

Plant Mediated RNAi Approach to Knock Down Ecdysone Receptor Gene of Colorado Potato Beetle

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Abstract : RNA interference (RNAi) has proved its usefulness in functional genomic research on insects recently and is considered potential strategy in crop improvement for the control of insect pests. The different insect pests incur significant losses to potato yield worldwide, Colorado Potato Beetle (CPB) being most notorious one. The present study focuses to knock down highly specific 20-hydroxyecdysone hormone-receptor complex interaction by using RNAi approach to silence Ecdysone receptor (EcR) gene of CPB in transgenic potato plants expressing dsRNA of EcR gene. The partial cDNA of Ecdysone receptor gene of CPB was amplified using specific primers in sense and anti-sense orientation and cloned in pRNAi-GG vector flanked by an intronic sequence (pdk). Leaf and internodal explants of Lady Olympia, Agria and Granola cultivars of potato were infected with Agrobacterium strain LBA4404 harboring plasmid pRNAi-CPB, pRNAi-GFP (used as control). Neomycin phosphotransferase (nptII) gene was used as a plant selectable marker at a concentration of 100 mg L⁻¹. The primary transformants obtained have shown proper integration of T-DNA in plant genome by standard molecular analysis like polymerase chain reaction (PCR), real-time PCR, Southern blot. The transgenic plants developed out of these cultivars are being evaluated for their efficacy against larvae as well adults of CPB. The transgenic lines are expected to inhibit expression of EcR protein gene, hindering their molting process, hence leading to increased potato yield.

Keywords : plant mediated RNAi, molecular strategy, ecdysone receptor, insect metamorphosis

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