J. Bangladesh Agril. Univ. 7(2): 253–258, 2009 **ISSN 1810-3030**

In vitro regeneration system in brinjal (*Solanum melongena* L.) for stress tolerant somaclone selection

A. Ferdausi, U. K. Nath, B. L. Das[1] and M. S. Alam
Department of Genetics and Plant Breeding and [1]Department of Biochemistry, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Brinjal is the second most important vegetable crop after potato in Bangladesh in respect of total areas and third in production. It also plays a vital role in the national economy as a cash crop. An experiment was conducted with two cultivars of brinjal namely Jhumky and Islampuri to observe the callus induction ability of different explants-shoot tip, hypocotyl and midrib in MS basal media supplemented with different concentrations and combinations of growth regulators. The rate of callus induction from shoot tip, hypocotyl and midrib were 82.78%, 74.88% and 78.71%, respectively. Highest rate of callus induction was found in shoot tip. Variety Islampuri showed higher rate of callus induction (80.62%). Among the treatments 2mg/l NAA showed the best performance in callus proliferation. Cytokinin (0.5 mg/l BAP) showed highest percentage of shoot regeneration (57.13%). For root induction, MS basal medium was proved to be better treatment for average number (12-15) of roots. The survival rate of transferred regenerated plantlets after hardening was higher in Jhumky (80%). Regenerated plantlets from callus of both the varieties exhibited 4-9 times higher proline, 2-3 times lower vitamin C and 2-3 times higher iron (Fe) content compared to their seed derived seedlings. This experiment showed that it is possible to develop shoot and fruit borer tolerance brinjal genotypes through somatic embryogenesis that was selected based on biochemical markers within the very short period of time. These findings will be helpful for further selection of the selected variants in field condition in the next phase of the study.

Keywords: Somaclonal variants, Biochemical markers, Brinjal

Introduction

Eggplant or brinjal (*Solanum melongena* L.) is an important solanaceous crop grown as vegetable. Among the solanaceous vegetables, brinjal is the most common and popular vegetable crop grown in Bangladesh. It is also known as aubergine, melongena and guina squash in different countries of the globe. The area under brinjal cultivation is estimated as 0.51 million ha with total production of 8,200,00Mt (FAO, 2005). Biotechnology is a recently developed novel approach, which includes a range of techniques. Using these techniques, remarkable successes have been demonstrated for the improvement of numerous economic and food crops during the last 20 years.

Now a day's 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. 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 present experiment was to select somaclonal variants of brinjal genotype tolerance to brinjal shoot and fruit borer insect based on biochemical markers. 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).

Materials and Methods

Plant materials

Two varieties namely Jhumky and Islampuri were selected as source of explants.

Explants

Shoot tip, hypocotyl and midrib of *in vitro* grown plants of the selected cultivars. The experiment was conducted following Completely Randomized Design (CRD) with three replications.

Callus induction and somatic embryogenesis

Explants were excised and cut into segments of 2-3 mm with the help of a scalpel. The explants were then placed on the MS medium (Murashige and Skoog, 1962) supplemented with various concentrations and combinations of 2, 4-D (0.0, 1.0 and 2.0 mg/l), NAA (0.0, 1.0 and 2.0 mg/l) and BAP (0.0, 0.5 and 1.0 mg/l).

Regeneration

Within 3-4 weeks of inoculation, calli were sub divided and cultured separately for further proliferation and root induction. The cultures were inoculated at 25±2°C under 16/8h light/dark condition. The plantlets with sufficient root systems were transferred to soil after hardening.

Biochemical parameters

a) Estimation of Proline

Free proline content of the leaves was determined following the method of Bates *et al.,* (1973). Five hundred milligrams of leaf tissue was homogenized in a mortar with pestle using 10 ml of 3% sulfosalicylic acid. Centrifuged at 1000 ppm for five minuets and then filtered through Whatman no. 1 filter paper. Two milliliter of the filtrate was pipetted into the test-tube and 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added to it and the mixture was shaken well. The test tubes were incubated for 1 hr at 100^0C in a hot water bath then transferred to an ice bath to terminate the reaction. Four milliliters of toluene was added to each of the test tube which was stirred vigorously for 15-20 seconds. The toluene containing the chromophore was separated from the aqueous phase and collected carefully. Absorbance of the collected toluene was measured at 520 nm in an UV spectrophotometer. A standard curve was prepared with analytical grade proline and based on this curve proline content of the sample was calculated.

