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# Root formation by microshoots of *Prunus insititia* L., rootstock GF 655/2 in an auxin-free medium

**(with 2 tables)**

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**Abstract.** Root formation by *Prunus insititia* L cv.GF 655/2 microshoots in an auxin-free medium (BM) after several subculture periods in a multiplication medium was studied. More roots were formed in shoots when subcultured for 31 weeks in a multiplication medium (MM) than in those with only 2 subcultures. In the first group, roots started to emerge eight days after the transfer of shoots to BM, while 24 days elapsed for those with only 2 periods in MM. All shoots rooted in the 31 group by day 16th, but only 46.7% shoots of group 2 had roots at the end of the experiment (30 days). No differences were found in the number of roots but a larger root system was observed in the group of the 31 subcultures. This difference in rooting ability is attributed to partial rejuvenation caused by the long period spent in a cytokinin containing medium.

**Keywords:** *Prunus,* rooting, *in vitro* culture, rejuvenation.

Micropropagation is generally considered a technique for rejuvenation of adult woody plants (3). This phase change is expressed, among other characteristics, by the rooting capability of the microshoots. The multiplication phase of protocols includes a cytokinin; this compound promotes shoot formation but, at the same time, inhibits root formation. For that reason, the explants are transferred to an auxin containing culture medium. There are reports

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the serial culture of shoots in the last medium increases the ng ability of the microshoots (14, 8, 16, 4 among others).

During micropropagation of *Prunus insititia* L., rootstock GF some shoots formed roots in the multiplication medium (10). ous trials with 2, 11 and 31 subcultures in that medium revealed rences in the rooting expression of the microshoots after being sferred to a medium without growth regulators (11). This effect be attributed to an increase of juvenility, caused by culture in dium with cytokinin during several periods.

The present paper describes responses of microshoots that after us culture periods in a multiplication medium were transferred normone free medium.

## MATERIAL & METHODS

Microshoots of *Prunus insititia* L., rootstock GF 655/2, about 2 ng, with two to four expanded leaves were employed. They had cultured for several multiplication periods in the medium ibed by Radice et al., (10), and then, transferred to a regulator- medium, composed by a modified MS medium [(Murashige & g (9)] with nitrates reduced by half and double Fe.EDTA; to which llowing organic compounds were added:(mg/L): myo-inositol, 100; mine HCl, 0,1; glycine, 2; pyridoxine HCl 0,1; Ca panthotenate nicotínic acid 0,1; biotin 0,1; riboflavin 0,5; ascorbic acid 10; se 30.000; regional agar 8.000. Aliquots (50 mL) of medium were nsed into 350 mL flasks. This medium will be called basal (BM). pH was adjusted to 5,8±0,02 with KOH 1 N before autoclaving 20° during 20'. The environmental conditions were 24±1°C, and operiod of 16 h light from cool-white fluorescent lamps. (57 $\mu m^{-2}.s^{-1}$). Three microshoots were placed in each flask and each ment consisted of fifteen shoots. Observations were made every days and the final count after thirty days.

During the culture period, half of the shoots were maintained a 16 h photoperiod, provided by cool white fluorescent lamps the other half was placed in darkness for the first week, and transferred to light.

Experiments were repeated thrice, with different number of plication periods before transfer to BM. During the culture d, data recorded were: percentage of rooted shoots, shoot length, rea, number of roots per shoot: root fresh and dry weights and root length. Data of rooting percentage were analyzed by $\times^2$ and Hoc for multiple comparisons, while the Tukey test analyzed the data.



## RESULTS & DISCUSSION

In previous experiments, the rooting ability after several subculture periods in BM revealed differences in the growth responses of the microcuttings. Here data after two (2) and thirty-one (31) subcultures are reported. While roots emerged after 24 days in BM in some microcruttings of the "2" group, those of the "31" group showed roots after 8 days (Table 1). In "2", only 46.7% rooted at the end of the experiment, while the entire "31" had rooted (same table). Shoots kept in darkness for the first week and thereafter placed under light conditions, showed lower rooting percentages that those maintained all the time under 16 h photoperiod. The maximum number of rooted shoots of "31" under both conditions was achieved at about day 16th. The number of rooted microcuttings of "2" increased till the end of the experiment. Also fewer shoots rooted of those held in the dark at the beginning of the rooting phase.

