COLLABORATIVE RESEARCH TOWARDS DEVELOPMENT OF TRANSGENIC BT EGGPLANT RESISTANT TO *Leucinodes orbonalis* GUENEE

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Evaluation of *cry1Ab* transgenic eggplant resistant to *Leucinodes orbonalis* at Tamil Nadu Agricultural University (TNAU), Coimbatore

Objectives:

❖To test the efficacy of *cry1Ab* transgenic eggplant in greenhouse and field conditions
❖To study the level of toxin expression in different tissues at different stages

Greenhouse evaluation of transgenic *cry1Ab* plants at TNAU, Coimbatore

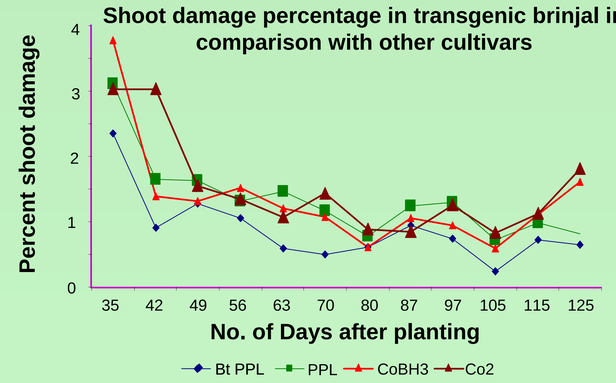
The larval survival and the fruit area damaged by first instar larvae of *Leucinodes orbonalis* in both Bt as well as non-Bt egg plants (70 days old) were recorded. The area of damage was found to be less than 7.58 per cent in 22 out of 25 Bt plants analyzed as against 84.34 per cent in non – Bt plant. Two plants showed no damage indicating the potential of *cry1Ab* gene in managing *L. orbonalis*.

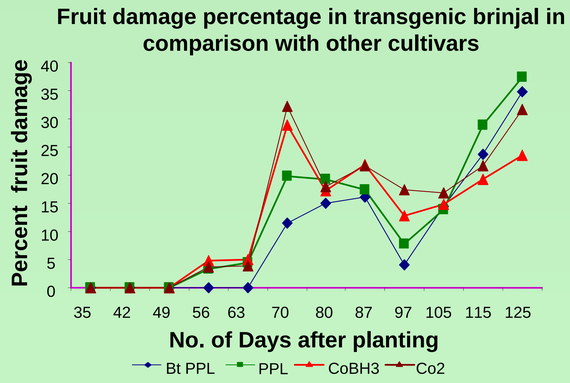
Cry1Ab protein expression in Bt egg plant parts at different stages of crop growth.

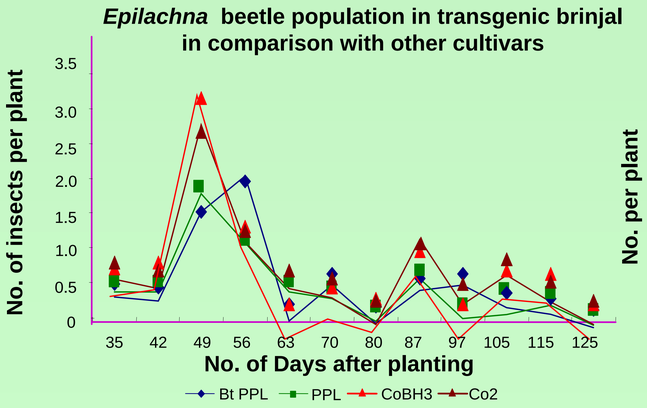
The Cry1Ab protein expression in leaves, shoots and fruits of Bt-eggplant cv. Pusa purple long was estimated using precoated ELISA kit (Agdia kit) with plant parts collected at different stage of crop.

