Plant Tissue Cult. & Biotech. **16**(1): 53-61, 2006 (June)

PTC&B

Multiple Shoot Formation in Eggplant (*Solanum melongena* L.)

R. H. Sarker, Sabina Yesmin and M. I. Hoque

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Regeneration, Multiple shoot, Eggplant

Abstract

Among the *in vitro* derived different explants such as cotyledonary leaf, hypocotyl, shoot tip and root of two local varieties, namely Singhnath and Kazla (BARI Begun-4) of eggplant (*Solanum melongena* L.) cotyledonary leaf was found to be the best for multiple shoot regeneration. High frequency direct organogenesis of shoots was achieved from cotyledonary leaf in MS supplemented with 1.0 mg/l BAP and 1.0 mg/l Kn. Anatomical studies using freezing microtome supported the formation of shoots through organogenesis. Proliferation and elongation of such shoots were obtained in hormone free MS. Moreover, the regenerated shoots produced healthy roots when they were cultured on MS without hormonal supplements. Following the formation of roots the *in vitro* raised plantlets were successfully established in soil. Viable seeds were obtained from the *in vitro* raised mature plants.

Introduction

Eggplant (*Solanum melongena* L.) is a vegetable crop of the family Solanaceae grown in the subtropics and tropics. It is one of the most popular vegetables in many parts of the world including Bangladesh. The crop is cultivated on small family firms and considered to be important source of nutrition and cash income for many resource-poor farmers. Eggplant can be cultivated grown round the year but the productivity and quality of this crop suffer due to its susceptibility to a number of diseases and insect pests. In South and South East Asia eggplant is extensively damaged by the infestation of a Lepidopteron insect, *Leucinodes orbonalis* commonly known as shoot and fruit borer. This insect is very much specific to this crop. During its cultivation the total loss caused by this insect pest is 5 - 20 % in shoot and 10 - 70 % in fruit (Das et al. 2000). The principal methods used for the improvement of this crop are selection from inbred lines and intervarietal crosses (Anisuzzaman et al. 1993). The progress towards the improvement of this crop for insect pest resistance is hampered mainly due to the wide prevalence of sterility in the progeny and occurrence of genetic incompatibility following intergeneric and interspecific crosses, respectively (Rao

1979 and Daunay et al. 1991). To overcome such problems of conventional breeding advanced biotechnological method such as genetic transformation can be applied as an alternative approach for the development of disease and pest resistance for this crop. An efficient and reproducible *in vitro* regeneration system is considered as an integral part of successful transformation. There are a number of reports available regarding the *in vitro* regeneration of eggplant from different explants *via* organogenesis (Kamat and Rao 1978, Fassuliotis et al. 1981, Sharma and Rajam 1995, Fari et al. 1995, Magioli et al. 1998) and somatic embryogenesis (Matsuoka and Hinata 1979, Gleddie et al. 1983, Yadav and Rajam 1998). Under the circumstances the present attempt was made to develop a suitable regeneration protocol for eggplant cultivars grown in Bangladesh.

Materials and Methods

Seeds of the two varieties of eggplant (*Solanum melongena* L.) namely, Kazla (BARI Begun-4) and Singhnath were collected from the Vegetable Seed Division of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur and East West Seed (Bangladesh) Ltd., respectively.

For germination seeds were first washed with detergent under running tap water for 3 - 5 mins. Floating seeds were considered to be empty and discarded. Later the seeds were dipped in 70 % alcohol for 20 sec followed by washing with distilled water. Then the seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 5 - 6 mins and finally washed five times with sterile distilled water. The seeds were then kept on a sterilized Petri dish containing sterile filter paper to soak excess of water droplets. The surface sterilized seeds were then inoculated into conical flask containing 50 ml agar solidified MS medium with 3% sucrose for supporting seed germination and seedling development. About 10 - 12 seeds were inoculated in each flask. Different explants such as cotyledonary leaf, shoot tip, hypocotyl and root were excised from 7 - 9 days old seedling. Cotyledonary leaf, hypooctyl and root explants were cut into 5 - 6 mm long pieces, on the other hand shoot tips were cut into 3.0 mm long pieces. These explants were then cultured on agar solidified MS supplemented with various concentrations and combinations of BAP, Kn and zeatin for the induction of multiple shoots. All media contained 3 % sucrose and 0.8 % agar with pH 5.8 adjusted before autoclaving. All *in vitro* raised seedlings and cultures were maintained under illumination on a 16 h photoperiod at 25 ± 2°C.

