

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.



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

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 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.



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

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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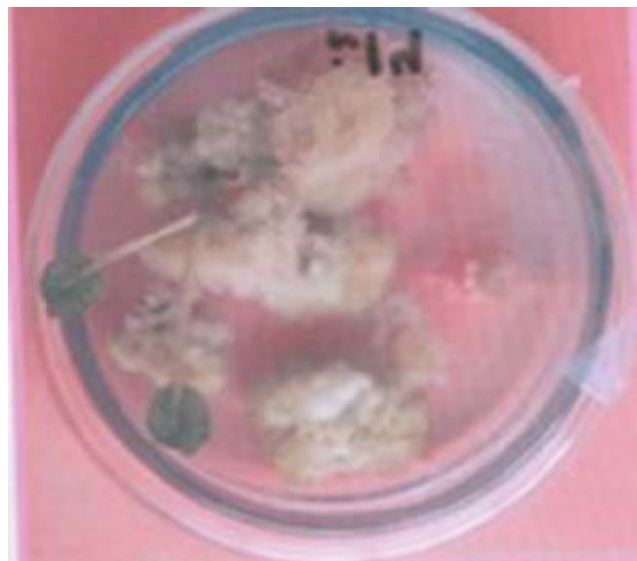


Figure 4. Direct regeneration from brinjal cv. Jhumki on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA

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	26.000 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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