darkness they became chlorotic with longer internodes and smaller leaves than the control. The increase of IBA concentration resulted gradually in an optimum level of root formation per shoot, fresh, and dry mass at 10 μM when shoots were grown in standard conditions (Table 2). When shoots were exposed to a period of darkness, an increment IBA concentration to 2.5 μM increased root formation per shoot, while higher levels (5 and 10 μM) reduced it. When shoots were exposed to a period of darkness, higher fresh and dry mass of roots was obtained on the media containing 5 μM IBA while the mean root length was highest on the media containing 1 μM IBA. Many plants are capable of producing adequate number of adventitious roots when the shoots are kept in the dark for a determined period. Druart et al. (1982) reported that darkness was more promotive to root formation of *in vitro* apple shoots at the period of root initiation. Beneficial effects of the dark treatment were found chiefly in difficult-to-root woody plants and have been demonstrated in many genera. Standardi (1979) found that the *in vitro* rooting of *Prunus* and *Malus* shoots was improved if the shoots were first cultured in darkness and then grown in normal growth room conditions. This result was confirmed by Hammerschlag (1987) who obtained 100% rooting of *Prunus cerasifera, Prunus insititia* and *Prunus domestica* shoots by incubating them in the dark for 2 and 6 weeks respectively. Zanol et al. (1997) reported that rooting of apple shoots was better when they were exposed to 3 days with darkness. Furthermore, Rugini and Verma (1982) reported that a dark treatment for 10 days enhanced rooting of *Prunus dulcis* shoots *in vitro*. The positive effect of dark treatment on rooting may be correlated to the faster metabolism of endogenous or exogenous auxins in the dark compared to the light (Maynard & Bassuk, 1987). However, darkness induced supra-optimal shoot elongation and leaf-stem chlorosis at all IBA levels, especially at the highest.

Table 1. Effect of IBA and mineral concentration of the culture medium on the rooting percentage, mean number of roots per shoot, mean length of roots, fresh, and dry mass of the PR 204/84 shoots after 24 days in culture.

Treatments	Rooting (%)	Number of roots	Length of roots (mm)	Fresh mass of roots (mg)	Dry mass of roots (mg)
MS + 0 μM IBA	0	0	0	0	0
MS + 2.5 μM IBA	70	2.0	9.3	338	44
MS + 5 μM IBA	100	8.1	10.5	506	56
MS + 10 μM IBA	100	15.2	9.6	1579	88
MS ½ + 0 μM IBA	0	0	0	0	0
MS ½ + 2.5 μM IBA	100	10.1	10.1	914	90
MS ½ + 5 μM IBA	100	14.5	9.7	1093	126
MS ½ + 10 μM IBA	100	14.5	9.2	2147	228
$LSD_{0.05}$		1.7	1.5	146	11

Table 2. The effect of irradiance and IBA concentration of the culture medium on rooting percentage, number of roots per shoot, mean length of roots, fresh, and dry mass of the PR 204/84 shoots after 24 days in culture.

Treatments	IBA	Rooting (%)	Number of roots	Length of roots (mm)	Fresh mass of roots (mg)	Dry mass of roots (mg)
(24 days in light)	0 μM	0	0	0	0	0
	1 μM	54	1.7	3.5	298	39
	2.5 μM	61	2.0	4.9	338	44
	5 μM	90	8.0	14.5	506	56
	10 μM	90	15.2	9.6	923	88
24 days (12 days darkness – 12 days light)	0 μM	0	0	0	0	0
	1 μM	90	7.2	12.6	783	99
	2.5 μM	90	10.1	9.7	808	100
	5 μM	85	8.5	8.9	890	115
	10 μM	84	8.4	8.9	810	95
$LSD_{0.05}$			1.86	1.94	84.3	11.4

The supra-elongated shoots were stressed by the light intensity when they were placed at standard growth room conditions, showing symptoms of tip burn. This happened because the elongated parts of the shoots exhibited juvenile characteristics with very sensitive tissues to the light conditions in the growth room. Further experimentation is needed for a shorter dark treatment to avoid problems of shoot chlorosis and excessive shoot elongation due to the long exposition of plants under dark conditions.

