Adenine Sulphate and L-Glutamine Enhance Multiple Shoot Induction from Cotyledon Explants of Melon (Cucumis melo L. cv. Swarna)

M. Muruganantham¹, A. Ganapathi¹, N. Selvaraj², R. Prem Anand¹, A. Vasudevan¹, and G. Vengadesan¹
¹Department of Biotechnology, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, and ²Department of Botany, Periyar E.V.R college, Thiruchirappalli - 620 023, Tamil Nadu, India. Email: ganabiotech@rediffmail.com

Introduction. Cucumis melo L. is a popular commercial as well as household vegetable in India. Among various uses, it is mainly known for fresh salad and pickling. However, this economically important crop is susceptible to a number of devastating insects and diseases (7) including papaya ringspot virus and cucumber green mottle mosaic virus, which seriously limit productivity. Hence, this crop deserves improvement in terms of disease resistance, abiotic stress and higher yields. Melon is known to be recalcitrant to regeneration (1,2). The development of tissue culture protocols is one of the solutions to address these problems. The present study was conducted to produce multiple shoots in a local cultivar of melon by using important additives, adenine sulphate (AdS) and glutamine, in the culture medium.

Materials and Methods. The Indian melon cultivar Swarna (Indo-American Hybrid Seeds (India) Pvt. Ltd., Bangalore, India) was used in the present study. Seeds were surface sterilized by the usual sterilization procedure (3). The seed coats were aseptically removed and the two cotyledons were separated from the embryonic axis. Mature cotyledons were used as explants. The cotyledonary pieces with intact proximal ends (0.5 cm) were inoculated in Murashige and Skoog (MS) medium (4) containing 0.8% bacto agar, 3% sucrose and combinations of the growth regulators BA, Kinetin (Kn), and the additives AdS and L-glutamine. Every treatment was tested at five different concentrations (mg/l): BA – 0.5, 1.0, 1.5, 2.0, 2.5, Kn - 0.5, 1.0, 1.5, 2.0, 2.5, AdS – 5, 10, 15, 20, 25, L-Glutamine - 5, 10, 15, 20, 25.

To facilitate shoot elongation, the regenerated shoots were grown in MS medium fortified with gibberellic acid (GA₃) (0.5mg/l). The cultures were maintained at 25 ± 2°C under white fluorescent light (Philips India Pvt. Ltd.) with a photon flux of (30µ mol m⁻² s⁻¹) at a 16h photoperiod.

Results and Discussion. Effect of cytokinins. Multiple adventitious shoot buds were induced from the proximal end of the cotyledons in MS medium containing BA (2.0mg/l) or Kn (1.5mg/l) after 10-12 days of inoculation. A maximum number of shoots (12.2/explants) was obtained after 3-4 weeks of culture supplemented with only BA (2.0 mg/l) (Table 1). Lower numbers of shoots were produced in medium containing Kn (1.5mg/l). Similar results were observed by others in melon (3,5) and in cucumber (6). The shoots were subcultured in medium with the same composition.

Effect of BA with additives. Multiple adventitious shoot buds (Fig. 1) were initiated from the proximal end of the explants after 2-3 weeks of inoculation in MS medium containing BA in combination with AdS and glutamine. A maximum number of 30.6 shoots/explant was produced with BA (2.0mg/l), AdS (15mg/l) and glutamine (15mg/l) in two subcultures (Table 1). Lower numbers of shoots were produced in medium containing Kn (1.5mg/l). Similar results were observed by others in melon (3,5) and in cucumber (6). The shoots were subcultured in medium with the same composition.

Effect of BA with additives. Multiple adventitious shoot buds (Fig. 1) were initiated from the proximal end of the explants after 2-3 weeks of inoculation in MS medium containing BA in combination with AdS and glutamine. A maximum number of 30.6 shoots/explant was produced with BA (2.0mg/l), AdS (15mg/l) and glutamine (15mg/l) in two subcultures (Table 1). Lower numbers of shoots were produced in medium containing Kn (1.5mg/l). Similar results were observed by others in melon (3,5) and in cucumber (6). The shoots were subcultured in medium with the same composition.
Multiple shoot development from cotyledon explant of *Cucumis melo* L.

*Fig 1: Shoot bud initiation*
*Fig 2: Shoot proliferation*
*Fig 3: Shoot elongation*
Table 1. Effect of plant growth regulators and additives on multiple shoot induction from mature cotyledon explants of melon (C. melo cv. Swarna) cultured on MS medium.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of shoots/explants</th>
<th>Shoot length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA 2.0</td>
<td>12.2 b</td>
<td>6.2 b</td>
</tr>
<tr>
<td>Kn 1.5</td>
<td>2.0 h</td>
<td>n.d.</td>
</tr>
<tr>
<td>BA + AdS 2.0 + 15</td>
<td>9.2 c</td>
<td>5.0 c</td>
</tr>
<tr>
<td>BA + L-Glutamine 2.0 + 15</td>
<td>8.0 d</td>
<td>4.3 d</td>
</tr>
<tr>
<td>BA + AdS + L-Glutamine 2.0 + 15</td>
<td>30.6 a</td>
<td>7.9 a</td>
</tr>
<tr>
<td>Kn + AdS 1.5 + 15</td>
<td>6.3 e</td>
<td>4.0 de</td>
</tr>
<tr>
<td>Kn + L-Glutamine 1.5 + 15</td>
<td>5.0 f</td>
<td>2.3 f</td>
</tr>
<tr>
<td>Kn + AdS + L-Glutamine 1.5 + 15</td>
<td>4.0 g</td>
<td>2.0 g</td>
</tr>
</tbody>
</table>

Means with the same letter in a column are not significantly different according to Duncan’s Multiple Range Test at the 5% level.

n.d. - not determined.

Literature cited


