BRIEF COMMUNICATION

# In vitro cultivation and regeneration of *Solanum melongena(L.)* using stem, root and leaf explants.

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## Abstract

The treatment combinations was BAP (0, 2.0, 3.0 and 4.0 mg/L) and NAA (0, 0.1, 0.5, and 1.0 mg/L). The rate of callus formation varied in different treatments. The highest amount of callus (48.66%) was produced on MS medium containing 2.0 mg/l BAP and 0.5 mg/l NAA from stem and 8.2 days required for callus induction. The number of shoot regenerated through callus from stem containing 2.0 mg/l BAP and 0.5 mg/l NAA was 3.4 (23.287%) and days required for 38.8 days.

**Key words:** Regeneration, BAP, NAA.

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## Introduction

Brinjal (*Solanum melongena L.*), belongs to the family Solanaceae, is one of the most popular, palatable and nutritious vegetable crop in Bangladesh. It is thought to be originated in Indian subcontinent with the secondary centre of origin in China (Zeven and Zhukovsky, 1975). Brinjal is cultivated throughout the entire tropics and sub-tropics. It has higher calorie, iron, phosphorus and riboflavin than tomato. Brinjal is the second most important vegetable crop after potato in respect of total acreage (million ha) and production (370,000 mt) in Bangladesh (BBS, 2003). It also plays a vital role in the national economy as a cash crop. Brinjal is highly susceptible to different insects, pests and diseases that exert a deleterious effect on yield, market quality, storability and international germplasm distribution. The seed-borne pathogens of previous years can be perpetuated over the generations with symptoms expressed. To overcome this situation, plant tissue culture offers an efficient method for pathogen free materials and germplasm preservation of plants. The potential value of tissue culture in plant breeding has been widely recognized, and it is generally used as useful tool for crop improvement. Regeneration of valuable economic plants through tissue culture based on the principle of totipotency, individual plant cell is capable of regenerating new plantlets. Anwar *et al*. (2002) cultured the aborigine leaf explants on MS media containing IAA, BA (benzyl adenine), IBA, NAA or 2,4-D at 2 mg/l. NAA produced greenish, fast-growing callus. 2, 4-D induced early callus production from the petiole, while BA induced green callus production from the upper surface of the lamina. The addition of NAA or IBA at 0.5 mg/l in BA supplemented medium increased the mass production of callus and shoot regeneration. The regeneration efficiency of the plant decreased in MS medium supplied with kinetin (2 mg/l) and NAA (0.5 mg/l).

The seeds of brinjal cv. Jhumki were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur and stem, root and leaf were used for establishment of culture. Healthy seeds of brinjal cv. Jhumki were collected from the BARI. The seeds were then washed thoroughly in running tap water. The instruments like scalpels, forceps, needles etc. were sterilized inside the Laminar Air Flow Cabinet. Other requirements like petridishes, distilled water and glassware were sterilized by an autoclave. The surface sterilization of these seeds was carried out by dipping

and flaming method under a Laminar Air Flow Cabinet and others were rinsed in 70% ethyl alcohol for one minute, and then thoroughly washed with sterilized distilled water. The alcohol treated seeds were sterilized with 0.1% $HgCl_2$ solution for 8-10 minutes, few drops Tween-20 per 100 ml was also added at that time. The seeds were then washed 5-6 times with sterilized distilled water. The seeds were then ready for placement into the media. Sterilized seeds were placed into seed germination medium in Petridish. Six seeds were placed in each Petridish. The culture was then incubated in dark till the germination of seeds. These were then transferred to 16 hours light for normal seedling growth. MS (Murashige & Skoog, 1962) basal medium with different concentrations and combinations of BAP (0, 2.0, 3.0 and 4.0 mg/l) and NAA (0, 0.1, 0.5 and 1.0 mg/l) were used. Six pieces (2-3 mm) of stem segments were arranged horizontally on each petridish and gently pressed into the surface of the sterilized culture medium with various concentrations and combinations of hormones like NAA and BAP. The petridish was covered and sealed with Para film. Leaf segment from each germinated seedling were cut into small pieces using sterilized scalpel under a Laminar Air Flow Cabinet. Six pieces of leaf segments were arranged on each petridish and gently pressed into the surface of the sterilized culture medium. The Petri dishes were covered and sealed with Para film. Root tip segments (0.5mm) were placed on a sterilized petridish under a Laminar Air Flow Cabinet. The petridish was covered and sealed with Para film.

