Agrobacterium-mediated Transformation in Indian Cotton (*G. hirsutum*) Cultivar with cry1A(b) Gene and Regeneration by Direct Shoot Organogenesis

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Abstract: Genotype independent transformation and regeneration of Indian cotton (*G. hirsutum* L.) cultivars were carried out with *Bt-cry*1A (b) gene by Agrobacterium-mediation. Apical meristem of elite Indian cotton cultivar Anjali (LRK-516) and LRA-5166 were co-cultivated with *Agrobacterium tumefaciens LBA* 4404 carrying synthetic *Bt-cry*1A (b) + *npt*-II genes. Putative transformants were regenerated by direct shoot organogenesis in the selection medium containing 100μg · ml^{-1} kanamycin. Bacterial concentration, duration of co-cultivation, stage and size of tissues, concentration of selection marker in the medium, media composition and growth hormones, all have influence on transformation efficiency and frequency, were optimized in our protocol. Integration and expression of the *Bt-cry* gene was confirmed by PCR, Southern blot analysis and ELISA test respectively. Southern analysis indicated the presence of 3～5 copy of the gene number in the transformed plants. However the CRY protein expression was found to be very low (0.003%～0.004% of leaf protein) and insect bioassay shown less or no effective on *Helicoverpa armegira*. Nevertheless, this protocol may be used to produce genotype independent transformation and regeneration to produce transgenic cotton plants with other *cry* genes or any economically important genes.

Key words: *G. hirsutum*; *Agrobacterium tumefaciens*; Bt-*cry*1A (b); genotype independent transformation; regeneration; kanamycin; Apical meristem