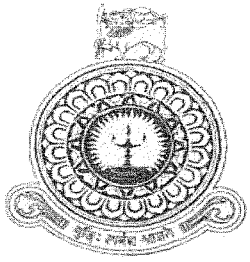

SUSTAINABLE INSECT RESISTANCE IN LOCAL RICE VARIETIES

A Thesis Submitted for the Degree of Doctor of Philosophy

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ABSTRACT

Rice is one of the most important and staple food for more than 50% of the world population. Demand for rice is increasing all over the world. Despite the demand rice productivity is affected by both the biotic and abiotic factors. Among the biotic factors, losses due to insect pests are substantial. Rice leaffolder (*Cnaphalocrosis medinalis*) is one of the important pests of rice in Asia which causes yield losses of 1 million tons of rice per year resulting in several billion dollars per year.

An efficient and reproducible *Agrobacterium* mediated transformation and regeneration procedure for local rice has not been established so far. We here report high frequency of *Agrobacterium tumefaciens* mediated transformation of embryogenic calli of mature rice seeds, and *in vitro* regeneration of rice for three varieties (Bg 350, Bg 94-1 and Bg 352). Embryogenic calli were transformed with *Agrobacterium tumefaciens* strain LBA 4404 harboring binary plasmid pCAMBIA 1301 containing *GUS* and *hpt II* driven by CaMV 35 S promoter and NOS terminator as marker genes. Cocultivation was for 2 days followed by callus induction (without hygromycin) for 2 weeks. Afterwards selection of transformants was carried out for 4 – 5 weeks with 30 mg/L hygromycin.

Exclusion of selection with hygromycin for 2 weeks (resting period) followed by selection for several weeks had enhanced the active proliferation of transformed calli and regeneration during selection. During 5th week of selection greening was observed and regenerating calli were transferred to shoot regeneration medium followed by rooting and acclimatized under contained environment in a green house. Transgene integration was confirmed through PCR analysis and transformation efficiencies of 28% for Bg 350, 17% for Bg 94-1 and 16% for Bg 352 were observed.

Developed and optimized transformation procedure for local rice was utilized for the introduction of Cry genes of *Bacillus thuringiensis* into a local rice variety. Embryogenic calli of sub species *indica* variety Bg 94 -1 were transformed with *Agrobacterium tumefaciens* strain GV 3101 carrying binary plasmid pCAMBIA 1305.1 with *Cry 1C* and *Cry 2A* transgene constructs. These genes were driven by CaMV 35S d promoter and NOS terminator together with *GUS Plus* and *hpt II* as marker genes. Transformation, regeneration and recovery of putatively transformed rice with *Cry* genes were carried out according to the optimized procedure for local rice.

The integration of the respective transgenes in the rice genome was observed through PCR analysis for *Cry 1C* and *Cry 2A*, sequencing of the genomic DNA of transformants and histochemical GUS analysis. Transformation efficiencies of 32% and 6% were observed for Cry 2A and Cry 1C. Insect Bioassays with rice leaffolder larvae in T_0 transgenic plants revealed high toxicity to rice leaffolder larvae where mortalities of 89% and 83% were observed for Cry 2A and Cry 1C transgenic plants.

The gene transfer technology developed by this project after appropriate biosafety measures could be adopted by Sri Lankan rice scientists in their efforts in enhancing insect resistance breeding in local rice varieties. Adoption of these Bt rice varieties would lead to reduction of the insecticide use in rice crop production.