IN VITRO PLANT REGENERATION STUDIES USING HYPOCOTYL EXPLANT OF BRINJAL (SOLANUM MELONGENA)

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Solanum Melongena
Shoot Regeneration
Root Regeneration

ABSTRACT
The present study was done to standardize the protocol of embryo rescue technique in which the seeds of indigenous collection of Solanum melongena were used as explants. The explants hypocotyle was cultured on MS media supplemented with different combinations of auxins and cytokinins such as BAP (1 to 2.5 mg/l)+ IAA (0.2 to 0.4 mg/l) and Kinnetin (1 to 2.5 mg/l)+IAA (0.2 to 0.4 mg/l) for shoot regeneration. Callus initiation and shoot initiation was observed after 15 - 20 days and 25 days of inoculation, respectively on media supplemented with BAP-IAA. While, it took 25 and 30-35 days for callus initiation and shoot initiation, respectively on media supplemented with Kinnetin-IAA. The highest shoot regeneration (65.12%) was observed on MS medium supplemented with 2mg/l BAP + 0.3mg/l IAA after 25 days of inoculation. For root regeneration, regenerated shoots were transferred to root regeneration medium having MS medium supplemented with different concentration of auxins IAA (0.5-1mg/l) and IBA (0.5-1mg/l), so as to obtain the complete plantlets. The highest root regeneration was observed when MS medium was supplemented with IBA (1mg/l). Hence the plant regeneration from hypocotyl of brinjal was observed to be influenced by different combinations and concentrations of auxins and cytokinins.

INTRODUCTION
Eggplant (Solanum melongena L., 2n = 2x = 24) is a widely adaptive and highly productive vegetable crop of tropical and subtropical regions world. The crop finds place in kitchen gardens besides being grown for commercial scale production. It is considered to be important source of nutrition and cash income. It suffers from various abiotic and biotic stresses particularly insect-pests (Sharma and Rajam, 1995; Kaur et al., 2004). These exert a deleterious effect on yield, market quality and storability of brinjal. In brinjal the cross incompatibility with wild resistant relatives (Solanum mammosum, Solanum incanum and Solanum grandiflorum) and inadvertent linkage drag of undesirable genes are problems in developing resistance in brinjal through conventional breeding methods (Baksh and Iqbal, 1979). Thus, use of biotechnological techniques can be an alternative approach to tackle such issues. However, transfer of genes depends upon the efficient and rapid regeneration protocol.

Several reports on in vitro regeneration in brinjal are available and in majority are of indirect plantlets regeneration through a callus phase (Sharma and Rajam, 1995; Franklin, et al., 2004; Singh et al., 2000; Mir et al., 2011; Bhatt et al., 2013; Sidhu et al., 2014). Various protocols on in vitro regeneration in egg plant have been carried out using various auxins and cytokinins either alone (Mukherjee et al., 1991; Magioli et al., 1998) or in combinations (Matsuoka and Hinata 1979) using various explants. In vitro plant regeneration studies using hypocotyl as explant was also carried out by various researchers (Bardhan et al., 2012 and Panwar et al., 2013). It is generally accepted that plant in vitro culture response is influenced by the donor genotype (Henry et al., 1994) and type of explants used (Sharma and Rajam, 1995). Although the present study was a part of standardization of embryo rescue technique, the aim was to get higher number of regenerated plants. Therefore, the present study was undertaken to standardize the rapid and efficient protocol for high frequency shoot regeneration of brinjal using hypocotyl as explants.

MATERIALS AND METHODS
Plant material
The present study was a part of standardization of protocol for embryo rescue technique, the plant material used were the seeds of indigenous collection of Solanum melongena L. IC 203585 obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. This genotype was quite responsive in wild hybridization with Solanum khasianum. Seeds were thoroughly washed in running tap water and then surface sterilized with 0.1 % mercuric chloride (HgCl2), followed by two to three times rinsing in sterile distilled water and in sterile distilled water overnight. These seeds were inoculated into half-strength MS medium (Murashige and Skoog1962) devoid of any growth regulators. Hypocotyl explant of brinjal was obtained from the 15 days old in vitro
grown seedlings. The explant were sterilized with 0.1% HgCl₂ for 60 seconds under laminar flow, followed by their 3-4 times washing with distilled water to remove traces of mercury chloride. The explant was cut into 1.0 cm size and then cultured on MS medium containing different concentrations of auxins and cytokinines. Basal MS medium containing all the macro and micro nutrients, vitamins, 100mg/L mesoinositol with 3% sucrose and 0.8% agar was used as cultural medium (Murashige and Skoog, 1962). After inoculation cultures were kept in the culture room at 26 ± 2°C with 16h photoperiod.

Culture media and shoot regeneration

For shoot regeneration, the explant was kept on MS medium supplemented with different combinations of auxins and cytokinines such as, MS + 1mg/L BAP + 0.2mg/L IAA, MS + 2mg/L BAP + 0.3mg/L IAA, MS + 2.5mg/L BAP + 0.4mg/L IAA, MS + 1mg/L kinnetin + 0.2mg/L IAA, MS + 2mg/L kinnetin + 0.3mg/L IAA, MS + 2.5mg/L kinnetin + 0.4mg/L IAA. Explants were evaluated for number of shoots per explant and percent shoot regeneration. After culturing the cultures were kept at 26 ± 2°C for 30 days and then process of sub culturing was followed on fresh medium for further shoot multiplication.

