# Characterization and expression of wheat proteins gliadin and glutenins in rice seeds: towards the development of wheat-like rice

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# ABSTRACT

Despite being a major rice consuming country, Sri Lanka has a relatively high consumption of wheat. However, due to the climatic conditions, wheat cannot be grown successfully in Sri Lanka. Wheat flour has unique properties for making dough during bread making. Gluten in wheat flour gives the high elasticity and extensity required for bread making. Gluten is a complex protein containing gliadin, LMW and HMW glutenin. Genetic engineering in plants can be a useful technology to improve rice grain quality via increasing the desired recombinant protein production or introducing new recombinant proteins in rice seeds. The aim of this project was to express all three genes (gliadin, LMW and HMW glutenin) of wheat gluten in individual rice plants under the control of the glutelin B-1 promoter.

To clone gliadin, LMW and HMW glutenin genes for expression in rice, genomic DNA was extracted from wheat leaves of *Triticum aestivum* wheat cultivar Dacke and PCR amplified gene products were cloned, sequenced and analyzed. Of the five α- gliadin containing clones, two were functional and three were pseudogenes. One functional gliadin gene (Pgli 79) was a novel gene. Three LMW glutenin clones were identified and two were found to be pseudogenes. The functional LMW glutenin gene (pLMWGN) was found to be a novel LMW-i type. Five HMW glutenin genes containing clones were identified, of which four were pseudogenes. The functional HMW glutenin gene (HMWGAx) is a Ax2* type HMW glutenin gene. The secondary structures of the functional α-gliadins, LMW and HMW glutenin of wheat cultivar Dacke was also analyzed.

Glutelins are the most abundant storage proteins in rice grain. Among the glutelin promoters, glutelin B-1 (GluB-1) has been widely studied. Therefore, to analyze the activity of the GluB-1 promoter, three different lengths of the GluB-1 promoter sequence (350 bp, 1308 bp and 2300 bp) from the Sri Lankan rice variety Bg 250 (GluB-1 $_{Bg\ 250}$) were studied. The promoter constructs were separately cloned into binary vector pCAMBIA 1391Z harboring the β-*glucuronidase* (GUS) reporter gene. Putative transgenic plants were regenerated by the *Agrobacterium* mediated gene transformation method and confirmed by PCR using nopaline synthase (Nos) terminator primers. The transformed plants of all three GluB-1 $_{Bg\ 250}$ promoter constructs showed expression of the GUS gene in the endosperm of $T_0$ seeds. However, the GUS expression was comparatively higher in the 1308 bp GluB-1 $_{Bg\ 250}$ promoter construct than the 350 bp and 2300 bp constructs.

Hence, the functional gliadin, LMW and HMW glutenin genes were separately cloned into binary vector pCAMBIA 1391Z under the control of rice GluB-1 $_{Bg\ 250}$ promoter for expression in rice endosperm. The genes were transformed into rice calli using *Agrobacterium* mediated and *in-planta* transformation methods. Gliadin, LMW and HMW glutenin genes were successfully transferred into Bg 250 rice variety and seeds were harvested. Gliadin and LMW glutenin protein expression were confirmed by SDS-PAGE and western blotting by using a custom generated antibody. Expected size of gliadin (30 kDa) and LMW glutenin (42 kDa) protein were observed in the western blot analysis. This is the first report on the expression of α-gliadin in rice seeds. The presence of cysteine residues, a high number of alpha helices and glutamine residues would likely have a positive influence on the dough forming ability of transgenic rice flour.