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Plant Regeneration from Seedling Derived Explants through Callus of Eggplant (*Solanum melongena L)*

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Abstract

Different concentrations and combinations of hormones were used in MS medium to observe callus induction and then plant regeneration using stem, leaf and root explants. The rate of callus formation varied in different treatments used. 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 after 8.2 days. The highest fresh weight of callus was 1.12, 0.5 and 0.48 g from stem, leaf and root, respectively. The highest percentage of regeneration (23.28%) was recorded in MS media containing 2.0 mg/l BAP + 0.5 mg/l NAA from stem after 38.8 days. However, 11.94% leaf the regeneration was obtained by 2.0 mg/l BAP + 0.5 mg/l NAA after 46.2 days. It was also found that 2.0 mg/l BAP + 0.5 mg/l NAA was the best stem regeneration. The protocol in 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 eggplant using biotechnological approach.

Keywords: 6-Benzyl amino purine (BAP), α-Naphthalene acetic acid (NAA), *Solanum melongena* , regeneration.

1. Introduction

Eggplant (*Solanum melongena* L.) cultivation is usually suffered from some bacterial and virus diseases and by insects. The fruit fly (*Leucinodes orbonalis*), which damages up to 30% of total yield (Shukla and Upadhyay, 2000). The seed-borne pathogens of previous year can be perpetuated over the generations with symptoms being expressed. Plant tissue culture offers an efficient method for pathogen free materials and germplasm preservation. Tissue culture techniques are widely used for the improvement of various crops. *In vitro* shoot induction from callus culture can induce genetic and epigenic changes in the regenerated plants. These genetic changes have been coined "Somaclonal variation" (Larkin *et al.,* 1981). Calli induction and subsequent plant regeneration through calli culture generate somaclonal variation. Therefore, reproducible protocol should be established on callus induction and its subsequent plant regeneration for using the technique of somaclonal variation of the studied genotype in eggplant. Selection could be performed based on phenotypic expression of a field experiment would be fluctuated with the environmental change. However, selection based on molecular and biochemical markers are very powerful tool in plant breeding (Ofori, 2008). The potential value of tissue culture in plant breeding has been widely recognized, and it is generally used as a tool for crop improvement. Somaclonal variations were observed by Hitomi *et al*. (1998) among plants through somatic embryogenesis

induced by NAA or 2, 4-D in eggplant. A high frequency of somaclonal variation was observed among plants from both methods. Embryogenesis with NAA was more efficient than 2, 4-D.

Anwar *et al*. (2002) cultured the aubergine leaf explants on MS media containing IAA, BA (benzyladenine), IBA, NAA or 2, 4-D at 2 mg/litre and found that NAA produced greenish and fast-growing callus. However, 2, 4-D induced early callus from the petiole, while BA induced green callus 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. Callus induction and plant regeneration ability have been studied in *nigrum* from various explants namely shoot tip, stem, leaf and root segments(Jahan and Hadiuzzaman 1996). Best callus induction was observed when the leaf segments are cultured on MS medium supplemented with 0.5 mg/l NAA and 2.0 mg/l BAP. Mohamed (2003) grew the shoot buds of landrace pepper in Murashige and Skoog's medium with or without benzyladenine (BA) alone or in combination with IAA. The highest number of shoots was produced in the medium containing 3 µM BA singly or in combination with 0.9 µM IAA, and in the medium with 5 µM BA singly or in combination with 1.5 µM IAA. In the present study, efforts have been made to establish a protocol for efficient plant regeneration from callus culture in eggplant, using different explants. The main purpose of the present experiment was to select somaclonal variants of eggplant genotypes tolerance to brinjal shoot and fruit borer insect based on biochemical markers.

2. Materials and Methods

Healthy seeds of eggplant cv. Jhumki were collected from Bangladesh Agricultural Research Institute (BARI). The seeds were then washed thoroughly in running tap water. The surface sterilization of these seeds was 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. Sterilized seeds were placed into seed germination medium in Petri dishes. Six seeds were placed in each Petri dish and then incubated in dark till the germination of seeds in Fig. 1. These were then transferred to 16 hours light for normal seedling growth.

2.1. Preparation of culture media

For the induction of callus and plantlets regeneration in eggplant a number of culture media have been advocated by scientists. Cardenas *et al*., 1997 reported MS (Murashige & Skoog, 1962) medium containing 2, 4-D at various concentrations promoted callus formation in stem and leaf explants of *Capsicum annuum*. Growth was better with 1.5 than 1.0 mg/l 2, 4-D. The experiment was conducted in a randomized complete block design with 16 treatments replicated. The details of the treatment combinations of this experiment were presented in Table 1.

