Abstract

An efficient Agrobacterium mediated rice transformation protocol was found to be effective in case of genotype independent three different *indica* rice cultivars. Optimisation of the gene transfer protocol was finally carried out with EHA105 strain of Agrobacterium species and gus as the marker gene in Badshahbhog cultivar with different in vitro media formulations. The improved transformation efficiency of the protocol optimized in case of a Badshahbhog cultivar line was then utilised in raising a large number of transgenic rice lines with a new version of *cry1Ab* gene that was found to contain a single nucleotide change at the 668th position with respect to the standard cry1Ab gene of Bt berliner. The new cry1Ab gene was discovered by this laboratory and named as Cry1Ab (BR). The toxin contained a high potency for mortality against YSB/ Yellow Stem Borer with a lower LC₅₀ value than the reported Cry1Ab. This was considered to effect mortality to YSB at a low dose level. Such a potent toxin gene was consequently reconstructed with rice plant preferred codons and put under simultaneously a maize ubiquitin 1 promoter and a new rice ubiquitin 1 promoter to develop plant expression vectors pMBREF1 and pRBREF2 respectively. The rice ubiquitin 1 promoter was also developed by this laboratory and for the first time made to use. Embryogenic calli from matured seeds were cocultivated independently with supervirulent EHA105 A. tumefaciens strain harbouring the plasmid pMBREF1 or pRBREF2. The transformants were screened on the basis of resistance to hygromycin (50mg/l), the selection marker. A transformation efficiency of 17% could be obtained giving rise to 74 and 68 primary transformant lines in case of pMBREF1 and pRBREF2 respectively. Southern blot analysis provided evidence for stable integration of the cry1Ab (BR) gene with copy number varying from 1-3. The level of Cry1Ab (BR) toxin in the different transformants was found to be high, with the toxin titre ranging from 0.3-0.4% in case of pMBREF1 transformed lines and 0.6% in case of pRBREF2 transformed lines. Bioassay with some of the highly expressive lines were found to be toxic to the neonate larvae of YSB showing 80-100% mortality within 5 days of feeding on transgenic plant tissue. Thus, the transgenic insecticidal rice lines endowed with the highly potent cry1Ab (BR) toxin gene under the RUBO 1 promoter are likely to be quite efficient in resistaing the damages caused by YSB in the rice field.