ISSN 0258-7122
Bangladesh J. Agril. Res. 36(3) : 397-406, September 2011

IN VITRO REGENERATION OF BRINJAL (*Solanum melongena* L.)

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Abstract

The effect of different explants and concentrations of BAP and NAA on induction of callus and plant regeneration of brinjal cv. Jhumki were investigated. The treatment combinations were 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 highest fresh weight of callus was 1.12g from stem and 0.48g from root. 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. All regenerated plantlets survived in normal environment.

Keywords: NAA, BAP, regeneration, brinjal.

Introduction

Brinjal is the second most important vegetable crop after potato in respect of total acreage and production (3, 70,000 mt) in Bangladesh (BBS, 2003). 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 sub-continent with the secondary centre of origin in China (Zeven and Zhukovsky, 1975). Brinjal is cultivated throughout the entire tropics and subtropics. It has higher calorie, iron, phosphorus, and riboflavin than tomato. 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, and storability. The seed-borne pathogens of previous year 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 gerrnplasm 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 (Krikorian and Berquam, 1969). The regeneration of plants from cell and tissue culture is an important and essential component of biotechnology that is required for the genetic manipulation of plants. High frequency regeneration of plants from *in vitro* cultured tissues and cells are a pre-requisite for successful application of

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tissue culture and genetic engineering technologies for crop improvement. Culture of explants produces calli that are suitable materials for genetic transformation. Many attempts have been made to enhance the frequency of plant generation from brinjal callus and a lot of research programmes have been devoted to investigate the factors affecting plant regeneration. Both callus induction and plant regeneration from explants require the presence of appropriate combinations and concentrations of plant growth regulators in the culture media. Somaclonal variations were observed by Hitomi *et. al.* (1998) among plants through somatic embryogenesis induced by NAA or 2, 4-D in brinjal. A high frequency of somaclonal variation was observed among plants from both methods. Embryogenesis with NAA was more efficient than 2, 4-D. Callus induction and plant regeneration ability have been studied in *Solanun nigrum* from various explants, namely shoot tip, stem, leaf, and root segments by Jahan and Hadiuzzaman (1996). Best callus induction was observed when the leaf segments were cultured on MS medium supplemented with 0.5 mg/l NAA and 2.0 mg/l BAP. Anwar *et al.* (2002) cultured the aborigine leaf explants on MS media containing IAA, BA (benzyladenine), IBA, NAA or 2, 4-D at 2 mg/I. 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 at 2 mg/l + NAA at 0.5 mg/l.

Materials and Method

Healthy seeds of brinjal cv. Jhumki were collected from BARI. The seeds were then washed thoroughly in running tap water. Special care was taken to avoid all types of injury. The surface sterilization of these seeds were carried out under a Laminar Air Flow Cabinet. The floated seeds were discarded and others were rinsed in 70% ethyl alcohol for one minute, and then thoroughly washed with sterilized distilled water. The alcohol treated seeds were immersed into 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 Petri dishes. Six seeds were placed in each Petri dish. 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 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 (2-3mm each piece) of stem segments were arranged horizontally on each Petri dish and gently pressed into the surface of the sterilized culture medium. The Petri dish was covered and sealed with Para film. Root tip segments (0.5mm)

were placed on a sterilized Petri dish under a Laminar Air Flow Cabinet. The Petri dish was covered and sealed with Para film.

Data collection

a) Days of callus initiation

Generally callus initiation started eight days of inoculation of explant. The number of callus initiated over a number of days was recorded.

b) Percent callus induction

Percent callus induction was calculated on the basis of the number of explant placed and the number of callus induced.

$$\text{Percentage callus induction} = \frac{\text{Number of explants induced calli}}{\text{Number of explants inoculated}} \times 100$$

c) Nature of callus

After three weeks of inoculation, nature ot callus was recorded and graded as 3 for compact, 2 for friable and 1 for looses of its texture.

d) Abundance of callus

After three weeks of inoculation, abundance of callus was recorded and graded as 3 for plenty, 2 for moderatem and I for poor.

e) Weight of callus

After three weeks of inoculation, the weight of callus was measured in gram (g) with the help of an electrical balance.

f) Days to shoot initiation

Shoot initiation was started after 25-30 days of inoculation of explants. The number of shoots proliferated over a number of days were recorded. The mean value of the data provided the days required for shoot initiation.

g) Days to root induction

The number of roots over a number of days was recorded. The mean value of the data provided the days required for root initiation.

h) Shoot length

After one weeks of shoot inoculation, the shoot length was measured in centimeter (cm) with the help of scale.

i) Number of callus with shoot (per cent of shoot or plant regeneration)

Number of callus with shoot was recorded and percentage of shoot regeneration was calculated as

$$\text{Percentage shoot regeneration} = \frac{\text{No. of inoculated calli}}{\text{No. of calli with plantlet}} \times 100$$

j) Number of shoots per callus (Average number of shoots per callus)

Some calli produced only single shoot while some produces multiple shoots. So, number of shoots per callus was recorded at twenty five days interval and the mean was calculated using the following formula: $\overline{X}\,\frac{\sum Xi}{n}$

Where.