For induction of roots, regenerated shoots (2.5 - 4 cm long) were excised and transferred to MS and half strength MS medium with 3 % sucrose without hormonal supplements. Another set of rooting experiments was conducted using MS supplemented with different concentrations of IBA, IAA and NAA. Following the development of sufficient root system plantlets were transferred to

small plastic pots containing sterilized soil. The *in vitro* raised plantlets were acclimatized and then transferred to the field.

Histological examinations during shoot formation were carried out using a freezing microtome (Coldtome, Sakura, Japan). Microtome slide preparation and observation were made following the methods as described by Sarker and Awal (1999).

Results and Discussion

Different concentrations of BAP (0.5 - 5.0 mg/l) and zeatin (1.0 - 5.0 mg/l) were used separately but BAP and Kn at various concentrations were used in combination to examine their effect on multiple shoot regeneration *via* organogenesis. Among the three cytokinin combinations of BAP and Kn showed better response in terms of number of shoots per explant in both varieties of eggplant. Further, it was observed that the shoot tips, among all other explants, were found not suitable for multiple shoot regeneration, more so majority of the shoot tip explants produced single shoot with variable amount of basal callus. They did not produce multiple shoot at any stage in either of the varieties Singhnath or Kazla of eggplant.

Hypocotyl explant was found to produce callus along its surface but the callus failed to produce shoot and gradually became brown in colour. Occasionally a few hypocotyl explants initiated the formation of shoots at the cut ends without the formation of callus. In most of the cases only 2 - 3 shoots were found to develop on MS supplemented with 2.0 mg/l BAP in Singhnath and 1.0 mg/l BAP + 0.5 mg/l Kn in Kazla. Magioli et al. (1998) also found that hypocotyl explants were less responsive towards regeneration using thidiazuron (TDZ) in a Brazilian eggplant variety. But earlier reports indicate that in most of the cases *in vitro* regeneration in eggplant occurs from either hypocotyl or leaf explant *via* organogenesis (Kamat and Rao 1978, Matsuoka and Hinata 1979, Sharma and Rajam 1995, Gleddie et al. 1983, Mukherjee et al. 1991).

Franklin et al. (2004) reported the efficient regeneration of plantlets from cultured root explants on MS supplemented with 0.45 μM TDZ and 13.3 μM BA. But in the present study root explants were not found to be suitable for regeneration in both the varieties of eggplant.

Out of the four explants cotyledonary leaf showed best response for multiple shoot regeneration. Effect of BAP and zeatin singly as well as the combined effect of BAP and Kn on multiple shoot regeneration from cotyledonary leaf explatns are shown in Table 1. Among the different concentrations of BAP maximum number of shoots (1.7 - 2.0) were observed in MS supplemented with 2.0 mg/l BAP in both the varieties Singhnatha and Kazla.

Table 1. Effects of various concentrations and combinations of BAP, zeatin and Kn on multiple shoot regeneration from cotyledonary leaf explant of eggplant.

