International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(4): 679-687 December 2014
DOI Number: 10.5958/2230-732X.2014.01375.8

BIOTECHNOLOGY

Biolistic transformation of *cry 1*Ac gene in eggplant (*Solanum melongena* L.)

M. K. Sidhu[1], A. S. Dhatt[2], J. S. Sandhu[3] and S. S. Gosal[4]

[1&2] Department of Vegetable Sciences, [3&4] School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab, India-141004

Corresponding author: mksidhu@pau.edu

Paper No. 256 Received: 16 August, 2014 Accepted: 15 November, 2014 Published: 20 December, 2014

Abstract

Among different explants, cotyledon showed highest regeneration and number of buds per explant on all media combinations. However, highest regeneration potential was observed on MS fortified with 3.0 mgl^{-1} BAP (80.36%), but number of buds per explant were highest (21.76) on MS media supplemented with 2.5 mgl^{-1} BAP+ 1.0 mgl^{-1} Kin. Plant regeneration of cotyledons was completely checked with a selection pressure of 15ppm to 25ppm hygromycin for untranformed explants. But the selection efficacy of transformed tissue was 19.56% with 20ppm and 66.66% with 15 ppm hygromycin. *GUS* gene was expressed in 76.92% bombarded explants with 2 to 7 *GUS* spots per explant confirming the presence of transformed tissue. *GUS* gene was also visualized in 66.66% regenerated shoots from bombarded tissue. PCR analysis of putative transgenic eggplant plants with the specific primers confirmed the presence of *Cry1*Ac gene with 1.2 KB product size (falling between 1KB and 2KB bands of 10KB DNA ladder) in two of the four plants.

Highlights

- Cotyledon of genotype BL-5 had highest regeneration potential and number of buds per explant on MS media supplemented with 2.5 mgl^{-1} BAP+ 1.0 mgl^{-1} Kin.
- Plant regeneration of cotyledons was completely checked with a selection pressure of 15ppm to 25ppm hygromycin for untranformed explants.
- The selection efficacy of transformed tissue was 19.56% with 20ppm and 66.66% with 15 ppm hygromycin.
- *GUS* gene was expressed in bombarded explants as well as in regenerated putative transgenic explants.
- PCR analysis of putative transgenic eggplant plants with the specific primers confirmed the presence of *Cry1*Ac gene.

Keywords: Eggplant, regeneration Bt-gene, biolistic gun, transgenic

Eggplant (*Solanum melongena* L., 2n=2x=24) is a widely adaptive and highly productive vegetable crop of tropical and subtropical regions, but shoot and fruit borer (*Leucinodes orbonalis* Guenee) is a serious pest that causes 37 to 71% reduction in marketable yield (Singh *et al.*, 2000 and Kaur *et al.*, 2004). To control the pest various biological and biochemical control measures have been recommended, but cryptic nature is a big hindrance in efficient management. Consequently, growers use excessive and unrecommended pesticides, which is a matter of concern for food safety, environmental

degradation, pest resistance and economics of the crop. For development of intrinsic plant resistance conventional breeding approaches face problems of non-availability resistance in cultivated, cross incompatibility with wild relatives (*S. mammosum*, *S. incanum* and *S. grandiflorum*) and inadvertent linkage drag of undesirable genes (Baksh and Iqbal 1979). To tackle these issues use of biotechnological techniques can be an alternative.

Genetic transformation of *Bt*-gene(s) is a single generation and established breeding approach to develop inbuilt plant resistance against shoot and fruit borer (SFB) in eggplant. This requires an efficient and reliable *in vitro* plant regeneration and genetic transformation protocols (Kumar *et al.*, 1998b). Eggplant is most amenable to cell, tissue and organ culture, but regeneration potential varies with genotype, explant and culture media (Dobariya and Kachhadiya, 2004). *Agrobactrium tumefaciens* and Biolistic gun method are commonly used for genetic transformation. *Agrobactrium* mediated transformation of *Cry*1Ac gene in eggplant, has been reported (Franklin and Sita, 2003; Soniya *et al.*, 2005), but Biolistic mediated is not there in literature. This method has been proved useful to deliver DNA into cells of wide range of plants (Klein *et al*., 1988) and used successfully vegetables like broccoli (Puddephat *et al.*, 1999), garlic (Sawahel, 2002) and potato (Craig *et al.*, 2005). Therefore, experiment was conducted on standardization of regeneration protocol, transformation of *Cry*1Ac gene using Biolistic gun and molecular evaluation of the putative transgenic plant.

