ABSTRACT

The purpose of this research study was to optimize a reproducible protocol for *Agrobacterium*-mediated transformation of chickpea (*Cicer arietinum* L. cv. PB-2008) against pod borer using cry gene. Three different types of explants (cotyledonary node, node and shoot tip), embryo axes and one month-old callus cultures derived from these explant types were selected for *in vitro* transformation study. These were treated with bacterial inoculum (*A. tumefaciens* strain LBA4404) with optical density (OD_{600}) of 0.2, 0.5, 0.8 or 1.0 with infection period of 15, 20, 25 or 30 minutes. Potentially infected plant tissues were co-cultivated for 2 or 3 days in light at 26±2°C. After antibiotic treatment with cefotaxime, plant tissues were cultured on regeneration and selection medium for transgenic plantlets. *In planta* transformation was achieved through seeds sowing and germination in soil enriched with leaf litter under natural environmental conditions, these seedlings were infected with bacterial cultures at meristem and nodal regions through a fine, sterilized syringe needle. Following PPT screening, stable transformants were subjected to transgene analysis through PCR using cry gene specific primers. Results of the transformation study revealed that the combination of 0.8 OD_{600} with 3 days of co-cultivation and 25 min of infection caused cotyledonary node explant and embryo axes to respond optimally. Callus cultures showed recalcitrancy to regeneration. Molecular analysis of *ex vitro* grown and *in vitro* regenerated transgenic plants confirmed the transgene integration in genome of transformants. Eighty percent transformation efficiency was obtained with twenty percent escapee rate. Thus introduction of cry gene in chickpea through *Agrobacterium*-mediated plant transformation offers environment friendly control of chickpea crop insect pest such as pod borer, since the chickpea crop is self-fertilized.