$\overline{X}$ = mean of shoot/callus

$\sum$ = Summation

Xi = Number of shoots/callus

n = Number of observations

k) Total number of shoots per petri dish

Some calli produce only single shoot while some produce multiple shoots Number of shoots per Petri dish was recorded at twenty five days interval and mean was calculated using the following formula: $\overline{X}\,\frac{\sum Xi}{n}$

Where.

$\overline{X}$ = mean of shoot/petri dish

$\sum$ = Summation

Xi = Number of shoots/petri dish

n = Number of observations

1) Number of shoots with root

Average number of shoots with root was calculated using the following formula: $\overline{X}\,\frac{\sum Xi}{n}$

Where,

$\overline{X}$ = mean of shoot with root

$\sum$ = Summation

Xi = Number of shoots with root

n = Number of observations

m) Number of regenerated plantlets

The established plants were calculated based on the number of plantlets placed in the pot and number of plants finally established or survived.

$$\text{Percent plant establishment} = \frac{\text{No. of established plantlets}}{\text{Total number of plantlets}} \times \text{I 00}$$

Statistical analysis of data

The data for the characters of the present study were statistically analyzed wherever applicable. The experiments were arranged in Completely Randomized Design (CR1). The analysis of variance for different characters was performed and means were compared by the Dunean's Multiple Range Test (DMRT).

Results and Discussion

In this experiment, effects of different explants and concentration of BAP and NAA on callus induction and regeneration of brinjal cv. Jhumki were studied. Stem and root segments were used as explants to observe their callusing response. Thirty explants were inoculated in each treatment. Among the explants used, stem as comparatively more responsive for callus induction than root 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 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 combinations 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 (plate 3) i.e. 14.600 and 11.600, respectively, out of 30 cultured explants. On the other hand, in case of' root the combination oI' 3.0 mg/l BAP + 0.5 mg/l NAA showed better callus induction. The explants cultured on MS medium without hormones did not produce any callus. It was also found that callus 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% (Table 1). The combination of 2.0 mg/l BAP H+0.5 mg/l NAA required 8.2 days for callus induction from stem explants. On the other hand, the combination of 2.0 mg/l BAP + 0.1 mg/l NAA needed 10.8 days for callus induction from root explants. So, callus induction from stem required minimum days.

Table 1. The effect of different combinations of BAP and NAA in MS medium on callus induction from stem and root explants of brinjal cv. ihumki. Thirty explants were placed in each treatment.

Treatment combinations			No. of expalnts showing callus induction	% of callus induction	Days required for callus inductions	Callus wt explant (g)
Treatment Explants	BAP (mg/l)	NAA (mg/l)				
	0	0	0.000 R	0.000 R	0.0001	0.000
		7 0.1	7.000 JKLMN	23.333 JKLMN	10.400 ABCDEF	0.220
		0.5	6.600 KLMNO	22.000 KLMN0	10.44 ABCDEF	0.240
	2.0	1.0	7.400 HIJKLM	24.066 HIJKIM	10.600ABCDEF	0.240
		0	6.200 LMNO	20.660 LMNO	10.400 ABCDEF	0.220
		0.1	8.600 FGHI	28.666 FGHI	9.600 EBCDEFG	0.280
		0.5	14.600 A	48.666 A	8.200 GH	1.120
Stem		1 .0	9.600 DEFG	32.000 DEFG	9.600 EFG	0.480
		0	6.800 KLMNO	22.660 KLMNO	11.200 ABCDE	0.260
	3.0	0.1	9.400 DEFG	31.330 DEFG	10.800 ABCDE	0.260
		0.5	10.200 DE	34.000 DE	9.800 DEFG	0.400
		1.0	9.200 DEFG	30.666 DEFG	10.600 ABCDEF	0.260
		0	6.600 KLMNO	22.000 KLMNO	11.400 ABCDE	0.220
	4.0	0.1	10.000 DEF	33.333 DEF	11.200 ABDE	0.340
		0.5	11 .600 C	38.666 C	10.000 CDEFG	0.240
		1.0	10.000 DEF	33.333 DEF	11.400 ABCDE	0.360
		0	0.000 R	0.000 R	0.000 I	0.000
	0	0.1	4.400 Q	14.666 Q	12.200AB	0.220
		0.5	6.400 KLMNO	21.333 KLMNO	12.400 A	0.280
		1.0	6.600 KLMNO	22.000 Kl.MNO	1l.800ABCD	0.260
		0	4.200 Q	14.000 Q	12.000 ABC	0.260
Root	2.0	0.1	6.600 KLMNO	22.000 KLMNO	11.400 ABCDE	0.320
		0.5	9.600 DEFG	32.000 DEFG	10.800 ABCDE	0.460
		1.0	8.200 GHlJ	27.330 GHIJ	11.600 ABCDE	0.400
		0	5.400 OPQ	18.000 PQ	11 .400 ABCDE	0.280
	3.0	0.1	7.800 HIJK	26.000 HIJK	11.000 ABCDE	0.280
		0.5	10.600 CD	35.330 CD	10.200 BCDEF	0.480
		1.0	8.800 EFGH	29.330 EFGH	11.600 ABCDE	0.420
		0	4.400 Q	14.666 Q	7.600 H	0.320
	4.0	0.1	6.800JKLMNO	22.66OJKLMNO	11.800 ABCD	0.220
		0.5	9.400 DEFG	31.330 I)EFG	10.400 ABCDE	0.260
		1.0	7.800 HIJK	26.00 HIJK	11.400 ABCDE	0.320