No differences in root number and either root or shoot length were found in the different treatments. The root system was longer in shoots kept for 31 subcultures in MM (Table 2). While there were differences in the fresh root weight of both groups under light conditions, no statistical differences in root dry weights of those shoots that were kept in darkness during the first week of rooting were found. There were no significant differences in leaf area.

Adventitious root formation in a cytokinin-containing medium was obtained in the longest shoots growing in a group of several buds (10). Similarly, Jordan et al. (7) found that low cytokinin concentrations can induce rooting in cultures of *P. avium* hypocotyls. It is possible that in microshoots with several subcultures in a medium with low cytokinin level, the induction of root formation is a consequence of the existence of buds and young leaves, both good auxin producers (13). This is the reason why the greatest and fastest rooting was observed in shoots from the 31st culture. In media with auxins, shoots with more subcultures had an increased rooting ability, similar to a young plant arising from seed (8). Sriskandarajah et al.,

| | Light | | Dark | |
|---|---|---|---|---|
| BM | 2 subc | 31 subc | 2 subc | 31 subc |
| 8 days | 0 | 46.7* | 0 | 13.3 |
| 16 days | 0 | 100* | 0 | 46.7* |
| 24 days | 33.3 | 100* | 12.5 | 46.7* |
| 30 days | 46.7 | 100* | 26.7 | 46.7* |

Table 1.- Rooting of shoots, as %, after subculture (subc) periods of 2 or 31 months, in a multiplication medium (MM) and then transferred to a basal medium (BM)

Light= 16 h photoperiod, 4 weeks; dark= first week dark + 3 weeks light.
* Data of different number of subcultures in the same light treatment are significantly different (5%) in Post-Hoc test

Table 2.- Root number, total root length (cm) and root fresh and dry weight (mg), shoot length (cm) and leaf area ($cm^2$) of rooted microshoots after 4 weeks in a hormone-free medium and being previously cultured for either 2 or 31 subcultures in a multiplication medium (n=15; averages ± S.E.)

| | Light | | Dark | |
|---|---|---|---|---|
| | 2 subcult. | 31 subcult. | 2 subcult. | 31 subcult. |
| Root number | 1.28±0.18a | 1.73±0.26a | 1.50±0.28a | 1.14±0.14a |
| Total root length | 4.42±0.79a | 8.40±1.20b | 8.32±0.62a | 7.10±0.71a |
| Shoot length | 2.82±0.18a | 3.52±0.20a | 2.22±0.04a | 3.90±0.22a |
| Leaf area | 4.36±1.82a | 3.14±0.21a | 2.23±0.13a | 3.06±0.48a |
| Root fresh weight | 20±0.2a | 30±0.2b | 16±0.1a | 13±0.3a |
| Root dry weight | 2±0.4a | 2±0.03a | 1.6±0.01a | 0.4±0.03b |

Light= 4 weeks under 16 h photoperiod; dark= first week dark + 3 weeks light
Among columns within same light treatment, different letters indicate significant differences (95 %) in Tukey test.

found in two apple cultivars high frequency of adventitious root mation only after several subculture periods in a medium with in. Hammatt & Grant (4) found that micropropagated *Prunus um* plantlets showed greater multiplication and rooting abilities h increasing number of subcultures; it was concluded that arently this was a sign of rejuvenation (4).

The presence of $GA_3$ in MM may contribute to a partial reversion a juvenile stage. Rogler & Hackett (12) observed that the addition $GA_3$ caused phase change in *Hedera helix*. Low $GA_3$ concentrations almond cultures increased the number of rooted shoots, but only after 3th subculture (1). Gibberellins have a dual effect on root formation: promoters, in some species or root inhibitors in others (5).

The lower number of rooted shoots in the treatment with one ek of initial darkness disagrees with reports of other authors [(2) ong others]. However, a stimulatory effect of a continuous light atment on rooting is reported (15).

From the results reported here it may be concluded that shoots n less subcultures had lower rooting ability, irrespective of light ditions. Rooting ability of shoots cultured in a medium without wth regulators was obtained with repeated subcultures in a tiplication medium. This increased capacity could mean the



achievement of an apparent rejuvenation, as postulated by Hackett (3). Similar conclusions have been drawn from cultures of M.9 apple, pear (6) and wild cherry (4), rootstocks (16).

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