b) Estimation of Vitamin C

According to 2, 6-dichlorophenol indophenol visual titration method 50 gm edible vegetable portion with 100 ml of 3% of Metaphosphoric acid solution was blended in a warring blender to yield homogenous slurry. The whole extract was then filtered through a piece of cloth and washed with 3% of metaphosphoric acid solution (MPA) to obtain a 250 ml extract. Ten ml aliquots of the filtrate in triplicate were titrated against the standardized dye. Vitamin C content calculated using formula:

mgs ascorbic acid per 100 gm sample = 50XY

Where, X= mgs of ascorbic acid equivalent to 1 ml dye solution.
Y= average ml dye solution used for titration of 10 ml aliquots of the filtrate

c) Estimation of Iron (Fe)

Iron content of the plant samples was estimated by atomic absorption spectrophotometer at the wave length of 324.8 nm. Iron was extracted with DPTA (Diethylene Tri Amine Pentachloro Acetic Acid) extracting reagent according to the method by PCARR (1983).

Results and Discussion

Callus induction

Callus was initiated within a period of 8-15 days of culture and a mass of callus was formed. Among the genotypes highest percentage (84.20%) of callus induced in Jhumky from shoot tip (Table 1, Plate 1a and 1b). The highest percentage (98.15%) of callus was obtained from 2mg/l NAA among all the hormonal combinations. The similar results were obtained by Anwar *et al.,* (2002) in their study.

Table 1. Effect of auxin in single and combination with cytokinin in MS media on callus induction from different explants of the genotypes Jhumky and Islampuri

Growth regulators (mg/l)		% of explants responded						Mean
		Jhumky			Islampuri			
		Midrib	Hypocotyl	Shoot tip	Midrib	Hypocotyl	Shoot tip	
Control	0	48.18	31.52	64.85	55.55	66.66	55.55	55.57
2, 4-D	1	48.18	51.93	88.89	66.66	81.52	77.77	69.16
	2	100	48.18	98.19	64.85	64.85	55.55	73.78
NAA	1	88.89	88.89	100	100	100	100	96.30
	2	100	100	100	100	100	88.89	98.15
BAP	0.5	100	77.77	88.89	77.77	77.77	77.77	83.33
	1	77.77	77.77	88.89	77.77	100	64.85	81.17
2, 4-D+BAP	1+0.5	100	66.66	100	77.77	66.66	88.89	83.33
	1+1	66.66	81.52	33.33	77.77	100	100	76.55
	2+0.5	62.80	44.44	88.89	66.90	44.44	66.66	62.35
	2+1	31.52	31.52	77.78	31.52	44.44	64.85	46.94
NAA+BAP	1+0.5	66.66	44.44	55.55	100	100	100	77.77
	1+1	88.89	66.66	77.77	100	100	100	88.89
	2+0.5	100	100	100	100	100	77.77	96.29
	2+1	100	100	100	77.78	88.89	100	94.44
Mean		78.64	67.42	84.20	78.29	82.35	82.71	