Materials and Methods

The experiments were conducted at Dr. Khush Laboratories, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, India.

Plant regeneration

Standardizing the protocol for regeneration, seeds of pure line BL-5 were surface sterilized with commercial bleach (50%) for 20 minutes and germinated on half strength MS (Murashige and Skoog, 1962) media. Cotyledon and hypocotyl were excised from 15-days old and leaves were excised from 30-days old *in vitro* grown seedlings. Different explants were cultured on MS medium having variable concentrations (2.0, 2.5 and 3.0 mgl^{-1}) of benzyl amino purine (BAP) alone or in combination with 1.0 mgl^{-1} kinetin (Kin.) for shoot regeneration at 16 hrs light/ 8 hrs dark cycles at 25 ± 2°C. Plant regeneration (%) was calculated from number of explants regenerated over the number of explants cultured and number of buds per explant was averaged from total buds in ten explants. The regenerated buds were elongated on ½ strength double agar MS medium containing 0.3mgl^{-1} BAP and the elongated plantlets cut aseptically and rooted on MS medium. The rooted plants were hardened on moist filter paper with 0.2% Carbendazim 50%WP solution for 20 days and planted in polythene bags for further growth at 25 °C, followed by shifting to bigger containers for growth and fruiting.

Hygromycin Sensitivity Assay

The best performing explant viz. cotyledon was subjected to hygromycin (selectable marker for transgenics) selection pressure on best regenerating medium. The best regeneration medium i.e. MS supplemented with 2.5 mgl^{-1} BAP+ 1.0 mgl^{-1} Kin was fortified with the different concentrations of hygromycin i.e. 5 to 25ppm and the concentration that inhibit 50% explant was used in transformation experiment. The selection efficiency was calculated from the number of explants showing regeneration over the total number of explants subjected to selection.

Biolistic transformation

The plasmid DNA containing *Cry1*Ac construct (Ubi promoter and NOS terminator) and *GUS* gene construct (CaMV35S promoter and NOS terminator) (Altosaar, USA). Competent cells were prepared from *E coli* strain JM 109. These competent cells were transformed with the plasmid having Cry1Ac construct through heat shock method and grown in SOB (Sub Optimal Broth: Bacto-trypton: 20 gl^{-1}, Bacto Yeast Extract: 5 gl^{-1}, NaCl: 0.5 gl^{-1}, KCl (250mM): 10

ml^{-1}, $MgCl_2.6H_2O$ (2M):5 ml^{-1}, pH: 7) medium with selection pressure of 100ppm ampicillin. Then the isolated DNA from these cells was confirmed for transformation through PCR (band size of 1.2 KB), and the confirmed competent cells were maintained in glycerol stock. Bacterial cultures (500ml for each sample with 100ppm ampicillin) of both the constructs were grown in LB medium (Luria bertani: Trypton: 10 gl^{-1} Bacto yeast extract: 5 gl^{-1} NaCl: 10 gl^{-1} pH: 7) overnight in an electrical incubator-cum-sha ker set at 37°C. The plasmid DNA of both the constructs was isolated by using Qiagan Maxi kit method. The isolated DNA was quantified with gel electrophoresis as well as spectrophotometer. The plasmid DNA of 1 microgram per micro-litre concentration was used for transformation. The Biolistic PDS-100 He-Gun (Bio Rad, Richmond, California) along with its accessories was used for bombardments. Plasmid DNA of *Cry1*Ac and *GUS* constructs (9:2 proportions) was mixed and coated on to the microcarriers. The coated micro-carriers (tungsten particles) were bombarded on to cotyledons arranged in the centre of target plate containing osmoticum medium (MS + 0.4M mannitol + 30gl^{-1} + 8gl^{-1} agar). The target plates were prepared four hours prior to the bombardments with plasmid DNA (*Cry1*Ac and *GUS*) coated tungsten particles. The DNA shot on target tissue placed in the centre of target plate at 9 and 11 cm distance with 900 and 1100psi pressure, respectively.

Selective Regeneration of Putative Transgenics

The bombarded cotyledons were shifted from osmoticum medium after 16 hours and allowed to regenerate on MS supplemented with 2.5 mgl^{-1}BAP and 1 mgl^{-1} Kin, where the explants were simultaneously subjected to different selection pressure of hygromycin (10-25 ppm) for two cycles of 15 day each, so that only bombarded cells grow into buds. The selected buds were again cultured on half strength MS supplemented with 0.3 mg BAP with lower selection pressure of hygromycin. Here the selected buds elongated, rooted and acclimatized to get putative transgenic plants.