2.2. Preparation of samples

Stem (2-3mm), leaf and root (0.5mm) segments from each germinated seedling were cut using sterilized scalpel under aseptic condition. Six pieces of each segment were arranged horizontally on each petridish and gently pressed into the surface of the sterilized culture medium with various concentrations and combinations of hormones. The Petridishes were covered and sealed with Para film.

Table 1. Treatment combination

Serial No.	Treatment combination	Serial No.	Treatment combination
	BAP mg/l + NAA mg/l		BAP mg/l + NAA mg/l
Treatment 1	0 + 0	Treatment 9	3.0 + 0
Treatment 2	0 + 0.1	Treatment 10	3.0 + 0.1
Treatment 3	0 + 0.5	Treatment 11	3.0 + 0..5
Treatment 4	0 + 1.0	Treatment 12	3.0 + 1.0
Treatment 5	2.0 + 0	Treatment 13	4.0 + 0
Treatment 6	2.0 + 0.1	Treatment 14	4.0 + 0.1
Treatment 7	2.0 + 0.5	Treatment 15	4.0 + 0.5
Treatment 8	2.0 + 1.0	Treatment 16	4.0 + 1.0

Fig. 1. Seed germination from eggplant cv. Jhumki on MS media without hormones at 7 days.

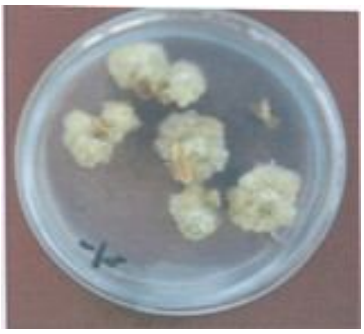

Fig. 2. Callus induction from eggplant cv. Jhumki on MS media with hormones (BAP and NAA).

2.3. Data collection

2.3.1 Days to callus initiation

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

2.3.2 Per cent callus induction

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

2.3.3 Color of callus

After three weeks of inoculation, the color of the callus was observed visually and were graded as 3 for green, 2 for creamy and 1 for yellow color.

a) Nature of callus

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

b) Abundance of callus

After three weeks of inoculation, abundance of callus was recorded and graded as 3 for plenty, 2 for moderate and 1 for poor.

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

b) Weight of callus

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

c) Days of shoot initiation

Shoot initiation 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.

d) 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

$$\% \text{ shoot regeneration} = \frac{\text{Number of calli with plantlet}}{\text{Number of inoculated calli}} x100$$

e) Number of shoot per callus

Some calli produced only single shoot while some others produced 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 shoot/callus
n = Number of observation

f) Total number of shoots per Petridish

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
X_i = Number of shoot/Petri dish
n = Number of observation

g) Number of shoot with root

Average number of shoot with root was calculated using the following formula:

$$\overline{X} = \frac{\sum Xi}{n}$$

Where,

$\overline{X}$ = mean of shoot with root
$\sum$ = Summation
X_i = Number of shoot with root
n = Number of observation

h) 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.

$$\% \text{ plant establishment} = \frac{\text{Number of established plantlets}}{\text{Total number of plantlets}} x100$$

i) 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 cow dung mixture. The plantlets were kept in diffused sunlight covered with polythene bag to prevent desiccation. Adequate moisture was supplied for 10 days and gradual exposure to air and light was allowed. After 30 days, the plantlets became 30 cm long and the survival rate was 75% Fig. 5.

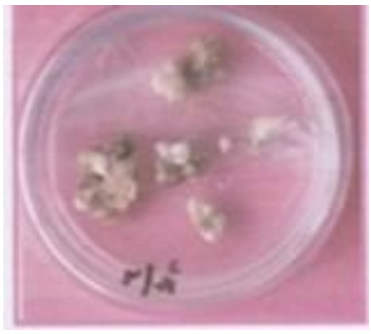

Fig. 3. Callus induction from eggplant cv. Jhumki on MS media with hormones (BAP and NAA).

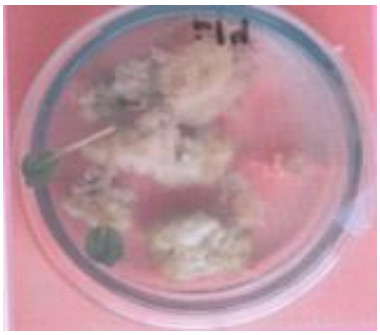

Fig. 4. Direct regeneration from eggplant cv. Jhumki on MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA.

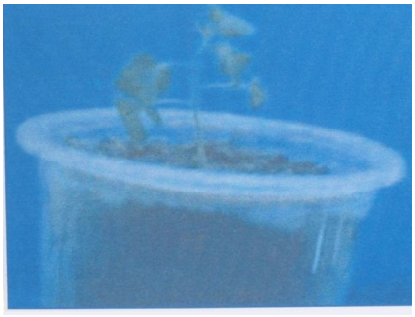

Fig. 5. plantlet of eggplant was transferred on earthen pot.