The combined effect of different combinations of BAP and NAA in MS medium on callus weight per explant has been presented in Table 1. Among the different combinations, 2.0 mg/l BAP + 0.5 mg/l NAA showed highest callus weight i.e., 1.12 g from the stem. In case of root, 3.0 mg/l BAP + 0.5 mg/l NAA showed highest callus weight i.e. 0.48 g.

The callus mass from which shoot developed was hard in texture. Among the explants, stem showed better performance in plant regeneration than root. Highest number of regenerated plants were found from stem (0.563) followed by root (0.138). Stem took minimum time (29.738 days) for regeneration. On the other hand, comparatively more time (29.938 days) was required for regeneration from root explants. The combined effect of different combinations of BAP and NAA in MS medium on plant regeneration from stem and root of brinjal cv. Jhumki has been presented in Table 2. Various combinations of supplements showed significant variation in shoot regeneration ability. Among the used combinations, 2.0 mg/l BAP + 0.5 mg/l NAA showed the highest shoot regeneration from stem (3.400) in Plate 4. Root showed lowest regeneration. The percentage i.e. 23.28% of shoot 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 i.e. 5.128% of shoot regeneration was recorded highest in MS media containing 3.0 mg/l BAP + 0.5 mg/l NAA from root and days required for regeneration was minimum (59.8 days). From the above discussion, we found that the best shoot regeneration was recorded from stem in media supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA (plate 4). The results of the main effect of different explants and different supplements (BAP and NAA) on shoot length have been presented in Table 2. The highest of shoot length was obtained from stem i.e., 2.50 cm, and shoot length of root explants was 1.50cm. The highest shoot length obtained from MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA was 2.50 cm. On the contrary, that was better performance of any other concentration of hormones.

In case of root initiation from stem explants, MS media containing 2.0 mg/l BAP + 0.5 mg/l NAA, better performance than root explants. Days required for rooting from stem explants was minimum (8 days) to 2.0 mg/l BAP + 0.5 mg/l NAA hormones with MS medium than root explants (Table 2).

Transplantation of *in vitro* grown plantlets to the soil

The plantlets with well-developed root system were removed from culture vessels with care and without damaging the roots. The agar was removed from the roots by washing with running tap water. After washing, the plantlets were transferred to small earthen pots filled with 1:2:1 of sand, soil and cowdung mixture (plate 5). The plantlets were kept in diffused sunlight covered with

polythene bag to prevent desiccation. Adequate moisture was supplied for 10 days and allowed gradual exposure to air and light. After 30 days, the plantlets became 30 cm long and ready for field condition and the survival rate was 75%. The protocol developed from the result of the study might be useful for the production of disease free and healthy plant materials and also it would be useful for genetic transformation of brinjal using modern biotechnological approach.

Table 2. Combined effect of different combinations of BAP and NAA in MS medium on plant regeneration from different explant. Thirty explants were placed in each treatment.