Plant regeneration from induced calli of brinjal through MS medium supplemented with different combinations of hormones was used. Stem, leaf, root segments were used as explants to observe their callusing response. Thirty explants were inoculated in each treatment. Among the explants used, stem was comparatively more responsive for callus induction than other explants. The combined effect of explants and different combinations of BAP and NAA on callus induction has been presented in Table 1.Stem showed the highest callusing mean (8.363) whereas leaf segments gave callusing mean (6.950) and root segments had the lowest callusing mean (6.688). The highest callusing was obtained in 2.0 mg/l BAP (8.567) and 0.5 mg/l NAA (9.333). Also minimum days (9.725) were required for callus induction from stem. Days required for callus induction from 2.0 mg/l BAP were 10.350 and days required for callus induction from 0.5 mg/l were 10.367. In case of stem, among the different combination of MS media containing 2.0 mg/l BAP + 0.5 mg/l NAA and 4.0 mg/l BAP + 0.5 mg/l NAA showed better callus induction i.e. 14.600 and 11.600 respectively out of 30 cultured explants in Fig. 2 and Fig. 3. On the other hand, in case of leaf the combination of 2.0 mg/l BAP + 0.5 mg/l NAA showed better callus induction i.e. 13.4. The explants cultured on MS medium without hormones did not produce any callus. It was also found that calli were induced in medium supplemented with BAP and NAA which is in support of the results obtained by Jayasree *et al*. (2001). The percentage of callus induction was highest in MS media containing 2.0 mg/l BAP + 0.5 mg/l NAA from stem i.e. 48.666% followed by callus induction in

Figure 1: Seed germination from brinjal cv. Jhumki on MS media without hormones at 7 days

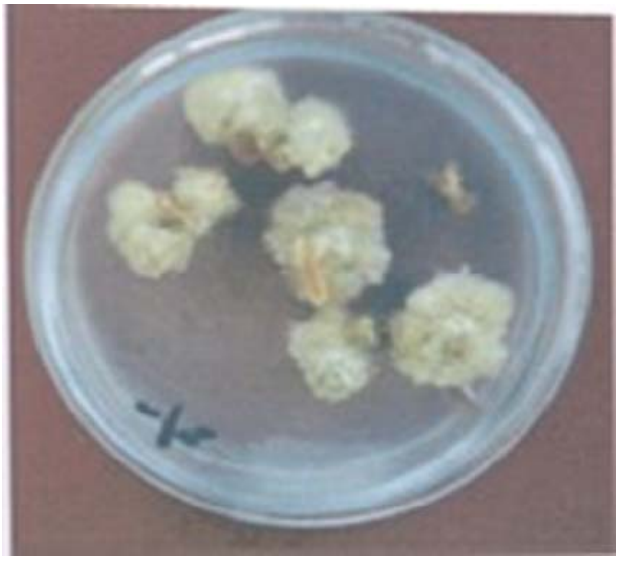

Figure 2. Callus induction from brinjal cv. Jhumki on MS media with hormones (BAP and NAA) at 22 days