Molecular Analysis

Histochemical *GUS* Assay was used to check the accuracy of the bombardments. The bombarded cotyledons were analyzed for the presence of *GUS* spots five days after the experiment. Also selected and elongated putative shoots were examined with this assay. Its expression was analyzed with histochemical *GUS* solution (X-gluc: 10 mg, Sodium phosphate (50 mM) : 500 µl, Triton X-100 (10%):50 µl, K4Fe(CN)6 (10 mM) : 100 µl, K3Fe(CN)6 (10 mM): 100 µl, DMSO: 200 µl, Water : 9 ml) as described by Jefferson *et al.* (1987). Rooted *GUS* positive plants and un-transformed plants as control were analyzed for the presence of *Cry*1Ac gene using PCR analysis. The genomic DNA was isolated from leaf tissue of hygromycin resistant plants using mini prep DNA extraction (1M Tris buffer pH 8, 0.5M EDTA pH 8, 5M NaCl, 10% SDS) method for the analysis. PCR analysis was carried out for the amplification of *Cry*1Ac gene using specific forward primer '5-TGG AGA ACG CAT TGA AAC CG-3' and reverse primer '5-TGT TGC TGA ATC CGG AAC GG -3'. The analysis was carried out in reaction volume of 25µl containing the template genomic DNA (100ng), Forward primer and reverse primer (70ng each) dNTPs (0.1mM), 1X PCR buffer (50mM KCl,1.5mM MgCl2, 10mM Tris, pH 9) and Taq polymerase (1unit). *In vitro* amplification using polymerase chain reaction was performed in a PTC-100 thermal cycler (MJ Research, MA). Amplification was performed at temperature profile: initial denaturation at 94°C for 4 minute, second denaturation at 94°C for 2 minute, annealing at 55°C for 1 min and extension at 72°C for 2.5 min and whole process of second denaturation to extension was repeated 35 times. Final cycle was carried out at 72°C for 7min. PCR amplification products were resolved by running 14 µl aliquot from each reaction through electrophoresis in 0.8% Agarose gel.

Statistical Analysis

At least three repeats were maintained for each treatment and data was recorded. Statistical analysis was done in CRD (factorial) design using $CPCS^{-1}$