Treatment combinations			No. of shoots/ explant through callus	Days required for shoot regeneration	Shoot length (cm)	Days to root induction
Explants	BAP (mg/l)	NAA (mg/l)				
		0	-	-	-	-
	0	0.1	-	-	-	-
		0.5	-	-	-	-
		1.0	-	-	-	-
		0	0.200 CD	39.200 G	1.12	12
	2.0	0.1	0.600 CD	39.800 G	1.50	11
		0.5	3.400 A	38.800 G	2.50	8
Stem		1.0	0.600 CD	39.000 G	1.20	9
		0	0.200 CD	39.400 G	1.36	9
	3.0	0.1	0.800 C	39.800 G	1.00	14
		0.5	0.800 C	39.800 G	1.06	15
		1.0	0.600 CD	39.600 G	1.10	10
		0	0.400 CD	40.000 G	1.00	18
	4.0	0.1	0.600 CD	40.000 G	1.45	10
		0.5	0.400 CD	39.800 G	1.75	12
		1.0	0.400 CD	39.60 G	1.10	13
		0	-	-	-	-
	0	0.1	-	-	-	-
		0.5	-	-	-	-
		1.0	-	-	-	-
		0	-	-	-	-
Root	2.0	0.1	-	-	-	-
		0.5	0.200 CD	60.200 A	1.00	16
		1.0	0.200 CD	60.000 A	1.03	14
		0	-	-	-	-
	3.0	0.1	0.200 CD	59.600 A	1.50	17
		0.5	0.400 CD	59.800 A	1.50	14
		1.0	0.200 CD	59.600 A	1.00	16
		0	-	-	-	-
		0.1	0.200 CD	60.400 A	1.00	13
	4.0	0.5	0.200 CD	59.400 A	1.00	17
		1.0	0.400 CD	60.400 A	1.20	10

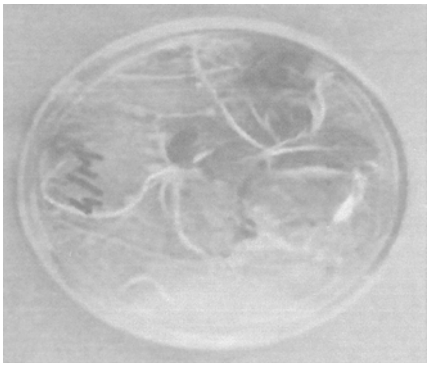

Plate 1. Seed germination from brinjal cv. Jhumki on MS media without hormones at 7 days.

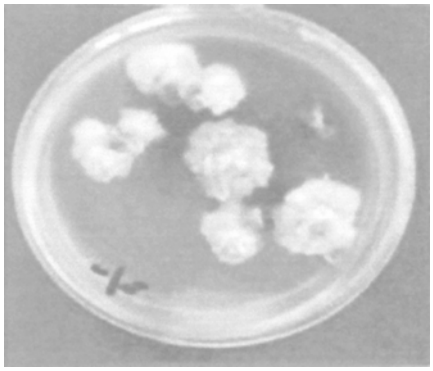

Plate 2. Callus induction from brinjal cv. Jhumki on MS media with hormones (BAP and NAA) at 22 days.

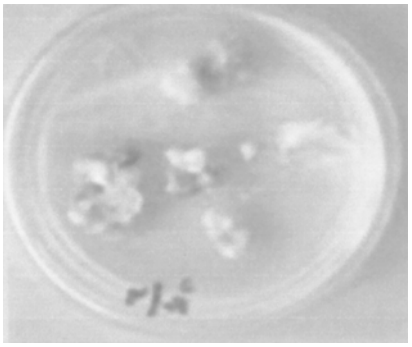

Plate 3. Callus induction in brinjal cv. Jhumki on MS media supplemented with hormones (BAP and NAA).

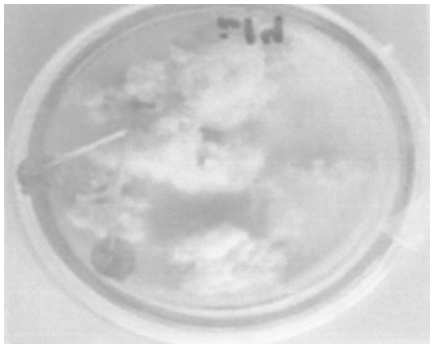

Plate 4. Direct regeneration from stem segment of brinjal cv. Jhumki on MS media supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA.

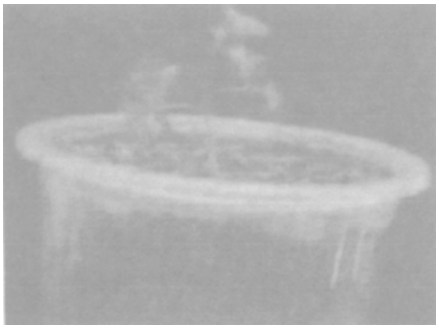

Plate 5. Planted of brinjal was Transferred on earthen pot

Stem and root segments were cultured on MS media containing 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. The highest fresh weight of callus was 1.120 g from the stem explants and 0.48 g from root. 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 root containing 3.0 mg/l BAP and 0.5 mg/l NAA were 0.4 (5.128%).

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