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Transformation of Tomato with *cry2AX1* gene of *Bacillus thuringiensis*

R. Bartakke Ruturaj[1†], A. Naveenkumar[1†], P. Nandeesha[2], R. Manikandan[3], N. Balakrishnan[4], V. Balasubramani[5], D. Sudhakar[6] and V. Udayasuriyan[7*]

ABSTRACT: *Tomato (Solanum lycopersicum L.,) is an important vegetable crop extensively damaged by lepidopteran insect pest, Helicoverpa armigera Hubner (tomato fruit borer). In order to mitigate this problem, an attempt was made to generate transgenic tomato plants resistant to fruit borer. In this study, a regeneration protocol was standardized for a local high yielding genotype, cv. PKM1. Out of nine different combinations of media tested, modified MS medium containing B5 vitamins along with zeatin exhibited higher regeneration efficiency. Cotyledonary explants of cv. PKM1 were transformed with an indigenous synthetic cry2AX1 gene encoding insecticidal crystal protein of Bacillus thuringiensis through Agrobacterium mediated transformation. Screening by PCR revealed presence of cry2AX1 gene in five out of nine putative transformants; two of the five PCR positive transformants showed expression of Cry2AX1 protein in qualitative ELISA. The two ELISA positive plants showed low level of mortality in H. armigera and significant reduction in feeding of leaf area, in detached leaf bioassay. Selection of cry2AX1 transgenic tomato plants by quantitative ELISA for high level of gene expression will be useful for getting plants with desirable level of insect protection.*

Key words: *cry2AX1, regeneration, Helicoverpa armigera, tomato transformation*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the major vegetables which has achieved tremendous popularity over the last century. It is grown worldwide either in the field, greenhouses or net houses. It is very versatile and is grown either for fresh fruits or for processing. At present, India contributes 11 per cent of world tomato production and it is grown in an area of 8,70,000 hectare with productivity of 18.24 MT/hectare in 2012 (FAO Statistical Database, 2014). Tomato is severely damaged by lepidopteran insect *Helicoverpa armigera* Hubner (fruit borer). Farmers apply numerous insecticidal sprays to manage the pest as it has developed resistance to insecticides (Anonymous, 1990; Kranthi *et al.*, 2000). In the recent past, tomato grower suffers heavy losses because of the very high incidence of *H. armigera*. Relying generally on chemical insecticides may not be viable as they provide ephemeral benefits, often with adverse side effects, and in some instances, actually worsen farmers' overall pest problems (Sharma and Ortiz, 2002). Thus, the major challenge is how to increase and sustain crop productivity with less use of insecticides. Evolving insect resistant crop varieties is one of the major breeding objectives of crop plants including tomato. Introduction of genes from wild relatives for resistance to various disease and pest in commercial cultivars of *S. lycopersicum* by conventional breeding techniques often meets serious difficulties due to high incompatibility barriers to hybridization (Kaul, 1991). Genetic engineering techniques may play a major role in the development of insect/disease resistant cultivars and they are being used in tomato varietal improvement programmes to act against these challenges.

Bacillus thuringiensis (Bt) is a spore-forming Gram-positive bacterium. During sporulation, the intracellular insecticidal crystal proteins (Cry proteins) are produced as inclusions. These proteins are toxic to lepidopteran, dipteran, and coleopteran insect larvae (Kuo and Chak, 1996). The Cry protein from Bt has been developed as one of the most

[1,3,4,5,6,7]Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641003, India.

[2] Present address: Indian Institute of Horticultural Research, Bengaluru 560089, India.