CONCLUSIONS

1. The results of this research suggested that reducing mineral concentration of MS medium to half the normal value increased the rooting percentage of PR 204/84 shoots when IBA concentration was 2.5 μM, and the mean root number when IBA concentrations were 2.5 and 5 μM. Root elongation was stimulated at all IBA levels on both full and half strength media, however means were not significantly different.
2. After 12 days of culture in a dark room followed by 12 days in standard conditions, rooting percentage of shoots increased in comparison to the control (24 days in standard growth room conditions) at IBA concentrations 1 and 2.5 μM. However, further experiments should be conducted adopting shorter dark treatments in order to avoid problems of shoot chlorosis and excessive shoot elongation. The differences in rooting percentage of shoots between the two experiments could be attributed to the different experimental periods.

REFERENCES

Amzallag, G. N., Lerner, H. R. & Poljakoff-Mayber, M. 1992. Interaction between mineral nutrients, cytokinin and gibberillic acid during growth of sorghum at high NaCl salinity. *J. Exp. Bot*. **43**, 81–87.

Dimassi-Theriou, K. & Economou, A. 1993. The effect of the explant type and the nutrient substrate composition on shoot production in *in vitro* cultures of the rootstock GF-677 (*P. persica* x *P. amygdalus*). In *Scientific Annals. Aristotle Univ. of Thessaloniki*, pp. 31–35, Greece.

Dimassi-Theriou, K. 1995. *In vitro* rooting of rootstock GF 677 (*P. persica* x *P. amygdalus*) as influenced by mineral concentration of the nutrient medium and type of culture-tube sealing material. *J. Hort. Sci*. **70**, 105–108.

Dimassi-Theriou, K. 1998. Response of increasing rates of NaCl or $CaCl_2$ and proline on 'Mr.S 2/5' (*Prunus cerasifera*) peach rootstock cultured *in vitro*. *Adv. Hort. Sci.* **12**, 169–174.

Driver, J. A. & Suttle, G. R. 1987. Nursery handling of propagles. In *Cell and Tissue Culture in Forestry.* (Bonga J. M. & Durzan D. J., eds.), pp. 320–335. Dortrecht, Netherlands.

Druart P., Kevers, C., Boxus, C. & Gaspar, T. 1982. *In vitro* promotion of root formation by apple shoots through darkness. Effect on endogenous phenols and peroxidases. *Z. Pflanzenphysiol*. **109**, 429–436.

Fouad, M. M., Gomaa, A. H. & El-Zaher, M. H. 1997. Factors influencing *in vitro* establishment and multiplication stages of peach. *Hort. Abst*. **67(1)**, 69.

George, E. F. 1996. *Plant propagation by tissue culture: in practice, Part II. 2nd edition.* Exegetics Limited Press, London.

Hammerschlag, F., Bauchan, G. & Scorza, R. 1987. Factors influencing *in vitro* multiplication and rooting of peach cultivars. *Plant Cell. Tissue Organ Cult*. **8**, 235–242.

Maynard, K.B. & Bassuk, N.L. 1987. Etiolation and banding effects on adventitious root formation. In *Adventitious Root Formation*. (Davies T., Haising B.E. & Sankhla N., eds.). Dioscourides Press, Portland, Oregon.

Moncousin, C. 1988. Adventitious rhizogenesis control: new developments. *Acta Hort*. **230**, 97–104.

Murashige, T. & Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant*. **15**, 473–497.

Rugini, E. & Verma, D. C. 1982. Micropropagation of difficult to propagate almond (*Prunus amygdalus*) cultivar. *Plant. Sci. Lett*. **28**, 273–281.

Ruzic, D., Rosati, P. & Marino, G. 1984. Effecto dei figoregolatori nella micripropagatione dell´ ibrido pesco X mandorlo GF 677. *Riv. Ortflorofrutt. It*. **68**, 413–422.

Standardi, A. 1979. In dagine preliminare sull' influenza della luce nela micropropagazione di alcume specie legnose. Alt. Inc. SOI 'Techniche di coltura in vitro', Pistoia. pp. 107–118.

Zanol, C., Fortes, G., Silva, J., Campos, A., Centellas, A., Muller, N. & Gottinari, R. 1997. Influence of the darkness and the indol-3-butyric acid on *in vitro* rooting and peroxidase activity of the apple rootstock Marubakaido. *Rev. Bras. Agr*. **3**, 23–30.

Tsipouridis, K. 2003. Annual report of the Pomology Institute of Naoussa, pp. 24. Pomology Institute, Greece.