PREFACE

Tomato (*Solanum lycopersicum* L.) is a major vegetable crop, ranking second in importance to potato in many countries, grown in about 4.7 million hectares worldwide with an annual production of 159 million metric tonnes including sizable contribution of 16.8 million metric tonnes in India during the year 2011 (FAOSTAT 2011). Tomato is one of the most consumed vegetables in the world and is the dietary source of vitamins, minerals and fibre, which are important for human nutrition and health. More importantly, with the increase in world population, the quantum of tomato consumption has considerably increased and farmers, agronomists and horticulturists have had to walk a tight rope to enhance yield without losing sight of the production quality to meet the demands of the fresh market and the processing industry. It is an important model plant system for studying molecular genetics, expression of heterologous genes and metabolic engineering in genetically modified (GM) lines through *in vitro* genetic manipulations.

Genetic improvement of this Solanaceous crop has been an on-going process with the objective of gaining high fruit yield, enhanced nutritive value, controlled fruit maturation and ripening, and developing resistance to polyphagous insects, microbial pathogens, and various abiotic stresses. Productivity of tomato is adversely affected due to abiotic stresses and pathogens including insect infestation particularly by tomato fruitworm *Helicoverpa armigera* which may inflict upto 30–50% damage. However, introduction of desired traits and genes into commercial varieties of *S. lycopersicum* often encounters serious difficulties due to high incompatibility barriers for hybridization, non-availability of these genes in existing germplasm and recalcitrant response of commercial varieties to *in vitro* manipulations. With the rapid progress in the area of plant molecular biology, identification of genes involved in crop improvement, their characterization and expression in related and unrelated plant species, offers an alternative and promising approach for crop improvement through recombinant DNA strategies. A number of requirements must be fulfilled in order to produce genetically modified transgenic plants. First, an efficient genotype-independent and reproducible tissue culture system; second, a complementary method to deliver foreign DNA into regenerative and transformation-competent plant cells and tissues; third an efficient procedure to screen and regenerate transformed cells and fourth, selection of promising *T₀* plants (independent
events) with high-level expression and Mendelian inheritance of transgene(s) in subsequent progenies.

Present methods of crop protection rely mainly on the use of synthetic agrochemicals, which have a significant drawback of environmental contamination and toxicity to non-target organisms, including humans. Pesticides are unable to control the insect and their frequent usage hazardously affects the yield and the market value of the fruit. Cloning of Bacillus thuringiensis (Bt) insecticidal crystal protein (ICP) genes and their expression in transgenic plants offer an alternative but complementary strategy, to conventional breeding practices for protection of crops against insect damage. The Bt-toxin is available within the transgenic plant and affects the susceptible insect pest right from the beginning and the system is environmentally safe because the product is retained in the plants and degraded rapidly when the plant decomposes. The routine recovery of transgenic events expressing high-level of Cry1Ac toxin is a rare, random and vexatious issue. Reports on the expression of both the full-length and truncated version of this gene, for effective resistance against target insects, have been documented. However, performance of Cry1Ac toxin encoded by full-length and truncated cry1Ac gene in plants is still not clear, and the question remains unaddressed that truncation of 3’ end of gene was documented and suggested for active insecticidal toxin production while the most successful transgenic event(s) of cotton for field performance are based on full-length cry gene. This situation has triggered to investigate the comparative study on toxicity of two versions of cry1Ac genes for development of stable transgenic plants of tomato and protection against Helicoverpa and comparison with transgenic tomato expressing cry1Ab gene. The present thesis entitled “Expression of insecticidal toxin coded by modified full-length and truncated Bt-cry1Ac gene in transgenic tomato for assessment of their stability and efficacy against target insects” encompasses the details of the studies undertaken and analysis of results obtained under 9 major chapters as described below.

1. **Chapter 1 - Introduction & Objectives:** This chapter includes a brief introduction and objectives identified for the present problem.

2. **Chapter 2 - Review of Literature:** This chapter summarizes the current status and scenario of in vitro regeneration, genetic transformation in plants including tomato, importance of full-
length and truncated versions of *Bacillus thuringiensis* crystal protein gene(s) in plant for insect resistance.

3. **Chapter 3 - Materials and Methods:** Includes the details of various experimental materials, procedures and techniques that were employed in order to accomplish the objectives.

4. **Chapter 4 - Comparative analysis of *Bacillus thuringiensis*-cry1A gene sequences and toxin characteristics:** This chapter includes various bioinformatics tools used to generate an idea of the integrity and stability of six Bt-Cry 1A genes.

5. **Chapter 5 - *Agrobacterium*-mediated transformation of tomato and selection of stable transgenic plants:** This chapter includes detailed procedure of *Agrobacterium*-mediated vegetative leaf tomato transformation of tomato, deployed for the study.

6. **Chapter 6 - Molecular characterization of insect-resistant transgenic tomato developed with full-length and truncated cry1A genes:** This chapter includes genetic transformation of tomato with modified full-length and truncated version of cry1Ac gene and also includes transformation with cry1Ab gene using a modified *Agrobacterium*-mediated leaf-disc transformation procedure. This chapter also includes molecular characterization and comparative expression analysis of Bt-cry genes.

7. **Chapter 7 - Comparative analysis of constitutive and synthetic promoter cassettes for enhanced transgene expression in tomato:** This chapter includes the use of synthetic promoters with defined regulatory sequences to enhance the expression and function of Bt-cry1Ac gene.

8. **Chapter 8 - Summary and Conclusions:** This chapter briefly summarizes the work that has been presented in this thesis and conclusions drawn there from.

9. **Chapter 9 - References:** This chapter contains citation of references used in the present investigation.