* *corresponding author: udayvar@yahoo.com*

[1†] Authors contributed equally to this work

successful biological agents in industry to control insect pests. As a good alternative to synthetic insecticides, these formulations are safe to the user and environment. Transgenic plants expressing Bt genes will be more effective on insect pests than Bt formulations (Chen *et al.,* 2005). The Bt gene *cry2A* produces insecticidal Cry protein that is active against both lepidopteran and dipteran insects. Besides it also has unique binding sites in the midgut of targeting insects as compared to other Cry toxins (Lee *et al.,* 1997). The difference in the structural and insecticidal mechanism of Cry2A protein leads it as a beneficial resource for insect-resistant transgenic plants (Morse *et al.,* 2001). In the present investigation, an attempt was made to standardize the regeneration protocol for a regional tomato variety PKM1. The tomato cv. PKM1 was considered to be a high yielding cultivar among farmers of Tamil Nadu, India. Hence, *Agrobacterium* mediated transformation of tomato cv.PKM1 with a novel synthetic *cry2AX1* gene (Accession No. GQ332539.1) consisting of sequences from *cry2Aa* and *cry2Ac* (Udayasuriyan *et al.,* 2010) was attempted to impart insect resistance to *H. armigera.*

MATERIALS AND METHODS

Plant Material and Culture Condition

Cotyledonary explants from 8-10 day old *in-vitro* germinated seedlings were used for transformation with novel synthetic *cry2AX1* gene. Breeder seeds of tomato cv.PKM1 were obtained from Horticultural College and Research Institute, Periyakulam, Tamil Nadu. Tomato seeds were washed thrice with sterile distilled water followed by a wash with 70 per cent ethanol for five minutes. Further the seeds were washed twice with sterile distilled water and surface sterilized with 4 per cent sodium hypochlorite solution containing two drops of Tween 20 for seven minutes. The seeds were rinsed with sterile distilled water for three times followed by air drying on sterile tissue paper. The sterilized seeds were germinated on half strength MS medium (Murashige and Skoog, 1962) under 48 hours dark period followed by 8/16 dark light cycle. A tissue culture room or growth chamber maintained at 25±1°C was used for seed germination and maintenance of pre-culture, co-cultivation and plant regeneration. From the ten day old seedlings, cotyledons were cut at the tip and base (to a size of approximately 0.5 cm^2) and placed on pre-culture medium (adaxial surface in contact with the medium) for one day. Explants were handled gently with sterile flat forceps to avoid any injury to them.

Regeneration Medium, Hormones and Antibiotics

MS medium (Murashige and Skoog, 1962) and modified MS medium containing B5 vitamins (MS-B5) (Gamborg *et al.,* 1968) with different combinations of growth regulators *viz.,* zeatin, BAP, NAA were evaluated for regeneration of tomato (Table 1). Dehydrated powder of MS and modified MS medium were obtained from Himedia, Mumbai. The growth regulators *viz.,* BAP (Duchefa) and zeatin (Duchefa) were initially dissolved in 1M sodium hydroxide (NaOH) and dimethyl sulfoxide, respectively, were further made up with sterile distilled water, whereas NAA (Sigma) and IBA (SD Fine chemicals) were prepared in sterile distilled water with the concentration of 1mg/ml and filter sterilized. Stocks of antibiotics, kanamycin 100 mg/ml (Duchefa) and cefotaxim 250 mg/ml (Omnatax) were prepared in sterile distilled water and filter sterilized, whereas rifampicin 20 mg/ml (Himedia) was dissolved in methanol. Media components were adjusted to pH 5.8 using 1M NaOH prior to addition of agar (Himedia Cat No. PCT0901) suitable for plant tissue culture 0.8% w/v and the contents were autoclaved after addition of agar for 20 min at 121°C. All the hormones and antibiotics were added to medium after it cooled to bearable warm temperature. The half strength MS medium supplemented with IBA 0.2-1.0 mg/l was studied for the root induction.

