TAXONOMY, POPULATION GENETICS AND GENOMICS OF *FUSARIUM* SPP.

Chairperson: John Leslie

FUSARIUM GENOMICS ACTIVITIES IN THE AUSTRIAN GENOME PROGRAM GEN-AU

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OBJECTIVES

Our working hypothesis is that *Fusarium graminearum* is able to overcome host resistance by producing small molecules suppressing defense gene expression. We intend to utilize the tools of genomics to identify fungal virulence factors and to use the knowledge gained to search for plant resistance mechanism which are able to at least partly antagonize the fungal virulence factors. The improved understanding of the interaction between plant and pathogen should be utilized for development of new chemical control strategies, development of new screening and selection tools for breeders, and in biotechnological approaches aiming to improve *Fusarium* resistance.

INTRODUCTION

In 2002 the Austrian Federal Ministry for Education, Science and Culture has established the national genome program GEN-AU (http://www.gen-au.at/). In the pilot project FUSARIUM (coordinated by G. Adam) an interdisciplinary team of researchers from BOKU and IFA Tulln, the TU Vienna, the Austrian Research Center Seibersdorf (ARCS) and from the wheat breeding company Saatzucht Donau (SZD) found together to approach the Fusarium head blight problem.

MATERIAL AND METHODS

As a first step towards the goal to get new insights into fungal virulence mechanisms by using the tools of genomics, we have supported the development of the *Fusarium graminearum* genome database by the subcontractor MIPS (http://mips.gsf.de/genre/proj/fusarium/). Several team members are involved in efforts to improve the available tools for functional genomics of *Fusarium* (e.g. repeated gene disruption using the Cre/lox system). Changes in the metabolite spectrum of *Fusarium* gene disruptions were characterized using HPLC-MS/MS techniques. Model organisms like yeast and *Arabidopsis thaliana* were used to characterize and identify toxin resistance mechanisms and to clone detoxification genes.

RESULTS AND DISCUSSION

As a first successful example we have identified a gene from the model plant *A. thaliana* encoding a UDP-glucosyltransferase, which is able to detoxify the known *Fusarium* virulence factor deoxynivalenol (Poppenberger *et al*., 2003). DON-glucoside is also formed in wheat (*Dall'Asta et al., 2004*), and this detoxification reaction seems to be a very important

resistance mechanism against *Fusarium* also in wheat (see contribution of M. Lemmens).

In collaboration with Prof. F. Trail (Michigan State University) we are currently investigating the effect of disruption of individual polyketide synthase (PKS) genes on virulence of *F. graminearum* and on its metabolite spectrum. Preliminary results suggest that inactivation of a PKS gene necessary for zearalenone (ZON) production leads to a moderate reduction in aggressiveness of the mutant on wheat heads. We have investigated the effect of ZON on the model plant *Arabidopsis thaliana*. Results of Affymetrix microarray experiments and characterization of signal transduction mutants indicate that ZON represses genes encoding proteins involved in cell wall modification/reinforcement, most likely by interfering with the ethylene signaling pathway. We have identified an *Arabiodpsis* gene encoding a UDP glucosyltransferase inactivating zearalenone (Poppenberger *et al*., in preparation).

We have furthermore identified a *Fusarium* ZON detoxification gene which seems to play a role in self protection (Mitterbauer *et al*., in preparation). Inactivation of the predicted sulfotransferase gene leads to loss of production of ZON and ZON-sulfate.

One result supporting the suppressor hypothesis is the recent identification of a new virulence gene. Deletion of this gene leads to highly pleiotropic changes in the metabolite spectrum of *F. graminearum* and a nearly complete loss in the ability to cause disease. We are currently working on the development of a bioassay allowing high throughput screening for inhibitors of this potential target for *Fusarium* control.

