Name of Scholar: Shabana Khan

Name of Supervisor: Prof. Arif Ali (Dept. Of Biotechnology, Faculty of Natural Sciences, JMI)

Name of Co-Supervisor: Dr. P. Ananda Kumar (Dept. Of NRC for Plant Biotechnology, IARI, New Delhi)

Name of Department: Department of Biotechnology, Faculty of Natural Sciences

Title of Thesis: Genetic Engineering of Tomato for Resistance to Fruit Borer (*Helicoverpa armigera*)

ABSTRACT

The benefits of transgenic insect resistant crops in terms of increased yields, reduced chemical inputs and, as a knock-on effect, improved farmer and consumer health. *Helicoverpa armigera* emigrating from other crops can infest tomatoes in large numbers and most of the fruits suffer extensive damage by the pest (Matthews, 1993). In the present study two synthetic *Bt* genes coding for novel chimeric Cry1Aabc and Cry1AcJJ proteins were constructed by domain swapping. *cry1Aabc* confers high expression in plant system and provide resistance against major polyphagous pest *Helicoverpa armigera*.

The salient features of the present investigation are:

- Two novel *Bt* toxins Cry1Aabc and Cry1AcJJ were designed by domain swapping.
- Before designing synthetic DNA sequence, protein 3D structure was developed by homology modeling, evaluated for quality of 3D model developed and finally tested for protein similarity. Prediction of 3D-structure by homology modeling and subsequent structural analysis shows that the designed proteins have general fold of *Bt* family.
- A synthetic *cry1Aabc* gene coding for chimeric Cry1Aabc toxin of Ist, IInd, IIIrd domain from *cry1Aa*, *cry1Ab* and *cry1Ac* respectively, similarly, *cry1AcJJ* gene synthesized by cloning the domains I from *cry1Ac* and domain II, III from *cry1J* genes.
- To test the efficacy of these synthetic genes in plant system, it was cloned in plant transformation vector pBinAR. Fully assembled (1.8 kb) *cry1Aabc* and *cry1AcJJ*

genes were cloned in plant transformation binary vector pBinAR, having constitutive 35S CaMV promoter and OCS terminator.

- After confirming cloning of the *cry1Aabc* and *cry1AcJJ* genes, these constructs have been mobilized into *A. tumefaciens* strain EHA 105 in order for further transformation plants.
- Molecular analysis of putative kanamycin resistant transgenic tomato was carried by PCR, Southern blotting, RT-PCR, Western blotting and ELISA.
- Out of 160 putative plants, 112 with the *npt*II and *cry1Aabc* specific gene primer. Out of total 36 plants analyzed for Southern blot, only 10 plants showed single copy number, 9 plants were double copy integration however, rest showing multiple copies, non-transgenic plant DNA did not show any hybridization signal. The RT-PCR resulted in amplification of an expected 1.2 kb fragment that confirmed the expression of cry1Aabc gene in putative transgenic tomato plants.
- Quantification of Cry1Aabc protein in transgenic plants showed efficient the translation of the *cry1Aabc* gene in transgenic lines.
- Cry1Aabc protein on western blot was performed using polyclonal antibodies (rabbit) raised against Cry1Ac toxin that gave ~65 kDa in putative transgenic tomato plants.
- Insect bioassay shows that *cry1Aabc* transgenic tomato provides resistance against major polyphagous pest, *H. armigera* neonate larvae.
- Self pollinated seeds of T_0 plants (plant no. 8, 26, 38, 59, 75, 88, 90, 91, 147, 222) were further analyzed in T_1 generation. Mendelian segregation (3:1) was observed.
- Molecular analysis of T_1 transgenic tomato was carried by PCR, Southern blotting, RT-PCR, Real Time PCR, Western blotting and ELISA which further confirmed the gene integration in T_1 progeny.
- Insect bioassay of T_1 tomato leaf and fruit showed 100 % mortality against *H. armigera*.

However, the results of the transgenics in open field conditions need to be carried out to critically evaluate the usefulness of these transgenic tomato plants vis-à-vis *H. armigera* infestation and damage.