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Functional Characterization of the Down-Regulated Genes in a *mat1-2*-Deleted Strain of *Gibberella zeae*, Using Microarray and Proteomics Analyses

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Gibberella zeae (anamorph *Fusarium graminearum*) is a homothallic (self-fertile) ascomycete with ubiquitous geographic distribution. This fungus is an important pathogen of wheat, barley, corn, and rice, causing disease symptoms such as head blight, scab, and stalk or ear rot. These diseases reduce grain yield and the harvested grain often is contaminated with mycotoxins, threatening to human and animal health. The importance of both ascospores (sexual spores) and macroconidia (asexual spores) produced by *G. zeae* in disease development had been recognized since the late nineteenth century. Especially, ascospore production caused by sexual development has been suggested to play important roles in completion of disease cycle of the head blight caused by this fungus. After over-wintering within the sexual fruiting body (perithecium) formed on plant debris, ascospores of *G. zeae* are forcibly discharged from the perithecium and initiate the primary infection in the next spring. Thus, a greater understanding of sexual reproduction in *G. zeae* is needed for a comprehensive disease control strategy.

Here we narrow down fungal sexual reproduction into the developmental processes genetically controlled by mating type locus (*MAT*), a single master regulator for mating. *MAT* loci, with two alternate idiomorphs (alleles) for heterothallic species, or with both idiomorphs for homothallic species are known to control the ability to mate (*i.e.* sexual reproduction) and ascospore/perithecium production in many filamentous ascomycetes. The *MAT* idiomorphs encoding confirmed or putative transcription factors are suggested to regulate the expression of genes specific to mating, via some signal transduction pathway. To identify the genes specifically controlled by the *MAT* locus during sexual development in *G. zeae*, we employed suppression subtractive hybridization between a *G. zeae* wild-type strain Z03643 and its isogenic self-sterile *mat1-2* strain T43ΔM2-2. Sequence analysis of 1,000 subtractive cDNA clones revealed 291 unigenes of expressed sequence tags. Both reverse northern and cDNA microarray analyses using these unigenes confirmed that 171 (58.8%) unigenes were significantly down-regulated in T43ΔM2-2, suggesting that the expression of these genes might be critical for sexual development

in *G. zeae*. Among these, 98 could be either manually or automatically annotated based on known functions of their possible homologs. A high frequency of the genes similar to those involved in signal transduction pathway, transcriptional regulation, protein degradation, and cellular differentiation indicates that many regulatory functions are required during the entire mating processes. Northern blot analysis revealed that all of the genes examined were down-regulated by *MAT1-2*, but needed for different stages during sexual development: specific to perithecial formation, vegetative stage, or no stage specific.

To select differentially expressed proteins under control of *MAT1-2* during the perithecial stage, the protein profiles of the strains Z03643 and T43ΔM2-2 were compared with each other by using two-dimensional electrophoresis. At least 13 different protein spots were differentially expressed between the two strains. Out of these proteins, 11 seemed to be involved in sexual stage. Especially, 2-nitropropane dioxygenase precursor, Hsp70, and cell division control protein 3 were found to be more abundant in the wild type strain. On the other hand, only two proteins, enloase (2-phosphoglycerate dehydratase) and UDP-N-acetylglucosamine pyrophosphorylase were found more abundant in the T43ΔM2-2.