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Agrobacterium tumefaciens-mediated Transformation of *Rosa hybrida* using the Green Fluorescent Protein (GFP) Gene

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Objectives

The goals of the present study were two fold. First to investigate the value of *gfp* as a reporter gene for localizing transgenic events to improve selection and recovery of transgenic rose plants. The second goal was to evaluate the effect of additional copies of *vir*E /*vir*G in *A. tumefaciens* LBA4404 on transformation efficiency in rose.

Materials and Methods

Maintenance of friable embryogenic tissues; Embryogenic calli was initiated from petioles according to the method of Marchant et al. (1996).

Agrobacterium-mediated transformation of embryogenic callus cultures; *Agrobacterium tumefaciens* strain LBA4404 containing the binary vector pBIN m-*gfp*-ER (Haseloff et al., 1997) was used. Additional copies of *vir*E/*vir*G were also introduced into *A. tumefaciens* LBA4404 containing pBIN m-*gfp*5-ER (Park et al. 2000). Transformation was performed as described by Firoozabady et al. (1994).

Visualization of GFP expression; For GFP expression, tissue was examined by observation under a fluorescence microscope (Axioskop I, Zeiss) using 475 nm excitation and 510 nm emission (Haseloff et al. 1997).

DNA isolation and southern hybridization analysis; Genomic DNA was extracted from leaf tissue (Paterson et al. 1993), and the isolated DNA was digested with *Hin*d III, separated on an 0.8% (wt/vol.) agarose gel, and then blotted onto a nylon membrane (Zeta-probe GT membrane, Bio-Rad, Hercules, CA) according to the manufacturers directions.

Results and Discussion

In conclusion, it was demonstrated that selection based on GFP expression can be utilized to identify and isolate transgenic plants of rose. The initial selective concentration of kanamycin required was lower than previously suggested for rose (Firoozabady et al. 1994; Marchant et al. 1998a). This study demonstrates that additional copies of *vir*E and *vir*G genes in *A. tumefaciens* enhance the transformation efficiency in rose. Plants are presently being grown to obtain seed to test the inheritance and stability of the transgenes in rose.

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