In Vitro Propagation of Malling 7 Apple Rootstock

E. M. Werner and A. A. Boe
Department of Plant and Soil Sciences, University of Idaho, Moscow, ID 83843

Abstract. Rapid in vitro propagation of Malling (M) 7 apple rootstock (Malus sylvestris Mill.) was obtained on a modified Murashige and Skoog (MS) medium using shoot tips as primary explants. Shoot regeneration was induced on a half-strength MS medium supplemented with 0.5 mg/liter benzylamino purine (BA). Rooting was induced by subculturing plantlets on a one-third strength MS medium containing 0.27% agar and supplemented with either 1.0, 2.0 or 3.0 mg/liter indolebutyric acid (IBA). Shoots subcultured on medium containing 2.0 mg/liter IBA rooted within 28 days.

Various investigators have cultured single apple shoots in vitro, but the induction of root formation has been difficult (2, 3, 4, 5, 8). Abbott and Whiteley (1) induced multiple shoot production from isolated meristems of apple seedlings and the cultivar ‘Cox’s Orange Pippin’, but root induction was also difficult. Jones et al. (6) induced shoot multiplication and rooting of M 26 apple rootstock from shoot tips using a culture medium containing phloroglucinol. The present paper describes a rapid propagation method for M 7 apple rootstocks.

Primary explants were isolated from shoot tips of M 7 apple rootstock in the field during June and July. Shoot tips were sterilized with a brief wash in wetting agent and immediately immersed in sodium hypochlorite (10% Clorox) for 20 min., followed by a rinse of 70% ethanol and 2 rinses of sterile distilled water. Explants 1 cm in length were cut aseptically from sterilized shoot tips and placed on a shoot multiplication medium in culture tubes.

The shoot multiplication medium consisted of a MS formulation (7) containing inorganic salts at one-half strength, 0.6% agar, and supplemented with 0.5 mg/liter BA. The pH was adjusted to 5.7. One hundred ml medium were dispensed into 0.95 liter jars and the medium was autoclaved. Shoot tips 0.5 to 1.0 cm in length were excised from proliferating cultures and transferred to rooting medium (Fig. 2). Shoot tips floated on the medium but were never submersed. After 30-40 days, plants were lifted from the medium and transplanted into peat pots containing a 1 sand:1 peat:1 perlite (by volume) medium supplemented with nutrients (Fig. 3). Potted plants were then placed in a growth chamber (2.0-3.0 klx at 20-25°C) for 7 days before transferring to a greenhouse.

A major problem in establishing the

![Fig. 2. Increase in shoots on second culture of A. andreanum stem section.](image)

**Literature Cited**


4. Whiteley (1) induced multiple shoot formation in callus tissues of Anthurium andreanum Lind.

5. Additional index words. tissue culture, Malus, micropropagation

initial apple explant was tissue browning. Primary explants cultured on the one-half strength MS salt multiplication medium showed little or no browning. Contamination rates were about 15% for primary explants and 5% for subcultures. Shoot multiplication began after 30 days. New shoots isolated from the primary culture continued to proliferate at a rate of 13 shoots per month. After about 4 months on multiplication medium several cultures showed BA toxicity. Periodic substitution of 5.0 mg/liter N6 \((\gamma^2\text{ isopentenyl})\) adenine (2iP) for BA reduced or eliminated toxicity in cultures. Cultures of M 7 apple rootstock have been maintained for over a year and have continued to proliferate.

Rooting was readily achieved on the low salt, low agar medium. Within 18 days, 88% of the shoots were rooted and within 28 days, all shoots were rooted on medium containing 2 mg/liter IBA (Table 1). Preliminary research involving IBA levels ranging from 1 to 80 mg/liter in low salt, low agar media showed rooting at all concentrations, but was less frequent and/or delayed at concentrations above 3 mg/liter. The low agar rooting medium used in this technique facilitated removal and transplanting with minimum root damage. Some transplant shock was observed, but was not severe. Transplanting results were best during the fall and winter.

With this technique, an exponential increase in shoot number could be achieved. Hypothetically, if all proliferating shoots were subcultured on a multiplication medium every 30 days, billions of shoots could be produced from a single explant annually. This method has potential not only in reducing the time required to increase stock material, but appears to be an attractive method of propagating these clones which are normally difficult to root by cuttings.

### Table 1. Effect of IBA level on rooting of Mailing 7 rootstock.

<table>
<thead>
<tr>
<th>IBA treatment (mg/liter)</th>
<th>18 days</th>
<th>28 days</th>
<th>35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73 (\text{a}^{2})</td>
<td>79 (\text{a}^{2})</td>
<td>79 (\text{a}^{2})</td>
</tr>
<tr>
<td>2</td>
<td>88 (\text{a}^{2})</td>
<td>100 (\text{b}^{2})</td>
<td>100 (\text{b}^{2})</td>
</tr>
<tr>
<td>3</td>
<td>36 (\text{b}^{2})</td>
<td>97 (\text{b}^{2})</td>
<td>100 (\text{b}^{2})</td>
</tr>
</tbody>
</table>

\(2\)Number of plants was 33, 24 and 39 for treatment 1, 2 and 3, respectively.

\(\text{Treatments not followed by the same letter are significantly different at the 5% level.}\)

### Literature Cited