Research Article

EFFECT OF BAP AND NAA ON In vitro MULTIPLICATION OF BANANA (Musa sp.) cv. CHENTHULUVAN

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Abstract
The present study was planned to investigate the micropropagation protocol for banana (Musa sp.) cv. Chenthuluvan by using shoot tip culture. Different concentration of BAP and Kinetin was used for multiplication of shoot and NAA for rooting. Maximum multiplication was observed in 3 mg/l of BAP and better rooting was observed in 1.5 mg/l of NAA. The rooted plants were acclimatized and successfully transferred to poly bag. After hardening, they were transferred to main field. In vitro propagated plants grew faster and produced better yield than the sucker-derived plants. The cultivator produced heavier bunch and could be harvested earlier.

Key words: Banana, BAP, NAA, Chenthuluvan and Micropropagation.

1. Introduction
Advances in Science and Agricultural technologies have delivered real benefits to farmers, processors and consumers and are continuing to increasingly provide new ways of tackling the challenges of reducing hunger malnutrition and food insecurity (CGIAR, 2005). Application of biotechnology has opened new frontiers in improving food production and the incomes of small holder formers in developing countries. Banana is one of the world’s most important horticultural Crops cultivated on five continents in about 120 countries. Current world production of banana is estimated at 97.5 million tons per year covering 10 million hectare (Kalloo, 2002; Singh, 2002). It is the largest produced and maximum consumed amongst the fruits cultivated in India. It is known as the ‘common man fruit’. It is highly nutritive and very delicious.

Bananas are propagated vegetative because almost all cultivated banana cultivars are triploid seedless or seed sterile. The materials used for Conventional Propagation include corms large and small suckers and sword suckers (Cronauer and Krikorian, 1984; Arias, 1992). However conventional Planting Materials are not the ideal propagate because they carry weevils, fungal pathogens nematodes and viruses (Arias, 1992; Sagi et al., 1996) and also suffer from slow multiplication, bulkiness and poor sanitary quality (Vuylsteke, 1989). Therefore, since 1985 Shoot tip culture has been increasingly used in some countries (Israel, the canary Islands Taiwan and South Africa) as an alternative to conventional plant material (Robinson, 1996).

Micropropagation of banana is highly efficient allowing a large turnover of plants in very short period of time with in very little space (Arranitoyannis et al., 2007). Tissue culture also plays a vital role in the distribution of germplasm conservation, safe exchange of internal planting
material and rapid propagation of newly selected hybrid cultivars. Several researchers have reported the regeneration of Musa sp. via micropropagation (Krishnamoorthy et al., 2001; Muhammad et al., 2004; Roels et al., 2005).

The extensive basic work on the in vitro propagation of banana had led to the technological development of in vitro mass production of different cultivars of banana (Kodym and Zapata, 1999; Nandwani et al., 2000). Plant tissue culture techniques can potentially overcome some of the factors limiting traditional approaches to improvement. Tissue culture technique produce 39% higher yield than conventional Sword suckers (Forahani et al., 2008).

In the present research work was undertaken to standardize efficient protocols for mass scale propagation of banana cv. Chenthuluvan using in vitro techniques to enhance yield per hectare of the crop.

2. Materials and methods

Banana cv. Chenthuluvan is tall, stout and a long duration variety. It requires heavy dose of manure for successful growth. It takes about 18 months to mature from time of planting to harvest stage. Stem tall, stout, greenish purple 14 to 16 feet height leaves large, oblong, slightly brittle, polished, veins and midrib prominent, petiole greenish purple. Inflorescence, peduncle pubescent. It has female persistent and deciduous male flowers, 6 - 10 hands, fingers rather compact, 11 - 25 in a hand, fruit tapersto apex, rind when ripe red, medium thick; pulp cream colored, sweet with good flavor.

Healthy and three months old sword suckers were selected for this study. After defoliation, roots were removed from the pseudostems and washed well in running water to removed soil particles. The sheathing leaf bases were removed one by one and the pseudostem trimmed to approx. 10 cm long and 5 cm diam. and immersed in 5% Sodium Chlorite and Bavistin for 45 minutes. The Surface Sterilized explants were immersed in antibiotics streptomycin 0.7 mg/l + cofotaxime 0.8 mg/l for 2 - 3 hrs to ensure contamination free culture.

After antibiotic treatment, the explants were transferred to laminar air flow chamber. Explants were surface sterilized with 10% Sodium Hypochlorite solution for 10 minutes and 0.1% HgCl₂ for 6 minutes and were washed with sterilized water for 3 - 4 times. All the explants were initiated to sterilized MS medium. The MS Medium Supplemented with different concentration of BAP and Kinetin individually (0, 1, 2, 3, 4, 5, 6 and 7 mg/l). After shooting, the isolated shootlets were transferred to rooting medium. The Rooting medium was supplemented with different concentration of NAA (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l). The pH of the Medium was adjusted to 5.8 before autoclaving and temperature was maintained at 26 + 2°C and 16 hrs photo period (4000 lux). The in vitro raised plants were transferred from bottles to net pots and kept in mist chamber maintained at 80 – 90% humidity. The humidity was gradually reduced and after 20 days transferred to polythene bags. The hardened plantlets were shifted to the field.