leaf. The combination of 2.0 mg/l BAP + 0.5 mg/l NAA required 8.2 days for callus induction from stem explants. On the otherhand, the combination of 2.0 mg/l BAP + 0.1 mg/l NAA needed 10.8 days for callus from root explants. So, callus induction from stem required minimum days. Among the supplements, the highest regeneration potentiality observed from 2.0 mg/l BAP (0.717) and 0.5 mg/l NAA (0.667). But there was no regeneration ability without hormones. The combined effect of different combinations of BAP and NAA in MS medium on plant regeneration from stem, leaf and root of brinjal cv. Jhumki have been presented in Table 2. Various combinations of supplements showed significant variation in regeneration ability. Among the used combinations, 2.0 mg/l BAP + 0.5 mg/l NAA showed the highest regeneration of plantlets from stem (3.400). The regeneration of plantlets was (1.6) from leaf in 2.0 mg/l BAP and 0.5 mg/l NAA combinations. Root showed lowest regeneration. The percentage(i.e. 23.28%) of regeneration was recorded the highest in MS media containing 2.0 mg/l BAP + 0.5 mg/l NAA from stem and days required for regeneration was minimum (38.8 days). The percentage (11.94%) of regeneration was the highest in 2.0 mg/l BAP + 0.5 mg/l NAA from leaf. i.e. 1.6 and percentage of regeneration from root is the lowest. Plant regeneration from leaf in 2.0 mg/l BAP + 0.5 mg/l NAA combination required minimum days (46.2 days). From the above discussion, we found that the best shoot regeneration was recorded from media supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA in Fig. 4.

Explants were cultured on MS media supplemented with different combinations and concentrations of BAP (0, 2.0, 3.0 and 4.0 mg/l) and NAA (0, 0.1, 0.5, and 1.0 mg/l). The highest amount of callus (48.66%) was produced on MS medium containing 2.0 mg/l BAP and 0.5 mg/l NAA from stem and 8.2 days required for callus formation. The growth of callus was faster on MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA from the stem. Maximum number of plant regeneration through callus from stem containing 2.0 mg/l BAP and 0.5 mg/l NAA were 3.4 (23.287%) and from leaf containing 2.0 mg/l BAP and 0.5 mg/l NAA were 1.6 (11.94%).

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Figure 3. Callus induction from brinjal cv. Jhumki on MS media with hormones (BAP and NAA) at 22 days.

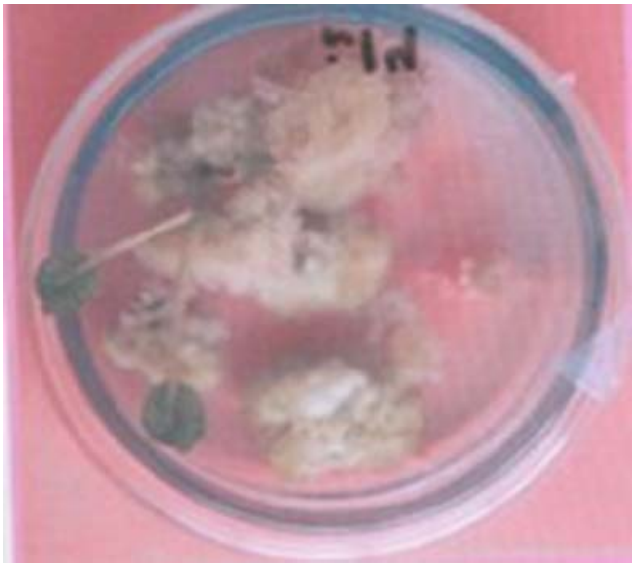

Figure 4. Direct regeneration from brinjal cv. Jhumki on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA

Table 1. The effect of BAP and NAA in MS medium on callus induction from different explants.

