BIOLOGIA PLANTARUM 48 (4): 629-631, 2004

BRIEF COMMUNICATION

# *In vitro* propagation of the peach rootstock: the effect of different carbon sources and types of sealing material on rooting

S. FOTOPOULOS and T.E. SOTIROPOULOS*

*N.AG.RE.F., Pomology Institute, P.O. Box 122, GR-59200 Naoussa, Greece*

## Abstract

The *in vitro* cultures of the PR 204/84 peach rootstock (*Prunus persica* × *P. amygdalus*) produced the higher rooting percentage, mean root number, mean root length, fresh, and dry mass of roots when grown on media containing 88 mM sucrose or 88 mM glucose. Parafilm, rubber and aluminum foil as sealing materials were not significantly different in terms of rooting percentage, fresh, and dry mass of roots after 24 d in culture. The use of cotton as sealing material induced lower root number per shoot, length of roots, fresh, and dry mass of roots than the rest treatments.

*Additional key words*: glucose, parafilm, *Prunus* micropropagation, rhizogenesis, sucrose.

The PR 204/84 peach 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. It is adapted 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 present study is the first attempt for its micropropagation. The induction of roots on explants from *in vitro* cultures is crucial in micropropagation process (Molassiotis *et al*. 2003/4). Rhizogenesis *in vitro* is an energy-demanding process and sugars must be added into the culture medium. The addition of different sugars in the culture medium may influence shoot proliferation potential of explants (Jain and Babbar 2003) as well as rhizogenesis (Vierskov 1987). Media containing 44 - 88 mM sucrose as carbon source have been used in most studies for rooting of *Prunus* species (George 1996). Another factor that affects rooting by modifying the composition of the gas phase of tissue culture vessels is the type of material used for sealing (Jackson 2003). The objectives of the present research were to study the effect of sucrose and glucose as carbon sources at various concentrations as well as the effect of different sealing materials (aluminum foil, cotton, rubber, parafilm) on the rooting response of the PR 204/84 shoots cultured *in vitro*.

The explants employed were shoots of the PR 204/84 peach rootstock (*Prunus persica* × *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 15 × 100 mm glass test tube containing 10 $cm^3$ of the Murashige and Skoog (1962; MS) nutrient medium supplied with 5 μM indole-3-butyric acid (IBA). Two experiments were conducted. In the first one, the effect of sucrose and glucose as carbon sources on rooting performance was investigated. Five concentrations of sucrose and glucose (0, 44, 88, 176, 352 mM) were added in the culture media. A total of 10 treatments were employed (2 sugars × 5 concentrations) with 20 replications (tubes) per treatment. In the second experiment, the effect of four types of sealing materials (aluminum foil, cotton, rubber, parafilm) on the rooting response of shoots was investigated. In both experiments, the pH of the media was adjusted to 5.8 before autoclaving at 121 °C for 15 min. The tubes were maintained in the growth room at temperature of 22 ± 1 °C and 16-h photoperiod (cool white fluorescent tubes, irradiance of 45 μmol $m^{-2}$ $s^{-1}$, 400 - 700 nm). Percentage of rooting was recorded after 24 d in culture. Furthermore, the mean root length, mean number of roots per shoot, fresh, and dry mass of roots were measured.

*Received* 19 April 2004, *accepted* 9 August 2004.
*Abbreviations*: IBA - indole-3-butyric acid; MS medium - Murashige and Skoog medium.
* Corresponding author; fax: +302332041178, e-mail: thosotir@alfanet.gr

The experiments were conducted twice, and the reported data are the means of the two experiments. The statistical design employed was the randomized complete block one. Differences between means were evaluated using LSD test at $P \leq 0.05$.

Inclusion of 88 mM sucrose in the culture medium significantly increased rooting percentage, mean number of roots per shoot, mean length of roots, fresh, and dry mass of roots in comparison to the rest treatments (Table 1). By increasing the concentration of sucrose in the medium from 0 to 88 mM, an increase of rooting percentage, mean length of roots per shoot, mean number of roots, fresh, and dry mass were measured. On the contrary, by increasing sucrose concentration from 88 to 352 mM, the previous parameters decreased gradually. Sucrose in concentrations 176 and 352 mM inhibited root elongation. At 352 mM sucrose in the medium, rooting percentage decreased to very low levels combined with leaf and shoot chlorosis and marginal leaf necrosis. Inclusion of 88 mM glucose in the medium significantly increased rooting percentage, mean number of roots per shoot, mean length of roots, fresh, and dry mass of roots in comparison to the rest treatments (Table 1). By increasing the glucose concentration of the medium from 88 to 352 mM, rooting percentage, mean length of roots per shoot, mean number of roots, fresh, and dry mass decreased. Low photosynthetic activity of plantlets cultured *in vitro* is considered one of the major limiting factors for the improvement of micropropagation efficiency. Photosynthetic rate of tissue-cultured plantlets is affected by various factors such as: explant type and size, stage of growth, $CO_2$ and $O_2$ concentrations, light quality and presence of exogenous sugars in the medium (Langford and Wainwright 1988, Desjardins 1995, Antonopoulou *et al.* 2004). Photoautotrophy may be induced in *in vitro* cultures by increasing irradiance and enriching the culture vessel atmosphere with $CO_2$ from an external source (Morini and Melai 2003/4). All carbon sources do not sustain rooting equally. For most plant species including *Prunus,* sucrose has been used for promoting rooting of shoots (George 1996). Dimassi-Theriou (1989) observed higher rooting percentage of GF-677 shoots on media containing 88 mM sucrose, although glucose resulted in satisfactory results, too. Mehta *et al*. 2000 reported that high additions of sugars (> 6 %) exert negative effects on growth of tamarind (*Tamarindus indica* L.) explants since they induce browning of media. Furthermore, high sugar contents induce an osmotic stress on explants (Hazarika 2003).