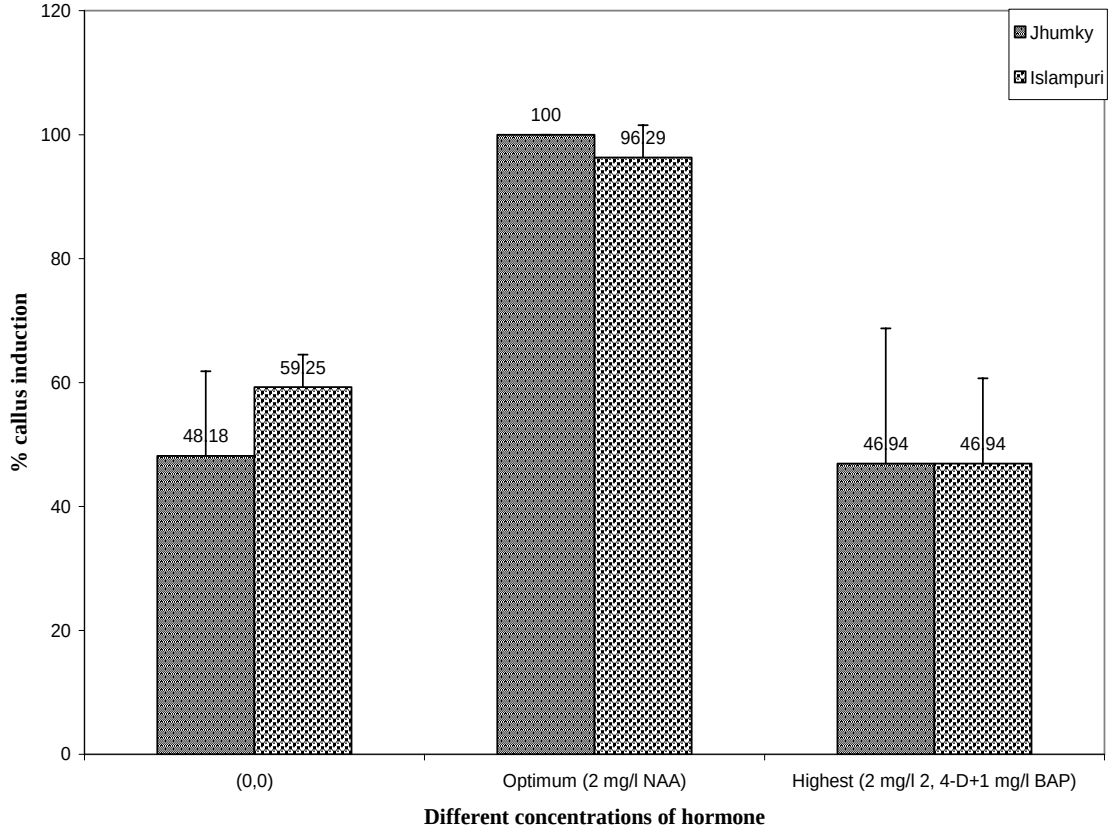

Fig 1. Effect of different hormones on percent callus induction of the genotypes Jhumky and Islampuri over all the explants cultured

Initiation of shoots

The calli were transferred to regeneration medium for shoot initiation after 28-30 days. The highest numbers of shoots were found in case of shoot tip (42) for both of the genotypes- Jhumky and Islampuri. Hypocotyl showed less shoot initiation in both of the genotypes (Figure 2, Plate 1d). Different hormonal combinations were used for shooting but the maximum number of shoots were found from the MS media supplemented with 0.5mg/l BAP in both Jhumky and Islampuri (Table 2). Similar results were found in the experiment of Hossain *et al*., (2007).

Table 2.Effect of different hormonal compositions on regeneration of shoot

Combination of growth regulators	Total number of shoot induced					
	Jhumky			Islampuri		
	Midrib	Hypocotyl	Shoot tip	Midrib	Hypocotyl	Shoot tip
2 mg/l 2, 4-D	4	3	7	3	2	6
2 mg/l NAA	3	2	5	3	1	5
0.5 mg/l BAP	7	5	13	8	4	12
1 mg/l BAP	4	4	6	5	3	8
1 mg/l 2, 4-D+0.5 mg/l BAP	5	3	5	4	2	6
1 mg/l NAA+0.5 mg/l BAP	4	4	6	3	2	5

