



ANALYSIS OF THE NEWLY SEQUENCED *FUSARIUM* GENOMES USING PHI-BASE AND ONDEX. A. Beacham¹, C. Cavenet², J. Taubert², M. Urban¹, J. Antoniw¹, C. Rawlings² and K. Hammond-Kosack¹. ¹Department of Plant Pathology and Microbiology. ²Department of Biomathematics and Bioinformatics, Rothamsted Research, Harpenden, AL5 2JQ, UK. E-mail: kim.hammond-kosack@bbsrc.ac.uk

The genomes of four economically important plant pathogenic *Fusarium* species have recently been sequenced. Each pathogen causes a different disease, attacks a different range of host species and occupies different ecological niches both on the plant and in the general environment. Our aim is to compare the repertoire of genes which may confer a disease causing ability to each species. To achieve this we have used the contents of the Pathogen-Host Interactions Database (<http://www.phi-base.org/>; Winnenbergh *et al.*, *Nucleic Acids Research Database issue*, 2008) which contains information on experimentally verified pathogenicity and virulence genes for multiple species, combined with the data integration system called ONDEX (Koehler *et al.*, *Bioinformatics* 22: 1383-1390, 2006). This analysis has revealed pathogenicity genes and gene families which have either expanded or have been lost from each species as well as the extent of sequence relationships.

IDENTIFYING THE SECRETOME OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*. A. Chakrabarti, P. Dodds and J. Ellis. CSIRO-Plant Industry, Canberra, Australia. E-mail: apra-tim.chakrabarti@csiro.au

Fusarium oxysporum f. sp. *vasinfectum* (*Fov*) is the causal pathogen of vascular wilt disease in cotton. No major gene resistance against *Fov* occurs in cotton and consequently no avirulence genes are defined in the fungus. Nevertheless in an attempt to identify proteins involved in virulence of *Fov* we have undertaken a large scale analysis of the genes expressed during the process of infection. Full length cDNA libraries have been prepared from *Fov* infected cotton root and stem tissues showing visible external symptoms of the disease. The libraries are presently being screened for the presence of *Fov* specific genes expressed during infection. Differentially expressed cDNAs will be subjected to bioinformatic as well as molecular analysis to confirm fungal origin. Subsequent analysis of the differentially expressed full length cDNAs will help us to predict secretory proteins that constitute the infection stage 'secretome' of *Fov* that may act as effector proteins important in virulence and host range. Findings from these experiments will be presented.

TAGGING PATHOGENICITY GENES IN *FUSARIUM GRAMINEARUM* USING THE TRANSPOSON SYSTEM *MIMP/IMPALA*. M. Dufresne¹, T. van der Lee², S.B. M'Barek^{1,2}, X. Xu^{2,3}, X. Zhang^{2,4}, T. Liu^{1,5}, C. Waalwijk², W. Zhang^{2,5}, G.H.J. Kema² and M.J. Daboussi¹. ¹Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay Cedex, France. ²Plant Research International B.V., P.O. Box 16, 6500 AA, Wageningen, The Netherlands. ³Plant Protection Institute, Liaoning Academy of Agricultural Sciences, Shenyang, 110161, China. ⁴Institute of Genetics, Jiangsu Academy of Agricultural Sciences, Zhongling Street 50, Nanjing, Jiangsu 210014, China. ⁵State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agriculture Sciences, Beijing 100094, China. E-mail: theo.vanderlee@wur.nl

