عنوان مقاله:

(High-Efficiency Agrobacterium-Mediated Transformation of Tobacco (Nicotiana tabacum

محل انتشار:

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نویسندگان:

Reza Heidari Japelaghi - *Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tabriz University, Tabriz, Iran*

Raheem Haddad - *Department of Agricultural Biotechnology, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran*

Mostafa valizadeh - *Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tabriz University, Tabriz, Iran*

Ebrahim Dorani Uliaie - *Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tabriz University, Tabriz, Iran*

خلاصه مقاله:

To improve Agrobacterium-mediated transformation of tobacco, factors influencing gene delivery, including genotype of the plant, bacterial strain, and Agrobacterium transformation procedure, were tested via direct somatic embryogenesis. Leaf tissue of three different tobacco genotypes (Nicotiana tabacum L. cvs. Samsun, and Xanthi, and N. benthamiana) were used as explant. Leaf explants were transformed using three Agrobacterium tumefaciens strains (EHA105, GV3101, and LBA4404) harboring the binary vector pCAMBIA1304 using three different types of transformation methods as named Agro-inoculation, Agro-infection and Agro-injection. Selection of hygromycin resistant shoots was conducted on MS medium containing 3.0 mgL-1 BAP and 0.2 mgL-1 IAA, 250 mgL-1 cefotaxime and 30 mgL-1 hygromycin. Hygromycin resistant shoots were then rooted on MS medium supplemented with 250 mgL-1 cefotaxime and 15 mgL-1 hygromycin. The results indicated that A. tumefaciens strain LBA4404 was more effective in gene delivery than EHA105 and GV3101 and Agro-infection method proved to be significantly better than two other methods. The highest transformation rate was obtained with the Agrobacterium strain LBA4404 and Agro-infection method with approximately 72.80%, 84.57%, and 93.33% for N. benthamiana, Samsun and Xanthi, respectively. Histochemical GUS assay confirmed the expression of gusA gene in putatively transformed plantlets. PCR and RT-PCR analysis using gene-specific primers confirmed the integration of the gusA and hpt genes and the expression of the gusA and hpt genes, respectively. Furthermore, Southern blot analysis confirmed stable integration .of the gusA gene in selected T0 transformants

کلمات کلیدی:

Agrobacterium tumefaciens, Direct somatic embryogenesis, regeneration, Tobacco, transformation

لینک ثابت مقاله در پایگاه سیویلیکا: