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Genetic Transformation of Tomato (*Solanum Lycopersicum*. L) Using *Cry1Ac* Gene to Impart Resistance Against Fruit Borer

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ABSTRACT

Particle gun-mediated genetic transformation was used to produce transgenic plants of tomato (Solanumlycopersicum L.) resistant to fruit borer. Cotyledon and leaf explants from *invitro* tomato seedlings of variety 'Punjab Upma' with *Cry1Ac* gene construct (pGEM-4Z) were driven by Ubi promoter and NOS terminator, and GUS gene construct (pWRG 1515) were driven by CaMV 35S promoter and NOS terminator. The constructs were maintained as plasmids in *E. coli* strain JM109. These results signify the successful introduction of the *Cry1Ac gene* into tomato plants. Further PCR analysis for the *Cry1Ac* gene of three putative transgenic plants were performed, but no amplification was observed.

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Introduction

Tomato (*Lycopersicon esculentum* Mill., 2n = 2x = 24) belongs to the family Solanaceae and is a major vegetable crop. It is widely grown across tropical, subtropical, and temperate regions worldwide. The cherry tomato (*L. esculentum* var. *cerasiforme*) is the most likely ancestor of cultivated tomatoes, originating in Central and South America (Vavilov, 1951) and spreading globally. Tomatoes were believed to be introduced to India in the third decade of the 19th century. Today, tomatoes are a significant fruit crop in the Americas, Southern Europe, the Middle East, and India, with increasing production in China, Japan, and Southeast Asia. Tomatoes gained popularity in the last six decades and are currently cultivated in 534.5 thousand hectares, representing 7.5% of the total vegetable area. The annual production is 9,361.8 thousand metric tonnes, or 8.6% of total vegetable production (Annon, 2006). Globally, tomatoes are the second most popular vegetable after potatoes.

Tomatoes are vital in promoting health and vitality, with antibiotic properties that aid wound healing. They are rich in vitamin C and are a good source of β-carotene, vitamin A, and

vitamin B. Due to their nutritional value, tomatoes are essential in both vegetarian and non-vegetarian dishes. They are widely consumed fresh in salads, fried in cooking, and processed into products like purée and soup. The crop is also interesting to genetic engineers because tomatoes serve as a model for introducing agronomically important genes into dicotyledonous plants (Wing et al., 1994). Genetic transformation and in vitro regeneration have successfully improved tomatoes (Lindsey, 1992). The key objectives of genetic modification include pest resistance, herbicide tolerance, and the production of edible vaccines and other novel bioproducts.

Effective genetic transformation requires an explant-based regeneration system. Therefore, establishing an efficient tissue culture protocol is crucial for exploiting cell and tissue culture in genetic improvement. Callus induction and regeneration have been reported in tomatoes using various explants, such as apical meristems, cotyledons, stems, petioles, leaves, anthers, and inflorescences (Sheeja et al., 2004). The most common method of tomato regeneration is through shoot organogenesis from callus, leaf, or cotyledon explants or directly from thin cell layers of the inflorescence (Compton and Veilleux, 1991). Factors affecting *in vitro* plant regeneration include explant type, genotype, growth medium composition, growth regulators, gelling agents, light intensity and quality, photoperiod, temperature, and cultivation vessels and their covers (Sheeja et al., 2004). Understanding the influence of these factors is essential for reliable transformation, particularly for traits like pest resistance.

Among the biotic stresses affecting tomatoes, late blight, early blight, and leaf curl virus are prevalent diseases, while the tomato fruit borer (*Helicoverpa armigera* Hubner) is a major insect pest. This polyphagous pest is one of the most widespread agricultural threats, laying eggs on leaves and flower buds, with larvae that bore into developing fruit, rendering it unfit for consumption. The fruit borer affects multiple crops in India, including tomato, cotton, pigeon pea, and chickpea, causing extensive damage across different regions (Kumar and Kumar, 2003). Pest management has been primarily chemical-based, but excessive pesticide use raises concerns about health hazards, environmental pollution, loss of beneficial organisms, pest resistance to insecticides, and increased production costs. Host-plant resistance, a more sustainable approach, can provide an economical, long-term, eco-friendly pest control solution.

