LOW COST STRATEGIES FOR MICROPROPAGATION OF BANANA

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To reduce the cost of micropropagation of banana, four experiments were conducted. In the first experiment, it was found that sucrose could be replaced by sugar cubes for multiplication and by even commercial grade sugar for rooting. In the second experiment, agar solidified medium was replaced by liquid medium supported with borosilicate glass beads in the rooting medium without affecting the 100% rooting. In the third experiment, IAA was removed from the rooting medium. The fourth experiment was conducted to replace the in vitro rooting by ex vitro rooting, but the results were poor. Cost of production has been further reduced by using the culture bottles of cheaper and re-usable soda-glass with polypropylene caps. The fast multiplication, perfect rooting and acclimatization further reduced the cost.

Key words: Banana, Ex-vitro rooting, Grande Naine, Low-cost, Micropropagation.

Although tissue culture is being used on commercial scale for several ornamentals and other horticultural crops, it is generally more expensive than the conventional clonal propagation (Pierik 1991). Since labour charges account for 60-70% of the cost of production in developed countries, they are either introducing automation (Vasil 1991) or setting up laboratories in developing countries, where labour cost comparatively very cheap (Bhojwani and Rajdan 1996). One estimate suggested that a 50% reduction in average would allow the market to be expanded to more than 10 times and that by decreasing production cost by 90%, the potential market would become 1000 times larger than at present (Kozai 1991). Thus, the future of micropropagation depends upon progress in adapting the procedure to industrial conditions, reduction in cost and coordinating production to markets (Hartmann et al. 2002).

There are several strategies to reduce the cost of micropropagation. One of them is reducing the resource cost i.e. the cost of the ingredients of the culture medium. There has been used sunlight instead of artificial light (Kodym and Zapata-Arias 2001), and tap water instead of distilled water and commercial grade sugar instead of purified sucrose (Ganpathi et al. 1995). The gelling agent, used to solidify the medium, has no nutritional role and is the costliest ingredient of the medium (Debergh 1983). Therefore, there are reports of use of low-cost alternatives like a starch / gelrite mixture (Kodym and Zapata-Arias 2001), sago and isubgol (Bhattacharya et al. 1994) and the use of liquid medium with support matrices like borosilicate glass beads (Anonymous 2003), filter paper, nylon cloth, polystyrene foam and glass wool cloth (Bhattacharya et al. 1994) or without support matrices (Alvard et al. 1993).

Like any other commercial tissue culture venture, the cost of production is the main concern in the case of banana also. Thus, there is an urgent need of improving and standardizing micropropagation of this important fruit so as to make it cost-effective (Anonymous 2003). Keeping this in mind, the investigation also attempts to reduce the cost of production of in vitro raised planlets. Now we have standardized a simple low-cost method for micropropagation of banana, Musa ‘Grande Naine’, a commercially important dessert.
banana of Giant Cavendish subgroup of AAA group. This cultivar has a great potential in international trade. For culture initiation shoot tips were employed. Multiplication of shoots followed the adventitious bud formation method without callusing. The shoots were rooted and the plantlets were acclimatized for field transfers.

**MATERIALS AND METHODS**

To establish the culture of banana, shoot tip explants (from suckers and rhizome) were collected from the campus of IFFCO, Phulpur, Allahabad. Following the standard methods of plant tissue culture, the explants were inoculated on solidified MS basal medium (Murashige and Skoog 1962) with 1 mg/l benzyl adenine purine (BAP). The cultures were incubated under controlled temperature (27 ± 2°C), light (2000-lux with 16-hour photoperiod) and humidity (70%) in a growth room. The buds, which started growing, were transferred to multiplication medium containing the MS basal medium with 2 mg/l BAP, 25 mg/l adenine sulphate (AdS) and 0.1 mg/l indole acetic acid (IAA) at 5.8 pH. The rate of multiplication was found ca. 9 fold in 6 weeks. The microshoots were rooted with 100% efficiency. The plantlets were acclimatized with 100% survival.

To reduce the cost of micropropagation of banana, four experiments were conducted.

**Table 1** Effect of replacement of sucrose on multiplication and rooting of banana 'Grande Naine'

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Shoot Multiplication (fold)</th>
<th>Rooting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sucrose 3%</td>
<td>8.77</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Sucrose 2%</td>
<td>6.93</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>Sugar cubes 3%</td>
<td>8.70</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Sugar cubes 2%</td>
<td>6.89</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Commercial sugar 3%</td>
<td>6.30</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>Commercial sugar 2%</td>
<td>4.13</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 2** Effect of replacement of agar on rooting of shoots of banana 'Grande Naine'

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Rooting (%)</th>
<th>Root Length (cm)</th>
<th>Shoot Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>100</td>
<td>4.56</td>
<td>4.69</td>
</tr>
<tr>
<td>2</td>
<td>Glassbead</td>
<td>100</td>
<td>4.58</td>
<td>4.98</td>
</tr>
</tbody>
</table>

