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Development of System-Wide Functional Analysis Platform for Pathogenicity Genes in Magnaporthe oryzae

Sook-Young Park^{1,2}, Jaehyuk Choi¹, Jaeyoung Choi¹, Seongbeom Kim¹, Jongbum Jeon¹, Seomun Kwon¹, Dayoung Lee¹, Aram Huh¹, Miho Shin¹, Kyungyoung Jung¹, Junhyun Jeon¹, Chang Hyun Kang³, Seogchan Kang⁴, and Yong-Hwan Lee^{1*}

¹Dept. of Agricultural Biotechnology, Fungal Bioinformatics Laboratory, Center for Fungal Genetic Resources, and Center for Fungal Pathogenesis, Seoul National University, ²Korean Lichen Research Institute, Sunchon National University, ²Dept. of Plant Biology, University of Georgia, USA, ³Dept. of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, USA

Null mutants generated by targeted gene replacement are frequently used to reveal function of the genes in fungi. However, targeted gene deletions may be difficult to obtain or it may not be applicable, such as in the case of redundant or lethal genes. Constitutive expression system could be an alternative to avoid these difficulties and to provide new platform in fungal functional genomics research. Here we developed a novel platform for functional analysis genes in Magnaporthe oryzae by constitutive expression under a strong promoter. Employing a binary vector (pGOF1), carrying $EF1\beta$ promoter, we generated a total of 4,432 transformants by Agrobacterium tumefaciens-mediated transformation. We have analyzed a subset of 54 transformants that have the vector inserted in the promoter region of individual genes, at distances ranging from 44 to 1,479 bp. These transformants showed increased transcript levels of the genes that are found immediately adjacent to the vector, compared to those of wild type. Ten transformants showed higher levels of expression relative to the wild type not only in mycelial stage but also during infection-related development. Two transformants that T-DNA was inserted in the promotor regions of putative lethal genes, MoRPT4 and MoDBP5, showed decreased conidiation and pathogenicity, respectively. We also characterized two transformants that T-DNA was inserted in functionally redundant genes encoding alpha-glucosidase and alpha-mannosidase. These transformants also showed decreased mycelial growth and pathogenicity, implying successful application of this platform in functional analysis of the genes. Our data also demonstrated that comparative phenotypic analysis under over-expression and suppression of gene expression could prove a highly efficient system for functional analysis of the genes. Our over-expressed transformants library would be a valuable resource for functional characterization of the redundant or lethal genes in M. oryzae and this system may be applicable in other fungi.

Keywords: the rice blast fungus, functional analysis, *Agrobacterium tumefaciens*-mediated transformation, over-expression, system development