Table 1
Regeneration of Cotyledonary Explants of Tomato PKM1 on Various Hormonal Combinations

Treatments	*Shoot regeneration (%)*
MS basal	0.0 (0.85) [g]
MS + 2 mg/l BAP + 0.2 mg/l NAA	8.00 (16.43) [f]
MS + 2.5 mg/l BAP + 0.2 mg/l NAA	12.00 (20.26) [e]
MS + 1.0mg/l zeatin	12.00 (20.26) [e]
MS + 1.5 mg/l zeatin + 0.2 mg/l NAA	16.00 (23.57) [d]
MS + 2.0 mg/l zeatin + 0.2 mg/l NAA	16.00 (23.57) [d]
MS + 2.5 mg/l zeatin + 0.2 mg/l NAA	20.00 (26.56) [c]
MS - B5 + 1.0 mg/l zeatin	88.00 (69.75) [a]
MS - B5 + 1 mg/l zeatin + 0.2 mg/l NAA	64.00 (53.13) [b]
SEd : 0.54 CD (P = 0.05) :1.14	

The values in parentheses are arc sine transformed values.
Means followed by a common letter are not significantly different at 5 % level by DMRT.

Construction of Plant Transformation Vector

The binary vector p23TPX *(pCAMBIA2300-EnCaMV35S-ctp-cry2AX1-Tnos)* was constructed (Fig 1). The binary vector p23TPX harbors the neomycin phosphotransferase (*nptII*) gene driven by

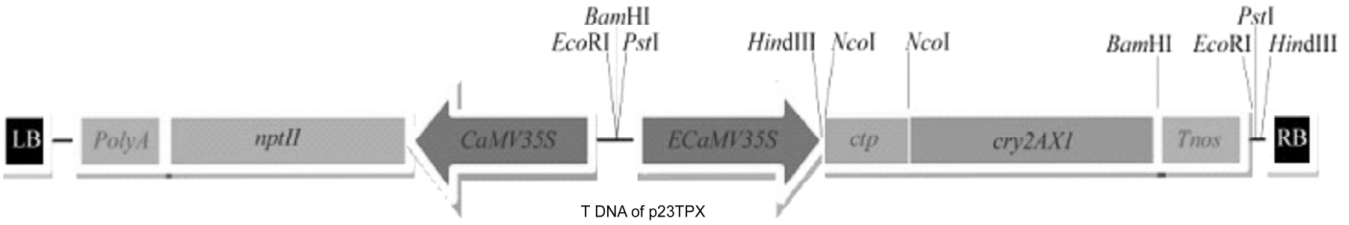

Figure 1: Schematic representation of T-DNA region of the plant transformation construct

LB: left border, poly A: CaMV35S terminator, *npt*II: neomycin phosphotransferase gene, CaMV35S: Cauliflower mosaic virus *35S* promoter, ECaMV35S: Enhanced Cauliflower mosaic virus *35S* promoter, *ctp*: chloroplast transit peptide, *cry2AX1:* gene of interest, *Tnos*: nopaline synthase terminator, RB: right border

CaMV35S promoter and terminator, which confers resistance to kanamycin used for plant selection. The chloroplast transit peptide (CTP) sequence from cotton *rbcS1b* was N-terminally fused in frame with plant codon optimized synthetic *cry2AX1* coding sequence. The p23TPX containing *ctp-cry2AX1* fusion gene driven by EnCaMV35S promoter and *nos-polyA* was mobilized into *Agrobacterium* strain LBA4404. *Agrobacterium* strain harboring the binary vector p23TPX was used for transformation of tomato, they were grown on YEP [1% Yeast extract, 1% Peptone and 0.5% NaCl pH 7] media containing kanamycin 50 µg/ml and rifampicin 20 µg/ml in a 28°C incubator shaker at 200 rpm for 48-72 hours.

Agrobacterium Mediated Transformation of Tomato

Initially to identify the lethal concentration of kanamycin for effective selection of transgenic tomato plants, sensitivity test was carried out using 30, 40, 50, 60 and 70 mg/l concentrations of kanamycin on shoot regeneration medium. The *Agrobacterium* strain harboring plant expression constructs grown on YEP medium at 28°C shaker and 200 rpm were harvested by centrifugation at 6,000 rpm for 10 min. The pellet was washed with 10 ml of MS broth and suspended in MS broth containing 100 mM acetosyringone. Density of the *Agrobacterium* in the suspension was maintained at 0.1OD at 600 nm and used for co-cultivation. The pre cultured explants were carefully submerged in the suspension (30 ml of co-cultivation suspension) in a sterile petri plate for 15 minutes. After the exposure, explants were blotted on sterile tissue paper and transferred to co-cultivation medium. Plates were kept under dark in a growth chamber for 48 hours. After the co-cultivation period, the explants were washed with a washing medium and blot dried. Later the co-cultivated explants were transferred to shoot regeneration medium containing kanamycin for the selection and maintained under 8/16 dark light cycle. The explants that responded well were sub cultured on to the fresh shoot regeneration medium for three times at every 15 days interval. The well-developed shoots were transferred to rooting medium. After profused rooting plants were hardened in a pot mixture containing sterile sand: soil: vermicompost in 1:1:1 proportion and covered with polythene cover for seven days in a culture room, and transferred to green house.

