Abstract

An efficient and reproducible protocol for *Agrobacterium tumefaciens*-mediated transformation of chickpea (*Cicer arietinum* L.) cv. CM-2008 against pod borer using cry2Ac7 gene has been achieved in this study. Two methods were tested for successful genetic transformation of chickpea using *A. tumefaciens.* Tissue culture based *Agrobacterium*-mediated transformation and *in-planta Agrobacterium*-mediated transformation was followed and both of these methods were found effective. The plant tissue of choice in this study were cotyledonary nodes, nodes, embryo and callus derived from explants. Seeds were treated with 70% ethanol, 70% bleach, 5% acetic acid along with a few drops of Tween^{20} for surface sterilization. The seven days old seedlings were used for callus induction as well as for *Agrobacterium*-mediated transformation. Genetic transformation of chickpea CM-2008 was optimized by experimenting on various variables namely; type of plant tissue used, optical density of bacterial inoculum, infection time and co-cultivation duration. Four different ODs_{600} of bacterial inoculum *i.e.* 0.2, 0.5, 0.8, 1.0 were used to infect these explants. Infection time for plant material was 20 and 30 min and co-cultivation time was 2 and 3 days. Phosphinothricin was used to eliminate non-transformed plant tissues. Co-cultivated plants were incubated on selection medium containing 2 mg/l PPT for *in vitro* transformation. *In-planta* transformed plants screened by painting leaves with 2, 4, 6, 8 and 10 mg/l PPT solution. For molecular analysis, DNA was isolated from screened plantlets as well as from control plantlets. PCR analysis was conducted to detect the presence of cry2Ac7 gene in transformed plants. About 66% of transformation efficiency was obtained by infecting cotyledonary node with 0.8 OD_{600} under infection time of 30 minutes and 2 days of co-cultivation. *In-planta* infected plants showed maximum transformation efficiency of 80%.