Root regeneration

The regenerated shoots developed from hypocotyl explants were transferred to root regeneration medium having MS medium with different concentration of auxins so as to obtain the complete plantlets. Different combinations used for root regeneration were basal MS medium + 0.05mg/L IAA, MS medium + 1.0mg/L IAA, MS medium + 0.05mg/L IBA and MS medium + 1.0mg/L IBA.

Hardening of the rooted shoots

In vitro developed plantlets were taken out and washed gently with distilled water to remove adhering agar medium in the laminar flow. The plants were then transferred in the tissue culture pots containing mixture of cocopeat: vermiculite: perlite (1:1:1) which was presterlized by autoclaving at 15lbs/inch² pressure for 1hour at 121ºC. The plantlets were then watered with ½ MS medium biweekly kept under varying conditions of humidity and light intensity and observed for growth.

Statistical Analysis

The statistical analysis was performed using sigma stat for windows. The values of number of shoot /explant were represented in terms of means for 5 random replicates

RESULTS

Shoot regeneration

MS medium with 2mg/L BAP + 0.3mg/L IAA was found to be optimum in obtaining high frequency of shoot regeneration in brinjal. After regeneration the hypocotyl explant (1.0 cm) were inoculated on various shoot regeneration medium supplemented with different combinations and concentrations of BAP-IAA and Kinetin-IAA. (Figure 1)

Effect of BAP-IAA on shoot regeneration

Three different concentrations (MS + 1mg/L BAP + 0.2mg/L IAA, MS + 2mg/L BAP + 0.3mg/L IAA, MS + 2.5mg/L BAP + 0.4mg/L IAA) of BAP-IAA were used for shoot regeneration. Callus initiation was observed after 10-12 days. Shoot initiation started after 25days. As depicted in the (Figure 2&3) maximum shoot regeneration (65.12%) and average number of shoots per explant (0.5) was observed on MS medium supplemented with 2mg/L BAP + 0.3mg/L IAA.

Effect of kinetin and IAA

Three different concentrations i.e. (MS + 1mg/l kinetin + 0.2mg/l IAA, MS + 2mg/l kinetin + 0.3mg/l IAA, and MS + 2.5mg/l kinetin + 0.4mg/l of kinetin) and IAA were used for shoot regeneration. The callus initiation was observed after 20 days and shoot initiation started after 30-35 days. Out of these three combinations maximum shoot regeneration (58.52%) and average number of shoots per explant (0.2) were observed on MS + 2mg/l kinetin + 0.3mg/l IAA. (Table 2 & Fig. 3)

Multiplication and elongation

The regenerated shoots from both the explants were separated and sub-cultured on same medium containing best shoot regeneration medium + growth regulators (2mg/l BAP + 0.3mg/l IAA). Elongation of shoots was obtained after 45-50 days after sub culturing.

Table 1: Effect of different concentrations of BAP-IAA on shoot regeneration

<table>
<thead>
<tr>
<th>Medium</th>
<th>Callus Formation</th>
<th>*Mean no. of shoot /explants</th>
<th>% shoot regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 1 mg/l BAP + 0.2 mg/l IAA</td>
<td>+</td>
<td>0.48 ± 0.101</td>
<td>32.08 ± 0.160</td>
</tr>
<tr>
<td>MS + 2 mg/l BAP + 0.3 mg/l IAA</td>
<td>++</td>
<td>0.52 ± 0.101</td>
<td>65.12 ± 0.121</td>
</tr>
<tr>
<td>MS + 2.5 mg/l BAP + 0.4 mg/l IAA</td>
<td>++</td>
<td>0.81 ± 0.100</td>
<td>48.16 ± 0.106</td>
</tr>
</tbody>
</table>

*values were calculated from 5 replicates (n = 5)

Table 2: Effect of different concentrations of Kinetin and IAA on shoot regeneration

<table>
<thead>
<tr>
<th>Medium</th>
<th>Callus Formation</th>
<th>*Mean no. of shoot /explants</th>
<th>% shoot regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 1 mg/l Kinetin + 0.2 mg/l IAA</td>
<td>+</td>
<td>0.52 ± 0.128</td>
<td>30.06 ± 0.104</td>
</tr>
<tr>
<td>MS + 2 mg/l Kinetin + 0.3 mg/l IAA</td>
<td>+</td>
<td>0.20 ± 0.100</td>
<td>58.52 ± 0.143</td>
</tr>
<tr>
<td>MS + 2.5 mg/l Kinetin + 0.4 mg/l IAA</td>
<td>+</td>
<td>0.15 ± 0.106</td>
<td>42.06 ± 0.138</td>
</tr>
</tbody>
</table>

*values were calculated from 5 replicates (n = 5)
Table 3: Effect of different concentrations of auxins on root regeneration