Table 1. Rooting percentage, mean number of roots per shoot, mean length of roots, fresh, and dry mass of the PR 204/84 rootstock, as influenced by sucrose, glucose and their concentrations in the medium after 24 d in culture. Means ± S.E., $n = 20$.

| Sugar | Concentration [mM] | Rooting [%] | Number [shoot$^{-1}$] | Length [mm] | Fresh mass [mg plant$^{-1}$] | Dry mass [mg plant$^{-1}$] |
|---|---|---|---|---|---|---|
| Sucrose | 0 | 80 | 4.5 ± 0.33 | 3.2 ± 0.20 | 1017 ± 47.2 | 99 ± 4.54 |
| | 44 | 90 | 4.5 ± 0.31 | 8.9 ± 0.71 | 1032 ± 48.4 | 101 ± 4.64 |
| | 88 | 100 | 8.0 ± 0.61 | 14.5 ± 1.36 | 1721 ± 79.23 | 139 ± 6.21 |
| | 176 | 81 | 3.6 ± 0.23 | 5.3 ± 0.40 | 989 ± 45.1 | 95 ± 4.21 |
| | 352 | 10 | 0.7 ± 0.04 | 0.8 ± 0.05 | 140 ± 6.11 | 12 ± 1.09 |
| Glucose | 0 | 80 | 4.5 ± 0.33 | 3.2 ± 0.20 | 1017 ± 47.2 | 99 ± 4.54 |
| | 44 | 88 | 5.9 ± 0.51 | 6.1 ± 0.48 | 1236 ± 53.9 | 125 ± 8.21 |
| | 88 | 100 | 10.8 ± 0.98 | 19.6 ± 0.42 | 1849 ± 88.9 | 189 ± 8.59 |
| | 176 | 91 | 5.3 ± 0.38 | 16.7 ± 0.31 | 1243 ± 54.1 | 118 ± 5.02 |
| | 352 | 62 | 1.7 ± 0.13 | 7.2 ± 0.54 | 530 ± 22.1 | 49 ± 2.27 |
| $LSD_{0.05}$ | | | 1.8 | 2.3 | 94.1 | 6.3 |

Table 2. Rooting percentage, mean number of roots per shoot, mean length of roots, fresh, and dry mass of the PR 204/84 rootstock, as influenced by the type of sealing material after 24 d in culture. Means ± SE, $n = 20$.

| Sealing material | Rooting 12 d [%] | Rooting 24 d [%] | Number [shoot$^{-1}$] | Length [mm] | Fresh mass [mg plant$^{-1}$] | Dry mass [mg plant$^{-1}$] |
|---|---|---|---|---|---|---|
| Parafilm | 83 | 100 | 11.6 ± 0.89 | 9.8 ± 0.61 | 1676 ± 81.99 | 164 ± 7.98 |
| Cotton | 42 | 58 | 3.8 ± 0.18 | 4.0 ± 0.20 | 754 ± 34.29 | 81 ± 2.98 |
| Rubber | 92 | 100 | 10.9 ± 0.68 | 8.2 ± 0.44 | 1649 ± 80.60 | 171 ± 8.11 |
| Aluminum foil | 100 | 100 | 7.8 ± 0.42 | 14.6 ± 1.21 | 1709 ± 84.10 | 177 ± 8.27 |
| $LSD_{0.05}$ | | | 2.55 | 2.11 | 107.1 | 14.2 |

