Effect of Growth Regulators on in vitro Propagation of *Citrus limon*, *Citrus sinensis*, *Fortunella* sp. and *Poncirus trifoliata*

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Received August 2000; accepted January 2002

Abstract

Tissue culture is a useful method for *Citrus* crop improvement and propagation. Cotyledonary nodes of *Citrus limon*, *C. sinensis*, *Fortunella* sp. and *Poncirus trifoliata* were cultured on Murashige and Skoog medium (MSM) supplemented with different concentrations of benzylaminopurine (BAP) and naphthalene acetic acid (NAA). The MSM was found satisfactory. Plant growth regulators were required at different concentrations for shoot multiplication and rooting. Roots were produced into shoots when transferred on sand.

Keywords: BAP, NAA, Cotyledonary nodes, Sand rooting

Introduction

*Citrus* fruits are highly demanded for their nutrition and commercial value. A lot of *Citrus* fruits are imported to Nepal and a small fraction of the Nepalese product goes to India (Shah 1992). Most of the citrus orchards of local varieties have been destroyed by bacterial and viral diseases. There is a demand of *Citrus* saplings. Most of the seedlings are prepared from seeds and very few are grafted. So selection of cultivars resistant to diseases and having high yield and improved quality are important. In any controlled breeding program designed to select scions and root stocks, it is important to have large number of clonal plants for screening (Chaturvedi & Mitra 1974, Barlass & Skene 1982). Conventional propagation methods are relatively difficult and commercially less feasible. Hence, it would be desirable to develop a rapid and efficient method of micro-propagation. The present work reports the effect of growth regulators on cotyledon culture and axillary shoot multiplication of *Citrus limon*, *C. sinensis*, *Fortunella* sp. and *Poncirus trifoliata*.

Materials and Methods

The seeds were extracted from fresh fruits *C. limon* and *C. sinensis* - collected from local market. *Fortunella* sp. - collected from green house grown plants) except the dry seeds of *Poncirus trifoliata* received from Horticulture Farm, Kirtipur. The seeds were surface sterilized with 0.1% mercuric chloride solution for 20 minutes after washing in running water for one hour. The surface sterilized seeds were inoculated on Murashige and Skoog (1962) basal medium. The cultures were incubated at 25.1 °C with 16 hour photo period under 3000 lux. The seeds were germinated after 3-8 weeks of culture.

The cotyledon explants were excised from 4-8 weeks old seedlings, and initially cultured in MS medium with the combination of BALP 1 mg/l and NAA 0.1 mg/l and casein hydrolysate 1000 mg/l (Niroula 1994). The cultures were incubated under the same condition as seeds. The axillary shoot buds from the cotyledonary node were allowed to develop to 2-3 cm in 4-6 weeks. Further sub-culture of shootlets were carried out on different concentrations of BAP ranging from 1-5 mg/l and NAA 0.01-0.1 mg/l. The combination of BAP and NAA which gave the high rate of proliferation and good development of shoot was selected for subsequent sub-culture. Shootlets measuring 3-4 cm were transplanted in sand for rooting.

Results and Discussion

After 4-6 weeks of cultures shoot developed from the nodal region of the cotyledon in BAP 1.0 mg/l and NAA 0.1 mg/l in all four species. The multiple shoots were excised and sub-cultured in the media supplemented with BAP 0.1-5.0 mg/l and 0.1 mg/l NAA. In higher
concentration of BAP (5mg/l) the explants became yellowish. The shoots developed in initial medium, but the proliferated shoot buds had stunted growth. In lower concentration of BAP (below 1mg/l) the number of proliferated shoots developed in 6-8 weeks of culture attaining height of 2-4 cm (Table 1).

Table 1. Growth response of Poncirus trifoliata shoots in MS medium with different concentrations of growth regulators

<table>
<thead>
<tr>
<th>Concentration of BAP+NAA mg/l</th>
<th>No. of shoots</th>
<th>Growth response</th>
</tr>
</thead>
<tbody>
<tr>
<td>5+0.1</td>
<td>–</td>
<td>Yellowish explant</td>
</tr>
<tr>
<td>1+0.1</td>
<td>20-40</td>
<td>Stunted growth</td>
</tr>
<tr>
<td>0.5+0.1</td>
<td>20-30</td>
<td>Stunted growth</td>
</tr>
<tr>
<td>0.25+0.01</td>
<td>20-30</td>
<td>Stunted growth</td>
</tr>
<tr>
<td>0.1+0.01</td>
<td>15-30</td>
<td>Shoots developed</td>
</tr>
</tbody>
</table>

Table 2. Successful combination and concentrations of growth regulators and additives for multiple shoot formation and growth

<table>
<thead>
<tr>
<th>Plant species</th>
<th>BAP mg/l</th>
<th>NAA mg/l</th>
<th>Sucrose (%)</th>
<th>Coconut milk (%)</th>
<th>Adenine sulphate mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poncirus trifoliata</td>
<td>0.1</td>
<td>0.1</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Citrus sinensis</td>
<td>0.5</td>
<td>0.01</td>
<td>3</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td>Fortunella sp.</td>
<td>0.1</td>
<td>0.01</td>
<td>3</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td>Citrus limon</td>
<td>0.5</td>
<td>0.01</td>
<td>5</td>
<td>15</td>
<td>50</td>
</tr>
</tbody>
</table>

The proliferated growth of shoots were found in lower concentration but the combination of growth regulators and other additives varied in different spieces (Table 2). The absence of NAA also reduced the growth of the shoots. For P. trifoliata, NAA 0.1 mg/l was required (Fig. 1), whereas for the rest NAA was reduced to 0.01 mg/l to avoid callus formation. For C. sinensis and Fortunella sp. 15% coconut milk was effective (Fig. 2,3). The regenerated shoots of C. limon were yellowish in color and soft and growth was slow in BAP0.5 mg/l and NAA0.01 mg/l. The addition of 15% coconut milk and 50 mg/l of adenine sulphate was effective for rapid growth and elongation (Fig. 4).

Fig. 1. Poncirus trifoliata

Fig. 2. Citrus sinensis
Fig. 3. *Fortunella* sp.

Fig. 4. *Citrus limon*

Tucker 1969). For rooting the 2-4 cm long shoots were transferred to sand which roots developed after 3-6 weeks. The cytokinin BAP is now increasingly used for the culture of woody species in *vitro*. In general maximum shoot production in *Citrus* culture has been achieved through the use of BAP and other cytokinin have been less effective (Grinblat 1972, Rajbhansali & Arya 1981, Barlass & Skens 1982). Instead of malt extract (Grinblat 1972) coconut milk is beneficial for the growth of the shoots. Addition of adenine sulphate was found to be very effective for the elongation of shoots in *C. limon*.

This study has shown that the salt mixture of MS has proved satisfactory for all genera we examined. For the cotyledonary explant, combination of cytokinin and auxin was common. The combination of growth regulators and other additives varies in different related genera with the system and mode of shoot multiplication. The cotyledons as explant of all the genera we examined had the capacity to regenerate plants in *vitro*. The cultures have maintained their ability to proliferate after regular sub-culture for four years. With further improvements in this method, the propagation of valuable *Citrus* plants can be achieved to satisfy or fulfil the huge demands of *Citrus* plantation in developing countries.

References


