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*S. Chen, L. Tao, L. Zeng, M. E. Vega-Sanchez, K. Umemura and G-L. Wang. 2006. A Highly Efficient Transient Protoplast System for Analyzing Defense Gene Expression and Protein-Protein Interactions in Rice. **Molecular Plant Pathology** 7(5): 417 – 428.*

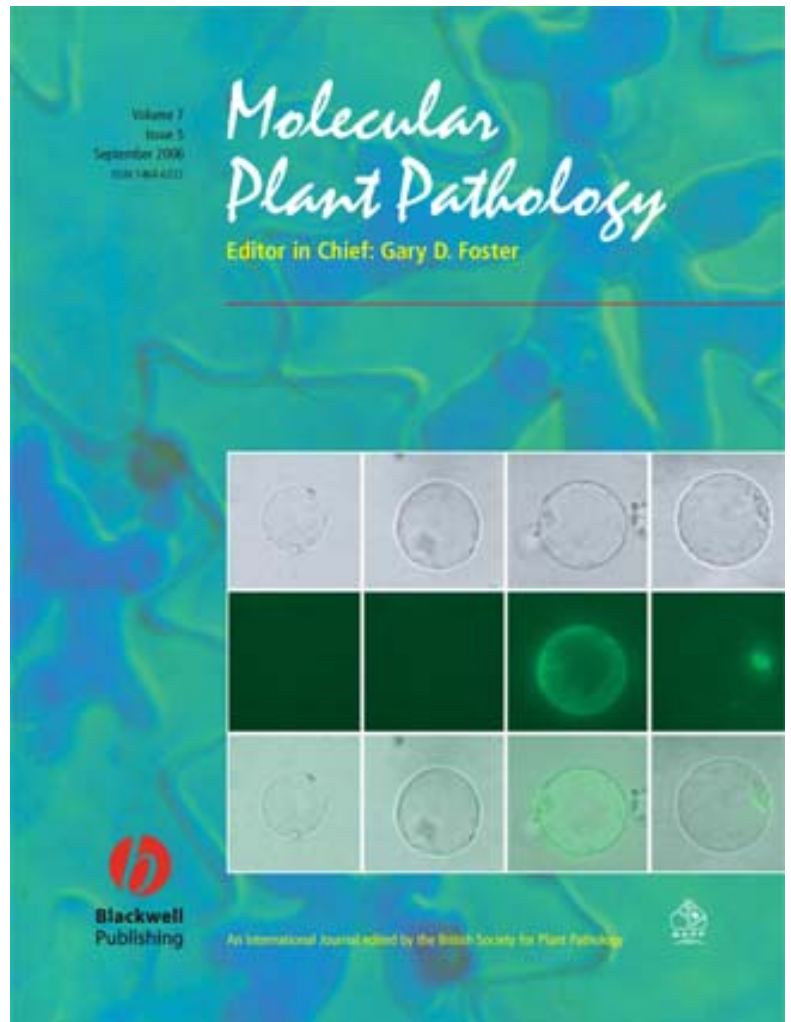
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housands of candidate genes for important agronomical traits, such as resistance to biotic and abiotic stresses, have been identified in crop plants by utilizing genome sequencing and expression analysis. One of the major challenges for plant biologists is to clarify the function of these agronomically important genes. Previous methods employed to reach this goal, including generation of knockouts and transgenic lines of target genes, are time-consuming and expensive. Transient assays provide a convenient tool to rapidly analyze the presumed function of the target genes. This method makes it possible to measure the biological effect of an expressed gene in cells immediately after transformation. In addition, transient expression of genes in a cell decays with time and is not inherited. With research support from the NRI Plant Genome Program, an improved transient expression method was developed for rapidly screening and characterizing candidate genes involved in defense signaling pathways. This process was applied to rice protoplasts. The researchers observed high-level co-expression of multiple genes and efficient suppression of exogenous genes, transgenes introduced into rice cells by transformation methods, and endogenous genes, those expressing from the rice genome. A transient green fluorescent protein (GFP) and a bioluminescent enzyme luciferase-based reporter system were established to analyze defense-related gene expression. A protoplast-based bimolecular fluorescence complementation (BiFC) system for the detection of protein-protein interactions in living rice cells was optimized. This system can now be used as an alternative to the yeast two-hybrid system for large-scale detection of protein-protein interactions in living plant cells. The application of these newly-developed methods will accelerate large-scale functional analysis of the completely sequenced genome of rice and other agronomically important crops, including corn, wheat, barley, and sorghum, to increase knowledge for crop improvement.

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