The number of predicted genes present in the genome of *Fusar-*

ium graminearum is estimated to be around 11,640. For many genes the function is yet unknown and consequently there is a need for a high-throughput method for functional analyses of genes. We applied a transposon mutagenesis strategy using a MITE (*mimp1*) activated *in trans* (or mobilized) by a transposase (*impala*) (Dufresne *et al.*, *Genetics* 175: 441-452, 2007) to obtain a large collection of transposon mutants. Using TAIL-PCR sequences and the annotated *F. graminearum* genome, we show that *mimp1* reinserted close to or within genes in 53% of the mutants. A collection of 331 mutants derived from *F. graminearum* isolate Fg820 was screened for growth on a wide set of media, for pathogenicity on wheat and for perithecia development. One mutant displayed altered growth characteristics, ten mutants showed altered virulence phenotypes and 11 were impaired in perithecia development. In one of these mutants (Fg820-6-11-r112) *mimp1* reinserted into an ORF encoding a transcription factor. The wild-type phenotype could be restored by complementation with a non-disrupted copy of the gene proving that the observed mutant phenotype is caused by the *mimp1* insertion. Our results indicate that *mimp1* is a powerful mutator that can be exploited for high-throughput analysis of *F. graminearum* and potentially other ascomycete fungi.

HIGHLY EFFICIENT SINGLE STEP CONSTRUCTION OF VECTORS FOR TARGETED GENOME MODIFICATIONS IN FILAMENTOUS FUNGI BY USER FRIENDLY CLONING. R.J.N. Frandsen, M. Bylov Kristensen, J.A. Andersson and H. Giese. Department of Ecology, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark. E-mail: raf@life.ku.dk

Functional genetics in filamentous fungi have always been dependent on the isolation or construction of mutant strains, either loss or gain of function. Targeted genome modifications, such as gene replacement or *in locus* overexpression, is typically achieved by double homologous recombination, between the genome target and an introduced recombinant DNA molecule. Where the recombinant DNA molecule contains a selection marker gene surrounded by two homologous recombination sequences, identical to sequences surrounding the target locus in the genome. Such DNA molecules are in general constructed by two consecutive directional cloning steps, each followed by screening and verification, which is a very laborious and time consuming process. We have therefore developed a new vector system that allows for single step construction of vectors for targeted modification, thereby reducing construction time to three days and removing half of the required screening and verification work. The vector system is dependent on the Uracil-Specific Excision Reagent cloning technology (USER Friendly™), which in its commercial version offers efficient directional cloning of a single PCR amplicon. However, our research shows that USER Friendly cloning also can be used for the simultaneous directional fusion of two PCR amplicons with two vector fragments, with an efficiency of 85%, thus allowing for single step construction of replacement vectors. In addition to the increased speed and reduced workload, the single step construction strategy also offers greater freedom of operation with respect to the placing of the homologous recombination sequences in the genome, as it is independent of restriction enzymes during cloning.

IDENTIFICATION OF THE GENE CLUSTER AND PATHWAY SPECIFIC TRANSCRIPTION FACTOR RESPONSIBLE FOR PERITHECIUM PIGMENT BIOSYNTHESIS IN *GIBBERELLA ZEAE*. R.J.N. Frandsen¹ and H. Giese². ¹Department of Ecology, Faculty of Life Science, Copenhagen University, Thor-



valdsensvej 40, opg.2, 1871 Frederiksberg C, Denmark. ²Faculty of Agricultural Sciences, Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark. E-mail: raf@life.ku.dk

The members of the genus *Gibberella* are characterized by their dark blue to black gibbous shaped perithecia, which can be observed in nature protruding from infected plants or plant debris. The function and chemical structure of the dark pigment is unknown; however previous studies have shown that its biosynthesis is dependent on *Fg-PKS3* (*Fg-PGL1*). In the current study we have identified a gene cluster, consisting of six genes, responsible for the biosynthesis of the pigment. The cluster consists of *PGL1*, *pglM* (monooxygenase), *pglJ* (O-methyltransferase), *pglV* (short chain dehydrogenase), *pglX* (zinc dependent oxidoreductase) and *pglR* (binuclear zinc cluster transcription factor). The genes are co-expressed during perithecium development (Affymetrix GeneChip data). Ectopic overexpression of *pglR* results in a constitutive overexpression of the six genes in vegetative mycelium and production of a chestnut coloured water soluble pigment. Overexpression of *PGL1* results in yellow water soluble pigment. Targeted replacement of the enzyme encoding genes results in a loss of production of the wild type perithecium pigment. The cluster encoded enzymes does not show homology to the enzymes previously described for DHN melanin biosynthesis in other fungal species, indicating that the *Gibberella* pigment is not DHN melanin. The gene cluster is conserved in *G. zeae*, *G. moniliformis* and *G. verticillioides*. This experiment shows that overexpression of pathway specific transcription factors allows for the biosynthesis of polyketides, which normally are only produced under very specific environmental or developmental conditions, allowing for the production of novel metabolites which can be chemically characterized and tested for bioactivity.

