EFFECT OF DIFFERENT EXPLANT AND CONCENTRATION OF ZEATIN RIBOSIDE FOR IN VITRO REGENERATION OF POTATO

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Abstract

The experiment was conducted at the Tissue Culture Laboratory of Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh in 2012. The different explants i.e. i. Shoot ii. Leaf iii. Node and iv. Internode were used in MS media supplemented with 1.0 mg L\(^{-1}\) Zeatin Riboside (ZR). The nodal explants were placed in MS media supplemented with different (0.0 mg L\(^{-1}\), 1.0 mg L\(^{-1}\), 2.0 mg L\(^{-1}\), 3.0 mg L\(^{-1}\), 4.0 mg L\(^{-1}\), 5.0 mg L\(^{-1}\), and 6.0 mg L\(^{-1}\)) to find out suitable concentration of Zeatin Riboside (ZR) and to investigate the direct in vitro regeneration performance of potato (Asterix). The higher percentage of explants produce shoot were recorded from shoot (66.28%), which is followed by the explants of node (64.75%), but nodal explants were suitable for higher number of shoot/explants (2.83), length of shoot (2.83), and number of per explants (2.07). The MS media supplemented with 4 mg L\(^{-1}\) to 5 mg L\(^{-1}\) ZR showed the best performance regarding in vitro direct regeneration of potato from the explants of node. The MS media + 4 mg L\(^{-1}\) ZR required minimum days for shoot appearance (17 days) which was statistically similar with 5 mg L\(^{-1}\) & 3 mg L\(^{-1}\) ZR. The highest explants producing shoot (94.67%) was recorded in 4 mg L\(^{-1}\) ZR. The maximum number of shoots per explant (12.93) was recorded in ZR 5 mg L\(^{-1}\). The maximum number of leaves 3.217 was noted from 5 mg L\(^{-1}\) ZR.

Key words: Zeatin Riboside in vitro regeneration, Potato

Introduction

In Bangladesh, it is cultivated over an area of about 0.4 million hectares with an annual production of about 6.0 million tons and ranks second after rice with respect to total production (Hossain et al., 2007). Generally farmers use seed tubers for multiplication and cultivation of potato as they are easy to plant and provide high yields with large uniformity (Gopal, 2004). However, use of seed tubers are labour and time consuming, have high risks of pests and bacterial and fungal diseases and a low rate of multiplication resulting in many field multiplications (Struik and Wiersema, 1999). Over the last three decades rapid Multiplication systems become an important technique to provide disease free propagules. These techniques yield in vitro plantlets transplants micro tubers and mini tubers which are used in the initial Phases of a seed tuber productions scheme (Jones 1988). Different approaches so far have been adapted to obtain efficient in vitro regeneration system in potato either from petioles with intact leaflets (Shirley et al., 2001) leaves (Anderson et al., 2003), tuber discs (Sheerman and Beaven, 1988), and from stem (Chang et al., 2002) after passing through callus phase. A long callus phase is a hindrance for the rapid development of elite potato cultivars and somaclonal variation is a problem associated with genetic and phenotypic instability of potato cultivars. Another problem is initiation of very low number of shoots from each callus. It is essential to circumvent the problems associated with potato regeneration from explants for the development of superior cultivars. The use of one of the important plant growth regulator, Zeatin Riboside (ZR) (Neelam and Mariam, 1995) in the regeneration media has allowed a considerably reduction of the callus phase, and accelerated transgenic bud formation. The present study was undertaken to investigate the direct in vitro regeneration performance of different explants of potato (Asterix), and optimize the concentration of Zeatin riboside (ZR) as a cytokinin in MS medium for efficient regeneration.

Materials and Methods

The experiment was conducted at the Tissue Culture Laboratory of Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh in 2012. The different explants i.e. i. Shoot ii. Leaf iii. Node and iv. Internode were used in MS media supplemented with 1.0 mg L\(^{-1}\) ZR to find out suitable explant for direct regeneration of potato. And to find out suitable concentration of ZR the nodal explants were placed in MS media supplemented with different (0.0 mg L\(^{-1}\), 1.0 mg L\(^{-1}\), 2.0 mg L\(^{-1}\), 3.0 mg L\(^{-1}\), 4.0 mg L\(^{-1}\), 5.0 mg L\(^{-1}\), and 6.0 mg L\(^{-1}\)) concentration of ZR.

Preparation of explants

In vitro plantlets of 25-30 days old at solid medium was considered for the explants collection. For stem explants, only the first 5-6 nodes from the top of the plantlet excluding shoot apex of the each plantlet were excised. Internodes and node segments were excised to avoid the auxiliary buds and divided into 0.5- 1.0 cm long segments. For leaf explants, thick and healthy
leaves from the upper nodes of the plants were used. The leaf tips and basal portions, including the petiole were discarded and the entire leaf was cut into 5 mm x 5 mm pieces. The leaf explants were placed upside down on the medium. For shoot explants, only 8-10 mm apex shoot were used where side leaves were discarded. All the explants were placed in test tubes and fuel paper were used and kept in a growth room at 25±2 °C. Only the first five internodes from the top of the plantlets were excised. Using alcohol, the laminar air flow chamber was thoroughly cleaned to maintain the aseptic condition. Under aseptic conditions, young and tender plantlets were taken out on a sterile glass plate using sterile forceps. The plantlets were not surface sterilized as those were already maintained under in vitro aseptic conditions. The roots of these plantlets were excised using a sterile scalpel and the leaves were removed.