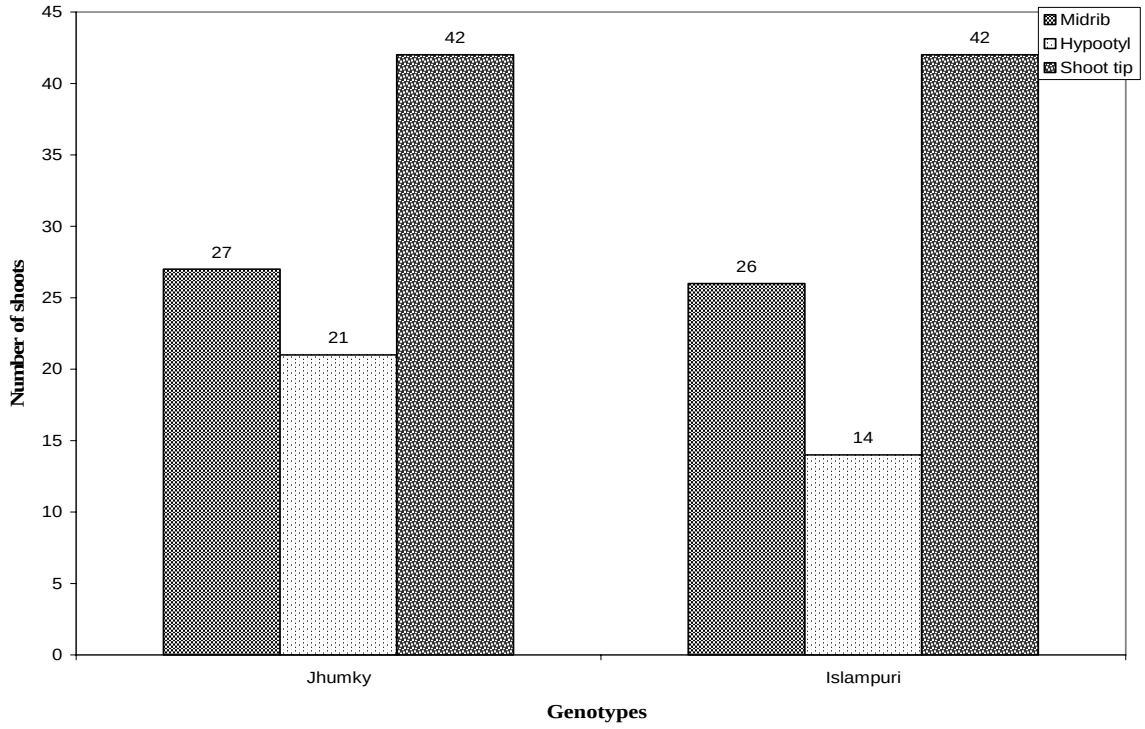

Fig 2. Effect of genotypes on number of shoots from different explants

Root induction

In case of Jhumky maximum numbers of roots (40) were obtained from shoot tip derived regenerates and minimum number (3) from those of hypocotyls. Days required for rooting in Jhumky varies from 7-12 days. And for Islampuri maximum number (38) was obtained from shoot tip (Table 2, plate 1e). Days required for rooting in case of Islampuri was 8-10 days. Somatic embryos germinated into plantlets with roots when transferred into MS medium devoid of growth regulators, which was found by Bastaki *et al.,* (1990) and by Jayasree *et al.,* (2001).

Table 3. Effect of different genotypes and explants on root induction in MS basal medium

Genotype	Explants	Total no. of roots	Days to root initiation
Jhumky	Midrib	10	7
	Hypocotyl	3	10
	Shoot tip	40	12
Islampuri	Midrib	4	10
	Hypocotyl	5	8
	Shoot tip	38	10

Establishment of plantlets

After sufficient development of shoots and root systems the plantlets were taken out from the culture vessels. Afterwards the plantlets were transplanted in plastic pots into growth chamber for proper hardening. Properly hardened plantlets were transplanted to earthen pots (Plate 1f). The survival rate was 80% in Jhumky and 60% in Islampuri.

Biochemical parameters

Proline content

Amount of proline was 2 times higher in regenerated Jhumky and 9 times higher in Islampuri than the seed derived plants (Table 3). The accumulation of free proline in a wide variety of species under various kinds of stresses and its possible involvement in adaptive mechanisms has been reported by Aspinall and Paleg (1981). However, Handa *et al.,* (1986) reported a high relationship between proline level and stress tolerance in cultured tomato cells and suggested a positive role for proline accumulation in adaptation of cells to changing internal water potential. More proline content is responsible for biotic and abiotic stress tolerance like insect infestation. During insect infestation plants combat to resist the infestation mechanism by producing more proline and making unpalatable to the insects.

Vitamin C content

Amount of Vitamin C was 2 times lower in regenerated Jhumky and 3 times lower in Islampuri than the seed derived plants (Table 3). High Vitamin C content is responsible for palatability to some biotic agents like insects and fungi. The results indicate that the regenerated plants were less palatable to insect infestations than the seed derived plants. Therefore, it could be concluded that the tissue culture regenerate of brinjal could be use a source of insect tolerance genotypes.

Iron content

Amount of iron was 2 times higher in regenerated Jhumky and Islampuri than the seed derived plants (Table 3), indicating that the tissue culture regenerate via somatic embryogenesis might be more harder and would be tolerance to insect infestation because iron makes the plants strong and tough, which reduces insect infestation.