Due to widespread chemical use, *H. armigera* has reportedly developed resistance to synthetic insecticides, including pyrethroids (Forrester et al., 1993). Effective and safe alternative pest control methods are needed to address these issues. A wild tomato species, *Lycopersicon hirsutum* f. *glabratum*, has shown high resistance to fruit borers (Salinas et al., 1993). However, transferring resistance genes through conventional breeding is time-consuming, resource-intensive, and susceptible to issues like linkage drag. Transgenic plants expressing *Bacillus thuringiensis* proteins, which are toxic to *H. armigera*, provide a sustainable and effective method to reduce crop losses. Since tobacco's first transgenic

plants expressing engineered foreign genes were produced (Christou, 1992), many other crop species have successfully been transformed.

The transgenic approach allows crops to produce their own insecticides, ensuring minimal disturbance to the recipient genome. This targeted strategy enables the transfer of specific genes, providing access to a broad gene pool across plants, animals, and microorganisms. Transgenic methods are commonly used for introducing insecticidal crystal protein (ICP) genes, also known as Bt genes, cloned from *Bacillus thuringiensis*, a soil-dwelling Gram-positive bacterium. These proteins, called δ-endotoxins, are highly toxic to Lepidoptera, Diptera, and Coleoptera insects, destroying the midgut epithelium, leading to starvation, paralysis, and insect death (Meiyalaghan, 2006). Gene transfer for economically valuable traits can be accomplished through vector-based and vectorless methods, such as the particle gun (biolistic) method, which simultaneously transfers multiple genes.

The main objective of this research was to produce transgenic plants resistant to fruit borer infestation.

Methods and Materials

The present investigation, titled "Genetic Transformation of Tomato (*Lycopersicon esculentum* Mill.) Using the Cry1Ac Gene to Impart Resistance Against Fruit Borer," was conducted in the Tissue Culture and Genetic Transformation Laboratory, Department of Plant Breeding, Genetics, and Biotechnology at Punjab Agricultural University, Ludhiana. The plant material included two tomato genotypes, Punjab Upma and IPA-3. Seeds for both genotypes were obtained from the Department of Vegetable Crops. Seeds were grown aseptically *in vitro*, and various explants such as cotyledons, leaves, and hypocotyls were cultured on Murashige and Skoog (MS) medium with different concentrations of growth regulators for direct shoot regeneration and somatic embryogenesis via callus formation. Antibiotic sensitivity tests were performed to determine the optimum concentration of hygromycin B for selective elimination of non-transformed tissues in particle gun-mediated genetic transformation. Sensitivity to hygromycin B was assessed by culturing explants of the tomato variety Punjab Upma on this antibiotic.

The selection efficiency (%) was calculated as follows:

Selection efficiency (%)=(Number of explants surviving on selection mediumTotal explants) ×100\text{Selection efficiency (\%)} = \left(\frac{\text{Number of explants surviving on selection medium}}{\text{Total explants}} \right) \times 100Selection efficiency (%)=(Total explantsNumber of explants surviving on selection medi um)×100

During the present study, cotyledon and leaf explants were transformed by co-bombarding them with GUS and Cry1Ac gene constructs using the Bio-Rad Gun (PDS-1000/He system). This particle gun system included microcarriers or tungsten particles (0.6 µm), rupture discs (1100 psi), rupture disc holders, macrocarriers (51 µm), macrocarrier launch

assemblies, target tissue shelves, and a helium gas cylinder to provide the necessary pressure. Explants were co-bombarded using the Cry1Ac gene construct (pGEM-4Z) driven by the Ubi promoter and NOS terminator and the GUS gene construct (pWRG 1515) driven by the CaMV 35S promoter and NOS terminator. These constructs were maintained as plasmids in *E. coli* strain JM109. All components were dissolved in Milli Q water to a final volume of 1 liter, adjusting the pH to 7.0 with NaOH. A measured volume (150 ml) of medium was dispensed into each jar and autoclaved. Once the medium cooled, 100 ppm ampicillin was added, and the bacterial culture was inoculated. LB medium with *E. coli* containing either the GUS or Cry1Ac plasmid construct was incubated overnight at 37°C with constant shaking at 120 rpm. Bacterial broth was harvested after 12-14 hours. Plasmid DNA from *E. coli* strain JM109 was isolated using the Qiagen plasmid DNA isolation kit.