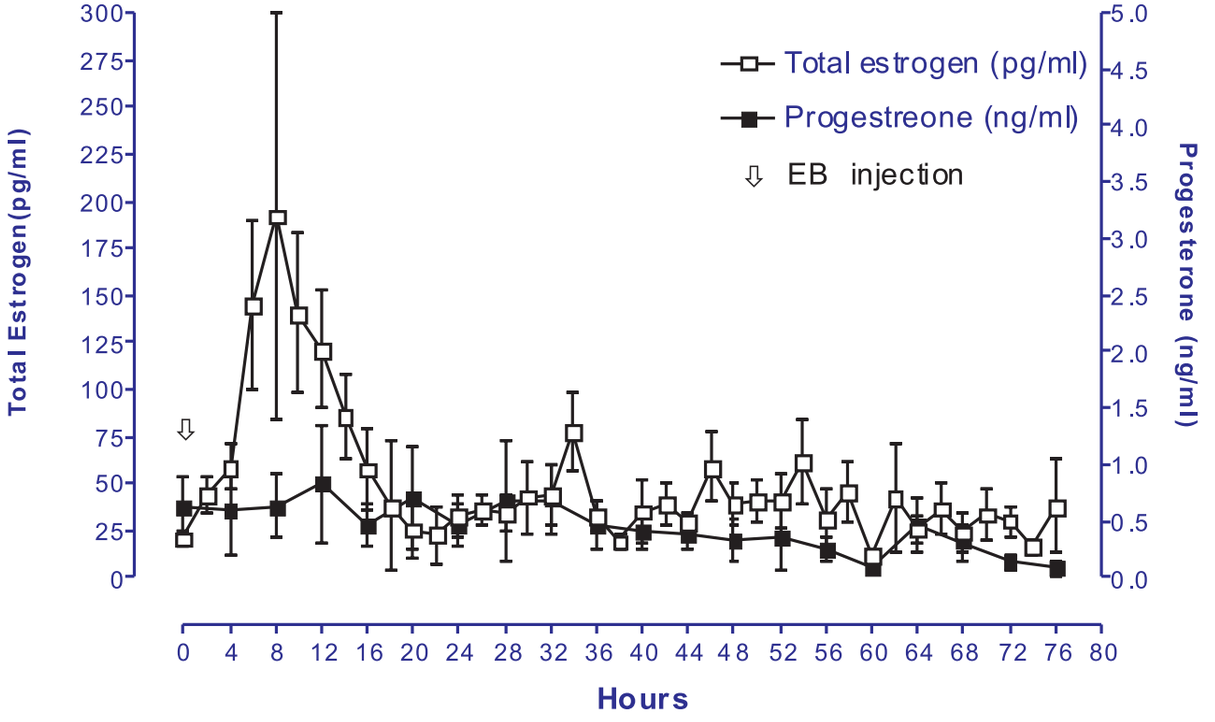

Plate 1: Regeneration in eggplant: (A) Stereoscopic view of regenerating cotyledon (B) Elongated plantlets, (C) Rooted plantlets (D) Hardening (E) Establishment of plants (F) Hygromycin sensitivity Assay

software package developed at Punjab Agricultural University by Cheema and Singh (1990). Least square differences (LSD) at 5% level of significance were calculated and interpreted.

Results and Discussion

Plant Regeneration

Among different explants, cotyledon showed highest regeneration and number of bud per explant on all media combinations. However, highest regeneration potential was observed on MS fortified with 3.0 mgl^{-1} BAP (80.36%), but number of buds per explant were highest (21.76) on MS media supplemented with 2.5 mgl^{-1} BAP+ 1.0 mgl^{-1} Kin (Figure 1). The potential of leaves and hypocotyl was significantly less on all media combinations for regeneration and number of buds per explant. This may be due to difference in requirement for exogenous auxin and cytokinin in the process of bud differentiation, which varies with the tissue system and apparently depends upon endogenous level of these hormones in the tissue (Razdan, 2000). Therefore, optimum ratio of cytokinin to auxins is required for bud formation and shoot regeneration (Taha and Tizan, 2002; Slater *et al.*, 2003; Sarker *et al*., 2006; Gisbert *et al.*, 2006; Kaur *et al*., 2011). The buds regenerated on best media combination were elongated, rooted and developed into plants for fruiting (Plate 1A to E).