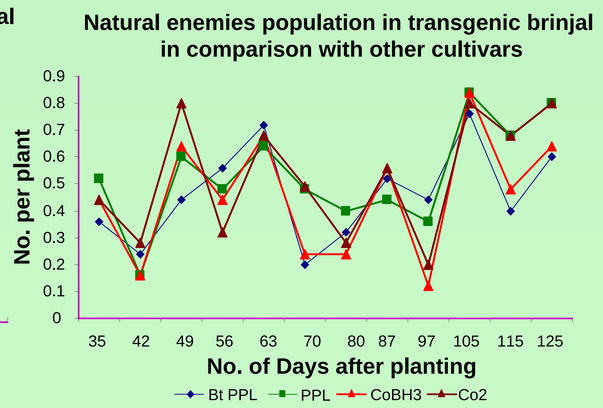
Days After Planting	Cry1Ab protein expression (ppm)*		
	Leaf	Shoot	Fruit
35	1.23	0.11	-
49	1.94	1.49	-
63	1.66	2.04	1.09
80	1.78	1.76	1.32
97	0.62	0.92	1.28
115	0.43	0.56	0.21
136	0.13	0.12	0.18
Mean	1.11	1.00	0.82

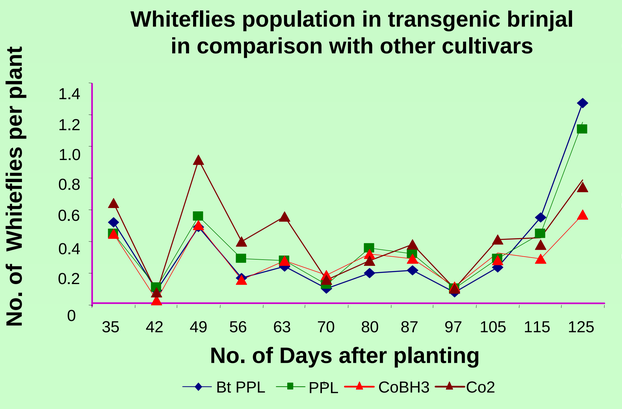
Field evaluation of transgenic *cry1Ab* plants at TNAU

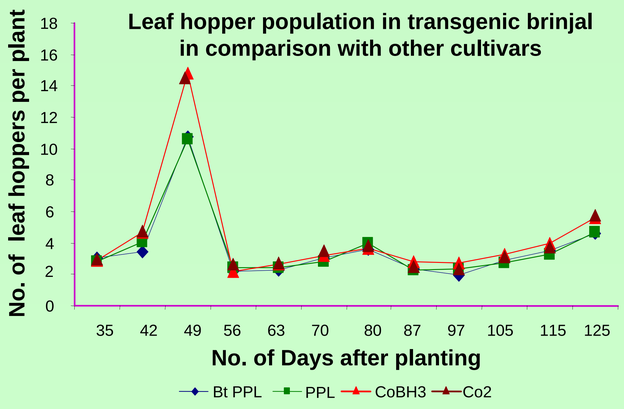












Since the reduction of pest damage is not sufficient in transgenic eggplant (Pusa purple long) developed at IARI, New Delhi with *cry1Ab* gene, use of other genes or pyramiding with other genes was felt necessary. Hence the second phase was initiated and the work is in progress

Development of marker free transgenic Bt eggplant for ESFB resistance

Objectives

❖Transformation of eggplant (Co2) with *cry2Aa* gene.
❖Search of insect resistance genes from plants
❖Development of antibiotic marker free transgenic eggplant lines expressing Bt genes

Toxicity analysis of Cry1Ac and Cry2Aa proteins against ESFB

Ten different bioassay experiments using potato discs and neonates of ESFB were carried out and significant level of mortality was observed in the neonates of ESFB with both the proteins tested.