Molecular and Biochemical Analysis of Putative Transgenics

Genomic DNA was extracted from the leaves of putative transgenic tomato plants. Pinch of leaf tissue (~100 mg) was ground in a 1.5 ml microfuge tube containing 300 µl CTAB buffer (0.2 M EDTA (pH 8.0); 4.0 M NaCl; 1.0 M Tris base (pH 8.0); 2.0% CTAB) and incubated in a water bath at 65°C for 1 hour. Equal volume of chloroform: isoamyl alcohol (24:1 ratio) was added, mixed well and then centrifuged at 12000 rpm for 10 minutes. The aqueous phase was treated with RNase for 1 hour at 37°C. The DNA was precipitated from the aqueous phase with equal volume of isopropanol at -20°C for 1 hour. The DNA was pelleted by centrifugation at 12,000 rpm for 20 minutes and washed briefly with 70 per cent ethanol and air dried at room temperature. The pellet was dissolved in 50 µl of 0.1X TE buffer (1mM Tris-HCl pH 8.0 and 0.1 mM EDTA pH 8.0). Initially, a 25 µl PCR reaction mixture containing 100 ng of template, 1X Taq buffer (10 mM Tris- HCl; pH: 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 75 µM each of dNTPs, and 1.5 Units of *Taq* DNA polymerase along with 50ng each of forward 5'-ACCTGATGAAGATCCTCAC and reverse primer 5'-ATCCTCCAATCCAGACAC specific to actin gene content of cv. PKM1 was performed. Presence of transgene was also confirmed by amplifying the transgene sequence using the pairs of forward primer 5'-AGCTTAAACCATGGCCTCCT; reverse primer 5'-GAAGAAGCTGGTAACCCTGA specific for *ctp-cry2AX1* gene. Qualitative estimation of Cry2AX1

proteins in putative transgenic tomato plants was done using commercially available Cry2A kit (DesiGen, Jalna, India) by following the manufacturer's instructions.

Insect Bioassay

Detached leaf bioassay was carried out to determine the degree of insect resistance in ELISA positive T_0 transgenic tomato plants under laboratory condition with *H. armigera*. Leaves detached from T_0 transgenic and control tomato plants were placed in a wet filter paper lined petri plates and used for bioassay. Three neonate larvae of *H. armigera* were released on each leaf. Five replications were maintained for each treatment. The experiment was carried out at 27±1°C with about 65 per cent relative humidity. Larval mortality was recorded after 48 hours at 24 hours interval for seven days.

RESULTS

Effect of Growth Regulators on Regeneration of Tomato

Nine different media composition with plant growth regulators at varying concentrations had been evaluated for regeneration using cotyledons as explants from ten days old *in vitro* seedlings of cv. PKM1 tomato. Among the different combinations of growth hormones tested, explants cultured on the MS-B5 medium with zeatin 1 mg/l responded well, which showed higher regeneration efficiency (88%) followed by MS-B5 containing zeatin 1 mg/l with 0.2 mg/l NAA (64%), whereas MS medium containing different concentrations of zeatin along with 0.2 mg/l NAA recorded only 12-20 per cent of regeneration. In addition, MS containing BAP with 0.2 mg/l NAA showed 8-12 per cent of regeneration only (Table 1). Regeneration efficiency obtained in MS-B5 medium containing zeatin was higher than both MS medium containing zeatin as well as MS medium containing BAP, respectively. The half strength of MS supplemented with IBA 1.0 mg/l gave profuse rooting within 10 days as compared to other treatments (Table 2).

