

PB-16

Verification of HIGS in Rice Blast Fungus and Application for Development of Anti-Fungal Rice

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[Introduction]

Host-Induced Gene Silencing (HIGS), has been successfully applied focusing on Western staple crops, including barely, corn and wheat. Although the importance of rice as food crop in Asia and potent feasibility of this technology in rice, the study of HIGS between rice blast fungus and rice is still remained passive. In this report, we established HIGS of rice blast fungus by visualizing silencing of fungal GFP expression in rice transgenic plants expressing *GFP* siRNAs. Moreover, we successfully applied this HIGS system into development of anti-fungal rice by suppressing various rice blast pathogenic genes.

[Materials and Methods]

Transgenic Arabidopsis and rice plants expressing small interfering RNAs (siRNAs) targeting fungal *GFP* and pathogenic genes, including *RGSI*, *MgAPT2*, and *LHS1*, were generated and challenged with rice blast fungus over-expressing *eGFP*, KJ201::*eGFP*. Suppression of fungal gene expression and disease symptoms were qualified by using confocal microscopy.

[Results and Discussions]

Transgenic Arabidopsis and rice plants expressing 35S::dsRNAi_ *eGFP* showed significant suppression of fungal *eGFP* expression during invasion, while apparent eGFP signal were observed in all fungal cells propagated on the leaves of negative control plants, suggesting successful HIGS in rice blast fungus. To develop the anti-fungal rice by using HIGS, we constructed dsRNAi vectors against a number of fungal pathogenic genes, such as *RGSI*, *MgAPT2*, and *LHS1*, and transformed into rice plants. Both 35S::dsRNAi_ *LHS1* and 35S::dsRNAi_ *MgAPT2* transgenic plants showed enhanced resistance to blast fungus, but, in contrast, 35S::dsRNAi_ *RGSI* plants showed hypersensitive phenotype compared to control plants. Moreover, developmental defects were observed in fungi infected in 35S::dsRNAi transgenic rices and these abnormal developments of blast fungi were identical to phenotypes of mutant fungi having the lesions in corresponding genes, suggesting the specific silencing of target genes. These results provide not only the evidences of HIGS in rice blast fungus but also the novel strategy for development of RNAi-based antifungal biotech-crops.

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