FUNCTIONS OF THE SEX PHEROMONES OF *GIBBERELLA ZEA*. J. Lee¹, J.F. Leslie¹ and R.L. Bowden². ¹Department of Plant Pathology, Kansas State University, Manhattan, Kansas 66506-5502, USA. ²USDA-ARS Plant Science and Entomology Research Unit, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506-5502, USA. E-mail: robert.bowden@ars.usda.gov

In heterothallic ascomycete fungi, idiomorphic alleles at the *MAT* locus control two sex pheromone/receptor pairs that function in recognition and attraction of strains with opposite mating types. In the ascomycete *Gibberella zeae*, the *MAT* locus is rearranged such that both alleles are adjacent on the same chromosome. Strains of *G. zeae* are self-fertile, but they can outcross facultatively. Our objective was to determine if pheromones retain a role in sexual reproduction in this homothallic fungus. Putative pheromone precursor genes (*ppg1* and *ppg2*) and their corresponding pheromone receptor genes (*pre2* and *pre1*) were identified in the genomic sequence of *G. zeae* by sequence similarity and microsynteny with other ascomycetes. *ppg1*, a homolog of the *Saccharomyces* α -factor pheromone precursor gene, was expressed in germinating conidia and mature ascospores. Expression of *ppg2*, a homolog of the α -factor pheromone precursor gene, was not detected in any cells. *pre2* was expressed in all cells, but *pre1* was expressed weakly and only in mature ascospores. Deletion mutations $\Delta ppg1$ or $\Delta pre2$ reduced fertility in self-fertilization tests. $\Delta ppg1$ reduced male fertility and $\Delta pre2$ reduce female fertility in outcrossing tests. In contrast, $\Delta ppg2$ and $\Delta pre1$ had no discernable effects on sexual function. $\Delta ppg1/\Delta ppg2$ and $\Delta pre1/\Delta pre2$ double mutants had the same phenotype as the $\Delta ppg1$ or $\Delta pre2$ single mutants. Thus, one of the putative pheromone/receptor pairs (*ppg1/pre2*) enhances, but is not essential for, selfing and outcrossing in *G. zeae*, whereas no functional role was found for the other pair (*ppg2/pre1*).

AN UPDATE OF THE GENETIC MAP OF *GIBBERELLA ZEA*. J.F. Leslie¹, J. Lee¹, J.E. Jurgenson² and R.L. Bowden³. ¹Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, Kansas 66506-5502, USA. ²Department of Biology, University of Northern Iowa, Cedar Falls, Iowa 50614, USA. ³USDA-ARS Plant Science and Entomology Research Unit, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506-5502, USA. E-mail: jfl@ksu.edu

We previously published a genetic map of *Gibberella zeae* (*Fusarium graminearum sensu lato*) based on a cross between Kansas strain Z-3639 (lineage 7) and Japanese strain R-5470 (lineage 6). In this study, that genetic map was aligned with the third assembly of the genomic sequence of *G. zeae* strain PH-1 (lineage 7) using seven structural genes and 108 sequenced AFLP markers. Several linkage groups were combined based on the alignments, with the nine original linkage groups reduced to six, and the total size of the genetic map reduced from 1286 to 1140 cM. Nine supercontigs, comprising 99.2% of the genomic sequence assembly, were anchored to the genetic map. Eight markers (four from each parent) were not found in the genome assembly and four of these markers were closely linked, suggesting that > 150 kb of DNA sequence is missing from the PH-1 genome assembly. The alignments of the linkage groups and supercontigs yielded four independent sets, which is consistent with the four chromosomes reported in this fungus. Two proposed heterozygous inversions were confirmed by the alignments; otherwise colinearity of the genetic and physical maps was high. Two of four regions with segregation distortion were explained by the two selectable markers employed in making the cross. Average recombination rates for each chromosome were similar to those previously reported for *G. zeae*. Despite an inferred history of genetic isolation between lineage 6 and lineage 7, their chromosomes remain homologous and are capable of recombination along their entire lengths, even within the inversions. This genetic map can now be used in conjunction with the physical sequence to study phenotypes, e.g., fertility and fitness, and genetic features, e.g. centromeres, and recombination frequency, that do not have a known molecular signature in the genome.