Regeneration and Selection of Transformants

The lethal dose of kanamycin for the cotyledonary explants was determined to check their intrinsic resistance. Various concentrations (30, 40, 50, 60, 70 mg/l) of kanamycin were tested. No morphogenic response was observed in all the tested concentrations of kanamycin and all the explants turned yellow to brown (Fig. 2). In consideration to various factors, a standardized regeneration protocol was developed with appropriate media composition and conditions (Table 3) to transform the local high yielding cv. PKM1 with the synthetic *cry2AX1* gene through *Agrobacterium* mediated protocol. The cotyledonary explants co-cultivated with *Agrobacterium* strain harboring p23TPX for 48 hours recorded 7.16 per cent regeneration efficiency on kanamycin selection (Fig 3). Regenerated putative transformants were analyzed for confirmation of transgene integration. Prior to the screening of transgene by PCR, actin gene was amplified in putative transformants to evaluate the suitability of DNA to screen for the transgene by PCR. Five out of nine putative transformants obtained from p23TPX showed the presence of transgene. The transformation efficiency under kanamycin selection was found to be 1.23 per cent. In order to determine the expression of Cry2AX1 protein in transgenic plants, qualitative ELISA was performed using

Table 2
Effect of Different Concentrations of IBA on Root Induction in Regenerated Shoots of Tomato

Treatment	*No. of shoots used*	*Rooting intiation*	
		After 10 days	*After 15 days*
½ MS	5	-	1
½ MS + 0.2 mg/l IBA	5	-	1
½ MS + 0.4 mg/l IBA	5	-	1
½ MS + 0.6 mg/l IBA	5	2	3
½ MS + 0.8 mg/l IBA	5	2	4
½ MS + 1.0 mg/l IBA	5	4	5

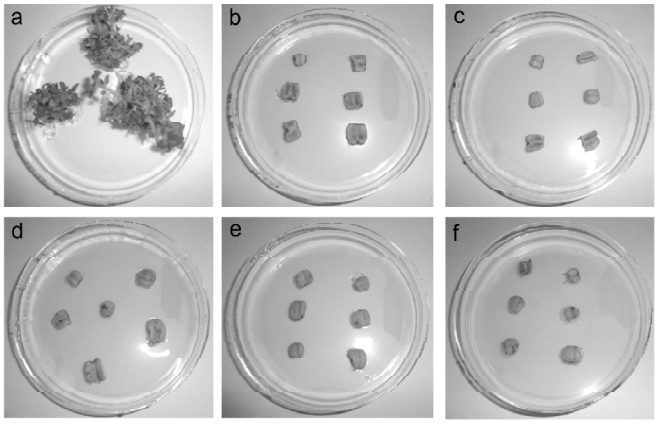

Figure 2: Kanamycin sensitivity assay for regeneration of tomato

Kanamycin sensitivity assay using the cotyledonary explants of PKM1, (a) Explants kept on the shoot regeneration medium without kanamycin, (b-f) Explants kept on shoot regeneration medium containing kanamycin at 30, 40, 50, 60 and 70 mg/l, respectively.

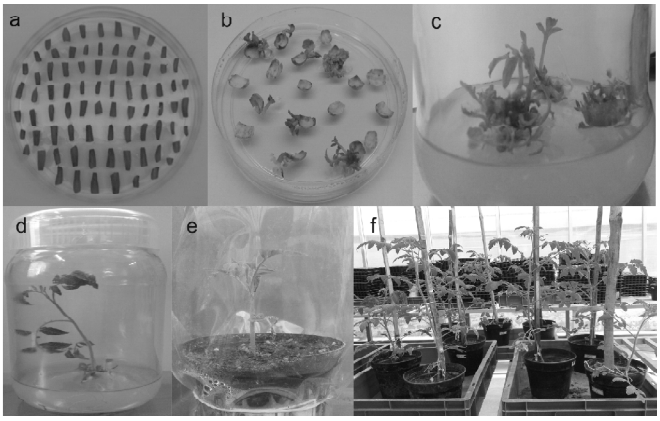

Figure 3: Different stages of *Agrobacterium* mediated transformation of tomato

(a) Cotyledonary explants of PKM1 on pre-culture medium (b) Co-cultivated explants on shoot regeneration medium (c) Explants showing regeneration of shoots (d) Elongated shoot on rooting medium (e) A hardened plant (f) Well established transformants at green house

