Development of sheath blight resistant transgenic rice by gene pyramiding

Abstract
Sheath blight, caused by the necrotrophic fungal pathogen *Rhizoctonia solani*, is the second most serious and destructive disease of the rice crop after blast. Screening of rice germplasm across the world failed to identify the rice variety resistant to *R. solani*. Thus conventional breeding could not be used to develop a rice variety for combating the disease and protect plants against yield loss. In this study, in order to improve sheath blight resistance, two different kinds of pyramided transgenic rice lines have been developed. The first pyramided rice line that overexpresses the rice oxalate oxidase 4 (*Osoxo4*) rice chitinase gene (*OsCHI11*) and second one that overexpress rice chitinase (*OsCHI11*) and Arabidopsis NPR1 (*AtNPR1*) gene. Transgenes were expressed under the control of two different green tissue specific-promoters: rice *P_{D54O-544}* and maize *P_{PEPC}*. Transgenic rice plants of each individual construct were screened via PCR, southern hybridization, activity assays, and expression analysis. Expression of PR genes increased significantly following pathogen infection in overexpressing transgenic plants. Following infection, transgenic plants exhibited elevated hydrogen peroxide levels, significant changes in activity of ROS scavenging enzymes and reduced membrane damage when compared to their wild type counterpart. In a *Rhizoctonia solani* toxin assay, a detached leaf inoculation test and an in vivo plant bioassay, transgenic plants showed a significant reduction in disease symptoms in comparison to non-transgenic control plants. Pyramiding of *OsCHI11* and *OsOXO4* genes have been done for the first time in a single vector background. It is evident from the study that green tissue-specific and simultaneous over expression of both the genes is able to degrade exogenous oxalate, and subsequently to produce H$_2$O$_2$ which activates plants defense system to enhance resistance of rice plant against the necrotrophic sheath blight pathogen *R. solani*. Co-expression of *OsCHI11* and *AtNPR1* genes in rice showed enhanced resistance by activating the SAR pathway related PR genes. It can be concluded from the study that green tissue-specific expression of *OsCHI11-AtNPR1* genes in rice is a promising strategy to enhance resistance against sheath blight pathogen without any concomitant phenotypic and agronomic cost.

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