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Cloning and characterization of glutelin B1 promoter from rice - towards the development of wheat-like rice

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Rice flour does not have dough-making properties and hence vast amount of foreign exchange is spent on importation of wheat for production of bread and other bakery products. Development of wheat-like rice would be beneficial as a substitute for wheat flour. Glutelin genes are selectively expressed in the rice endosperm. Thus, the glutelin promoter is an ideal candidate to drive seed specific expression of trans-genes in rice. The objective of this study was to clone the minimal glutelin B1 (Glu B1) promoter from two varieties of *Oryza sativa* subsp. *indica*. The Glu B1 promoter of Bg 300 and Bg 350 varieties were initially amplified by polymerase chain reaction (PCR) using a set of primers(F1-5´CTCAAGCATAAGACGTTTATG3´ R1-5´CGCCATAGCTATTTGTACTTC3´) flanking the promoter region and followed by nested PCR (F2-5´GGGGAATTCACATA TTAAGAGTATGGACAGAC3´, R2-5´GGGGGATCCTTAAGCTAATGATGGGTTC3´) to amplify the minimal promoter of 262 bp. The PCR primers were designed based on the published Glu B1 promoter sequence of the *japonica* sub species. Single PCR-amplified products of the expected size were obtained for both *indica* rice varieties. Subsequently these fragments were cloned into pUC19 plasmid. Positive transformants were analyzed by colony PCR and Pvu II restriction digestion, which confirmed the presence of the Glu B1 promoter region. The PCR amplified fragment of Bg 300 was sequenced and a BLAST search was performed against the available sequences in the NCBI data base. This revealed approximately 82% sequence similarity to Glu B1 promoter sequence derived from *japonica* sub species. Further analysis of the obtained sequence revealed the presence of AACA, GCN4, PROL and ACGT motifs, which are conserved in many seed storage protein genes and are crucial for seed specific expression. These results indicate that the amplified sequence corresponds to the authentic Glu B1 promoter region from *Oryza sativa* subsp. *indica*. This cloned Glu B1 promoter will be used in a subsequent study to develop an expression vector to drive endosperm specific expression of wheat glutenin and gliadin trans genes in rice.

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