THE TRANSCRIPTION FACTOR FGSTUA INFLUENCES SPORE DEVELOPMENT, PIGMENTATION, PATHOGENICITY AND TRICHOECENE PRODUCTION IN *FUSARIUM GRAMINEARUM*. E. Lysøe¹, M. Pasquali², S.S. Klemsdal¹ and H.C. Kistler^{2,3}. ¹Bioforsk-Norwegian Institute of Agricultural and Environmental Research, Ås, Norway. ²Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA. ³USDA ARS Cereal Disease Laboratory, St. Paul, MN 55108. E-mail: hckist@umn.edu

Members of the APSES family of fungal proteins regulate morphogenesis and virulence in ascomycetes. We deleted the *FgStuA* gene in *Fusarium graminearum* and demonstrate its involvement in several different processes. *FgStuA* is closely related to *FoStuA* in *F. oxysporum* and *StuA* in *Aspergillus*. Unlike *FoStuA* mutants in *F. oxysporum*, the *FgStuA* mutants were greatly reduced in pathogenicity both on wheat and apple slices. Reduced pathogenicity may be due to decreased levels of trichothecene production (<1% the levels of wild-type). Mutants were also diminished in growth and the aerial mycelium becomes more "wettable" than the wild-type. Several putative hydrophobin genes were shown to be highly down-regulated in the mutant, which may explain the loss of hydrophobicity. *FgStuA* mutants also were greatly reduced in asexual sporulation and produced no perithecia. Microarray analysis during conditions when the wild-type

produced asexual spores and the mutant produced no spores, showed that genes encoding several groups of cell-wall related proteins such as chitinases, glycanases and GPIs, mostly were down-regulated in the mutant. The *FgStuA* mutant has a white phenotype compared to the red wild-type, and 17 continuous genes, including the known aurofusarin genes, were virtually completely turned off in the mutant. Also eight continuous genes, including genes encoding a putative PKS-NPS hybrid, were found to be down-regulated in the mutant during "spore-production". In general it seems like the *StuA* protein in *F. graminearum* function as an activator of the genes discussed here.

EXPRESSION OF DEFENCE-RELATED GENES IN FOUR BANANA CULTIVARS INFECTED WITH *FUSARIUM OXYSPORUM* f. sp. *CUBENSE*. C. Munro¹, A.A. Myburg², N. van den Berg¹ and A. Viljoen^{1,3}. ¹Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002, South Africa. ²Department of Genetics, FABI, University of Pretoria, Pretoria 0002, South Africa. ³Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa. E-mail: alius@sun.ac.za

Bananas are an important source of nutrition for millions of people worldwide. The crop, however, is highly vulnerable to diseases such as Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Genetic improvement offers the most sustainable means to control Fusarium wilt. In recent years, biotechnological approaches for developing disease resistant banana cultivars have become popular, as conventional breeding with this crop has many obstacles. Elucidating factors related to defence mechanisms, thus, has become an important aspect for improving existing high-yielding but susceptible cultivars. In this study, gene expression profiles were investigated in the highly susceptible Cavendish cultivar "Williams", the tolerant hybrid FHIA 17 and the resistant varieties Rose and Calcutta IV. After root inoculation, RNA was collected 0, 6 and 72 h post inoculation for cDNA Amplified Fragment Length Polymorphism (AFLP) analysis. cDNA-AFLP fragments differentially expressed were selected based on their up-regulation in the resistant and tolerant plants and not in the susceptible plants. Transcript-derived fragments (TDFs) were then excised from the polyacrylamide gels and identified. Genes isolated included those with an unknown function and identity, general metabolic and photosynthetic functions, but some had functions which could be involved in the Fusarium wilt defence response. These include genes associated with cell wall strengthening such as *S-adenosylmethionine synthase* (SAMS) and *isoflavone reductase*, as well as transcription factors such as a putative *WRKY6* and *bZIP*. Quantitative reverse-transcriptase real-time PCR (qRT-PCR) confirmed the expression patterns of selected TDFs on the cDNA-AFLP gels.

THE USE OF SSR MARKERS TO PREDICT FUSARIUM HEAD BLIGHT RESISTANCE IN CHOSEN SPRING BARLEY GENOTYPES. Z. Nesvadba¹, I. Jeziskova², I. Polisenka¹ and L. Tvaruzek¹. ¹Agrotest Fyto, Ltd., Havlickova 2787, 76701 Kromeriz, Czech Republic. ²Faculty Hospital Ostrava, Medical Genetics Ward, 17. Listopadu 1790, 70852 Ostrava, Czech Republic. E-mail: nesvadba.zdenek@vukrom.cz

A set of 60 microsatellites was used to test the genetic diversity of parental genotypes of spring barley with declared resistance or susceptibility to FHB. The analysis of parental genotypes identified

four alleles at three loci of microsatellite markers that were specifically amplified only in the group of the genotypes with declared susceptibility (Bmag0353 and Bmag0382) or declared resistance to FHB (Bmag0382 and EBmac0806). Twenty-three DH lines were examined for the presence of the allelic variants of 112 bp and 116 bp at the locus of the marker Bmag0382 (chromosome 1H), 124 bp allele of Bmag0353 (4H) and 176 bp allele of EBmac0806 (6H). For 14 of them markers predicting FHB susceptibility were found in DH lines with paternal genotypes PI383933 and Foster. The presence of markers predicting resistance to FHB was confirmed in DH lines with maternal genotypes PEC210 and Zao Zhou3. The identified SSR marker EBmac0806, which was specifically amplified only in parental genotypes with declared resistance to FHB, is localised at the 119 cM distance based on the linkage map of chromosome 6H. It is in the region, where Ma et al. (2000) identified one of QTLs for resistance to FHB and also a QTL for low DON concentration in kernels. SSR marker Bmag0382, is localised on chromosome 1H at the distance of 97 cM. Ma et al. (*Phytopathology* 90: 1079-1088, 2000) have identified the QTL for low DON concentration in kernels in this region as well.

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GENE EXPRESSION PROFILING AND FUNCTIONAL ANALYSIS OF SPORE GERMINATION IN *FUSARIUM GRAMINEARUM*. M. Pasquali, K.Y. Seong, Y. Dong, U. Güldener and H.C. Kistler. Department of Plant Pathology, University of Minnesota and USDA-ARS Cereal Disease Laboratory, St. Paul, MN USA. E-mail: matias.pasquali@gmail.com

A full genome study on conidia and ascospores has been carried out using *F. graminearum* Affymetrix GeneChips to compare gene expression during germination in complete medium at 0, 2, 8, 24 h and after 10 days of drought stress. The majority of genes that are annotated with known function have a common expression pattern in both conidia and ascospores. Genes involved in primary metabolism and energy production are expressed similarly in conidia and ascospores, indicating shared fundamental biological processes during germination of these different spore types. Interestingly the greatest differences in gene expression between conidia and ascospores were found upon desiccation. Desiccated ascospores contained 6,801 ($p < 0.001$) expressed genes, a surprisingly high number (similar to the other germination stages) compared to the 2,915 genes expressed in desiccated conidia ($p < 0.001$). Gene expression changes reflect, and to a certain degree, probably determine differences in the biological behaviour of the spore types: desiccated ascospores are more viable and pathogenic on wheat than conidia after drought stress. Moreover expression profiles of ascospore and conidial germination showed some unique patterns of genes (some likely determinative of particular developmental states for each spore type). To identify genes with functional consequence in spores, a transcription factor (*FgStuA*) was observed to be constitutively expressed in all stages, but in ascospores transcript levels were at least 2 fold more than in conidia. Site-specific mutagenesis showed that *FgStuA* has a crucial role in both conidiation and perithecial development as well as in other cellular activities.