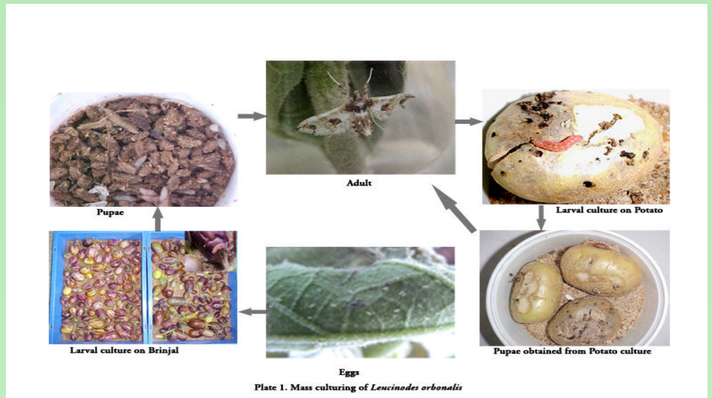


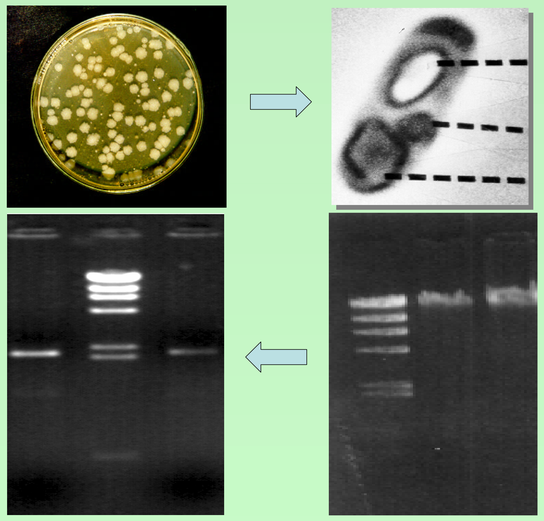
Plate 1. Mass culturing of *Leucinodes orbonalis*

Purified Bt proteins were verified for toxicity against *L.orbonalis* (neonate larvae) at TNAU

Treatment	Percent mortality
Cry1Ac	77.0 ± 1.6
Cry2Aa	86.5 ± 2.9

Cloning, sequencing and expression of *cry2Aa* gene from an Indian isolate of *Bacillus thuringiensis*

In TNAU, a *cry2Aa* gene was cloned from an Indian isolate of Bt and expressed in an acrystalliferous strain of Bt, 4Q7. Transformed acrystalliferous Bt strain expressing the *cry2Aa* gene produced Cry2Aa protein of about 65 kDa. Nucleotide sequencing and codon optimization of the *cry2Aa* gene were carried out at the Virginia Tech, USA. The codon optimized *cry2Aa* gene will be used to produce Bt egg plant through *Agrobacterium* mediated transformation.



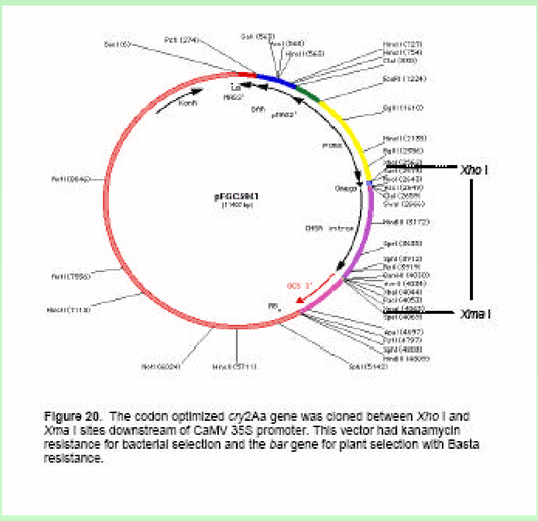


Figure 20. The codon optimized cry2Aa gene was cloned between Xho I and Xma I sites downstream of CaMV 35S promoter. This vector had kanamycin resistance for bacterial selection and the bar gene for plant selection with Basta resistance.

Isolation of plant derived genes from wild *Solanum* spp.

The cysteine protease inhibitor (CPI) gene from the *S. viarum* was cloned by degenerate oligo primed polymerase chain reaction. An alignment of tomato and potato CPI gene was used to design degenerate primers in highly conserved region. The cloned PCR amplified gene fragment contained about 377 bases. Sequence comparison to nucleotide databases revealed significant homologies with several cysteine protease inhibitors like multicystatin and cystatin gene sequences.

Development of antibiotic marker free transgenic eggplant lines expressing Bt genes.

Eggplant plants are being co-transformed with *Agrobacterium* strain that contains two binary vectors one carrying gene of interest and the other a selectable marker gene (kanamycin resistance gene) with a view to develop marker free transgenic eggplant expressing *Bt* genes. Gene constructs are being developed with a view to generating marker-free plants. The studies are in progress.

Future Plan

❖Evaluation of eggplant lines expressing *cry2Aa* for stable expression of toxin and resistance
❖Field evaluation of these lines for resistance to EFSB
❖Isolation of defense genes from diverse plant sources against pest, disease and nematodes affecting eggplant
❖Development of IPM strategy for Bt eggplant which is expected to be released for commercial cultivation in India shortly.

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