STUDIES ON TRANSFER OF *CRY2* GENE FROM *BACILLUS THURINGIENSIS* TO *INDICA* RICE (*ORYZA SATIVA* L.) USING *AGROBACTERIUM*-MEDIATED BINARY VECTOR SYSTEM AND ELECTROPORATION

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Thesis

Submitted in partial fulfillment of the requirements

for the degree of

MASTER OF PHILOSOPHY

in the

POSTGRADUATE INSTITUTE OF AGRICULTURE

of the

UNIVERSITY OF PERADENIYA

SRI LANKA

December, 1999

ABSTRACT

Rice (*Oryza sativa* L.) is by far the world's most important crop species. However, stress and pests have become the major constraints for the enhancement of rice yields. The most significant advancement in crop improvement, complementing the conventional tools of plant breeding in overcoming the pest problems has been the development and utilization of gene transformation techniques.

Attempts were made to clone lepidopteran toxic *cry* gene of locally isolated *Bacillus thuringiensis* strain into *Agrobacterium* binary vector. Also *indica* rice (*Oryza sativa* L.) was transformed using *Agrobacterium*- mediated binary vector system and electroporation.

Extracted plasmid DNAs of *Bacillus thuringiensis* var. *aizawai* HD-133 were digested with restriction enzyme *Dra*I and resolved by agarose gel electrophoresis. The 3.8 kb intact *cry* gene was isolated and probe was prepared by DIG-DNA labelling method.

Identification of *cry* gene of locally isolated *Bacillus thuringiensis* strain 6e was done by the southern hybridization using DIG-labelled 3.8 kb *cry* gene probe. The results revealed that the intact *cry* gene containing *Dra*I fragment of *Bacillus thuringiensis* strain 6e was around 3.5-4.0 kb.

The intact *cry* gene of *Bacillus thuringiensis* 6e was cloned into *Xba*I site of the *Agrobacterium* binary vector pABK01 via *Xba*I synthetic adaptor and this recombinant vector was introduced into *Agrobacterium* helper strain LBA4404 using electroporation and

freeze-thaw methods. Plasmid DNAs of resulted colonies obtained from electroporation were subjected to dot blot analysis. Results indicated that the stable incorporation of bin vector pABK01 into *Agrobacterium tumefaciens* host strain LBA4404, but no ligation of *cry* gene occurred with bin vector.

The presence of intron-*gus* reporter gene in bin vector pABK01 was evaluated and *gus* reporter gene did not express its gene activity in *Agrobacterium tumefaciens* cells.

Transformation of rice seeds of the *indica* variety BG 450 attempted with the *Agrobacterium tumefaciens* strain LBA4404, which contains a binary vector pABK01. Callus induction from seeds was considerably inhibited by the presence of cefotaxime in callus induction medium, nevertheless there was no effect of cefotaxime on callus proliferation.

Rice calli derived from scutella co-cultivated with the above strain were selected on the medium supplemented with hygromycin B and transformed calli were screened for its *gus* gene activity. Hygromycin resistant calli were regenerated on N6 rice regeneration medium.

Attempts were made to isolate protoplasts from suspension cells of *indica* rice variety BG 450. Low yield of protoplasts were released by the enzyme treatments and they were cultured as mixed nurse culture with tobacco or chilli protoplasts as a source of nurse cells and in liquid medium. However, rice protoplasts were failed to form protocolonies by the division of protoplasts.

As an alternative to the use of *Agrobacterium* as a gene vector, intact cells of rice were transformed by introducing a bacterial *hpt* gene conferring resistance to hygromycin B, by electroporation. Roots and shoots were emerged within few weeks from the hygromycin resistant calli on N6 rice regeneration medium.