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Assessment of Conditions Affecting Agrobacterium -Mediated Soybean Transformation Using Glufosinate Selection in the Cotyledonary-Node Method

Alkesh Hada, Amit Kumar Gupta, Mansi Punjabi, Veda Krishnan, Monica Jolly, [2]Andy Ganapathi and Archana Sachdev

Division of Biochemistry, Indian Agricultural Research Institute (IARI), New Delhi, [2]Department of Biotechnology and Genetic Engineering, School of Biotechnology, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India

Abstract The production of transgenic plants is an essential component of many methodologies used to investigate the function of genes. The progress in both transgenic improvement and functional genomics research in soybean has however been constrained as it has been regarded as recalcitrant to transformation for many years. To develop an efficient gene transfer system, we investigated the parameters, including different hormone concentrations, co-cultivation time periods, pH of co-culture media, age of explants, types of genotypes and explants, optical density of *Agrobacterium* culture media, mechanical wounding of explants during the infection with *Agrobacterium.* The concentrations of selection agents the effect of pre-culturing of explants on the efficiency of transformation and the comparison of different *Agrobacterium* strains for efficiency of gene transformation were also evaluated. The present study provides a detailed procedure for *Agrobacterium*- mediated transformation of soybean using the cotyledonary node method with *bar* gene as a selectable marker coupled with glufosinate as a selective agent. The efficiency of transformation significantly increased from an average of 0.9% to 3.5 % by the combining strategies. Wounded cotyledonary-node explants were inoculated with *A. tumefaciens* carrying a super-binary plasmid and co-cultivated in the presence of mixtures of the thiol compounds, L-cysteine, dithiothreitol, and sodium thiosulfate. Transformed shoots showed elongation within six weeks of co-cultivation. Southern analysis confirmed the integration of the T-DNA into genomic DNA and revealed no correlation between the complexity of the integration pattern and the thiol treatments applied at co-cultivation. All T_0 plants generated were fertile and a majority of these lines transmitted the β-glucuronidase (GUS) phenotype in 3:1 ratio to their progenies.