Original Article

*In Vitro* propagation of Banana (*Musa acuminate* L.) cv. Cavandish Dwarf.

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Received 18 June 2013; accepted 20 August 2013

Abstract

In the present investigation, *in vitro* propagation of *Musa acuminate* L. cv. Cavandish Dwarf was carried out and it was mainly aimed at the development of simple and effective medium composition for overall success in the micro propagation of the banana cultivar studied. The basal MS medium was supplemented with various concentrations of BAP for shoot induction and the best result was obtained at conc.2.0mg L$^{-1}$ BAP. For multiple shoot formation the MS and BAP were also added with various concentrations of Kn and the good result was achieved in MS medium fortified with 2.0mg L$^{-1}$ BAP and 1.0mg L$^{-1}$ Kn. For rooting of micro shoots they were cultured on $\frac{1}{2}$MS medium supplemented with different concentrations of IBA and the best rooting was observed in $\frac{1}{2}$MS containing IBA at 0.5mg L$^{-1}$. © 2013 Universal Research Publications. All rights reserved

Key words: *In vitro* propagation, Banana cultivar, MS medium, BAP, Kn, IBA.

Introduction

Banana is the most important and most widely grown fruit crop in the world. It ranks as the fourth major crop after rice, wheat and maize and is considered as a poor man’s apple in tropical and subtropical countries (Sweunen et al., 2000; Jain and Sweunen, 2004). Generally, banana cultivars are good sources of carbohydrates, proteins, vitamins and minerals. As the banana cultivars are having high degree of sterility and polyplody the conventional breeding methods are difficult in banana improvement. Many pests and diseases are also threatening the good production of banana cultivars. In order to augment convetional breeding and to avoid constraints imposed by pests and pathogens, transgenic and *in vitro* approaches are being considered (Tripathi, 2003). Though several in vitro approaches have been made on many banana cultivars for their overall improvements the present attempt aims at the formation of simple and cost effective protocol for the *in vitro* propagation of the banana cultivar studied.

Materials and Methods

As shoot tip culture is the most common practice for in vitro plant regeneration of banana and plantain, in the present study, meristem tip cultures of banana cultivar “Cavandish Dwarf” were derived from shoot apexes. Explants obtained from decapitated shoot apexes of suckers were surface sterilized with 70% ethanol for 20 seconds, then incubated in 5% Sodium hypochloride solution for 20 minutes followed by rinse in sterile water for three times. The explants were then inoculated aseptically in MS (Murashige and Skoog, 1962) medium containing 30g/L sucrose and 8g/L Difco Bacto-agarand supplemented with various concentrations of 6-benzyl amino purines (BAP) as shown in table. The PH of the medium was adjusted to 5.7 with 1M NaOH before autoclaving and cultures were maintained at 24±2°C under 16 hours cool white fluorescent tube light (4000Lux) (Dooley,1991). For multiple shoot formation, subculturing was carried out at 30–40 days intraval on freshly prepared same medium with addition of various concentrations of Kn (cytokinin) All treatments were preformed on three replications of 20 explants in experiments employing a completely randomized design. For rooting, *in vitro* developed healthy shoots were separated and transferred to MS medium supplemented with various concentrations of indole -3 – butyric acid (IBA) as shown in table3.

For hardening, the rooted plantlets were subjected to gradual exposure to sunlight for a week to ten days, then plantlets were carefully washed with tap water to remove the adherent traces of agar and then transferred to pots containing a mixture of composed humus and garden soil (1:1) and kept under shade house at a relative humidity of 80 – 90%. Finally, the hardened plants were transferred to natural field.

Results and Discussion

In the present study, the effects of different concentrations of BAP (0.5,1.0,1.5,2.0,2.5 and 3.0 mg L$^{-1}$) along with MS basal medium were tested for effective *in vitro* shoot induction and propagation of the banana cultivar studied. A morphogenic response of the cultured shoot tips was visible as a swelling and greenish colour after...
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Table – 1. Effect of BAP concentrations along with MS basal medium in shoot induction.

<table>
<thead>
<tr>
<th>Treatments MS + BAP (mgL⁻¹)</th>
<th>Number of shoot induction</th>
<th>Shoot length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 0.5</td>
<td>2.37</td>
<td>1.98</td>
</tr>
<tr>
<td>MS + 1.0</td>
<td>4.50</td>
<td>3.83</td>
</tr>
<tr>
<td>MS + 1.5</td>
<td>5.18</td>
<td>6.68</td>
</tr>
<tr>
<td>MS + 2.0</td>
<td>5.26</td>
<td>9.03</td>
</tr>
<tr>
<td>MS + 2.5</td>
<td>4.52</td>
<td>4.00</td>
</tr>
<tr>
<td>MS + 3.0</td>
<td>4.40</td>
<td>6.66</td>
</tr>
</tbody>
</table>

Table – 2. Effect of BAP and Kn concentrations along with MS basal medium in multiple shoot formation.

<table>
<thead>
<tr>
<th>BAP + Kn (mgL⁻¹)</th>
<th>Number of Ex plant inoculated</th>
<th>Response of multiple shoot formation (%)</th>
<th>No. of well developed multiple shoot formation</th>
<th>Number of days required</th>
<th>Growth result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 + 0.2</td>
<td>20</td>
<td>10.0</td>
<td>2.3 ± 0.3</td>
<td>35 – 40</td>
<td>#</td>
</tr>
<tr>
<td>2.0 + 0.6</td>
<td>20</td>
<td>22.6</td>
<td>3.3 ± 0.2</td>
<td>25 – 30</td>
<td>##</td>
</tr>
<tr>
<td>2.0 + 1.0</td>
<td>20</td>
<td>86.7</td>
<td>5.2 ± 0.1</td>
<td>20 - 25</td>
<td>###</td>
</tr>
<tr>
<td>2.0 + 1.4</td>
<td>20</td>
<td>52.0</td>
<td>3.2 ± 0.2</td>
<td>27 - 35</td>
<td>#</td>
</tr>
<tr>
<td>2.0 + 1.8</td>
<td>20</td>
<td>40.0</td>
<td>2.5 ± 0.2</td>
<td>36 - 43</td>
<td>#</td>
</tr>
</tbody>
</table>

# = Poor; ## = Good; ### = Very good

Table – 3. Effect of IBA concentrations along with ½MS medium in root development.

<table>
<thead>
<tr>
<th>½MS + IBA (mgL⁻¹)</th>
<th>Number of shoot inoculated</th>
<th>Percentage of root induction</th>
<th>Number of well developed roots</th>
<th>Number of days required</th>
<th>Growth response</th>
</tr>
</thead>
<tbody>
<tr>
<td>½MS + 0.1</td>
<td>20</td>
<td>22.2</td>
<td>3.4 ± 0.3</td>
<td>15 - 25</td>
<td>#</td>
</tr>
<tr>
<td>½MS + 0.2</td>
<td>20</td>
<td>32.5</td>
<td>4.2 ± 0.2</td>
<td>15 - 22</td>
<td>##</td>
</tr>
<tr>
<td>½MS + 0.3</td>
<td>20</td>
<td>57.1</td>
<td>4.6 ± 0.1</td>
<td>14 - 21</td>
<td>##</td>
</tr>
<tr>
<td>½MS + 0.4</td>
<td>20</td>
<td>76.3</td>
<td>5.3 ± 0.1</td>
<td>14 - 20</td>
<td>###</td>
</tr>
<tr>
<td>½MS + 0.5</td>
<td>20</td>
<td>94.3</td>
<td>6.1 ± 0.1</td>
<td>12 - 18</td>
<td>###</td>
</tr>
<tr>
<td>½MS + 0.6</td>
<td>20</td>
<td>66.7</td>
<td>4.5 ± 0.2</td>
<td>11 - 20</td>
<td>#</td>
</tr>
<tr>
<td>½MS + 0.7</td>
<td>20</td>
<td>46.7</td>
<td>3.6 ± 0.3</td>
<td>12 - 20</td>
<td>##</td>
</tr>
<tr>
<td>½MS + 0.8</td>
<td>20</td>
<td>33.3</td>
<td>2.7 ± 0.3</td>
<td>12 - 22</td>
<td>##</td>
</tr>
</tbody>
</table>

# = Poor; ## = Good; ### = Very good

10 to 15 days of inoculation and the best result in terms of shoot induction and proliferation was observed in medium containing BAP at 2.0 mg L⁻¹ and the concentrations below 1.5 mg L⁻¹ did not improve shoot regeneration. Likewise the BAP concentrations above 2.2 mg L⁻¹ also suppressed the shoot induction and proliferation. Hence, The MS medium enriched with BAP at 2.0 mg L⁻¹ was set as optimum level for good shoot proliferation. Frequency of shoot proliferation was increased with the increase of subculture in the same medium. In early studies, optimum BAP concentrations were found to be 22.2 µm by cronauer and Kakorian (1984) and Jarret et al (1986) and 20 µm by Vuylsteke (1989), Wong (1986) stated that 44.4 µm BAP reduced shoot multiplication. Arinative et al (2000) stated that shoot proliferation is cultivar dependent.

Multiple shoot formation

Based on the relatively good responses resulting from the early step, synergism of cytokinin was demonstrated when the regenerated shoot was excised and then cultured in MS medium containing BAP 2.0 mg L⁻¹ and different concentrations of Kn. The synergism resulted in multiple shoots and cluster formation. The optimum multiplication frequency and the highest number of multiple shoot formation were archived in MS medium fortified with 2.0 mg L⁻¹ BAP and 1.0 mg L⁻¹ Kn (Table2). The rate of multiple shoot formation observed in the present study is higher than reported by Uddin et al., 2006.

Root induction

For in vitro rooting of micro shoots developed earlier, they were sub-cultured on ½MS medium added with different concentrations of IBA to find out their effect and optimum level. The highest percentage of rooting was observed on ½MS medium containing IBA at 0.5 mg L⁻¹ (Table3) Rooting can be stimulated when individual shoots were transferred to a basal medium ( Cronauer and Keikorian,1984;Jarret et al, 1986). However, auxins may induce further root initiation (Vuylsteke, 1989). The optimum IBA concentration was found to be 1 µm by Vuylsteke and Langhe (1985).

Acclimatization

About 95% of in vitro raised plantlets were well acclimatized in open field condition.

Conclusion

The micropropagation method developed in this study can serve as a convenient method for large scale, disease free, homogenize development of banana cultivar studied and can help the enhancement of economic benefit of the farmer.

References


Source of support: Nil; Conflict of interest: None declared