3. Result and Discussion

The result of this study showed that shoot tips obtained from the sword suckers of banana cv. Chenthuluvan were cultured on MS medium supplemented with 0, 1, 2, 3, 4, 5, 6 and 7 mg/l BAP and kinetin alone for induction of multiple shoot productions. After 30 days of culture for organogenesis all culture was refreshed on same media. Among the various concentrations of BAP, 3 mg/l yielded best shoot proliferation response (55.67 shoots on 120th day) the length of longest shoot (7.66 cm) with 6.6 cm long leaf was recorded on 3.0 mg/l BAP. The lowest number of shoots (8.66) was observed in control and lowest shoot length (3.06 cm) was observed in control.

The best result was obtained in 3 mg/l of BAP. The concentration of BAP increased or decreased the number of shoots and length of shoots was decreased. Shoot proliferation of kinetin under various concentrations tested, produced 44 shoot and longest shoot (6.33) was observed in 3 mg/l concentration. Among the two tested cytokinins, BAP (3 mg/l) performed superior to kinetin.
Table – 1: Effect of Cytokinins on Shoot Initiation of Banana cv Chenthuluvan

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration of Cytokinins (mg/l)</th>
<th>Number of shoot (after 120th day)</th>
<th>Length of shoot (after 120th day) (cm)</th>
<th>Length of leaf (after 120th day) (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BAP</td>
<td>KN</td>
<td>BAP</td>
<td>KN</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>8.66 ± 0.47</td>
<td>5.33 ± 0.47</td>
<td>3.06 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>19.33 ± 0.47</td>
<td>12.67 ± 0.47</td>
<td>4.86 ± 0.16</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>36 ± 0.81</td>
<td>26 ± 0.81</td>
<td>5.9 ± 0.08</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>55.67 ± 0.47</td>
<td>44 ± 0.81</td>
<td>7.66 ± 0.12</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>42.33 ± 0.47</td>
<td>32.33 ± 0.47</td>
<td>5.03 ± 0.16</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>31 ± 0.81</td>
<td>23 ± 0.81</td>
<td>4.33 ± 0.12</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>20.33 ± 0.47</td>
<td>15 ± 0.81</td>
<td>3.46 ± 0.20</td>
</tr>
</tbody>
</table>

Table – 2: Effect of NAA on Root Initiation of Banana cv. Chenthuluvan

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration of NAA mg/l</th>
<th>Number of roots</th>
<th>Length of roots (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1.66 ± 0.47</td>
<td>2.4 ± 0.21</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>3 ± 0</td>
<td>4.43 ± 0.16</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>4.66 ± 0.47</td>
<td>5.2 ± 0.08</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>7.66 ± 0.47</td>
<td>6.86 ± 0.16</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>4.33 ± 0.47</td>
<td>5.23 ± 0.24</td>
</tr>
<tr>
<td>6</td>
<td>2.5</td>
<td>3 ± 0.81</td>
<td>4.16 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>2.33 ± 0.47</td>
<td>3.06 ± 0.12</td>
</tr>
</tbody>
</table>

BAP – Benzylaminopurine  KN – Kinetin  NAA – Napthaleneaceticacid

Effect of BAP on days taken for shoot initiation  
Effect of Kinetin on days taken for root initiation  
Effect of NAA on days taken for root initiation
Plantlets were shifting the rooting medium on the 30th day the data was recorded. 1.5 mg/l concentrations of NAA produced 7.66 roots. The longest root was (6.86 cm) observed in the same concentration. The root initiation started on 4th day after shifting the shootlets in to rooting medium. The lowest number of roots (1.66) was observed in control.

The high performance of BAP over other cytokinins in the multiplication of shoot tips has also been reported in different cultivar of banana by Gilmar et al. (2000). Abeyaretne and Lathiff (2002) states that 2 - 3 mg/l of BAP with basal media is an advisable concentration for banana shoot tip culture. Rahman et al. (2002) reported that the BAP was effective in shoot proliferation than other cytokinins. Higher concentration of
BAP and kinetin beyond optimum levels were also reported to cause necrosis and reduction in shoot formation during in vitro multiplication of banana cv. Nendran (Madhulatha et al., 2004).

Sholi et al. (2009) suggested that Musa cultivars require different levels of plant growth regulators; BAP is more effective in multiple shoot generation. Cytokinins such as benzyl aminopurine (BAP) and kinetin are generally known to reduce the apical dominance and induce both axillary and adventitious shoot formation from meristematic explants in banana and the most established banana shoot tip culture system was achieved by using BAP as a supplement to basal media (Jafari et al., 2010). Shirani (2010) reported that scalp produced by kinetin treatments was small compared to BAP treatments for banana cultivars in shoot tip culture of banana variety Basari, the frequency of bud formation doubled and the fresh weight increased about four times higher in media with BAP at 3 mg/l which was compared to media supplemented with 7 mg/l of BAP. Studies conducted by Choudhary et al. (2013). Three mg/l of BAP and 0.2 mg/l of NAA medium showed good results both for shoot initiation and multiplication. BAP alone was found to be effective for establishment than the combination of cytokinins and auxins. BAP and NAA were used successfully in multiplication of banana cultivars in many studies (Doreswamy and Sahijiram, 1989; Ganapathi et al., 1999). Similarly, BAP in combination with NAA induced maximum shoot multiplication, when shoot tips were used as explants as observed by various workers (Suprasanna et al., 1999; Rahman et al., 2004).

4. Conclusion

The current research investigation concluded that the study of micropropagation protocol is successful for mass scale production of Banana cv. Chenthuluvan. This protocol can further be employed for the germplasm storage and production of disease free planting material.

5. Reference


