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Construction of a fusion cassette for the introduction of a lysine rich gene into rice (*Oryza sativa* L.) genome

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Lysine is the least available amino acid among the eight essential amino acids needed for human growth and function. Protein deficiency disorders in human are particularly severe, especially in Asia where rice is the major staple food. One way to address this problem is enhancing both lysine and total protein content in rice by genetic engineering. The present study was carried out to increase total protein content including lysine in rice (*Oryza sativa* ssp *indica*) by introducing a pollen specific lysine-rich gene, *SBgLR*, from potato (*Solanum tuberosum*) under the control of a rice seed-specific globulin promoter. Genomic DNA was extracted from rice leaves (*Oryza sativa* ssp *indica*) and promoter region of globulin was amplified by PCR using globulin promoter-specific primers. The amplified product of 1024 bp was sub cloned into pGEM®-T Easy vector. Recombinants were screened by rapid screening method, colony PCR and restriction digestion. Sequencing was performed for further confirmation of 1024 bp fragment. Recombinant clone was cleaved with *Pst*1 and *Bam*H1 restriction enzymes and the resulting fragment was cloned into pCAMBIA1391Z for the construction of pCAMBIA1391Z-*Glb* recombinant vector. The lysine rich gene cloned previously (pCR®2.1-TOPO-*SBgLR*) was cleaved and cloned into pCAMBIA1391Z-*Glb* recombinant vector for the construction of pCAMBIA1391Z-*Glb*-*SBgLR* fusion cassette. Recombinants were identified and isolated. Current study is in progress to transform *Glb*-*SBgLR* fusion cassette to rice calli by *Agrobacterium* mediated gene transfer.

Key words: Globulin (Glb) promoter, *SBgLR* gene, *pCAMBIA1391Z* vector

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