Abstract

Oryza sativa essentially a major food crop has been used as food by a large population throughout the world. However, high glycemic index and low resistant starch content of processed rice often appear as an impediment to continuing its use as staple food for a large population; especially who are at the risk of diabetes type 2. To address this problem the present study was aimed at developing an improved variety of *O. sativa* var. Swarna with increased amylose through RNA interference of rice starch branching enzyme 3 (RBE 3) gene involved in starch biosynthesis pathway in order to facilitate increased resistant starch production after cooking Therefore, a fragment of *rbe* 3 gene of 540 bp was set apart and characterized by the presence of the conserved peptide motifs responsible for its substrate binding and catalytic activity in the derived amino acid sequence. Additionally, a minimal *GluB* 1 promoter of 264 bp with endosperm-specific motifs was isolated and its efficacy was determined. Introduction of RNAi inducing gene silencing cassette under *gluB* 1 promoter linked with hygromycin resistant gene through *Agrobaterium* mediated transformation in calli from mature embryos of rice resulted in the production of four transgenic lines as revealed by PCR amplification and Southern hybridization. The endogenous *rbe* 3 gene was found to be downregulated in varying quantum as revealed by northern hybridization as well as by its enzymatic activity in the independent transgenic. A direct correlation between down-regulation of RBE 3 and high apparent amylose content in rice was observed between transgenic lines with the highest increment of ~ 2 fold compared to control. Increase in amylose content in the transgenic lines resulted changes in granule surface morphology, granule size, types of crystal polymorph, gelatinization and retrogradation properties as well as the increment of resistant starch. Maximum increment in resistant starch was observed in $T_0$63 line by ~ 2.4 fold. Thus, the present study suggested that the suppression of *rbe* 3 gene is an efficient method to improve rice in terms of increased resistant starch production. Furthermore, the presently developed transgenic rice plants have the perspective to become useful for consumption by the people suffering particularly from diabetics and could be considered to have medical implication.

Keywords: *Oryza sativa*, amylose, crystallinity of starch, transgenics, resistant starch