RESEARCH ARTICLE

DEVELOPMENT OF SUGARCANE PLASTID TRANSFORMATION SYSTEM USING PARTICLE BOMBARDMENT

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Abstract: Chloroplast transformation has number of advantages over nuclear transformation like high-level of transgene expression, transgene containment and lack of gene silencing. The present work carried out to develop a chloroplast transformation protocol for sugarcane. Embryogenic calli of sugarcane variety Co86032 used as target tissue for transformation. Chloroplast specific transformation vector pZE39 having *NPTII* and *GFP* genes flaked by *trnG* and *pzbZ* of chloroplast sequence used for transformation. Selection of transformants were carried out at callus, shoot and rooting stages with Geneticin ranging from 25 to 75 mg/l during different selection cycles. Most of the regenerated shoots turned albino during selection. Among different bombardment parameters tested, rupture discs pressure at 1350 psi, distance between target tissue and stopping screen at 8 cm and expose of target tissue to light for 8 days before bombardment found prominent in producing more number of green and resistant plants on selection medium. Molecular analysis revealed that out of 146 plants tested, 44 plants are found PCR positive. Four of eleven PCR poitive plants showed positive by Southern hybridization and five of ten plants are showed positive signals for GFP. This is the first report on an attempt to develop a chloroplast transformation protocol for sugarcane.

Keywords: Chloroplast transformation, Co86032, NPTII, GFP, Particle bombardment

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