Agrobacterium -meditated Genetic Transformation of an Upland Cotton (*Gossypium hirsutum* cv Coker 310) Using a Novel Bt Gene *Cry 2 Ac*

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The development of transgenic cotton varieties resistant to bollworms has been a major success of applying plant genetic engineering technology to agriculture, evidenced by phenomenal increase in the cultivable area under (*B. thuringiensis*) Bt cotton in recent years worldwide. Of late, there are reports of insects developing resistance against the most commonly used Bt toxin *Cry1Ac*. Hence, there is an urgent need to broaden the source of resistance by employing new genes in order to reduce the chances of insects developing Bt resistance. Keeping this objective in view, cotton (*Gossypium hirsutum* cv Coker 310) plants expressing a novel insecticidal crystal protein *Cry2Ac* were developed in the present study. Embryogenic calli as explants derived from cotyledonary discs of Coker 310 were used to co-cultivate with *Agrobacterium* LBA 44O4 harboring the vector plasmid pCAMBIA2301, which contained the gene of interest, *Cry2Ac* (isolated from the local strain of *B. thuringiensis*), and the selectable marker gene *npt*II. Co-cultivation was carried out on MS medium supplemented with 100 M of acetosyringone under dark for 3 days. The co-cultivated embryogenic calli were later transferred onto selection medium (MS salts + 1.9 g • L^{-1} KNO_3 + 25 mg • L^{-1} Kanamycin) for somatic embryo induction. After three rounds selection, each at 15 days interval, kanamycin-resistant embryogenic calli were formed while non-transgenic calli turned brown and subsequently died. Upon further subculture on the same selection medium, the calli produced somatic embryos after 6 weeks, and they differentiated into globular, heart, and torpedo stages. Putative transgenic plants were recovered when the cotyledonary embryos were transferred onto the regeneration medium (MS + 0.1 mg • L^{-1} GA_3 + 1.0 mg • L^{-1} IAA) without selection. PCR analysis showed the amplification of the expected size of 800 bp for *Cry2Ac* gene confirming the presence of the gene in few transgenic lines tested. All the T_0 lines expressing *Cry2Ac* protein were subjected to generation advancement to obtain homozygous lines. Bioassay studies and molecular analyses are in progress to identity event-specific best performing transgenic lines.