DEVELOPMENT OF BEAN (*PHASEOLUS VULGARIS*) PLANT RESISTANT TO BEAN FLY (*OPHIOMYIA PHASEOLI*, BRAZHNIKOV) ATTACK BY THE TRANSFER OF *CRY* GENE FROM THE DIPTERAN TOXIC *BACILLUS THURINGIENSIS*

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ABSTRACT

Grain legumes provide a leading source of plant proteins for human consumption. Considering the importance of common bean (*Phaseolus vulgaris*) in human nutrition, efforts should be made for its genetic improvement by using tissue culture and molecular biological techniques. Engineering plants with the crystal protein gene of *Bacillus thuringiensis* is being widely done to produce insect resistant plants. The *in vitro* culture of bean variety Topcrop was done to develop a suitable regeneration protocol. Plants were regenerated from cotyledonary explants via organogenesis. Attempts to obtain callus regeneration were unsuccessful. The protein profiles of the standard Bt isolates, HD 133 ; Bt *Kurstaki* and the local Bt isolates Bt 6E ; Bt 4 showed similarity. The evaluation of the larvicidal activities of the Bt isolates showed that the local isolate was highly toxic to *Anopheles tessellatus*. The *cry* gene residing in the 3.8 kb *Dra* 1 fragment of Bt 6E was ligated to the binary vector PBI 121. The *E.coli* colonies transformed with the ligation mixture were selected on media containing 75 µg/ml of kanamycin. The non-radioactive random primed DNA labelling with digoxygenin-dUTP was used for labelling the 3.8 kb fragment of HD 133 and the hybrids were detected by enzyme immunology. The recombinant vector $PBIR_{12}$ was electroporated into the *Agrobacterium* strain AGL 1. The cotyledonary explants transformed with AGL 1 ($PBIR_{12}$) produced shoots on media containing 50µg/ml kanamycin and 12µM BA. The root induction media contained 2µM NAA and 75 µg/ml kanamycin. The rooted plantlets were analysed by dot blot analysis and callus induction assay. The hybridisation of the plant DNA to the DIG-labelled plasmid DNA of Bt 6E and the production of callus on MS media containing

0.02μM NAA, 0.01μM Kinetin, 0.01 μM 2,4-D and 100 μg/ml kanamycin confirmed the presence of *cry* gene in the transformed bean plants.