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-Short Communication

IN VITRO REGENERATION OF BRINJAL (*SOLANUM MELONGENA* L.) USING STEM AND LEAF EXPLANTS

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Brinjal (*Solanum melongena* L.), belongs to the family Solanaceae, is one of the most popular, palatable and nutritious vegetable crop in Bangladesh. 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 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 germplasm preservation of plants.

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 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 regeneration from brinjal callus and a lot of research programmes have been devoted to investigate the factors affecting plant regeneration.

Healthy seeds of brinjal cv. Jhumki were collected from Bangladesh Agricultural Research Institute (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 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 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 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. Stem segments from each germinated seedling were cut into 2-3 mm pieces using sterilized scalpel under a Laminar Air Flow Cabinet. Six pieces 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.

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The Segment of explants were placed on MS Media supplemented with different concentration of 0, 2.0, 3.0 and 4.0 mg/l BAP and 0, 0.1, 0.5 and 1.0 mg/l NAA. Stem, leaf 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 highest callusing means (8.36) whereas leaf segments gave callusing mean 6.95. The highest callusing was obtained in 2.0 mg /l BAP (8.56) and 0.5 mg/l NAA (9.33). Also minimum days (9.72) were required for callus induction from stem. Days required for callus induction from 2.0 mg /l BAP were 10.35 and days required for callus induction from 0.5 mg /l NAA were 10.36. 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. 14.60 and 11.60 respectively out of 30 cultured explants. 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. 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. 48.66% followed by callus induction in leaf. The combination of 2.0 mg /l BAP + 0.5 mg /l NAA from stem days required for callus induction was 8.2 days. On the otherhand, the combination of 2.0 mg /l BAP + 0.1 mg /l NAA from leaf days required for callus induction was 8.6 days. So, callus induction from stem required minimum days.

Shoots developed as light green spots on the surface of the callus after seven weeks of culture initiation when the explants were completely covered by the callus. Sometimes leaf like structure had been developed. The callus mass from which shoot developed was hard in texture. Among the explants stem showed better performance in plant regeneration. Leaf showed poor performance for regeneration. Highest number of regenerated plant was found from stem (0.56) followed by leaf (0.40). Stem took minimum time (29.73 d) for regeneration. On the other hand, comparatively more time (36.40 d) was required for regeneration from leaf. Different supplements of BAP and NAA in MS medium were used to observe the effect on plant regeneration.

Among the supplements, the highest regeneration potentiality observed from 2.0 mg /l BAP (0.71) and 0.5 mg/l NAA (0.67). 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 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 (3.40). The regeneration of plantlets was (1.6) from leaf in 2.0 mg /l BAP and 0.5 mg /l NAA combinations. 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). 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%). Plant regeneration from leaf in 2.0 mg /l BAP + 0.5 mg /l NAA combination required minimum days (46.2 days). In an experiment Jayasree *et al*. (2001) found that leaf sections when cultured on MS media supplemented with 2, 4-D + BA and NAA + BA, nodular embryogenesis callus developed from the cut ends of explants on MS media containing 2, 4-D and BA, whereas, compact callus developed on media containing NAA and BA. The cotyledon stage embryos developed into complete plantlets on hormone free MS medium. From the above discussion, we found that 2.0 mg /l BAP + 0.5 mg/l NAA combination in stem is best regeneration.

Table 1. The combined effect of different combinations with BAP and NAA on MS medium from stem and leaf of brinjal. Jhumki have been presented.

Explants	Treatment combinations		No. of explants showing callus induction	% of callus induction	Days required for callus induction
	Treatment				
	BAP (mg/l)	NAA (mg/l)			
Stem	0	0.1	7.0	23.33	10.4
		0.5	6.6	22.00	10.4
		1.0	7.4	24.06	10.6
	2.0	0	6.2	20.66	10.4
		0.1	8.6	28.66	9.6
		0.5	14.6	48.66	8.2
		1.0	9.6	32.00	9.6
	3.0	0	6.8	22.66	11.2
		0.1	9.4	31.33	10.8
		0.5	10.2	34.00	9.8
		1.0	9.2	30.66	10.6
	4.0	0	6.6	22.00	11.4
		0.1	10.0	33.33	11.2
		0.5	11.6	38.66	10.0
		1.0	10.0	33.33	11.4
Leaf	0	0.1	5.8	19.33	11.0
		0.5	6.0	20.00	10.8
		1.0	9.2	30.66	10.6
	2.0	0	4.6	15.33	11.0
		0.1	7.4	24.66	10.2
		0.5	13.4	44.60	8.6
		1.0	9.8	32.66	10.8
	3.0	0	4.4	14.66	10.8
		0.1	6.4	21.33	10.8
		0.5	6.2	20.66	11.2
		1.0	10.0	33.33	10.8
	4.0	0	4.4	14.66	11.4
		0.1	7.6	25.33	11.6
		0.5	7.4	24.66	11.6
		1.0	8.6	28.66	11.2

Table 2. Combined effect of different combinations of BAP and NAA in MS medium on plant regeneration from stem and leaf of brinjal cv. Jhumki have been presented.

Treatment combinations			No. of plants regenerated through callus	% of regeneration	Days required for regeneration
plan	BAP (mg/l)	NAA (mg/l)			
Stem	0	0.1	-	-	-
		0.5	-	-	-
		1.0	-	-	-
	2.0	0	0.2	3.22	39.2
		0.1	0.6	6.97	39.8
		0.5	3.4	23.28	38.8
		1.0	0.6	6.25	39.0
	3.0	0	0.2	2.94	39.4
		0.1	0.8	8.51	39.8
		0.5	0.8	7.84	39.8
		1.0	0.6	6.52	39.6
	4.0	0	0.4	6.06	40.0
		0.1	0.6	6.00	40.0
		0.5	0.4	3.44	39.8
		1.0	0.4	4.00	39.6
Leaf	0	0.1	-	-	-
		0.5	-	-	-
		1.0	-	-	-
	2.0	0	0.4	8.69	48.8
		0.1	0.6	8.11	48.0
		0.5	1.6	11.94	46.2
		1.0	0.6	6.12	49.0
	3.0	0	0.4	9.09	48.8
		0.1	0.4	6.25	48.4
		0.5	0.6	9.67	47.4
		1.0	0.4	4.00	48.2
	4.0	0	0.2	4.54	49.0
		0.1	0.4	5.36	49.0
		0.5	0.4	5.41	50.2
		1.0	0.4	4.65	49.2

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

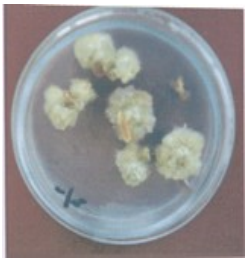

Plate 2. Callus induction from brinjal cv. Jhumki on MS media with hormones (BAP and NAA) at 22 days

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

Plate 4. *Direct regeneration o brinjal cv. Jhumki at 38 days o MS medium supplemented wit 2.0 mg/l BAP + 0.5 mg/l NAA.*

Reference

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