Abstract

Pigeonpea [*Cajanus cajan* (L) Millsp], a high-quality protein-rich grain legume of semiarid tropics (SAT), is severely affected by lepidopteran insect pests. For sustainable resistance against this pests, chloroplast targeted synthetic version of the bioactive core component of a crystal protein (Syn cry1Ab) of *Bacillus thuringiensis* was expressed in pigeonpea under the control of green-tissue specific ribulose1, 5 bisphosphate carboxylase/oxygenase small subunit (*rbcS*) gene promoter. *Agrobacterium*-mediated transformed plants generated with the expression cassette (*cry1Ab-lox-bar-lox*) showed high insect mortality rate (90%) *in-vitro* against *Helicoverpa armigera* in the T_1 generation, indicating the insecticidal potency of Syn cry1Ab. Alongside, another vector with chimeric *cre recombinase* gene under the constitutive (2x35S) promoter was designed for the elimination of selectable marker *bar* (bialaphos resistance) gene. Crossing experiments were performed between T_1 plants with single insertion site of *cry1Ab-lox-bar-lox* T-DNA and one T_1 plant with moderate expression of cre recombinase with linked hygromycin resistance (*hpt*II) gene. Marker gene excision was achieved in hybrids with up to 35.71% recombination efficiency. Insect-resistant transgenic lines, devoid of the selectable marker (*syn bar + cre-hpt*II), were established in the subsequent generation through genetic segregation.

Key words: Pigeonpea, *In-vitro* culture, *Syn cry1Ab, Cre,* Marker-free transgenic pigeonpea