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DNA CHIP FOR MONITORING EXPRESSION OF *FUSARIUM SPP*. SECONDARY METABOLITES IN CEREALS

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ABSTRACT

We have developed a DNA chip to facilitate expression studies of genes involved in the synthesis of secondary metabolites in *Fusarium graminearum* and related species. The genome sequence from *F. graminearum* was used to design a PCR probe that corresponds to 9 genes from the trichothecene cluster, 8 ABC transporters, 15 polyketid synthases, 21 peptidsynthetases, and 6 genes from the aurofusarin cluster. As an internal standard for fungal growth, probes corresponding to the constitutive expressed genes, b-tubulin and glyceraldehydes-3-phosphate, gpd, were generated. The DNA chips were spotted using a Genetix Q-pix Robot (contact spotting) and scanned using a ArrayWoRx scanner (Applied Prescision Inc.). The chip design included one replication of the target genes and several copies of the internal standards.

Secondary metabolites are expressed relatively late in the fungal life cycle and under stress. Some of these substances have a detrimental effect on the quality of RNA and the amounts that can be purified. Contaminating compounds or degradation of RNA prepared from older cultures has resulted in a lowered efficiency of the incorporation of Cy3/Cy5. Several different methods are tested to overcome this problem. Analyses of aurofusarin deficient mutants indicate that this pigment may in part be responsible for the problem. Preliminary results from comparison of 3 different *F. culmorum* isolates showed a significant difference in the regulation of two ABC transporters and *Tri*101 (trichothecene 3-O-acetyl transferase). To determine how gene expression correlates to the presence of secondary metabolites a time series experiment using *F. culmorum* is performed. The trichothecenes are analysed by HPLC and the expression profile of the *tri* genes determined by DNA chip analyses. To increase the sensitivity of the assay allowing the use of smaller amounts of RNA from infected plant material a Gensisphere 3DNA array 900™ kit is tested.

FUNCTIONAL ANALYSIS OF TRICHOTHECENE BIOSYNTHETIC GENES VIA HETEROLOGOUS EXPRESSION IN A TRICHOTHECENE-NONPRODUCING *FUSARIUM* SPECIES

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ABSTRACT

The biosynthesis of trichothecene mycotoxins by *Fusarium sporotrichioides* and *F. graminearum* involves a complex biochemical pathway that begins with the cyclization of farnesyl pyrophosphate to the sesquiterpene hydrocarbon trichodiene and continues with multiple oxygenation, cyclization and esterification reactions. While almost all of the steps in the pathway have been identified, there are still some questions regarding gene function, particularly of several of the P450 enzymes involved in the oxygenation steps. In previous studies using *F. sporotrichioides*, disruption of the P450 monooxygenase-encoding gene *Tri4* blocked trichothecene production and led to the accumulation of trichodiene. Therefore, trichodiene is the likely substrate of the TRI4 protein. To further elucidate the function of the TRI4 protein, we heterologously expressed the *F. graminearum Tri4* (*FgTri4*) in *F. verticillioides*, which does not produce trichothecenes. Transgenic *F. verticillioides* carrying *FgTri4* under the control of a fumonisin biosynthetic gene (*FUM8*) promoter converted exogenous trichodiene to isotrichodermin. Conversion of trichodiene to isotrichodermin requires seven steps. Previous studies indicate that two of these reactions are non-enzymatic, and feeding studies done here indicate that wild-type *F. verticillioides* can convert isotrichodermol to isotrichodermin. Thus, the remaining four oxygenation reactions required for the conversion of trichodiene to isotrichotriol must be catalyzed by the TRI4 protein, suggesting that it is a multifunctional monooxygenase. Using a similar strategy, we analyzed the expression of FgTRI1 and FsTRI1. We have shown the usefulness of using a transgenic expression system to determine function of unknown genes which should be helpful in analyzing the many genes that are being identified in genomic projects.