<table>
<thead>
<tr>
<th>Medium</th>
<th>Mean no. of roots</th>
<th>% root regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS + 0.05mg/L IAA</td>
<td>4.6</td>
<td>62.08</td>
</tr>
<tr>
<td>MS + 1mg/L IAA</td>
<td>10.06</td>
<td>72.16</td>
</tr>
<tr>
<td>MS + 0.05mg/L IBA</td>
<td>9.81</td>
<td>75.08</td>
</tr>
<tr>
<td>MS + 1mg/L IBA</td>
<td>11.08</td>
<td>91.06</td>
</tr>
<tr>
<td>CD</td>
<td>1.06</td>
<td>1.13</td>
</tr>
</tbody>
</table>

(Table & Fig. 3) maximum percentage of root regeneration (91.06%) was observed on MS media containing IBA 1mg/L. After root regeneration the completely formed plantlets were transferred to pre sterilized mixture of cocopeat: vermiculite: perlite (1:1:1) in pots. (PLATE-2).

DISCUSSION

Plant regeneration is a crucial aspect of plant tissue culture methodology that is boon for the production of genetically engineered plants, somaclonal variants and the rapid propagation of difficult to propagate species. Effective in vitro regeneration is required for genetic improvement of brinjal through tissue culture and genetic engineering approaches. The aim of present investigation was to determine high frequency shoot regeneration in the brinjal. Callus mediated regeneration with the use of different concentration of BAP (cytokinins) and auxins (IAA or IBA) have been reported in hypocotyls in brinjal by (Sagare and Mohanty 2012). However some of the earlier workers obtained direct or indirect organogenesis from hypocotyl explants by manipulating the concentrations of growth regulators (Alicchio, 1982; Magioli and Mansur 2005). The present investigation was a part of study which aimed at the regeneration of brinjal plantlets from immature ovules. In these hypocotyls of brinjal seedlings were used as explants for the regeneration, it was observed that regeneration was mediated through callus formation. In several other experiments a range of kinetins and TDZ either alone or in combination with NAA or IAA had been used which resulted in callus mediated response in brinjal as reported by (Alicchio et al., 1982; Magioli et al., 1998; Khatun et al., 2006; Bardhan et al., 2012). Combination and balance of auxins and cytokinins is the main criteria in growing medium which determine the morphogenesis of callus. Furthermore, intrinsic plant growth regulator levels in explants make it respond better on a particular ratio and concentration of plant growth regulators supplemented in culture medium depends upon genotype and crop (Franklin et al., 2004). Hence there is wide response of different combinations of plant growth regulators on MS medium supplemented with different auxins and cytokinines.

Gradual increase in the regeneration frequency and number of shoots per explant was observed with the increasing concentration of BAP and highest shoot regeneration (65.12%) was observed on MS medium supplemented with 2mg/L BAP + 0.3mg/L IAA after 25 days of inoculation, however it was observed that with increase in concentration of BAP, there was 26 % decrease in shoot regeneration in the present study. Zayova et al. (2000) and Bhat et al. (2013) had also observed higher regeneration in brinjal with increased BAP concentrations i.e. 0.5, 1 and 2mg/L. However, they reported that higher BAP level had negative effect on organogenesis leading to shoots vitrification. Kaur et al. (2011) had also observed that increase in BAP concentration above 2.5mg/L resulted in decreased regeneration capacity and number of buds on all the explants. Pawar et al. (2012) and Sagare and Manty (2012) also observed the shoot regeneration mediated through callus on medium containing BAP@ 1.0 - 2.0 mg/L with highest shoot regeneration at 2.0 mg/L BAP.

When the hypocotyl was cultured on MS medium
supplemented with kinetin, an increase in regeneration efficiency of shoot was observed with maximum (58.52%) at MS media supplemented with 2 mg/L Kinetin + 0.3 mg/L IAA. These findings are in line with (Sagare and Manty, 2012; Bhat et al., 2013) who also observed the increase in shoot regeneration with increase in kinetin level using cotyledonary leaves as explant. On the contrary to this Kaur et al. (2011) had reported that addition of kinetin decreased the regeneration capability and number of buds on all the explant.

Different auxins i.e. IAA, IBA of various concentrations was used for root regeneration from in vitro developed shoots of brinjal. Root regeneration was observed after 20 days in culture room in media containing IAA and IBA. IBA is widely used for efficient root regeneration in brinjal (Sharma and Rajam, 1995; Singh et al., 2000) although several suggested IAA as efficient root regeneration hormone (Bardhan et al., 2012) In the present study, highest root regeneration (91.06%) was observed when MS medium was supplemented with IBA 1mg/L. These findings are similar to findings of (Sharma and Rajam, 1995; Hossain et al., 2007; Chakravarthi et al., 2009; Panwar et al., 2013), who also observed higher root regeneration with addition of IBA.

In the present study it has been observed that an efficient plant regeneration protocols would make a platform for exploitation of useful somaclonal variations, mutation breeding, induction of di-haploids and genetic transformation with economically important genes for the improvement of eggplant by transfer of economically important traits.

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REFERENCES


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