2.4. Statistical analysis of data

The data for the characters of the present study were statistically analyzed wherever applicable. The analysis of variance for different characters was performed and means were compared by the Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

Plant regeneration from induced calli of eggplant in MS medium supplemented with different combinations of hormones was used. Among the explants used, stem was comparatively more responsive for callus induction than other explants such as leaf and root. The combined effect of explants and different combinations of BAP and NAA on callus induction are presented in Table 2. In case of stem, among the different combinations 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 in Fig. 2. 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 in Fig. 2. 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 agreement with that reported by Jayasree *et al*. (2001). The highest percentage of callus induction was found in MS media containing 2.0 mg/l BAP + 0.5 mg/l NAA from stem i.e. 48.666% followed by leaf in Fig. 3.

Table 2. Combined effect of different combinations of BAP and NAA in MS medium on callus induction from stem, leaf and root explants of eggplant cv. Jhumki.

Explants	Treatment combinations		No. of explants showing callus induction	Percent of callus induction	Days required for callus induction
	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

The combination of 2.0 mg/l BAP + 0.5 mg/l NAA from stem days required for callus induction was 8.2 days. While it required 8.6 days for callus induction from leaf explants with 2.0mg/l BAP + 0.1mg/l NAA. So, callus induction from stem required minimum days.

Stem and leaf segments were used as explants to observe their callus weight. The highest fresh weight of callus was obtained from stem i.e. 0.321 g and callus weight of leaf was 0.291 g. Different concentrations of BAP and NAA influenced the average fresh weight of callus. The highest callus weight obtained from MS medium supplemented with 2.0 mg/l BAP was 0.407 g and 0.5 mg/l NAA was 0.417 g. On the contrary, explants cultured on MS media containing without hormones did not produce any callus. Among the different combination of 2.0 mg/l BAP + 0.5 mg/l NAA showed highest callus weight from the stem i.e. 1.12 g. In case of leaf, 2.0 mg/l BAP + 0.5 mg/l NAA showed highest callus weight from the leaf i.e. 0.500 g.

The combined effect of different combinations of BAP and NAA in MS medium on plant regeneration from stem, leaf and root of eggplant cv. Jhumki are presented in Table 3. Various combinations of supplements showed significant variation in regeneration ability. Among the 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 the lowest regeneration. The percentage of regeneration was recorded the highest in MS media containing 2.0 mg/l BAP + 0.5 mg/l NAA from stem. i.e. 23.28% and days required for regeneration is minimum (38.8 days) in Fig. 4. The percentage of regeneration was the highest in 2.0 mg/l BAP + 0.5 mg/l NAA from leaf. i.e. 1.6 (11.94%) and percentage of regeneration from root was the lowest. Plant regeneration from leaf in 2.0 mg/l BAP + 0.5 mg/l NAA combination required minimum days (46.2 days). So, we found that 2.0 mg/l BAP + 0.5 mg/l NAA combination in stem is the best for regeneration in eggplant.

Table 3. Combined effect of different combinations with BAP and NAA in MS medium on plant regeneration from stem, leaf and root of eggplant cv. Jhumki

Treatment combinations			No. of plants regenerated through callus	Percent of regeneration	Days required for regeneration
Explants	BAP (mg/l)	NAA (mg/l)			
Stem	0	0	-	-	-
		0.1	-	-	-
		0.5	-	-	-
		1.0	-	-	-
	2.0	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	0.600 CD	6.25 CD	39.000 G
	3.0	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	0.600 CD	6.521 CD	39.600 G
	4.0	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	0.400 CD	4.00 CD	39.600 G
Leaf	0	0	-	-	-
		0.1	-	-	-
		0.5	-	-	-
		1.0	-	-	-
	2.0	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	0.600 CD	6.122 CD	49.000 CD
	3.0	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	0.400 CD	4.00 CD	48.200CDE
	4.0	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	0.400 CD	4.651 CD	49.200 BC
Root	0	0	-	-	-
		0.1	-	-	-
		0.5	-	-	-
		1.0	-	-	-
	2.0	0	-	-	-
		0.1	-	-	-
		0.5	0.200 CD	2.083 CD	60.200 A
		1.0	0.200 CD	2.439 CD	60.000 A
	3.0	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	0.200 CD	2.272 CD	59.600 A
	4.0	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	0.400 CD	5.128 CD	60.000 A

4. Conclusions

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 after 8.2 days. 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 0.5g from the leaf. The 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%). The best 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.

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