Bombarded tissue plates were incubated overnight at 28°C. After 16 hours, the tissues were transferred to a proliferation medium. Following two days of growth, tissues were moved to a selection medium containing the appropriate selective agent, with two cycles of selection lasting two weeks each. Tissues that grew on the selection medium were transferred to the shoot regeneration medium containing the selective agent. Cotyledon and leaf explants were pre-cultured on proliferation medium for 1-2 days, arranged at the center of a target plate containing fresh proliferation medium. The laminar airflow cabinet walls, macro carrier launch assembly, target plate shelf, and vacuum chamber were thoroughly sterilized with cotton soaked in rectified spirit and exposed to UV radiation for at least half an hour. Rupture discs (1100 psi), rupture disc holders, macrocarriers (51 µm), macrocarrier holders, and stopping screens were sterilized by dipping in 70% ethanol and then dried on sterile filter paper before bombardment.

The particle-mediated transformation procedure was optimized by testing various distances between the microcarrier launch assembly and target tissues (2.5 cm, 5.0 cm, and 7.5 cm). Bombarded explants were cultured on a selection medium with hygromycin B at concentrations of 10, 20, and 30 mg/L for two cycles of two weeks each. Hygromycin B-resistant tissues were regenerated into plants. GUS assays of explants were performed by immersing them in X-gluc solution and incubating them at 37°C in the dark. GUS expression frequency was recorded after 24 hours and again after 15 days.

Histochemical GUS assays on selected explants were conducted to assess the introduction and expression of the marker gene. Eighteen randomly selected cotyledon explants were tested, of which 10 showed positive GUS expression, yielding a rate of 55.55% (Table 5). Similarly, GUS expression in leaf explants was approximately 42.85%. Positive explants were destained using glacial acetic acid and ethanol in a 1:3 ratio, providing a clear view of GUS expression. PCR analysis of To plants for the Cry1Ac gene was performed using leaves from *in vitro*-grown plants, following the CTAB method of DNA isolation. A total of three plants were screened for the presence of the Cry1Ac gene; however, no amplification was observed.

Results and Discussion

In vitro plant regeneration from tissues and organ culture is a basic process for the application of plant biotechnology. Biolistic gene transfer, popularly known as "Particle Gun," involves accelerating foreign DNA-coated gold or tungsten particles into target plant cells. Particle bombardment is an important tool for generating transgenic plants. In this approach, DNA of interest is coated onto metal particles (tungsten or gold), which are then bombarded into plant cells. Particles thus serve as vehicles for DNA delivery and are an important variable in bombardment-mediated plant transformation. Some experiments were conducted to determine factors affecting "Particle Gun"-mediated genetic transformation. In this experiment, explants were placed at three different positions in the particle gun.

Table 1. Percent GUS expression in cotyledonary explant of tomato genotype Punjab Upma at different distances from microcarrier launch assembly.

Distance between assembly and cotyledons	Total number of cotyledons bombarded	No. of cotyledons incubated in X-gluc	No. of cotyledons showing GUS expression	% GUS expression
2.5	53	12	5	41.25
5.0	46	11	9	49.23
7.5	39	13	8	33.21

Cotyledon and leaf explants of the genotype Punjab Upma were placed in the center of the target plate in a circle of about 1 cm diameter. These explants were bombarded at varying distances, such as 2.5 cm, 5.0 cm, and 7.5 cm, from the microcarrier launch assembly with 6 µl of DNA suspension. Maximum GUS expression was 49.23% in the case of cotyledons and 43.25% in the case of leaf explants when kept at a 5 cm distance, as presented in Table 1. Puddephat et al. (1991) reported the highest transient GUS expression in broccoli with cotyledon explants placed at a 6.0 cm distance. Kaur (2004), however, recorded maximum GUS expression in calli kept at a 5.0 cm distance in sugarcane.