Shoots cultured in test tubes covered with aluminum foil produced the maximum rooting percentage (100 %) after 12 d in culture (Table 2). When the tubes covered with cotton the rooting percentage was very low (41.8 %). Rooting percentage of shoots grown in tubes covered with cotton was significantly lower in comparison to the rest treatments after 24 d in culture. The use of cotton as a sealing material caused a decline in rooting percentage, shoot chlorosis, and abscission of leaves. The mean number of roots per shoot was significantly higher when the test tubes were covered with parafilm or rubber than cotton or aluminum foil. However, the maximum mean length of roots was observed on shoots grown in test tubes covered with aluminum foil, while the rest treatments produced shorter roots. The use of cotton as sealing material induced the lowest mean root number per shoot, mean length of roots, fresh, and dry mass of roots, than the rest treatments. The type of material used to seal the culture tubes is a factor that affects rooting by modifying the composition of the gas phase of tissue culture vessels ($O_2$, $CO_2$, $C_2H_4$ concentrations and relative humidity) (Nelson *et al.* 1980). Dimassi-Theriou (1995) reported that the type of sealing material affected rooting of GF-677 peach rootstock. The previous researcher concluded that rubber capping proved superior to parafilm with respect to rooting percentage, number, and fresh mass of roots. However, mean root length declined with rubber as capping material in comparison to parafilm. Cotton plugs have been used in research laboratories but the medium in tubes covered with cotton plugs dries out during the incubation period, affecting adversely the growth of the cultured tissues (George 1996). In the present research, culture medium in tubes sealed with cotton was obviously started to dry out by the 19$^{th}$ day from the initiation of the experiment. Closures such as parafilm are permeable to gases such as $O_2$, $CO_2$, and $C_2H_4$. As pointed out by Dimassi-Theriou *et al.* (1993) ethylene accumulation in the culture vessels if not above the detrimental level, promotes rooting *in vitro*. Aluminum foil has the disadvantage of not providing a tight seal and act more or less like parafilm but reduces the availability of light. Parafilm allows light penetration through from above in contrast to aluminum foil and rubber.

## References


Antonopoulou, C.A., Dimassi, K., Therios, I., Chatzissavvidis, C.: The influence of radiation quality on the *in vitro* rooting and nutrient concentrations of peach rootstock. - Biol. Plant. **48**: 549-553, 2004.

Desjardins, Y.: Photosynthesis *in vitro* – on the factors regulating $CO_2$ assimilation in micropropagation systems. - Acta Hort. **393**: 45-62, 1995.

Dimassi-Theriou, K.: Factors affecting the *in vitro* culture of peach-almond hybrid GF-677 in the stage of multiplication and rooting. - Ph.D. Thesis. Aristotle University of Thessaloniki, Thessaloniki 1989.

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

Dimassi-Theriou, K., Economou, A., Sfakiotakis, E.: Promotion of petunia (*Petunia hybrida* L.) regeneration *in vitro* by ethylene. - Plant Cell Tissue Organ Cult. **32**: 219-225, 1993.

George, E.F.: Plant Propagation by Tissue Culture: in Practice. - Exegetics Limited Press, London 1996.

Hazarika, B.: Acclimatization of tissue-cultured plants. - Curr. Sci. **85**: 1704-1712, 2003.

Jackson, M.B.: Aeration stress in plant tissue cultures. - Bulg. J. Plant Physiol. **28**: 96-109, 2003.

Jain, N., Babbar, S.B.: Effect of carbon source on the shoot proliferation potential of epicotyl explants of *Syzygium cuminii*. - Biol. Plant. **47**: 133-136, 2003.

Langford, P.J., Wainwright, H.: Influence of sucrose concentration on the photosynthetic ability of *in vitro* grown rose shoots. - Acta Hort. **227**: 305-310, 1988.

Mehta, U., Krishnamurthy, K., Hazra, S.: Regeneration of plants via adventitious bud formation from zygotic embryo axis of tamarind (*Tamarindus indica* L.). - Curr. Sci. **78**: 1231-1234, 2000.

Molassiotis, A., Dimassi, K., Therios, I., Diamantidis, G.: Fe-EDDHA promotes rooting of rootstock GF-677 (*Prunus persica* x *P. amygdalus*) explants *in vitro*. - Biol. Plant. **47**: 141-144, 2003/4.

Morini, S., Melai, M.: $CO_2$ dynamics and growth in photoautotrophic and photomixotrophic apple cultures. - Biol. Plant. **47**: 167-172, 2003/4.

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

Nelson, N.D., Isebrands, J.G., Rietveld, W.J.: Ethylene loss from the gas phase of container-seal systems. - Physiol. Plant. **48**: 188-190, 1980.

Tsipouridis, K.: Annual report of the Pomology Institute of Naoussa. - Pomology Institute, Naoussa 2003.

Vierskov, B.: Relation between carbohydrates and adventitious root formation. - In: Davies, T., Haising, B.E., Sankhla, N. (ed.): Adventitious Root Formation. Pp. 70-78. Dioscourides Press, Portland 1987.