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# High-expression of transgene in rice seeds via RNA silencing of the glutelin multigene family

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## Objectives

We have tried to augment recombinant protein by suppression of storage protein gene using RNAi and obtainedtransgenic glutelin RNAi rice.

## Materials and Methods

1. Material

Plant - Rice (Oryza sativa)

Agrobacterium strain - LBA4404

2. Methods

Plsmid construction - The homologous region(229bp)of glutelin multifamily genes was subcloned into RNAi vector, pMJC-SGH using gateway cloning system.

Transformation - Transformation experiments were performed using the rice cultiva Nakdongbyeo according to Fukuoka *et al.*

DNA and RNA gel blot analysis - Genomic DNA and total RNA were prepared from leaves and developing seeds (DAF 16), respectively. DNA and RNA gel blot analyses were performed according to Sambrook *et al.*

Reverse Transcriptase(RT) PCR - For nested reverse transcriptase PCR, cDNA was made from total RNA isolated at 16days after flowering using the SuperScript first-strand synthesis system(Gibco BRL). The number of PCR cycle was reduced to 13 that enabled quantification of mRNA.

## Results and Discussion

We have investigated if the expression a transgene(RFP) in rice seeds could be enhanced by RNAi of an endogenous seed storage protein(glutelin). The seeds of transformants having RFP gene and glutelin that was suppressed by RNAi showed brighter fluorescence than the seeds transformed with RFP gene only. DNA gel blot confirmed integration of RNAi vector for glutelin into the genome of transgenic rice plants. RNA gel blot analyses indicated that endogenous glutelin gene was severely suppressed in transgenic rice, whereas it was fully expressed in non-transgenic rice. Reverse transcriptase-mediated PCR revealed that *GluA* and *GluB* were suppressed by this approach. It suggests that RNAi technology for endogenous storage protein could be of great utility to favor high expression of transgene.

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