Abstract:

In order to transform a marker-free transgenic rice with improved salinity and drought stresses tolerance, expression vectors contains "*choline oxidase*" gene named as pABRII-Chl and pABRII-Cyt (contained leader sequence and lacked, respectively) were constructed from pChl and pCyt and pTRA132 to be used in 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. In order to confirm accuracy of construction, recombinant plasmids which named as pABRII-Chl and pABRII-Cyt were analyzed by digestion and PCR analyses. The recombinant vectors (pABRII-Chl or pABRII-Cyt) and pTRA132 (containing *hph* gene) introduced into embryogenic calli derived from the mature seeds of a rice cv. Hashemi by biolistic transformation method. Then Putative transformant were screened after 3 rounds selection on N6 medium containing different concentration of hygromycin B that increased in each subculture from 60 to 80 mg/1. Finally, hygromycin resistant calli were regenerated on MS medium supplemented with 50 mg/l hygromycin B(decrescent from 60 to 80 mg/1). Putative transgenic rice plants gained were analyzed by PCR analysis. Then, 10 of transgenic plants were analysis by dot blot and Southern blot. It was shown that each transgenic plant received at least 1 copy number of both *choline oxidase* and *hph* gene. The high frequency of transformation rate in the study showed that co-transformation method is a reliable method for stable transformation and to make marker-free transgenic plants in subsequent steps.

Key words: ***choline oxidase,*** **Co-transformation, rice cv. Hashemi, *hph* gene, Biolistic.**

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Transformation of Rice with "Choline oxidase" Gene, Using Gold Nanoparticles

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