Table 4. Amount of free proline, Vitamin C and Iron (Fe) content in regenerated and in seed derived (control) seedlings

Genotype	Amount of free proline (%)		Amount of Vitamin C in mg%		Iron (Fe) content in ppm	
	Control	Regenerated	Control	Regenerated	Control	Regenerated
Jhumky	0.02	0.04	41.05	24.80	0.2015	0.5531
Islampuri	0.007	0.093	47.19	17.69	0.2509	0.5007

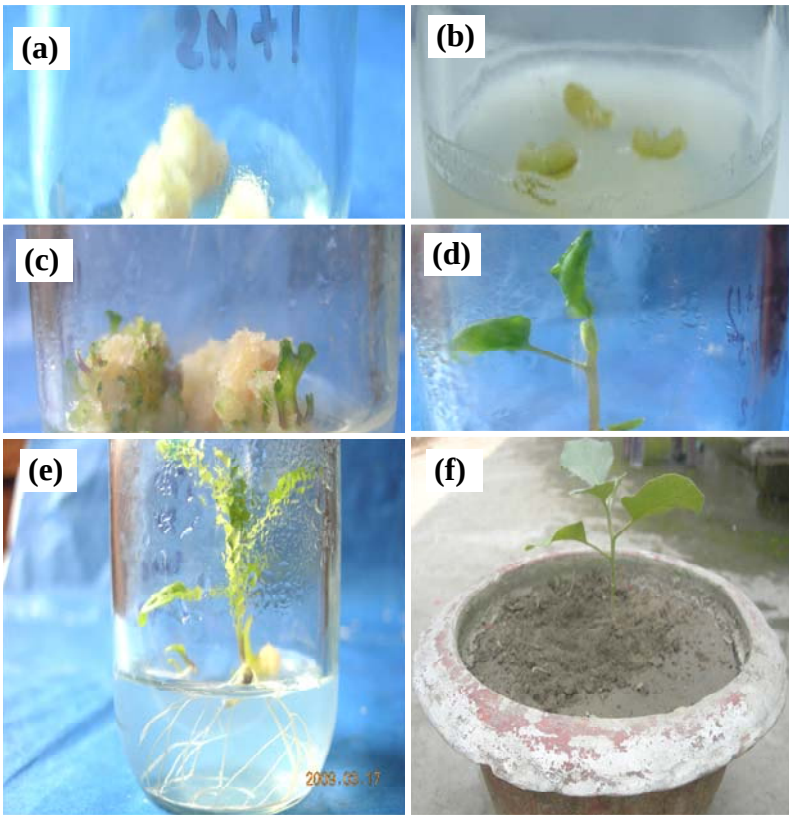

Plate 1. a) callus induction in Jhumky; b) callus induction in Islampuri; c) proliferation of callus; d) initiation of shoot from the proliferated callus; e) root induction from the regenerate; f) establishment of plantlet

References

Anwar, S.D., Sabana, S.A., Siddiqui, A. and Shahead, S. 2002. Clonal propagation of brinjal (*Solanum melongena* L.) through young petiolated leaf culture. *Bionotesx*. 4(3):61.

Aspinall, D. and Paleg, L.G. 1981. Proline accumulation and physiological aspects. *In: Physiology and Biochemistry of Drought Resistance in Plants 205-207 Academic Press, New York.*

Bastaki, S., Nil, M. A. and Awadi, A. 1990. Factors affecting embryogenesis in eggplant cultures. *Plant Tissue Culture*. 4(1): 65-68.

FAO (Food and Agriculture Organization). 2005. FAO Yearbook Production. Food and Agriculture Organization of the United Nations. Rome, Italy. 57:223.

Handa, S., Handa, A., Hagegawa, K., Paul, M. and Bressan, R.A. 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiology*. 80: 938-945.

Hossain, M.J., Rahman, M. and Bari, M.A. 2007. Establishment of cell suspension culture and plantlet regeneration of brinjal (*Solanum melongena* L.). Journal of Plant Science. 2(4): 407-415.

Jayasree, T.V., Paban, M., Ramesh, A.V. and Reddy, K.J.M. 2001. Somatic embryogenesis from leaf cultures of potato. *Plant Cell Tissue Organ Culture*. 64(1): 13-17.

Larkin, P.J. and Scowcroft, W.R. 1981. Somaclonal variation- a novel source of variability from cell cultures for plant environment. *Theory of Applied Genetics*. 60: 197-214.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiology*. 15: 473-497.

Ofori, A., Heiko, C. and Becker, 2008. Breeding of Brassica rapa for biogas production: Heterosis and combining ability of biomass yield. *Bioenergetic Research*. 1:98-104.