Varieties	MS supplemented with hormones (mg/l)	% of responsive explant	Days required for initiation of regeneration	Mean No. of shoots/explant after 60-70 days
Singhnath	BAP			
	0.5	65	10 - 11	1.0
	0.75	70	10 - 11	1.5
	1.0	80	10 -12	1.8
	2.0	90	10 - 11	2.0
	5.0	60	10 - 12	0.85
	Zeatin			
	1.0	100	12 - 13	2.0
	2.0	90	11 - 12	2.5
	3.0	95	11 - 12	2.0
	5.0	90	12 - 13	0.75
	BAP + Kn			
	0.5 + 0.5	90	12 - 13	1.3
	1.0 + 0.5	90	11 - 12	2.0
	1.0 + 1.0	100	10 - 12	4.4
	2.0 + 1.0	100	10 - 12	1.5
	2.0 + 2.0	90	10 - 11	0.75
Kazla	BAP			
	0.5	70	10 - 12	0.93
	0.75	75	10 - 11	1.0
	1.0	90	10 - 12	1.5
	2.0	95	8 - 10	1.7
	5.0	60	9 - 10	0.75
	Zeatin			
	1.0	100	12 - 13	1.5
	2.0	100	11 - 12	1.6
	3.0	95	11 - 12	0.80
	5.0	90	12 - 13	0.60
	BAP + Kn			
	0.5 + 0.5	85	13 - 14	1.2
	1.0 + 0.5	90	11 - 12	1.4
	1.0 + 1.0	95	10 - 12	3.5
	2.0 + 1.0	100	8 - 10	1.0
	2.0 + 2.0	95	10 - 11	1.0

Among the different concentrations of zeatin, 1.0 and 2.0 mg/l in MS were found to be most effective in case of Singhnath (2.0 shoot/explant) and Kazla (1.6 shoot/explant), respectively. Guri and Sink (1988) also reported low

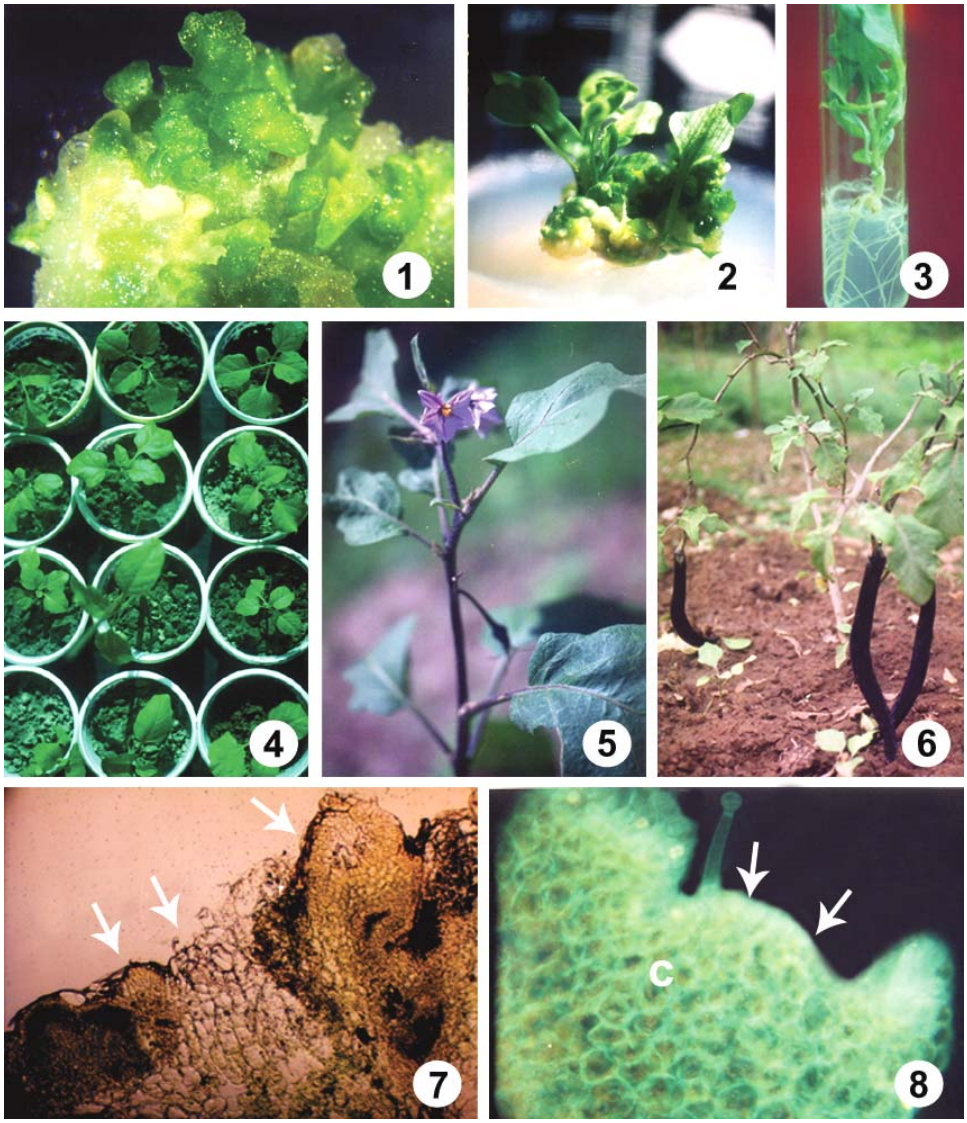
regeneration frequencies using 2.0 mg/l zeatin in eggplant cv. Black Beauty for the production of transgenic shoot using leaf explant.