Preparation of media, inoculation of explants and incubation

To evaluate the performance of different types of explant for direct regeneration the composition of MS media supplemented with 1.0 mg L⁻¹ ZR. And to find out the suitable concentration of ZR the media was supplemented with different concentration (0.0 mg L⁻¹, 1.0 mg L⁻¹, 2.0 mg L⁻¹, 3.0 mg L⁻¹, 4.0 mg L⁻¹, 5.0 mg L⁻¹, and 6.0 mg L⁻¹) of ZR. Fine agar powder of Loban, India brand was used. After pH adjustment 8 g L⁻¹ agar was added to the solution to solidify the media. An amount of 10 ml media were dispensed into each test tube. The test tubes were autoclaved at 121°C for 20 minutes at 1.2 kg/m² and thereafter stored at 25°C. After 2-3 days, the media was checked for any type of contamination and the explants were inoculated after performing several sequential steps. The explants were placed in each plate for each treatment horizontally in a plate using sterile forceps. The plantlets were not discarded. All the explants were performed statistically and means were separated by Least Significance Difference method.

Results and Discussion

The higher percentage of explants produce shoot were recorded from shoot (66.28%), which is followed by the explants of node (64.75%). This superiority of shoot may be due to Presence of the living shoot at every explants. Number of shoot per explants were produced statistically similar by shoot and node explants (Table 1).

The length of shoot was higher at the explants of node (2.83 cm) which was followed by the explants of shoot (1.67 cm), may be due to more hormonal activity. The higher number of leaf per shoot were recorded from the nodal explants (2.07 cm) which was statistically followed by the explants of shoot (1.30 cm); may be due to the higher length of shoot (Table-1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Explants producing shoot (%)</th>
<th>Number of shoot/explants</th>
<th>Length of shoot (cm)</th>
<th>Leaf/shoot</th>
<th>Callus formation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot</td>
<td>66.28</td>
<td>2.67</td>
<td>1.167</td>
<td>1.30</td>
<td>0.00</td>
</tr>
<tr>
<td>Node</td>
<td>64.75</td>
<td>2.83</td>
<td>2.83</td>
<td>2.07</td>
<td>0.00</td>
</tr>
<tr>
<td>Internode</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>86.97</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.96</td>
<td>8.18</td>
<td>6.36</td>
<td>12.69</td>
<td>26.28</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td>1.281</td>
<td>0.253</td>
<td>0.141</td>
<td>0.016</td>
<td>11.53</td>
</tr>
</tbody>
</table>

Days required for shoot appearance

Days required for shoot appearance was significantly influenced due to the different levels of ZR. The explants observed with ZR 4 mg L⁻¹ which was statistically similar with ZR 5, 3 & 2 mg L⁻¹. The lowest explants producing shoot (70%) were recorded in ZR 6 mg L⁻¹ (Table 2).

Table 1. Effect of different explants on ZR to direct regeneration of potato (Asterix)

Eco-friendly Agril. J.
The maximum number of shoots per explant (12.93) was recorded in ZR 5 mg L⁻¹. On the other hand, the minimum number of shoots per explant (1.00) was counted in ZR 0 mg L⁻¹ (Table 2). Doo and Boe (2001) reported that MS medium supplemented with 7 mg L⁻¹ IAA and 3 mg L⁻¹ ZR produced maximum shoots.

**Length of shoot**
Significant difference was observed among the treatments as to the length of shoot. The maximum shoot length (3.5 cm) was measured in ZR 5.0 mg L⁻¹ containing MS medium followed by ZR 4 mg L⁻¹ (3.083 cm) and ZR 3 mg L⁻¹ (2.7 cm) (Table 2). However, minimum length of shoot (1.0 cm) was recorded ZR of 6 mg L⁻¹. The reason for higher shoot length might be due to early shoot initiation resulting longest shoot.

**Number of leaves per shoot**
Distinct variation was observed in number of leaves per shoot due to different level of ZR concentration. The maximum number of leaves 3.217 was noted from ZR 5 mg L⁻¹ followed by ZR 2, 3 & 4 mg L⁻¹. However, the minimum number of leaves 1.10 was recorded in ZR 6 mg L⁻¹ which was statistically similar with ZR 0 & 1 mg L⁻¹ (Table 2). There is no significantly difference among the treatment ZR 2, 3, and 4 mg L⁻¹ for leaves production. The reason for maximum number of leaves per shoot might be due to longest shoot witch produce maximum number of leaves. The result of the study reflected that longest shoot produced maximum number of leaves.

**Conclusion**
From the above discussions, it may be concluded that for direct regeneration of potato (Asterix), the nodal explants is suitable for direct regeneration and 4 mg L⁻¹ to 5 mg L⁻¹ Zeatin Riboside (ZR) supplemented with MS media showed the best performance regarding in vitro direct regeneration of potato from shoot explants.

**References**