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MOLECULAR CLONING OF RICE ENDOSPERM SPECIFIC GLOBULIN PROMOTER AND THE 5' UNTRANSLATED REGION

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Rice seed, as biofactories for the production of useful recombinant proteins has proven to address the micronutrient deficiencies and human health requirements. Seed-specific promoters can be used to target transgene expression specifically in the seed. Globulin is a rice seed storage protein and the globulin content of the rice seed is approximately 12 % of the total seed storage protein. 26 kDa globulin promoter directs the endosperm specific expression of globulin protein which is the most abundant protein encoded by a single gene. Therefore globulin promoter is suitable for the expression of useful recombinant proteins in transgenic rice grain.

Globulin promoter and the 5' untranslated region (5'UTR) was amplified by Polymerase Chain Reaction (PCR) using primer FP: 5'-NNNCTGCAGGCCCGTGGCGCCTGGA-3' and primer RP: 5'NNNGGATCCAGACGACCTTGCTAGCCATTGATG-3'. Amplified region was designated as glb promoter. It was cloned into *pCR®2.1-TOPO®* vector. Colony PCR and restriction digestions were used to confirm the presence of the recombinant plasmid. The resulting plasmid was then sequenced and analyzed using bioinformatics tools. A search of non redundant nucleotide sequence data base at National Center for Biotechnology Information (NCBI) showed the highest homology of 99 % with *Oryza sativa indica* whole genome shotgun sequence (Accession number: AAAA02017563.1) and *Oryza sativa japonica* globulin gene promoter (Accession number: AC11332.2, AC130605.3, and D50643.1). A signal scan of contig globulin sequence obtained from sequencing was done using plant cis acting DNA regulatory elements (PLACE) and the presence of potential promoter motifs were confirmed. Purified glb promoter from *pst*I and *BamH*I digested recombinant *pCR®2.1-TOPO®* vector was successfully cloned in pCAMBIA1391Z producing a new recombinant plasmid called pCAMgpu and it was transformed into *Escherichia coli* DH5α strain. The presence of recombinant plasmid was confirmed by colony PCR and restriction digestions. After confirmation and genetic manipulation of recombinant pCAMgpu plasmid in *Escherichia coli*, it was successfully transformed into *Agrobacterium tumefaciens* GV3101 strain. Antibiotic resistance and Colony PCR were used to confirm the successful transformation of pCAMgpu plasmid.

These results revealed the successful cloning of the correct fragment of *Oryza sativa indica* 26 kDa globulin promoter along with the 5'UTR, into the pCAMBIA1391Z plant expression vector. The expression of a transgene in rice grain using cloned sequence will confirm the success of cloning.

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