In the present investigation high percentage of regeneration response in Singhnath and in Kazla was obtained on MS supplemented with 1.0 mg/l BAP and 1.0 mg/l Kn. Stereomicroscopic view of initiation of multiple shoot from the cotyledonary leaf explant is shown in Fig. 1. It was also noticed that from a single explant the development of shoots was found to continue for an extended period. It was observed that during the formation of new shoot buds a small amount of callus was also produced simultaneously. Under the circumstance the elongation of shoots and control of callus formation at the base of the developing shoots are possible when the explants with small shoots and shoot buds were isolated and transferred to hormone free MS basal medium after 20 - 25 days of inoculation. Fully developed multiple shoots on hormone free MS is shown in Fig. 2. Regenerated shoots were isolated within 45 days of culture for rooting. Franklin and Sita (2003) reported that the hormone free MS medium was sufficient for shoot elongation. Mukherjee et al. (1991) used 2.0 mg/l Kn to induce direct organo-genesis without the intervention of callus.

For the induction of roots, shoots of 2.5 - 4.0 cm in length were excised and cultured on both half and full strength MS containing 3% sucrose without hormonal supplements as well as on MS supplemented with 0.1 mg/l IAA, 0.1 mg/l IBA and 0.1 mg/l NAA. The results of these experiments are presented in Fig. 9. In hormone free MS about 75 % of the shoots of both Shingnath and Kazla produced roots. Fully developed roots at the base of regenerated shoot on MS with 3 % sucrose is presented in Fig. 3. MS basal medium was also reported to be effective for root induction and growth by Taha and Tijan (2002) for a Malaysian eggplant variety. Moreover, MS supplemented with 0.1 mg/l IBA was also effective for root induction. NAA and IAA were found to be ineffective for root induction in eggplant varieties used in this study. Magioli et al. (1998) reported the induction of roots using half strength of MS supplemented with 0.6 μM IAA. Earlier Miyoshi (1996) reported that shoot regenerated through microspore culture of eggplant in media containing BA and NAA showed initiation of root primordia in most of the shoots.

After the sufficient development of root the plantlets obtained from two eggplant varieties were successfully transplanted into small plastic pots containing soil (Fig. 4). Following proper acclimatization the plantlets were transferred to the field. These plants flowered within three months after transplantation (Fig. 5) and developed fruits (Fig. 6). Viable seeds were produced with 40 - 50 days.

Anatomical and histochemical studies are required to understand the mechanism of *in vitro* morphogenesis (Yeung 1999). Only a few reports are available regarding the histological studies on regeneration in eggplant (Tarre et al. 2004, Picoli 2002). Longitudinal section from leaf explant showed the