A COMPREHENSIVE PHOSPHOPROTEOMICS APPROACH TO IDENTIFY REGULATORY MECHANISMS OF DEOXYNIVALENOL SYNTHESIS IN *FUSARIUM GRAMINEARUM*. C. Rampitsch¹, G. Subramaniam² and S. Djuric-Ciganovic¹. ¹*Cereal*

Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg MB, Canada. ²Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa Ontario K1A 0C6, Canada. E-mail: crampitscb@agr.gc.ca

The steps comprising the synthesis of deoxynivalenol (DON) and its derivatives by *Fusarium graminearum* are well understood, but its regulation at the molecular level is not. We are investigating a potential role for protein phosphorylation in initiating DON synthesis during nitrogen starvation *in vitro*. Multidimensional separation and analysis ('GeLCMS') was used to probe the phosphoproteome of *F. graminearum* after the onset of DON synthesis. Proteins were first separated by SDS-PAGE; following a trypsin digest, peptides were extracted from gel slices and phosphopeptides enriched from these by immobilized metal affinity nano-scale chromatography. Enriched samples were analyzed by LC-MS using a tandem mass spectrometer performing neutral loss scans on the most intense ions in the eluant to facilitate the detection and identification of phosphopeptides. The Mascot search engine was used to query the *F. graminearum* database for the identification itself. Fifty-three unique phosphopeptides were identified in 33 samples from *F. graminearum* grown on nitrogen-poor media at t = 0, 6 h and 12 h (11 gel slices per time-point). Phosphorylation sites were assigned to all of them and confirmed manually by observing the presence of a neutral loss of 98 in the mass spectra. The peptides were from proteins involved in the regulation of protein synthesis, general metabolic enzymes, biosynthetic enzymes and proteins of unknown function. Many contained consensus kinase sequences. The biological role of some of these proteins in regulating DON synthesis will be assessed *in vivo* by producing *F. graminearum* mutants and measuring both their virulence and ability to produce DON.

NONHOMOLOGOUS END JOINING AND DNA REPLICATION PARTICIPATE IN DNA INTEGRATION DURING TRANSFORMATION OF *FUSARIUM GRAMINEARUM*. R.J. Watson, S. Burchat and J. Bosley. Research Branch, Agriculture and Agri-Food Canada, Ottawa, Ontario K1A 0C6, Canada. E-mail: watsonrj@agr.gc.ca

Transformants of *Fusarium graminearum* were derived using plasmid DNA linearized using different restriction enzymes. The plasmids were designed to replace the trichodiene synthase gene, a cutinase gene or a xylanase gene with a hygromycin-resistance marker cassette by homologous recombination between 1-kbp segments of flanking DNA. The transformants did not exhibit the DNA structure expected to arise if the marker cassette was introduced by double recombination at the homologous ends. Instead, they contained complete linearized plasmids joined end-to-end and integrated into the genome. Transformant types included ectopic integrations and integrations at the target site with or without removal of the targeted gene. We have analyzed a large number of transformants using cloning, PCR and DNA sequencing to determine the structures of their integrated DNA. The data indicate that 1-3 copies of input DNA are first joined end-to-end to produce either linear or circular structures, probably mediated by the nonhomologous end-joining (NHEJ) system. The end joins typically have 1-5 nucleotides in common and are near or within the original cleavage site of the plasmid. For ectopic integrations the linear DNA is joined to two ends of genomic DNA with the same join characteristics. Integration at the target site involves replication around circularized input DNA, beginning and ending within the flanking homologous DNA, resulting in the integration of multiple copies of the entire structure. This results in deletion or duplication of the target site, or leaves one copy at either end of the integrated multimer.