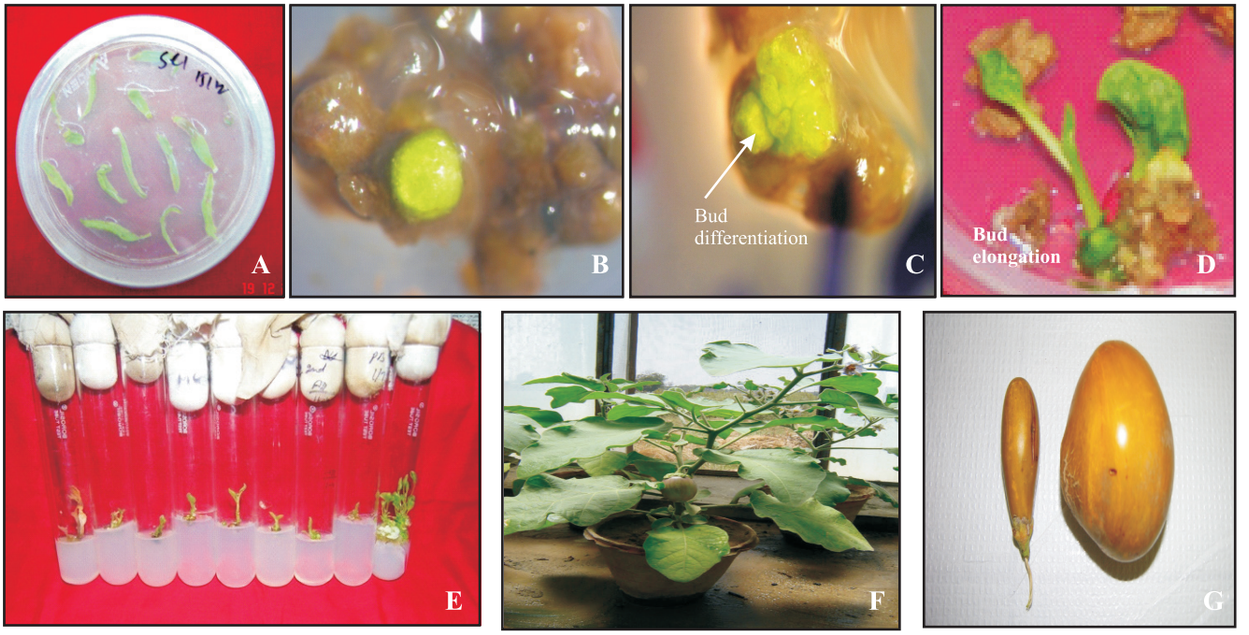

Plate 2 : Biolistic transformation in eggplant: A. First selection (15ppm), B. Bud formation, C. Bud differentiation, D. Elongation (10ppm), E. Selected plantlets with -ve and +ve controls, F. Fruit bearing plant, G. Control and transferred fruit

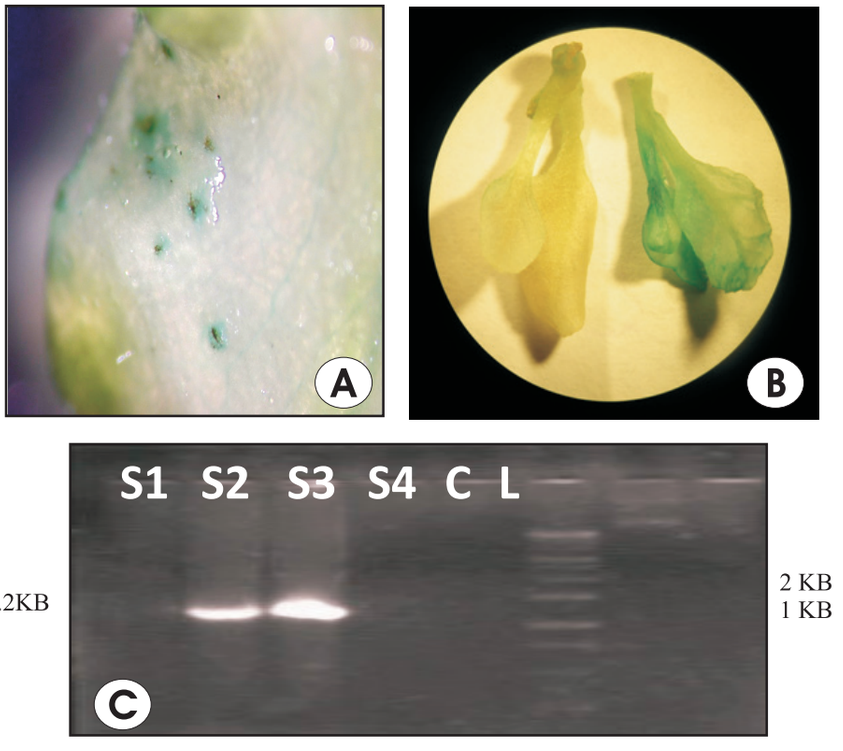

Plate 3: Analysis of Putative Transgenic Plants: (A). GUS spots on target cotyledon (B). GUS +ve plantlet with control (C). confirmation of Cry1Ac gene in positive plants.

Hygromycin Sensitivity Assay

There was no regeneration of cotyledons at a selection pressure of 15ppm to 25ppm hygromycin, even, at conc. of 10ppm 6.90% buds were produced, which turned pale afterwards and did not elongated further. However, at 5ppm regeneration was 54.62% compared with 87.14% in control (Figure 2). It was observed that under selection pressure of hygromycin a small part of the explant was able to regenerate, whereas in control whole explant turned into regenerating buds after expanding (Plate 1F). There were nodular structures on the cotyledons under the selection pressure of hygromycin. When these were sub-cultured on elongation medium, shoots were emerged from these structures and grown into the plantlets. The high selection pressure on elongating buds resulted in wilting symptoms within a week and turned brown afterwards. Regeneration differences of different eggplant genotypes to hygromycine (7.5 to 10 mgl^{-1}) selection pressure was observed by Picoli *et al.,* (2000). Selection pressure of 15 ppm Hygromycin that checkss the regeneration of untransformed tissue can further be used for selective regeneration of bombarded tissue.