MONITORING OF FHB USING PCR FOR QUALITATIVE AND QUANTITATIVE DETECTION OF *FUSARIUM* SPP

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ABSTRACT

Since the 1990ies Fusarium head blight (FHB) and the resulting mycotoxin (mainly DON) contamination of the crops are a great problem in winter wheat production in Bavaria. Main objective is to elaborate a forecasting system for FHB and DON production based on the correlation of actual epidemiological data gained in the field and the corresponding meteorological parameters captured by weather stations in spatial proximity. As a first step in our project field trials using *Fusarium graminearum* infected maize stubbles as inoculum were conducted in 2004. Two different cultivars of winter wheat were planted at two different locations and the development of FHB was monitored throughout the growing period. Samples were taken two times a week. For qualitative and quantitative determination of *Fusarium* in these samples PCR-based methods were elaborated. For the quantitative approach realtime PCR using SYBR green and Taqman ® probes are chosen. First results with the newly designed primers and probes are shown. As a prerequisite for the risk assessment of DON production in a next step a test for expression of the tri 5 gene, coding for the key enzyme in the production of thrichothecene mycotoxins, is to be established.

CROSS FERTILITY OF *GIBBERELLA ZEAE*

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ABSTRACT

O'Donnell *et al.* (2000) divided *Gibberella zeae* into seven phylogenetic lineages and this was extended to eight lineages by Ward et al. (2002). Recently, the lineages were extended to nine and given species rank by O'Donnell et al. (2004). Bowden and Leslie (1999) had previously shown cross fertility between some strains later placed in different species in the *Gibberella zeae* clade by O'Donnell et al. The objective of this study was to estimate the potential for genetic exchange between these lineages or species by quantifying cross fertility in the laboratory. Crosses were conducted on carrot agar as described by Bowden and Leslie (1999). Three strains of *G. zeae* lineage 7 with an insertion in the *MAT1-2* locus that renders them heterothallic were used as females (Lee et al., 2003). Standardized suspensions of macroconidia from strains of each of the nine lineages were used as males to fertilize the females. At least two male strains were used for each lineage except lineage 1. On day 10 after fertilization, carrot agar plates were inverted in a 40 cm spore settling tower made of 10 cm PVC pipe. Fertility was measured by counting ascospores deposited overnight on water agar plates at the bottom of the spore settling tower. Homothallic cultures and unfertilized heterothallic strains served as controls. Cross fertility was highly variable and differed for the three female strains. All males from all lineages produced viable progeny with at least one lineage 7 female strain. Individual pairings of lineage 7 females with males of lineages 1, 4, 5, 6, and 9 showed fertility levels comparable to lineage 7 x 7 crosses. Pairings with representatives of lineages 2, 3, and 8 are in progress. No evidence for consistent fertility barriers between the lineages (or species) of O'Donnell et al. (2004) and lineage 7 has been found.

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GENETIC MAPPING IN *GIBBERELLA ZEAE*

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ABSTRACT

Fusarium graminearum is the asexual conidial stage of a haploid fungus that can self-fertilize to produce the sexual ascospore stage, *Gibberella zeae*. Although predominantly an inbreeder, *G. zeae* has been shown to outcross in laboratory studies (Bowden and Leslie, 1999). Crosses can be fertile both within and between the lineages of *G. zeae* described by O'Donnell et al. (2000). A difficulty in genetic studies of fungi with mixed mating systems is distinguishing selfings from outcrosses. One solution is to use pairs of complementary auxotrophic markers, such as nitrate nonutilizing mutants, to detect recombinants among random ascospore progeny (Bowden and Leslie, 1999). In this fungus, all asci in individual perithecia result from one fertilization event and contain either selfed or outcrossed ascospore progeny, but not both. When the ascospores from an individual perithecium are collected, single segregating markers can be used to detect outcrosses. Another solution is to create obligate outcrossing strains by deleting portions of the mating type (*MAT*) locus (Lee et al., 2003). Segregation occurs in the F_1 generation in haploid fungi so recombinant population development can be rapid.

