AGROBACTERIUM MEDIATED TRANSFER OF BACILLUS THURINGIENSIS 6E CRY GENE TO IXORA ODORATA

By

THUSHARA CHATHURANGA BAMUNUARACHCHIGE

Thesis

Submitted in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in the

POSTGRADUATE INSTITUTE OF AGRICULTURE

of the

UNIVERSITY OF PERADENIYA

PERADENIYA

May, 2003

ABSTRACT

Ixora species are quite popular among landscape architects and gardeners as hedge plants and potted ornamentals. However, infestations by pests such as *Ixora* leaf webber, flower webber, green horned caterpillar and looper caterpillar have become a threat to the commercial exporter, especially with strict quarantine regulations under which presence of a single pest egg may spell disaster. Most chemical methods adopted have hazardous effects on the environment and other beneficial insects and therefore, are unpopular among the buyers. As an alternative *Ixora odorata* var. *vulcan* was transformed with the *cry* gene of *Bacillus thuringiensis* strain 6e by *Agrobacterium* mediated gene transfer. In the process, an effective plant regeneration procedure and a gene transfer system were developed. The *cry* gene of *Bacillus thuringiensis* strain 6e was isolated, purified and cloned into the *Xba*I site of the T-DNA region of the *Agrobacterium* vector pLG121Hm *via* an adapter. Recombinant pLG121Hm was transferred to *Agrobacterium* strain LBA4404 by electroporation. Positive *cry* clones were confirmed by Dot blot analysis of the plasmid DNA (extracted from the electroporated strains) with Dig labeled *cry* probe.

In vitro grown *Ixora odorata* shoot tips (2cm) were co-cultivated with LBA4404, harboring the Bt 6e *cry* cloned binary vector pLG121Hm. Co-cultivated explants were transferred to shoot multiplication medium (½ MS with 2mg/l BAP and 500mg/l cefotaxime) and incubated at 2500lux with a 16h photoperiod. After 4 weeks the axillary shoots were screened on selective medium (½ MS supplemented with 200mg/l hygromycin). Hygromycin positive plants were used for the PCR analysis and Southern

blot analysis. PCR analysis of the putative transformants, carried out with *gus* specific primers, produced the expected 1.680kb fragment in all the hygromycin positive transformants. Southern blot analysis of these with the Dig labeled Bt 6e *cry* probe produced positive results. The positive results of both, the PCR analysis and the Southern blot confirmed genomic integration of the Bt 6e *cry* gene. The transformation efficiency was 20% for the shoot tips whereas it was 40% and 30% for callus and leaf disk transformations respectively but the inability of proper regeneration made both these techniques redundant.