Figs. 1-8: 1. Stereomicroscopic view of multiple shoot initiation from cotyledonary leaf explant in Singhnath on MS medium with 1.0 mg/l BAP + 1.0 mg/l Kn (× 18). 2. Fully developed multiple shoots along with a number of developing shoots on hormone free MS medium in Singhnath. 3. Fully developed roots at the base of regenerated shoots on MS medium with 3% sucrose in Singhnath. 4. Regenerated plantlets of Singhnath transferred to soil in small plastic pots. 5. Regenerated plant flowered after three months following transplantation. 6. Fully developed fruits on tissue culture derived plant of Singhnath. 7. Longitudinal section of cotyledonary leaf explant from Singhnath on MS medium containing 1.0 mg/l BAP and 1.0 mg/l Kn showing the formation of meristematic zones (Arows) within the explants tissue (× 36). 8. Fluorescent micrograph from longitudinal section of fully developed shoot primordia showing the epidermis (Arrows) and developing cortical (c) tissue (× 82).

formation of numerous meristematic zones within their tissue (Fig. 7). These meristematic zones subsequently converted into shoot buds. The formation of shoot buds was characterized by the appearance of shoot apex with the developing leaf primordia (Fig. 8). Prior to the formation of shoot buds they were found to organize vascular tissues during the course of their development. Further, the results presented here demonstrated the accumulation of higher amount of protein within the developing shoot primordia. Similar findings were reported in peanut and chickpea (Sarker and Islam 2000, Sarker and Awal 1999). These anatomical and histological studies indicate the nature of morphogenesis during *in vitro* regeneration.

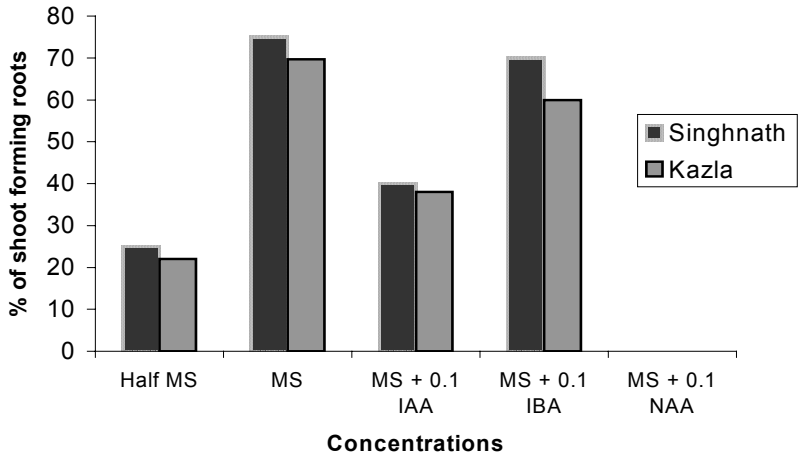


Fig. 9. Effect of different auxins on root formation in two varieties of eggplant (Singhnath and Kazla).

The *in vitro* regeneration protocol described here is easily, reproducible, requires minimum hormonal supplements and genotype independent. In here the efficiency of regeneration system is further demonstrated by obtaining viable seeds from the regenerated plants. Moreover, the regeneration of plantlets achieved without the intervention of callus and this clearly indicates the possibility of obtaining true-to-type plantlets. This present protocol can effectively be used for the development of desired plant types following future genetic transformation in eggplant varieties.

Reference

Anisuzzaman M, Kamal AHM, Islam R, Hossain M and **Joarder OI** (1993) Genotypic differences in somatic embryogenesis from hypocotyl explants in *Solanum melongena* L. Plant Tissue Cult. **3**(1): 35-40.

Das GP, Ramaswamy S and **Bari MA** (2000) Integrated crop management practices for the control of the brinjal shoot and fruit borer in Bangladesh. DAE-DANIDA stengthening plant protection Services (SPPS) project. Dept. of Agriculture Extention, Khamarbari, Dhaka. pp. 29.

Daunay MC, Lester RN and **Laterrot H** (1991) The use of wild species for the genetic improvement of eggplant (*Solanum melongena* L.) and tomato (*Lycopersicon esculentum*). *In:* Hawkes J.C., Lester R.N., Nee M. and Estrada, N. (eds.). Solanaceae III: Taxonomy, Chemistry, Evolution, Vol. **27**: 389-413. Royal Botanic Gardens Kew and Linnean Soc., London.