| Treatment combinations | | | No. of explant showing callus induction | % of callus induction | Days required for callus induction |
|---|---|---|---|---|---|
| Explants | Treatments | | | | |
| | BAP (mg/l) | NAA (mg/l) | | | |
| Stem | 0 | 0 | 0.000 R | 0.000 R | 0.000I |
| | | 0.1 | 7.000 JKLMN | 23.333 JKLMN | 10.400 ABCDEF |
| | | 0.5 | 6.600 KLMNO | 22.000 KLMNO | 10.44 ABCDEF |
| | | 1.0 | 7.400 HIJKLM | 24.066 HIJKIM | 10.600 ABCDEF |
| | 2.0 | 0 | 6.200 LMNO | 20.660 LMNO | 10.400 ABCDEF |
| | | 0.1 | 8.600 FGHI | 28.666 FGHI | 9.600 EBCDEFG |
| | | 0.5 | 14.600 A | 48.666 A | 8.200 GH |
| | | 1.0 | 9.600 DEFG | 32.000 DEFG | 9.600 EFG |
| | 3.0 | 0 | 6.800 KLMNO | 22.660 KLMNO | 11.200 ABCDE |
| | | 0.1 | 9.400 DEFG | 31.330 DEFG | 10.800 ABCDE |
| | | 0.5 | 10.200 DE | 34.000 DE | 9.800 DEFG |
| | | 1.0 | 9.200 DEFG | 30.666 DEFG | 10.600 ABCDEF |
| | 4.0 | 0 | 6.600 KLMNO | 22.000 KLMNO | 11.400 ABCDE |
| | | 0.1 | 10.000 DEF | 33.333 DEF | 11.200 ABCDE |
| | | 0.5 | 11.600 C | 38.666 C | 10.000 CDEF\G |
| | | 1.0 | 10.000 DEF | 33.333 DEF | 11.400 ABCDE |
| Leaf | 0 | 0 | 0.000 R | 0.000 R | 0.000 ABCDE |
| | | 0.1 | 5.800 NOP | 19.333 NOP | 11.000 ABCDE |
| | | 0.5 | 6.000 MNO | 20.000 MNO | 10.800 ABCDE |
| | | 1.0 | 9.200 DEFG | 30.666 DEFG | 10.600 ABCDF |
| | 2.0 | 0 | 4.600 PQ | 15.330 PQ | 11.000 ABCDE |
| | | 0.1 | 7.400 IJKLM | 24.660 IJKLM | 10.200 BCDEF |
| | | 0.5 | 13.400 B | 44.660 B | 8.600 FH |
| | | 1.0 | 9.800 DEF | 32.666 DEF | 10.800 ABCDE |
| | 3.0 | 0 | 4.400 Q | 14.666 Q | 10.800 ABCDE |
| | | 0.1 | 6.400 KLMNO | 21.333 KLMNO | 10.800 ABCDE |
| | | 0.5 | 6.200 LMNO | 20.660 LMNO | 11.200 ABCDE |
| | | 1.0 | 10.000 DEF | 33.333 DEF | 10.800 ABCDE |
| | 4.0 | 0 | 4.400 Q | 14.666 Q | 11.400 ABCDE |
| | | 0.1 | 7.600 HIJK | 25.333 HIJK | 11.600 ABCDE |
| | | 0.5 | 7.400 IJKLM | 24.661 IJKLM | 11.600 ABCDE |
| | | 1.0 | 8.600 FGHI | 28.660 FGHI | 11.200 ABCDE |
| Root | 0 | 0 | 0.000 R | 0.000 R | 0.000 I |
| | | 0.1 | 4.400 Q | 14.666 Q | 12.200 AB |
| | | 0.5 | 6.400 KLMNO | 21.333 KLMNO | 12.400 A |
| | | 1.0 | 6.600 KLMNO | 22.000 KLMNO | 11.800 ABCD |
| | 2.0 | 0 | 4.200 Q | 14.000 Q | 12.000 ABC |
| | | 0.1 | 6.600 KLMNO | 22.000 KLMNO | 11.400 ABCDE |
| | | 0.5 | 9.600 DEFG | 32.000 DEFG | 10.800 ABCDE |
| | | 1.0 | 8.200 GHIJ | 27.330 GHIJ | 11.600 ABCDE |
| | 3.0 | 0 | 5.400 OPQ | 18.000 PQ | 11.400 ABCDE |
| | | 0.1 | 7.800 HIJK | 26.000 HIJK | 11.000 ABCDE |
| | | 0.5 | 10.600 CD | 35.330 CD | 10.200 BCDEF |
| | | 1.0 | 8.800 EFGH | 29.330 EFGH | 11.600 ABCDE |
| | 4.0 | 0 | 4.400 Q | 14.666 Q | 7.600 H |
| | | 0.1 | 6.800 JKLMNO | 22.660 JKLMNO | 11.800 ABCD |
| | | 0.5 | 9.400 DEFG | 31.330 DEFG | 10.400 ABCDEF |
| | | 1.0 | 7.800 HIJK | 26.00 HIJK | 11.400 ABCDE |

