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Czech J. Genet. Plant Breed.

S.K., Manikandan R.:
The effect of different antibiotics on the elimination of *Agrobacterium* and high frequency *Agrobacterium*-mediated transformation of indica rice (*Oryza sativa* L.)

Czech J. Genet. Plant Breed., 48 (2012): 120-130

We report here the suitable explant with high efficiency of transformation and the positive effects of timentin over other antibiotics like carbenicillin and cefotaxime on the elimination of *Agrobacterium tumefaciens* during the genetic transformation of popular indica rice (*Oryza sativa* L.). The tissues

assayed were embryogenic calli, embryos with endosperm contamination, intact seeds, leaf blades, leaf bases and coleoptiles. The frequency of transient β -glucuronidase (GUS) expression as revealed by histochemical assay was 90% for embryogenic calli, which was the highest among the explants used. On the basis of disc-diffusion assay, the maximum zone of inhibition (29 mm) at 250 mg/l was obtained for timentin. In tissue culture conditions the frequency of *Agrobacterium* recurrence after 20 days of infection was minimum (2.3%) at 200 mg/l of timentin. At 250 mg/l of timentin there was no *Agrobacterium* growth, besides, there were no negative effects on the callus growth unlike other antibiotics, hence it was selected as the optimum concentration for high frequency callus proliferation and regeneration. The effect of the parameters evaluated was determined by the callus proliferation during selection, reduction in browning, transient GUS expression and stable transformation efficiency (23.3%). The resultant plants were stable transformants as confirmed by a molecular analysis of the *gus* and *hpt* genes. The developed

transformation protocol will be very helpful for the information on indica rice cultivars in general and on IR 64 in particular.

Keywords:

Agrobacterium-mediated transformation; *Agrobacterium* elimination; *gus* reporter gene; hygromycin phosphotransferase selectable marker gene (*hpt*); rice

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