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

TIHRUVENNGADAM V¹, RASIIMI J A¹, UDYASURIAN V², BALASUBRAMANIAN P¹, RAVEENDRAN T S¹
(1, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India; 2, Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India)

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 4404 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 nptII. 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 KNO₃, | 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 GA₃ + 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 T₂ 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.