Table 3
Standardized Conditions for *Agrobacterium* Mediated Transformation of Tomato cv. PKM1

Ingredients	*PCM*	*CCS*	*CCM*	*WM*	*SRM*	*RM*
MS-B5 medium	1x	1x	1x	1x	1x	0.5x
Sucrose (g/l)	30	30	30	30	30	15
Agar (tissue culture tested) %	0.8	0	0.8	0	0.8	0.8
zeatin (mg/l)	1	—	1	—	1	—
IBA (mg/l)	--	—	--	—	--	1.0
Kanamycin (mg/l)	--	—	--	—	50	30
Cefotaxim (mg/l)	-	—	--	250	250	250
Acetosyringone (µM)	--	100	100	—	--	—
Agrobacterium density	—	OD 600=0.1	—	—	—	—
Incubation time at 25±1°C	1 day	15 min	2 days (dark)	5 min	Interval of 15 days	20 days

PCM: Pre culture medium, CCS: Co-cultivation suspension, CCM: Co-cultivation medium, WM: Washing medium; SRM: Shoot regeneration medium, RM: Rooting medium

antibodies specific to Cry2A protein and the presence of Cry2AX1 was observed in two transgenic plants, TPX1 and TPX2 (Fig 4).

Toxicity Analysis of Transgenic Plants against Fruit Borer

Detached leaf bioassay was performed for the T_0 transformants of tomato along with a suitable *in vitro* regenerated control to assess the insecticidal activity towards *H. armigera*. Thirteen per cent larval mortality was recorded in *cry2AX1* transformants of tomato, whereas the control plants showed no mortality. Even though, the level of mortality was less, growth inhibition in surviving larvae and reduction in leaf area feeding were observed in both the ELISA positive plants (Fig 5). Thus it can be suggested that the level of expression of Cry2AX1 protein in tomato transformants generated in the present study may not be sufficient for causing higher mortality in *H. armigera.* Hence there is necessity to generate and screen large number of transformants to identify an effective event.

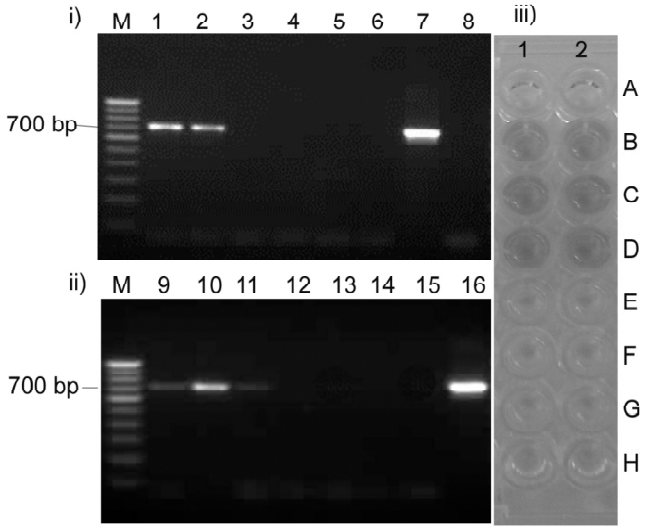

Figure 4: Screening of putative transformants of tomato by PCR (i&ii) and ELISA (iii)