Table 2: Percent GUS expression in tomato genotype Punjab Upma leaf explants at different distances from the microcarrier launch assembly

Distance between assembly and leaf	**Total number of leaf-bombarded**	**No. of leaf incubated in X-gluc**	**No. of leaf showing GUS expression**	**% GUS expression**
2.5	41	10	3	35.21
5.0	39	11	6	43.25
7.5	31	9	4	29.56

For this experiment, explants of Punjab Upma were placed in the center of the osmoticum medium contained in a petri dish in a circle of about 1 cm diameter 4 hours before bombardment. The standard amount of DNA suspension used per bombardment was 1 µg/µl. The plasmid DNA carrying the gene of interest, Cry1Ac, along with the selectable marker (hpt), was mixed (3:1) with a separate plasmid construct containing the GUS reporter gene. This mixture was coated on tungsten particles and bombarded onto cotyledons and leaves of the Punjab Upma genotype.

Table 3. Genetic transformation of tomato genotype Punjab Upma by bombarding cotyledon explants with Cry1Ac gene

Experiment	The total number of explants bombarded	No. of explants showing regeneration on 20 mg/l hygromycin B	Percent regeneration
1	140	12	8.58
2	130	10	7.69
3	125	8	6.40
4	120	9	7.50
5	115	14	12.18
Total	**630**	**53**	**8.41**

The selection efficiency after two cycles and throughout the process was also investigated. A total of 630 cotyledon explants of the Punjab Upma genotype were bombarded with the Cry1Ac construct, as shown in Table 3. Fifty-three cotyledon explants were selected out of 630 on hygromycin B (20 mg/L) after two complete cycles of selection for two weeks each, showing an average regeneration rate of 8.41%. These explants were transferred to a regeneration medium containing (MS + kin 0.5 mg/L + BAP 0.5 mg/L), where three explants regenerated into shoots, exhibiting a regeneration rate of 5.60%. The regenerated leaf and stem were incubated in X-gluc, which showed a blue color, indicating gene transformation.

Similarly, 466 leaf explants of the Punjab Upma genotype were bombarded with the Cry1Ac construct, as shown in Table 4. Thirty-four explants were selected out of 466 on hygromycin B (20 mg/L) after two complete selection cycles for 15 days each, showing an average regeneration rate of 7.51%. These explants were transferred to the regeneration medium containing (MS + kin 0.5 mg/L + BAP 0.5 mg/L), but no shoot regeneration was observed.

Table 4. Genetic transformation of tomato genotype Punjab Upma by bombarding leaf explants with Cry1Ac gene

Experiment	The total number of explants bombarded	No. of explants showing regeneration on 20 mg/l hygromycin B	Percent regeneration
1	110	7	6.36
2	85	7	8.23
3	95	6	6.31
4	93	7	7.52
5	83	8	9.63
Total	**466**	**34**	**7.51**

Table 5.GUS expression (%) in cotyledon and leaves explant with particle bombardment

Treatment	Total number of explants incubated in X-gluc	No. of explants showing GUS expression	% GUS expression
Cotyledons	18	10	55.55
Leaves	14	6	42.85

Histochemical GUS assay of selected explants was performed to confirm the introduction and expression of the marker gene. Eighteen cotyledon explants were randomly selected and tested, of which 10 showed GUS positivity, with a GUS expression rate of 55.55%, as shown in Table 5. Similarly, in leaf explants, GUS expression was about 42.85%. The positive explants were destained with glacial acetic acid and ethanol in a ratio of 1:3, which provided a clearer view of GUS expression. PCR analysis of To plants for the Cry1Ac gene was carried out by taking leaves from in vitro-grown plants using the CTAB method for DNA isolation. A total of three plants were screened for the presence of the Cry1Ac gene, but no amplification was observed.