Biolistic Transformation

The mixed DNA (1µgµl-1) of Cry1AC with selectable marker (hpt) and GUS reporter gene was coated on tungsten particles and bombarded onto the cotyledon explants of genotype BL-5 (Plate 2). Among the rupture disks (900 and 1100-psi) and target distances (9cm ad 11cm) investigated (Table 1), 900-psi rupture disk at 9.0 cm distance showed best results for plant regeneration, whereas, 1100-psi rupture disk caused acute injury to the cotyledonary explants and, reduce their regeneration ability. Moreover, holes in the cotyledons indicated that particles has been passed through and not embedded in the tissues. This can be a reason for less regeneration of the explants on the selective medium. The particle bombardments using variable parameters have been detailed by Heiser, (1995). In broccoli, consistently high rate of transformation was obtained using M17 Tungsten particles fired at 900 psi with a gap distance of 20 mm and target distance of 55 mm (Puddephat *et al.,* 1999).

Selective Regeneration of Putative Transgenics

The selection efficiency at each cycle was conducted in three sets using different concentrations of hygromycin, where, concentration of hygromycin was reduced at each succeeding cycle (Table 2). In Set-A, bombarded cotyledons were subjected to 25ppm hygromycin and selection efficacy was 8.62% of explants having one or two nodules like structures only. These nodules did not elongate into shoot upon shifting to elongation medium (1/2 MS + 0.3 mgl^{-1}BAP+double agar +20 ppm Hygromycin), but converted into brown callus. In Set-B, the initial selection pressure was reduced to 20 ppm, where selection efficacy was 19.56% and formed the nodule like structures. These nodules like structures viz. direct somatic embryos, when transferred to the elongation-cum-selection medium (15 ppm), started differentiating into shoots (66.66%). The further

Table 1. Standardization of target distance for particle bombardments on cotyledon explants of eggplant

Rupture disk (psi)	Target distance (cm)	Total number of cotyledons bombarded	Cotyledons showing severe injury	Cotyledons regenerated at 15ppm hygromycin	Selection efficiency	Remarks (%)
1100	9	18	5	3	16.66	Presence of holes on the explant
	11	15	2	5	33.33	Presence of holes on the explant
900	9	20	0	7	35.00	Reduced injury level
	11	17	0	5	29.41	Reduced injury level

Table 2. Selection efficiency of transformed in 3 selection cycles (after bombardment) at various concentrations of hygromycin in eggplant

Selection Cycles	Number of explants / plant	Number of selected explants/plants	Selection efficiency at each cylce (%)	Overall Selection efficiency (%)	Remarks
Set A					
1st at 25ppm hygromycin in regeneration medium	58	5	8.62	0.00	Buds formed after selection did not elongate and got converted into callus
2nd at 20ppm hygromycin in elongation medium	7	0	0		
3rd at 15ppm on elongation medium	-	-	-		
Set B					
1st at 20ppm hygromycin in regeneration medium	138	27	19.56	7.97	Buds formed after selection elongated slowly and developed into small shoots
2nd at 15ppm hygromycin in elongation medium	27	18	66.66		
3rd at 10ppm on elongation medium	18	11	61.11		
Set C					
1st at 15ppm hygromycin in regeneration medium	147	55	34.01	17.68	Buds formed after selection elongated slowly and developed into small shoots
2nd at 10ppm hygromycin in elongation medium	55	39	70.90		
3rd at 5ppm hygromycin in elongation medium	39	26	66.66		

Table 3. GUS expression (%) in cotyledon explants and elongating shoots in eggplant after transformation

Treatment tissue	Total number of cotyledons/ shoots	GUS response in cotyledon /shoots	Number of spots on cotyledon	Chimeric GUS expression in shoots
Cotyledon	13	10(76.92%)	2-7 spots	--
Elongating shoot	21	14(66.66%)	--	5(23.80%)

reduction in selection pressure in 3rd cycle at 10ppm lowered the selection efficiency to 61.11%. In Set-C, initial selection pressure was reduced to 15ppm and 34.01% explants selected on regeneration medium. Upon transferring to 10ppm selection pressure, regenerating buds developed into 70.90% little shoots, whereas in third cycle with 5 ppm Hygromycin 66.66% shoots were selected (Plate 2A to C). Similarly, hygromycin resistant plants from nearly 1.6% of the bombarded cotyledons were recovered on regeneration medium containing 10-25 mgl^{-1} hygromycin (Deng *et al.,* 2001). The overall selection efficiency was highest in Set-C with initial selection pressure of 15 ppm (17.68%) followed by

Set-B with 20 ppm (7.97%); however, in Set A with 25 ppm hygromycin no plantlet was recovered. The selected buds elongated under hygromycin selection pressure (Plate 2D and E) and developed into plants (Plate 2F) having misshaped fruits (Plate 2G).

