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Cloning of soybean ferritin gene towards iron-fortification of rice

M D H Perera¹, N Govindaraj¹, K G W W Bandara², N V Chandrasekharan¹ and S G Senaratne²

¹*Department of Chemistry, Faculty of Science, University of Colombo, Colombo 03*

²*Industrial Technology Institute, 363, Bauddhaloka Mawatha, Colombo 07*

Iron deficiency is a severe problem encountered by around 30% of the world's population, especially in third world countries such as Sri Lanka. Most individuals suffering from iron deficiency are below the poverty line and depend on their food staples as the sole source of nutrition. Rice which is the staple food in Sri Lanka is deficient in iron. Increasing the iron content of rice by genetic engineering may help to solve the problem of dietary iron deficiency. Ferritin is an iron storage protein which stores upto 4500 iron atoms in the central cavity. The objective of this study was to increase the overall iron content of rice by increasing its ferritin content through transformation of soybean (*Glycine max*) ferritin gene to rice seeds. A seed specific globulin (Glb) promoter will be used to drive the expression of the ferritin gene.

Total RNA was isolated from 5 day old soybean cotyledons and cDNA was synthesized by reverse transcription using oligo(dT) primers. The synthesized cDNA was amplified using ferritin gene specific primers. The amplified product was purified and cloned in pCR®2.1-TOPO® vector. Recombinants were screened using colony PCR. Recombinant clones were also subjected to restriction digestion with EcoR I. A restriction fragment of expected size (800 bp) was observed, confirming the successful cloning of the ferritin gene. The clone was then sequenced and analyzed. A search of the non redundant nucleic acid database at National Center for Biotechnology Information (NCBI) with the cloned sequence revealed significant similarities to a number of sequences cloned previously (Acc. No. AK 245798.1, BT097559.1, EF05589.1). A soybean clone (Acc. No. BT097559.1) displayed the highest degree of homology both at the nucleotide (99%) and deduced amino acid levels (100%).

Currently, work is underway to clone the ferritin gene under the control of the seed specific globulin promoter in pCAMBIA1391Z for *Agrobacterium* mediated transformation into rice.