Table 2. The effect of BAP and NAA in MS medium on plant regeneration from different explants.

| Treatment combinations | | | No. of plants regenerated through callus | % of regeneration | Days required for regeneration |
|---|---|---|---|---|---|
| Explants | BAP (mg/l) | NAA (mg/l) | | | |
| Stem | 0 | 0 | - | - | - |
| | | 0.1 | - | - | - |
| | | 0.5 | - | - | - |
| | | 1 | - | - | - |
| | 2 | 0 | 0.200 CD | 3.222 CD | 39.200 G |
| | | 0.1 | 0.600 CD | 6.976 CD | 39.800 G |
| | | 0.5 | 3.400 A | 23.287 A | 38.800 G |
| | | 1 | 0.600 CD | 6.25 CD | 39.000 G |
| | 3 | 0 | 0.200 CD | 2.94 CD | 39.400 G |
| | | 0.1 | 0.800 C | 8.510 C | 39.800 G |
| | | 0.5 | 0.800 C | 7.843 C | 39.800 G |
| | | 1 | 0.600 CD | 6.521 CD | 39.600 G |
| | 4 | 0 | 0.400 CD | 6.060 CD | 40.000 G |
| | | 0.1 | 0.600 CD | 6.000 CD | 40.000 G |
| | | 0.5 | 0.400 CD | 3.448 CD | 39.800 G |
| | | 1 | 0.400 CD | 4.00 CD | 39.600 G |
| Leaf | 0 | 0 | - | - | - |
| | | 0.1 | - | - | - |
| | | 0.5 | - | - | - |
| | | 1 | - | - | - |
| | 2 | 0 | 0.400 CD | 8.695 CD | 48.800 CD |
| | | 0.1 | 0.600 CD | 8.108 CD | 48.000 DE |
| | | 0.5 | 1.600 B | 11.940 B | 46.200 F |
| | | 1 | 0.600 CD | 6.122 CD | 49.000 CD |
| | 3 | 0 | 0.400 CD | 9.090 CD | 48.800 CD |
| | | 0.1 | 0.400 CD | 6.25 CD | 48.400 CDE |
| | | 0.5 | 0.600 CD | 9.677 CD | 47.400 E |
| | | 1 | 0.400 CD | 4.00 CD | 48.200CDE |
| | 4 | 0 | 0.200 CD | 4.545 CD | 49.000 CD |
| | | 0.1 | 0.400 CD | 5.361 CD | 49.000 BC |
| | | 0.5 | 0.400 CD | 5.405 CD | 50.200 C |
| | | 1 | 0.400 CD | 4.651 CD | 49.200 BC |
| Root | 0 | 0 | - | - | - |
| | | 0.1 | - | - | - |
| | | 0.5 | - | - | - |
| | | 1 | - | - | - |
| | 2 | 0 | - | - | - |
| | | 0.1 | - | - | - |
| | | 0.5 | 0.200 CD | 2.083 CD | 60.200 A |
| | | 1 | 0.200 CD | 2.439 CD | 60.000 A |
| | 3 | 0 | - | - | - |
| | | 0.1 | 0.200 CD | 2.564 CD | 59.600 A |
| | | 0.5 | 0.400 CD | 5.128 CD | 59.800 A |
| | | 1 | 0.200 CD | 2.272 CD | 59.600 A |
| | 4 | 0 | - | - | - |
| | | 0.1 | 0.200 CD | 2.947 CD | 60.400 A |
| | | 0.5 | 0.200 CD | 2.127 CD | 59.400 A |
| | | 1 | 0.400 CD | 5.128 CD | 60.000 A |

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