(i, ii) Screening of putative transformants of tomato by PCR. Lane M: 100bp ladder, Lane 1-5 & Lane 9-12: Putative transformants of tomato TPX1-9 respectively, Lane 6, 13, 14: Non-transformed control plants, Lane 7,16: Plasmid control, Lane 8,15: Water control. (iii) Screening of PCR positive plants by Cry2A Qualitative ELISA. Lane 1-2 are the replication of each other, A: Kit Negative, B: Positive control, C: TPX1, D: TPX2, E: TPX6, F: TPX7, G: TPX8, H: Control Plant

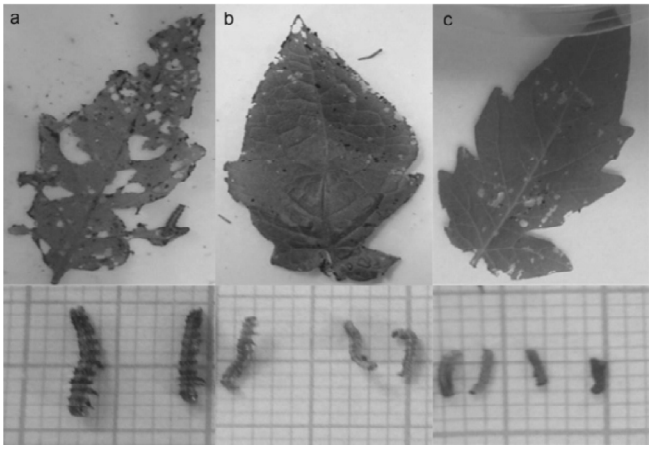

Figure 5: Detached leaf bioassay for transformants of tomato against *H. armigera*

(a) Non- transformed control plant and its survivors, (b, c) Transformants of tomato TPX1 and TPX2 and their respective survivors.

DISCUSSION

A wide range of plant growth regulators at varying concentrations had been used along with different explants from different cultivars of tomato in various studies for regeneration and the choice of the explants was found to be genotype dependent (Park *et al.,* 2003; Bhatia and Ashwath, 2008; Sarker *et al.,* 2009; Mamidala *et al.,* 2011). Standardization of regeneration protocol for tomato is still essential as different tomato cultivars vary in their response to specific treatment. It is reported earlier that cotyledonary explants showed good regeneration in tomato (Fillati, 1987; Ellul, 2003). Velcheva *et al.* (2005) observed higher regeneration (54.1%) in cotyledons of the tomato cv. Daniela 144 when compared to hypocotyls (7 %). In the present study cotyledons from ten days old *in vitro* seedlings of tomato cv. PKM1 were used as explants for evaluating various combination of regeneration medium supplemented with different concentration of auxin (NAA) and cytokinins (zeatin, BAP). Though earlier workers (Hu and Phillips, 2001; Sarker *et al.,* 2009) reported the successful regeneration in MS medium supplemented with different hormonal combinations, this study reports the regeneration efficiency obtained in MS-B5 medium with zeatin 1 mg/l was higher than MS medium with zeatin 1 mg/l and this may be due to presence of B5 vitamins in the former. In this connection, Kaur and Bansal (2010) also observed optimum regeneration frequency using MS-B5 medium supplemented with zeatin 0.5 mg/l and IAA 0.5 mg/l, however number of shoots produced per explant was found to be highest when zeatin 1 mg/l alone was used. Hormones are not essential for rooting as regenerated tomato contains endogenous phytohormones (Mensuali–Sodi *et al.,* 1995). However, Gunay and Rao (1980) and Oktem *et al.* (1999) reported that profused rooting could be induced by using IAA at 0.5 mg/l and 0.2 mg/l, respectively. Loc *et al.* (2011) used IBA 0.5 mg/l for induction of rooting in transgenic tomato plant. The half strength of MS supplemented with IBA 1.0 mg/l gave profuse rooting within 10days.

Kanamycin, a widely used selection agent for plant transformation tends to inhibit regeneration strongly even at low doses (Yepes-Martinez and Aldwinckle, 1994). The lethal dose of kanamycin for the cotyledonary explants was determined to check their intrinsic resistance. All five different doses of kanamycin tested between 30 and 70 mg/l showed yellowish and eventually brown explants without any regeneration. Hu and Phillips (2001) had reported regeneration was inhibited with increasing kanamycin dose and high level regeneration efficiency at kanamycin 50 mg/l. In this study also kanamycin 50 mg/l was used to select the transformants.