These genetic techniques make genetic mapping possible in this fungus. To date, we are aware of three genetic maps of *G. zeae* (Bowden et al, 2002; Jurgenson et al., 2002; and Gale and Kistler, http://www.broad.mit.edu/annotation/fungi/fusarium/maps.html). Markers utilized to construct the maps include Amplified Fragment Length Polymorphisms (AFLP), Restriction Fragment Length Polymorphisms (RFLP), Cleaved Amplified Polymorphic Sequences (CAPS), Derived Cleaved Amplified Polymorphic Sequences (dCAPS), and Simple Sequence Repeats (SSR). The genomic sequence of *G. zeae* (http://www.broad.mit.edu/annotation/fungi/fusarium/index.html) has been a valuable tool for development of some of these markers. A variety of software has been used for haploid linkage analysis including Map Maker, MapManager QTX, and JoinMap.

The genetic maps of *G. zeae* are useful for many purposes. For example, the linkage map of Jurgenson et al. (2002) was used to select an unbiased set of unlinked AFLP markers for population diversity studies of *G. zeae* (Zeller et al., 2003, 2004). Genetic maps can be used to assist or validate the genomic sequence assembly. Comparative mapping is useful for understanding differences in genome organization between species, lineages, or strains. Perhaps most importantly, genetic maps are valuable tools to dissect the genetic basis of important traits of the pathogen such as toxin production, fertility, or aggressiveness.

The map of Jurgenson et al. was based on a cross between a Japanese barley strain (R-5470, lineage 6) and a Kansas wheat strain (Z-3639, lineage 7). This cross was chosen because it was polymorphic for trichothecene toxin type (nivalenol vs. deoxynivalenol), toxin amount, colony color, female fertility, and AFLP banding patterns. The map contains 1048 polymorphic markers, primarily AFLPs, which map to 468 unique loci on nine linkage groups. The total map length is approximately 1300 cM with an average interval of 2.8 map units between loci. The map was used to position the deoxynivalenol/nivalenol switch in the trichothecene gene cluster. This was corroborated when the *Tri13* gene in the trichothecene cluster was proved to be the switch (Lee et al. 2002). We also located a major gene for toxin accumulation (*TOX1*) which was tightly linked to female fertility (*PER1I*) and colony color (*PIG1*)

In collaboration with colleagues at the University of Hohenheim, we conducted a QTL analysis of the aggressiveness of the progeny of the Jurgenson et al. mapping cross (Cumagun et al., 2004). We identified two major QTLs for pathogenicity or aggressiveness. One locus (*PATH1*) maps on linkage group IV near *TOX1*, which controls toxin amount. Progeny producing little or no detectable trichothecene toxin had very low pathogenicity, an effect that had previously been reported (Proctor et al., 1995). The other QTL maps on linkage group I and is centered on the trichothecene gene cluster that contains the deoxynivalenol vs. nivalenol switch. Progeny producing deoxynivalenol were, on average, twice as aggressive as those producing nivalenol. Both the high aggressiveness and high pathogenicity alleles were from the Kansas parent. It was interesting that no transgressive segregation for aggressiveness was detected in this cross between lineages 6 and 7.

The release of the genomic sequence of *G. zeae* strain PH-1 by the Broad Institute provided an opportunity to align the sequence assembly with our linkage map. We used 7 sequenced structural genes and 130 sequenced AFLP markers from all nine linkage groups of the genetic map (Lee et al., 2004). One hundred and fifteen markers were associated with nine supercontigs of the genomic sequence. The alignments of linkage groups with supercontigs allowed the assembly of four putative chromosomes that anchored 99% of the genomic sequence. Co-linearity of the physical and genetics maps was very high, though there was some evidence of inversions on two chromosomes. The map by Gale and Kistler also showed a high degree of co-linearity and anchored 99.8% of the genomic sequence. The supercontigs joined by the two maps are in agreement. These results validate both the genomic sequence assembly and the linkage maps.