Molecular Analysis

The expression of the *GUS* gene was visualized 5 days after bombardment of the cotyledonary explants. Total 13 explants were subjected to this assay. *GUS* gene was expressed in 10 explants (76.92%). The number of *GUS* spots varied from 2 to 7 per explant (Plate 3A). This assay confirmed the presence of transformed tissue that can be regenerated into putative transgenic plant. The assay was also performed after one and half month of bombardment on the elongating shoots (Plate 3B). Out of 21 shoots, *GUS* gene was visualized in total 14 shoots (66.66%). However, Chimeric *GUS* expression was observed in 5 shoots (23.80%) as shown in Table 3. The seed from mature transgenic fruits were again checked for GUS expression and seedling were selected through selective marker. There are reports, where histochemical *GUS* assay using *GUS* enzyme activity confirmed the presence of the *GUS* gene (Franklin and Sita, 2003; Deng *et al.,* 2001; Soniya *et al.,* 2005). PCR analysis of putative transgenic eggplant plants with the specific primers confirmed the presence of *Cry1*Ac gene with 1.2 KB product size (falling between 1KB and 2KB bands of 10KB DNA ladder) in two of the four plants (Plate 3C). These PCR positive plants were grown, selfed and seed was extracted from mature fruits to raise next homozygous generation. PCR analysis used for confirmation of putative transgenic has also been used by Deng *et al.,* (2001); Sawahel *et al.*,(2002) ; Soniya *et al.,* (2005); Kumar *et al.,* (1998a); Donzella *et al.* (2000); Frijters *et al.,* (2000); Goggin *et al.,* (2006) and Prabhavathi *et al.,* (2002).

The biolistic method of genetic transformation of biological material has been proved a valuable technique for delivering DNA into cells of plants (Klein *et al.,* 1988). This is applicable to a wide range of plant genotypes, especially those in which embryogenic cell cultures are available (Kikkert *et al.,* 2005). This has already been used in other vegetables like broccoli (Puddephat *et al.,* 1999), garlic (Sawahel, 2002) and potato (Craig *et al.,* 2005).

Conclusion

This can be concluded that cotyledon explant of eggplant is successfully transformed with *Cry 1AC* gene using biolistic gun method. PCR analysis confirmed putative transgenic plants. Upon selfing, homozygous plants can further be used for protein estimation of transferred gene followed by field trials against target insect.

References

Baksh S and M. Iqbal. 1979. Compatibility relationship in some of tuberous species of *Solanum*. *Journal of Horticultural Sciences* **54**: 163.

Cheema H S and B. Singh. 1990. A user's manual to CPCS-1. A Computer Programme Package for the Analysis of Commonly used Experimental Designs, pp-1, PAU, Ludhiana.

Craig W, C. Gargano, N. Scotti, T.T. Nguyen, N.T. Lao, T.A. Kavanagh, P.G. Dix and T. Cardi. 2005. Direct gene transfer in potato: a comparison of particle bombardment of leaf explants and PEG-mediated transformation of protoplasts. *Plant Cell Reports* **24:** 603-611.

Deng X.Y., Z.M. Wei and H.L. An. 2001. Transgenic peanut plants obtained by particle bombardment via somatic embryogenesis regeneration system. *Cell Research* **11:**156-160.

Dobariya K L and J.R. Kachhadiya. 2004. Role of genotype, explant, and culture medium on *in vitro* morphogenesis in brinjal (*Solanum melongena* L.). *The Orissa Journal of Horticulture* **32:** 52-54.

Donzella G., A. Spena and G.L. Rotino. 2000. Transgenic parthenocarpic eggplants: superior germplasm for increased winter production. *Molecular Breeding* **6:** 79-86.

Franklin G. and G.L. Sita. 2003. *Agrobacterium*-mediated transformation of eggplant (*Solanum melongena* L.) using root explants. *Plant Cell Reports* **21:** 549-554.

Frijters A., G. Simons, G. Varga, N. Quaedvlieg and M. de Both. 2000. The *Mi-1* gene confers resistance to the root knot nematode *Meloidogyne incognita* in transgenic eggplant. Plant and Animal genome VIII conference. Town and Country Hotel, San Diego, CA, January 9-12.

