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Application of co-transformation for *Choline oxidase* gene transfer into rice genome

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A B S T R A C T

In order to produce a marker-free transgenic rice with improved tolerance to salinity and drought stresses, expression vectors pABRII-Chl and pABRII-Cyt containing "*choline oxidase*" gene (with or without leader sequence respectively) were constructed from pChl and pCyt and pTRA132 for co-transformation. The pChl and pCyt vectors were digested with *Hin*dIII-*Bam*H and *Bam*HI-*Eco*RI enzymes. Then the resulting sequences were ligated and inserted into expression vector pTRA132, in which the *Hind*III-*Eco*RI fragment (*hph* gene) had been deleted. The constructs pABRII-Chl or pABRII-Cyt and pTRA132 (containing *hph* gene) were introduced into embryogenic calli derived from the mature seeds of a rice cv. Hashemi by biolistic transformation method. Then putative transformants were screened after 3 rounds of selection on N6 medium containing increasing concentrations of Hygromycin B from 60 to 80 mg/L. Finally, Hygromycin resistant calli were regenerated on MS medium supplemented with 50 mg/L Hygromycin B. Putative transgenic rice plants were analyzed by polymerase chain reaction PCR. Then, four of the transgenic plants were analyzed using Southern blotting. Each transgenic plant received one copy number of both *choline oxidase* and *hph* genes. Expression of the transgene was confirmed by reverse transcription PCR. The high frequency of transformation rate in this study showed that co-transformation method is a reliable method for stable transformation with the goal to make marker-free transgenic plants in subsequent steps.

Key Words

Choline oxidase, Glycine Betaine, Co-transformation, Rice, *hph* Gene, Biolistic.