

## Molecular Characterization of Pathogenesis-Related Genes in the Cereal Head-Blight Pathogen *Fusarium graminearum*

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*Fusarium graminearum* (teleomorph: *Gibberella zeae*) is a homothallic (self-ferile) ascomycete with ubiquitous geographic distribution. Sexual spores (ascospores) of the fungus which are produced on plant debris start to infect cereals under the favorable condition and cause disease symptoms such as scab, head blight, and stalk or ear rot. Recently severe epidemics of scab disease occurred in several countries including Korea and the United States, causing serious economic losses. In addition, *G. zeae* produces mycotoxins on diseased crops and has been a potential threat to human and animal health. Breeding the crops resistant to the disease has been an important subject. The conventional breeding has not been, however, successful because of no existence of true plant resistance. Therefore, the molecular breeding has been recently considered for development of cereal crops against this disease. Our goals are to massively isolate pathogenesis-related genes from the genome of *Fusarium graminearum*, the causal agent of *Fusarium* head blight of important cereal crops including rice using functional genomics and to select genes from the collection, which would be useful for construction of transgenic rice plants resistant to *Fusarium* head blight.

### Genetic diversity of *F. graminearum* isolated from rice in Korea

A total of 269 isolates were obtained from Southern provinces of Korea during 2001-2002. A phylogenetic tree of the isolates was constructed by using amplified fragment length polymorphism. Population structure of the rice isolates consists of a single lineage VI. Frequency of female fertility among these isolates was relatively low (37%) compared to that among lineage VII isolates from Korean corn. The chemical analysis revealed that most isolates (260) are nivalenol (NIV) chemotypes and 9 isolates are deoxynivalenol (DON) chemotypes.

### Pathogenesis-related genes from *F. graminearum* by insertional mutagenesis

The two representative strains (lineage VI and VII) were mutagenized using restriction enzyme-mediated integration (REMI). In total, 20,000 REMI transformants have been collected from the two strains. Out of 20,000 transformants screened, 115 strains have been selected as mutants defective for virulence, sexual development, or mycotoxin production. Molecular analyses revealed that 77% of the mutants examined carried a REMI vector at a single *Hind*III site of the genomes. BLAST analysis showed that 70% of the vector insertion sites examined were located within an ORF, or flanking regions (5' or 3') of an ORF. More than 30 mutations tagged with the

insertional vector, confirmed by a newly developed outcross method, have being analyzed for their specific roles in the pathogenesis by *F. graminearum*: functions of 13 genes have been determined so far.

### **Toxin biosynthesis genes involved in virulence**

We cloned and sequenced a *Tri13* homologs from DON and NIV chemotypes. The *Tri13* from a NIV chemotype was located in the trichothecene gene cluster whereas the *Tri13* from a DON chemotype carried several mutations. To confirm the roles of *Tri13* gene in trichothecene production, we altered toxin production in the two chemotypes. The targeted deletion of *Tri13* from the genome of NIV chemotype caused DON production rather than NIV. Heterologous expression of the NIV chemotype *Tri13* gene conferred on DON chemotype the ability to synthesize NIV. The functional analysis of the *Tri13* gene provides the first clear evidence for genetic basis of the DON and NIV chemotypes in *F. graminearum*. We also isolated genes encoding a type I polyketide synthase (PKS) and a putative laccase, all of which are required for aurofusarin biosynthesis. While screening insertional mutants of *F. graminearum* generated by REMI procedure, we selected a mutant (S4B3076) that was unable to produce aurofusarin. Genomic DNA region of the vector insertion site [a *HindIII* site 38 bp upstream the ORF encoding a putative laccase, designated *Gip8*] in S4B3076 was identified as contig 1.116 in the *F. graminearum* genome databases. On a 30 kb region of the insertion site, close to *Gip8*, are 10 more ORFs including putative transcription regulators and an ORF (designated *Gip5*), showing high similarity to the type I fungal PKS genes involved in pigment biosynthesis. Targeted gene deletion analysis confirmed that both *Gip8* and *Gip5* were responsible for aurofusarin production by *F. graminearum*.

### **Shifting fungal reproductive mode by manipulation of mating-type genes**

Fungi capable of sexual reproduction use heterothallic or homothallic mating strategies. In most ascomycetes, a single mating type locus, *MAT*, with two alternate forms (*MAT1-1* and *MAT1-2*) called idiomorphs, controls mating ability. In heterothallic ascomycetes these alternate idiomorphs reside in different nuclei. In contrast, most homothallic ascomycetes carry both *MAT1-1* and *MAT1-2* in a single nucleus, usually closely linked in *G. zeae*. We asked if *G. zeae* could be made strictly heterothallic by manipulation of *MAT*. Targeted gene replacement was used to differentially delete *MAT1-1* or *MAT1-2* from a wild type haploid *MAT1-1;MAT1-2* strain, resulting in *MAT1-1;mat1-2*, *mat1-1;MAT1-2* strains that were self-sterile, yet able to cross to wild type testers and more importantly, to each other. These results indicated that differential deletion of *MAT* idiomorphs eliminates selfing ability of *G. zeae*, but the ability to outcross is retained. They also indicated that both *MAT* idiomorphs are required for self fertility. To our knowledge, this is the first report of complete conversion of fungal reproductive strategy from homothallic to heterothallic by targeted manipulation of *MAT*. Practically, this approach opens the door to simple and efficient procedures for obtaining sexual recombinants of *G. zeae* that will be useful for genetic analyses of pathogenicity and other traits, such as ability to produce mycotoxins.

### **Two class V chitin synthase genes *FgChsv1* and *FgChsv2* are required for virulence and female fertility**

To study the genetic bases of sexual development by this fungus, a tagged mutation leading to no perithecia formation, which was generated by REMI procedure, was characterized. The REMI mutant showed altered phenotypes in other mycological traits such as reduced hyphal growth without apparent aerial hyphae, swelling of hyphal tip, and reduced conidiation. These phenotypes were, however, partially recovered when osmotic stabilizers were exogenously supplied. Moreover, this mutant showed reduced virulence toward on host plants. Molecular analysis revealed that an insertional vector integrated at a *HindIII* site within an ORF (*FgChsV1*) showing 48% amino acid identity to *F. oxysporum* class V chitin synthase gene (*ChsV*). Interestingly, another *ChsV* homolog (*FgChsV2*) was found 5kb upstream region of *FgChsV1*. Functional analysis using gene replacement strategy revealed that both *FgChsV1* and *FgChsV2* were responsible for the altered phenotypes including virulence (Fig. 2). Scanning probe microscopic analysis indicated that both genes were essential for cell wall rigidity, but *FgChsV1* contributed to cell wall rigidity more than *FgChsV2*.