Gisbert C., J. Prothens and F. Nunez 2006. Efficient regeneration in two potential new crops for subtropical climates, the scarlet (*Solanum aethiopicum*) and gboma (*S.*

macrocarpon) eggplants. *New Zealand Journal of Crop and Horticultural Sciences* **34:** 55–62.

Goggin F.L., L. Jia, G. Shah, S. Hebert, V.M. Williamson and D.E. Ullman. 2006. Heterologous expression of the *Mi-1.2* gene from tomato confers resistance against nematodes but not aphids in eggplant. *Molecular Plant-Microbe Interactions* **19:** 383-388.

Heiser W. 1995. Optimization of biolistic transformation using the helium driven PDS-1000/He system. *US/ES Bulletin* **1688:** 1-8.

Jefferson R.A., T.A. Kavanagh and M.V. Bevan. 1987. GUS fusions: *b*-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO Journal* **6:** 3901-3907.

Kaur M, A.S. Dhatt, J.S. Sandhu and S.S. Gossal (2011) *In vitro* plant regeneration in brinjal from cultured seedling ex plant. Indian Journal of Horticulture **68(1):** 61-65.

Kaur S., S.S. Bal, G. Singh, A.S. Sidhu and T.S. Dhillon. 2004. Management of brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee through net house cultivation. *Acta Horticulturae (ISHS)* **659:** 345-350.

Kikkert J.R., J.R. Vidal and B.I. Reisch. 2005. Stable transformation of plant cells by particle bombardment/ biolistics. *Methods of Molecular Biology* **286:** 61-78.

Kumar P.A., Mandaokar A.D. and R.P. Sharma. 1998b. Genetic engineering for improvement of eggplant (*Solanum melongena* L.). *Agricultural Biotechnology News Information* **10:** 329-332.

Kumar P.A., A. Mandaokar, K. Sreenivaasu, S.K.Chakarbarti, S. Bisaria, S.R. Sharma, S. Kaur and R.P. Sharma. 1998a. Insect resistant transgenic brinjal plants. *Molecular Breeding* **4:** 33-37.

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

Picoli E.A.T., W.C. Otoni, P.R. Cecon and M. Fari. 2000. Influence of antibiotics on NAA induced somatic embryogenesis in eggplant (*Solanum melongena* L. cv. Embu). *International Journal of Horticultural Sciences* **6:** 88-95.

Prabhavathi V., J.S. Yadav, P.A. Kumar and M.V. Rajam. 2002. Abiotic stress tolerance in transgenic eggplant (*Solanum melogena* L.) by introduction of *bacterial mannitol phosphodehydrogenase* gene. *Molecular Breeding* **9:** 137-147.

Puddephat I.J., N. Thompson, H.T. Robinson, P. Sandhu, J. Henderson 1999. Biolistic transformation of broccoli (*Brassica oleracea* var *italica*) for transient expression of the *β*-glucuronidase gene. *Journal of Horticultural Science and Biotechnology* **74:** 714-720.

Razdan M.K. 2000. An Introduction to Plant Tissue Culture. Pp 81-87. Oxford and IBH Publishing Co Pvt Ltd., New Delhi.

Sarker R.H., Y. Sabina and M.I. Hoque. 2006. Multiple shoot formation in eggplant (*Solanum melongena* L.). *Plant Tissue Culture and Biotechnology* **16:** 53-61.

Sawahel W.A. 2002. Stable genetic transformation of garlic plants using particle bombardment. *Cell Molecular Biology Letter* **7:** 49-59.

Singh S.V., K.S. Singh and Y.P. Malik. 2000. Seasonal abundance and economic losses of shoot and fruit borer (*Leucinodes orbonalis*) on brinjal. *Indian Journal of Entomology* **52:** 247-252.

Slater A., N. Scott and M. Fowler. 2003. Plant Biotechnology: The Genetic Manipulation of Plants. pp 42. Oxford University Press Inc, New York.

Soniya E.V., P. Kannapiran, S.C. Unni, J. Nisha and E.K. Radhakrishnan. 2005. Transformation and regeneration of *Solanum melongena* using *Agrobacterium tumefaciens*. *Journal of Plant Biology* **32:** 29-32.

Taha R.M. and M. Tijan. 2002. An *in vitro* production and field transfer protocol for *Solanum melongena* L. plants. *South African Journal of Botany* **68:** 447-450.