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Construction of rice endosperm specific expression cassette containing Glu-B1 promoter- GFP reporter-Nos terminator by overlap extension PCR

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Glutelins are major rice proteins expressed under endosperm specific glutelin promoters. Glu-B1 is such a promoter that has shown to be active in rice endosperm specific expression. Glu-B1 was cloned and characterized in a previous study as part of an on going project to express the wheat protein, glutenin in rice. The present study reports the construction of a Glu-B1-GFP-NosT expression cassette in view of evaluating the promoter activity of the isolated Glu-B1promoter of rice.

Fusion PCR was employed to combine rice Glu-B1 promoter gene with green fluorescent protein (GFP) gene containing nopaline synthase terminator. GFP gene is a reporter gene and was used to test the activity of the Glu-B1 promoter. Rice glutelin promoter (Glu-B1) was amplified from previously cloned Glu-B1 promoter. Reverse primer for Glu-B1 amplification was designed to contain 24 nucleotides of the 5' GFP sequence. GFP gene with nopaline synthase (Nos) terminator was amplified from the pCAMBIA 1302 vector using specific primers. The two amplified gene products were fused using overlap extension PCR. Amplification resulted in the fusion product (1350bp). The partial sequence of the fusion product (5'Glu-BI promoter and the 3' GFP –Nost region) was analysed with Clustaw multiple sequence alignment program. The 5' end (260bp) of the fusion product showed 93% similarity to Glu-B1 promoter and contained all potential cis-element motifs published for Glu-B1 promoter sequence (AACA-2 motif, GCN4 motif, A/G box, AACA-1, and TATA box). A 397 bp region obtained for the 3' end of the fusion product showed 98% similarity to the 3' end of the GFP gene containing the nopaline synthase 3'UTR sequence and the Poly A signal. The sequence analysis confirms the fusion of the two Glu-B1 promoter to the GFP reporter gene.

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