Conclusion

The particle gun-mediated transformation protocol was optimized by standardizing the distance between the microcarrier launch assembly and the target plate. Cotyledon and leaf explants of the tomato genotype Punjab Upma were bombarded with GUS and Bt gene constructs and selected on hygromycin B (20 mg/L). After two complete selection cycles of two weeks each, the regeneration efficiency for cotyledons and leaf explants was 8.41% and 7.51%, respectively. The explants were transferred to a regeneration medium containing MS + kin (0.5 mg/L) + BAP (0.5 mg/L), where three explants regenerated into shoots, with a regeneration rate of 5.60%. GUS expression was highest in cotyledon explants at 55.55% and in leaf explants at 42.85%. Regenerated leaf and stem tissues, incubated in X-gluc solution, exhibited blue coloring, indicating gene transformation. PCR analysis of three putative transgenic plants for the Cry1Ac gene was performed, but no amplification was detected.

Conflict of Interest: The author(s) declared no conflict of interest.

References

Anonymous (2006) INDIA STAT Agriculture Data http://apps.indiastat.org

Christou, P. (1992). Genetic transformation of crop plants using microprojectile bombardment. *The Pl J.* 2, 275-81.

Forrester, N. W., Cahill, M., Bird, L. J., & Layland, J. K. (1993). Management of pyrethroid and endosulfan resistance in Helicoverpa armigera (Lepidoptera: Noctuidae) in Australia. *Management of pyrethroid and endosulfan resistance in Helicoverpa armigera (Lepidoptera: Noctuidae) in Australia.*, (Supplement No. 1).

Gosal, S. S., & Wani, S. H. (2018). Plant genetic transformation and transgenic crops: methods and applications. *Biotechnologies of Crop Improvement, Volume 2: Transgenic Approaches*, 1-23.

Kumar, H., & Kumar, V. (2004). Tomato expressing Cry1A (b) insecticidal protein from Bacillus thuringiensis protected against tomato fruit borer, Helicoverpa armigera (Hübner)(Lepidoptera: Noctuidae) damage in the laboratory, greenhouse and field. *Crop Protection*, 23(2), 135-139.

Kaur, A. (2004). *Genetic transformation in sugarcane varieties* (Doctoral dissertation, Plant Breeding, PAU, Ludhiana).

Lindsey, K. (1992). Genetic manipulation of crop plants. *Journal of biotechnology*, 26(1), 1-28.

Meiyalaghan, S., Jacobs, J. M. E., Butler, R. C., Wratten, S. D., & Conner, A. J. (2006). Transgenic potato lines expressing cry 1Ba1 or cry 1Ca5 genes are resistant to potato tuber moth. *Potato research*, 49, 203-216.

Puddephat, I. J., Thompson, N., Robinson, H. T., Sandhu, P., & Henderson, J. (1999). Biolistic transformation of broccoli (Brassica oleracea var. italica) for transient expression of the b-glucuronidase gene. *The Journal of Horticultural Science and Biotechnology*, 74(6), 714-720.

Sheeja T E, Mondal B and Rathore R K S (2004). Efficient plantlet regeneration in tomato (*Lycopersicon esculentum*). *Pl Tiss Cult*, 14, 45-54.

Salinas, H., Vallejo Cabrera, F. A., & Estrada S, E. I. (1993). Evaluation of resistance to the tomato fruit borer Neoleucinodes elegantalis (Guenée) in material of Lycopersicon hirsutum Humb. & Bonpl. and L. pimpinellifolium (Just.) Mill. and its transfer to cultivated material of tomato, L. esculentum Mill. *Ade-Agronomica-Universidad- Nacional-de-Colombia,* 43, 1-4.

Wing, R. A., Zhang, H. B., & Tanksley, S. D. (1994). Map-based cloning in crop plants. Tomato as a model system: I. Genetic and physical mapping of jointless. *Molecular and General Genetics MGG*, 242, 681-688.

Vavilov N I (1951) The origin, variation, immunity and breeding of cultivated plants. *Chronica Botanica,* 1, 364.