Fari M, Nagy I, Csanyi M, Mityko J and **Andrasfalvy A** (1995) *Agrobacterium*-mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv. "Kecskemeti Lila') Plant Cell Rep. **15**: 82-86.

Fassuliotis G, Nelson BV and **Bhatt DP** (1981) Organogenesis in tissue culture of *Solanum melongena* cv. Florida Marker. Plant Sci. Lett. **22**: 119-125.

Franklin G, Sheeba CJ and **Sita GL** (2004) Regeneration of eggplants (*Solanum melongena* L.) from root explants. Plant Cell Rep. **40**(2): 188-191.

Franklin G and **Sita GL** (2003) *Agrobacterium tumefaciens*-mediated transformation of eggplant (*S. melongena* L.) using root explant. Plant Cell Rep. **21**: 549-554.

Gleddie S, Keller WA and **Setterfield G** (1983) Somatic embryogenesis and plant regeneration from leaf explants and cell suspensions of eggplant (*Solanum melongena* L.). Can. J. Bot. **61**: 656-666.

Guri A and **Sink KC** (1988) *Agrobacterium* transformation of eggplant. Plant Physiol. **133**: 52-55.

Kamat MG and **Rao NA** (1978) Vegetative multiplication of eggplant (*Solanum melongena* L.) using tissue culture technique. Plant Sci. Lett. **13**: 57-65.

Magioli C, Rocha APM and **Oliveria DE de** (1998) Efficient shoot organogenesis of eggplant (*S. melongena* L.) induced by thidiazuron. Plant Cell Rep. **17**: 661-663.

Matsuoka H and **Hinata K** (1979) NAA-induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena* L. J. Expt. Bot. **30**: 363-370.

Miyoshi K (1996) Callus induction and plantlet formation through culture of isolated microspores of eggplant (*Solanum melongena* L.). Plant Cell Rep. **15**: 391-395.

Mukherjee SK, Rathnasbapathi B and **Guptya N** (1991) Low sugar and osmotic requirements for shoot regeneration from leaf pieces of *Solanum melongena* L. Plant cell Tiss. Org. Cult. **25**: 12-16.

Picoli EA (2002) *In vitro* morphogenesis and *Agrobacterium tumefaciens*-mediated transformation of eggplant (*Solanum melongena* L. cv. Embu). Genetics and Molecular Biology **25**(4): 501-502.

Rao NN (1979) The barriers to hybridization between *Solanum melongena* and some other species of *Solanum*. *In:* Hawkes, J.G., Lester, R.N. and Skelding, A.D. (eds.). The Biology and Taxonomy of the Solanaceae (pp. 605-614). Acad. Press, London.

Sarker RH and **Awal ST** (1999) *In vitro* morphogenesis in chickpea (*Cicer arietinum* L.). Plant Tissue Cult. **9**(2): 141-150.

Sarker RH and **Islam A** (2000) Direct organogenesis from leaflet explants of peanut (*Arachis hypogaea* L.). Bangladesh J. Bot. **29**(2): 107-114.

Sharma P and **Rajam MV** (1995) Genotype, explant and position effects on organogenesis and embryogenesis in eggplant (*Solanum melongena* L.). J. Expt. Bot. **46**: 135-141.

Taha RM and **Tijan M** (2002) An *in vitro* production and field transfer protocol for *Solanum melongena* plants. South African J. Bot. **68**: 447-450.

Tarre E, Magioli C, Pinheiro MM, Martins, GS, Mansur E and **Fernandes DRS** (2004) *In vitro* somatic embryogenesis and adventitious root initiation have a common origin in eggplant (*Solanum melongena* L.). Revista Brasil. Bot. **27**: 79-84.

Yadav JS and **Rajam MV** (1998) Temporal regulation of somatic embryogenesis by adjusting cellular polyamine content in eggplant. Plant Physiol. **116**: 617-625.

Yeung EC (1999) The use of histology in the study of plant tissue culture system -some practical comments. In vitro Cell Dev. Biol. Plant **35**: 137-143.