The transformation efficiency of tomato depends upon many factors such as the cultivar, type of explant and its age, pre culture time, density of *Agrobacterium* suspension, co-cultivation time and regeneration medium (Davis *et al.,* 1991; Madhulatha *et al.,* 2007). Preculture of explants prior to co-cultivation has improved transformation efficiency in *Arabidopsis* (Sangwan *et al.,* 1992) and it has been reported in earlier transformation procedures of tomato also (Hu and Phillips, 2001; Sharma *et al.,* 2009). Hence in this study, explants were placed on pre culture media for about 24 hours and then subjected for co-cultivation. Most published protocols for tomato transformation (McCormick *et al.,* 1986; Hamza and Chupeau, 1993; Vidya *et al.,* 2000; Pozueta-Romero *et al.,* 2001; Ellul *et al.,* 2003) describe co-cultivation of explants with various *Agrobacterium* strains (LBA4404, C58C1, GV311SE or A208) for 48 hours with variable bacterial densities. In this study, *Agrobacterium* density at 0.1OD was used for the co-cultivation of explants. Sun *et al.* (2006) observed more than 40 per cent transformation efficiency in tomato cv. Micro-Tom, while El-Siddig *et al.* (2011) observed 3 to 7 per cent transformation efficiency using cotyledon as explants in tomato cv. Summer Set. In the present study, PCR screening revealed the presence of transgene sequence in putative transformants of p23TPX, which leads to the transformation efficiency of 1.23 per cent under kanamycin selection.

A wide range of Bt protein expression in transgenic plants has been reported by several workers. Independent plants of same genetic background and gene construct show greater differences in the level of expression (Breitler *et al.,* 2002; Hussain *et al.,* 2002; Ramesh *et al.,* 2004). In order to test the expression of Cry2AX1 protein in transgenic plants, qualitative ELISA was performed using antibodies specific to Cry2A protein and the presence of Cry2AX1 protein was detected in green leaves. Insect bioassay on transgenic plant expressing *cry* gene is effective tool to assess efficacy of Cry protein towards targeted insect pest. Mandokar *et al.* (2000) recorded 100 per cent mortality in five events of seven transgenic tomato plants expressing Cry1Ac protein against second instar larvae of *H. armigera.* Kumar and Kumar (2004) also observed Bt tomato plants expressing a Cry1Ab protein of *B. thuringiensis*

suffered significantly lower damage by *H. armigera* than the non-transgenic control plants in the laboratory, greenhouse and field. In the present study, insect bioassay of T_0 transformants of tomato recorded only thirteen per cent mortality, whereas the control plants did not show any mortality. In spite of low mortality, severe growth inhibition of surviving larvae and significant reduction in leaf area feeding was observed in transgenic plants. Chen *et al.* (2005) observed that the protein concentration is directly related to the level of insect resistance. Schuler *et al.* (1998) suggested that high level of insecticidal crystal protein expression in plants is necessary for an effective integrated pest management (IPM) strategy. Onarici *et al.* (2009) reported that delivering effective doses of the protein to the targeted insect pest at the threshold level of damage may provide the most effective strategy for IPM. Hence it is predicted that the expression of Cry2AX1 protein in the transformants of tomato is low and insufficient to cause significant mortality to *H. armigera*. So, further studies on developing more number of transformants through the standardized protocol and identification of high expression lines by quantitative ELISA and insect bioassay to identify an effective event of tomato will be useful to impart high level toxicity to fruit borer.

To conclude, through the present investigation a standardized regeneration protocol was developed for the local tomato variety cv. PKM1. *Agrobacterium* mediated transformation of tomato with *cry2AX1* gene resulted with transformants of tomato showing expression of *cry2AX1* gene and insecticidal activity to *H. armigera.* Generation and screening of more number of *cry2AX1* transformants of tomato may be useful to identify transgenic tomato plants with higher level of gene expression and protection against target pests.

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