

Transformation of PsCIPK and PsCBL Genes in Indica Rice to Enhance Abiotic Tolerance

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Abstract—*Agrobacterium tumefaciens* mediated transformation of PsCIPK and PsCBL genes in six indica rice (*O. sativa* L.) were conducted to enhance abiotic stress tolerance. Seeds and seed derived calli from selected rice cultivars were co-cultured with *Agrobacterium tumefaciens* harboring PsCIPK and PsCBL genes that confer stress tolerance. For callus initiation, rice seeds were treated with different concentrations of 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and maximum calli were observed $82 \pm 2.29\%$ when N6 medium supplemented with 3 mg/L 2, 4-D in Kalijira. The fresh growing calli were selected using 50 mg/L kanamycin after co-cultivation and transferred to regeneration media. The highest regeneration frequency ($66 \pm 3.38\%$) was observed using N6 media supplemented with BAP 3.0 mg/L and NAA 1.5 mg/L from the fresh growing calli of Kalijira. Maximum GUS positive response ($88.8 \pm 2.25\%$) was found using 150 μ M acetosyringone from the transformed calli of Kalijira. However, the other cultivars used in the current study showed less response in terms of calli initiation rate, GUS positive response, and regeneration frequency than the Kalijira. In case of in planta transformation, the highest percentage ($58.3 \pm 0.5\%$) of transformed seeds was observed from mature seeds of Kalobiruin. The portion of transformed shoot and root were cut into pieces and their GUS positive activity were recorded as dark blue spots using microscope. PCR amplification of GUG gene (600bp) confirmed the transformation of PsCIPK and PsCBL genes. In vitro evaluation of salt and submerge tolerance capacity of the regenerated transgenic plantlets showed better resistance than the control.

Keywords: Rice, Callus, In planta transformation, GUS assay, PCR.