SV-2

Isolation and characterization of pathogenesis-related genes from *Gibberella zeae* by insertional mutagenesis

Sung-Hwan Yun(1) and Yin-Won Lee(2)
(1) Division of Life Sciences, Soonchunhyang University, Asan, 336-745 Korea; (2) School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea.

Background

Gibberella zeae (anamorph: *Fusarium graminearum*) is a homothallic ascomycete with ubiquitous distribution. The fungus causes disease symptoms such as scab, head blight, and stalk or ear rot in corn, barley, wheat, and rice. In 1990s, severe epidemics of this disease occurred in several countries including Korea, Japan, and the United States, causing serious economic losses. Recently, the head blight of rice by the fungus was frequently occurred in southern provinces of Korea. In addition to economic losses, this fungus produces mycotoxins on diseased crops and has been a potential threat to humans and animals. The objectives of this study are to massively isolate pathogenesis-related genes from the genome of *G. zeae*, the causal agent of the *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 the *Fusarium* head blight.

Genetic diversity of *G. zeae* 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 *G. zeae* by insertional mutagenesis

The two representative strains (lineage VI and VII) were mutagenized using the restriction enzyme-mediated integration (REMI) procedure. 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 *Hin*dIII 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 *G. zeae*: functions of 13 genes have been determined so far.

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 *Hin*dIII 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. Scanning probe microscopic analysis indicated that both genes were essential for cell wall rigidity, but *FgChsV1*contributed to cell wall rigidity more than *FgChsV2*.

Hetrotrimetric G proteins transmit signals for regulating deveopment and virulence

We isolated three putative Ga subunits, designated as *FgGPA1*, *FgGPA2* and *FgGPA3*, respectively. Deletion of each Ga subunit affected pathogenecity, sexual development, and secondary metabolism such as mycotoxin production and pigmentation. The *FgGPA1* is homologous to the *fadA* gene in the model fungus *Aspergillus nidulans*, and to the *magB* in the rice blast fungus *Magnaporthe grisea*. The *FgGPA1*deletion mutant could not make fruiting body, leading to female sterility. Deletion of *FgGPA2*, which encodes *GanB* homolog of *A. nidulans*, resulted in normal perithecia formation but altered pigmentation and irregular colony margin. Furthermore, *FgGPA2* deletion mutant failed to cause disease on barley, indicating that *FgGPA2* plays an important role for pathogenicity. *FgGPA3* deletion resulted in decreased aerial hyphae, while perithecia formation seems to be greater than wild type, suggesting that the *FgGPA3* involves in activating hyphal growth but repressing sexual development.

Toxin biosynthesis genes involved in virulence.

We cloned and sequenced a *Tri13* homologs from DON and NIV chemotypes. Unlike the *Tri13* ORF of NIV chemotype, that of DON carried several mutations. To confirm the roles of the *Tri13* gene in trichothecene production, we altered toxin production in the two chemotypes by gene manipulation. 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 *G. zeae*. 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 *G. zeae* generated by REMI procedure, we selected a mutant (S4B3076) that was unable to produce aurofusarin. Genomic DNA region of the vector insertion site [a *Hin*dIII site 38 bp upstream the ORF encoding a putative laccase, designated *Gip1*] 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 *Gip1*, are 10 more ORFs including putative transcription regulators and an ORF (identified as *PKS12*), showing high similarity to the type I fungal PKS genes involved in pigment biosynthesis. Targeted gene deletion analysis confirmed that both *Gip1* and *PKS12* were responsible for aurofusarin production by *G. zeae*.

Conclusion

We have screened 20,000 REMI transformants, and 115 strains have been selected as mutants defective for virulence, sexual development, or mycotoxin production. We have determined the functions of 13 genes responsible for disease occurrence. The candidate genes for molecular breeding against the head blight fungus could be trichothecene-biosynthesis genes. Trichothecenes are known to be involved in virulence. Among the *Tri* genes, *Tri11* and *Tri12* are efflux pump gene and immunity gene, respectively. Transgenic rice resistant to head blight could be achieved by transforming these genes into rice.

References

Kim, H. S., T. Lee, M. Dawlatana, S.-H. Yun and Y.-W. Lee. 2003. Polymorphism of trichothecene biosynthesis genes in deoxynivalenol- and nivalenol-producing *Fusarium graminearum* isolates. Mycol. Res. 107: 190-197.

Kommedahl, T., and C. E. Windels. 1981. Root-, stalk-, and ear-infecting *Fusarium* species on corn in the USA. In: Nelson, P. E., T. A. Toussoun, R. J. Cook (eds) *Fusarium* diseases, biology, and taxonomy. The Pennsylvania State University Press, University Park, pp 94-103.

Lee, T., Y.-K. Han, K.-H. Kim, S.-H. Yun, and Y.-W. Lee. 2002 *Tri13* and *Tri7* determine deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae*. Appl. Eniviron. Microbiol. 68: 2148-2154.

Lee, J., T. Lee, Y. W. Lee, S. H. Yun, and B. G. Turgeon. 2003. Shifting fungal reproductive mode by manipulation of mating type genes: obligatory heterothallism of *Gibberella zeae*. Mol. Microbiol. 50:145-152.

McMullen, M., R. Jones, D. Gallenberg. 1997 Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.

O'Donnell, K., H. C. Kistler, B. K. Tacke, and H. H. Casper. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. USA 97: 7905–7910.

SV-3

Investigation of the molecular mechanisms involved in the resistance response during ripening stages of nonclimacteric pepper fruit against *Colletotrichum gloeosporioides*

Boung-Jun Oh

Jeonnam Biotechnology Research Center, Gwangju 501-180, Korea.

Fruit ripening represents a genetically synchronized system that involves developmental process unique to plant species (Giovannoni, 1993). The feature that ethylene hastens ripening distinguishes climacteric fruit such as apple and tomato from nonclimacteric fruit such as pineapple and strawberry, in which the progress of ripening appears to be independent of ethylene. Ripe fruits generally exhibit increased susceptibility to pathogen infection (Swinburn, 1983). However, in several nonclimacteric