**Table 3** Effect of removal of hormone on rooting of shoots of banana 'Grande Naine'

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>Rooting (%)</th>
<th>Root Length (cm)</th>
<th>Shoot Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>In vitro rooting</td>
<td>100</td>
<td>4.57</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ex vitro rooting with 0.1 mg/l IAA</td>
<td>60</td>
<td>3.84</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Ex vitro rooting without IAA</td>
<td>25</td>
<td>3.08</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4** Ex vitro rooting in shoots of banana 'Grande Naine'

Besides using the cheaper and re-usable soda-glass bottles with polypropylene caps as the culture vessels. In the first experiment sucrose was either reduced to 2% or replaced by sugar cubes and commercial grade sugar in the multiplication and rooting medium. In the second experiment, borosilicate glass beads replaced the agar in the rooting medium. In the third experiment, IAA was decreased or removed from the rooting medium. In the fourth experiment, the *in vitro* rooting stage was skipped and *ex vitro* rooting was tried during transplantation stage.

**RESULTS AND DISCUSSION**

Table 1 shows effect of replacement of sucrose with sugar cubes or commercial grade sugar on the multiplication and rooting. It was found that shoot multiplication was more sensitive than rooting with reference to changes in sucrose. Shoot multiplication remained almost the same, when sucrose was replaced with sugar cubes but decreased when it was replaced with
commercial sugar. Decrease from 3% to 2% sucrose/sugar cube decreased the shoot multiplication. But, rooting remained unaffected, whether sucrose was decreased or replaced with sugar cubes or commercial grade sugar. It can be suggested that sucrose could be replaced by sugar cubes for multiplication and by commercial grade sugar even at lower concentration (2%) for rooting. The tissue culture grade sucrose is a costly ingredient. It is used as a carbon source to compensate the photosynthesis. There are reports of use of the low-cost alternatives of sucrose. Ganapathi et al. (1995) have reduced the cost, without affecting the quality, of the medium for ‘Basrai’ banana cultures by using tap water, instead of distilled water and commercial grade sugar as carbon source in place of purified sucrose for shoot multiplication.

Table 2 shows the effect of replacement of agar by liquid medium supported with borosilicate glass beads in the rooting medium. It was found that rooting remained the same both on agar-solidified medium as well as I liquid medium supported with borosilicate glass beads. Root length was almost the same. Shoot length increased a little more in liquid glass bead medium than agar medium. Although borosilicate glass beads are costly, but because they can be used repeatedly, in long run it will

be much cheaper than agar. Reduction of cost of ‘Dwarf Cavendish’ banana micropropagation has been reported by developing a liquid medium with a substrate of borosilicate glass beads and by enhancing multiplication rate (1200 plantlets / mother culture flask / year) and 100% transplantation survival (Anonymous 2003). Bhattacharya et al. (1994) have used sago and isubgol as gelling agents and filter paper, nylon cloth, polystyrene foam and glass wool cloth as support matrices for liquid medium for chrysanthemum. Sago and isubgol cost about 1/18th and 1/10th respectively as compared to agar (Sigma). The cost of matrices was also less than that of agar. There are reports of the use of liquid medium with or without support matrices (Alvard et al. 1993, Bhagyalakshmi and Singh 1995). Alvard et al. (1993) have investigated, 5 different liquid media for ‘Grande Naine’ banana micropropagation and recommended the ‘temporary immersion system’ for multiplication. Kodym and Zapata-Arias (2001) reported a 90% resource cost reduction in tissue culture of banana by replacing tissue culture grade sucrose and gelrite in the medium with locally available commercial grade sugar and a starch / gelrite mixture and by using sunlight instead of artificial light. Table 3 shows the effect of decrease of IAA on rooting percentage. It was found that rooting remained 100% with 0.1 mg/l IAA and even without IAA. Root length remained almost the same but shoot length was a little higher in hormone free medium. Hormone-free rooting is perhaps the ‘carry-on’ effect of hormones used at shoot multiplication stage. It is not only cheaper, but it also prepares the plant better for transplantation. This is evident from the longer shoots in the hormone-free medium. Ray (2001) has also reported longer shoots in hormone-free medium.

Table 4 shows the effect of replacement of the in vitro rooting by ex vitro rooting. It was found that rooting decreased from 100% in vitro to 60% ex vitro with 0.1 mg/l IAA to 25% ex vitro without hormone. Ex vitro rooting needs more experimentation because this will reduce one step of micropropagation and simultaneously the labour cost also.

It has been found that the cost of tissue cultured raised (TC) plant is high e.g. Rs. 36.00 per plant for TC banana against Rs. 22.00 per plant for conventional banana (Anonymous 2005). However, even if the cost of TC plants is higher than conventional propagules, this cost is compensated by the quality and productivity e.g. the crop duration is shorter, 12 months rather than 18 months and the productivity is higher, average bunch weight is 25 kg against 15 kg for conventional banana (Anonymous 2005). The field performance of micropropagated plants has been found better in comparison to conventional plants in respect of growth and harvest for Grande Naine (Cabrera et al. 1998) and for ‘Basrai’ and ‘Shrimanti’ (Nandi et al. 1998).

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