603/E2

Molecular cloning and in silico analysis of *Oryza sativa indica* rice endosperm specific GluB-1 promoter

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The rice seed system, as a bioreactor for the production of recombinant proteins has proven to be a success. To facilitate expression of foreign genes specifically in rice seed at a satisfactory level a strong promoter such as GluB-1 is required. The GluB-1 promoter region of *Oryza sativa japonica* has been successfully used to express foreign genes in rice seed in many previous studies especially in Japan. South Asian countries such as Sri Lanka mainly cultivate the *Oryza sativa indica* variety. The primary objective of this research was to clone the GluB-1 promoter from *Oryza sativa indica* and to carry out a bioinformatics analysis on the motifs.

Since the sequence of *Oryza sativa indica* GluB-1 promoter was unavailable, a region with high degree of homology to *Oryza sativa japonica* GluB-1 sequence was identified within the *Indica* genome. The sequence of this region was used to design primers and a 1.3 kb region of GluB-1 promoter of *Oryza sativa indica* was successfully amplified by PCR. The promoter region was then cloned into TOPO cloning vector PCR2.1. The presence of recombinant clone containing GluB-1 insert was confirmed by colony PCR and restriction enzyme digestion analysis. The cloned region of GluB-1 of *Oryza sativa indica* was sequenced and analyzed by using plant promoter analyzing PLACE online software. This analysis confirmed the presence of motifs required for seed specific expression such as ACAA, ACGT, PROL box and GCN4. The locations of core promoter elements such as TATA box were confirmed within the proximal region of the promoter. Interestingly *indica* promoter sequence contained four types of other motifs (SORLIP 1, SORLIP 2, NtBBF1, and MybSt1), an additional GATA box and E-box compared to *japonica* promoter region. Previous studies in seed specific promoters suggest that these motifs have the potential to increase the level of expression and confer tissue specificity. To our knowledge this is the first *in silico* analysis of the *indica* GluB-1 promoter. The cloned region in this study can be potentially used as a GluB-1 promoter to express transgenes in *indica* rice variety.

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