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ANALYSIS OF INTRA- AND INTER-SPECIES GENETIC EVOLUTION OF EUROPEAN WHEAT PATHOGENIC *FUSARIUM* FUNGI

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ABSTRACT

Fusarium head blight (FHB) is an important disease of wheat, barley and maize world-wide. *Fusarium* fungi exhibit an extraordinary degree of biodiversity with respect to morphological, physiological and ecological characteristics. This study used AFLP analysis, to examine the inter- and intra-species genetic diversity of 80 *Fusarium* wheat-pathogenic isolates representing five species (*F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. nivale*) and originating from Ireland, the UK, Hungary and Italy. Nine other *F. graminearum* isolates representing the different geographic lineages and isolates of seven other *Fusarium* species were included in this study. Isolates were identified morphologically and by species-specific PCR analysis. At the intra-species level, UPGMA cluster analysis of AFLP data revealed that the *F. avenaceum, F. culmorum* and *F. graminearum* isolates showed the highest level of genetic similarity. The most genetically diverse species were *F. poae* and *M. nivale*. Principal coordinate analysis of AFLP data generally confirmed the same cluster profile as did the dendrograms. The present study also found a relationship between genetic diversity and country of origin of the isolates within certain species; no relationship was found between genetic diversity and growth or pathogenicity of the isolates.

PHYSICAL MAPPING OF THE *FUSARIUM GRAMINEARUM* GENOME

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ABSTRACT

Fusarium head blight, caused by *Fusarium graminearum,* is a major disease in wheat and barley. To enable genomics studies of *F. graminearum*, we developed a bacterial artificial chromosome (BAC)-based physical map and integrated it with the genome sequence and genetic map. We developed two complementary genomic libraries with average insert sizes of 107 and 95 kb, which were estimated to exhibit 23-fold genome coverage. We fingerprinted 4,224 BAC clones and developed a physical map consisting of 112 contigs. Using lower stringency parameters for contig assembly, 112 contigs were assembled into 26 contig groups covering 36.4 Mb. The physical map was confirmed by comparing our map to the genome sequence posted on the *F. graminearum* database website (http://www.broad.mit.edu/cgi-bin/annotation/fusarium). Our results show high consistency of the physical map with the genome sequence. Among the 112 contigs used for BAC assembly, 3 contigs did not match with any of the genomic sequences registered in database. To further validate the physical map and to integrate the physical map and the genetic map, we selected 30 genetic markers evenly covering the whole genome and conducted PCR-based screening of the BAC clones. The physical map was consistent with the genetic map throughout the entire genome. Our current physical map, integrated with the genome sequence and genetic map, will enable advanced studies such as gene cloning, comparative mapping, and elucidation of *F. graminearum* genome organization.

POLYMERASE CHAIN REACTION BASED ASSAYS FOR THE DETECTION AND IDENTIFICATION OF *FUSARIUM* SPECIES IN MYCELIAL CULTURES AND GRAINS

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OBJECTIVES

1. To use species-specific PCR assays to identify nine *Fusarium* species collected from cereals in Canada.

2. To screen cereal samples for Fusaria using PCR and whole seed plating techniques.

INTRODUCTION

Fusarium head blight (FHB) is one of the major fungal diseases of cereals in Canada and worldwide. *Fusarium graminearum* Schwabe is the predominant FHB pathogen in Manitoba and southeastern Saskatchewan (Clear and Patrick, 2000). The other principal FHB pathogens in Canada are *F. avenaceum* (Corda ex Fr.) Sacc. and *F. culmorum* (W.G. Smith) Sacc. Several other common *Fusarium* species also infect cereal grains in Canada, including *F. acuminatum* Ell. & Ev., *F. equiseti* (Corda) Sacc., *F. poae* (Peck) Wollenw., and *F. sporotrichioides* Sherb. (Clear et al., 2000a, Clear et al., 2000b, Clear and Patrick 1993).

The traditional diagnostic method for the detection and identification of *Fusarium* species in pure culture or in infected grain is usually based on the observation of the micro and macro morphological features of cultures developing on a nonselective agar medium. Besides requiring a growth period of several days, many of the diagnostic characters can be altered by the culture conditions. In addition, when culturing fungi from seed, only viable fungi that are able to compete well enough to be visually detectable are recorded. Polymerase chain reaction (PCR) is a sensitive and rapid method that can be used for detection and screening of *Fusarium* species in infected grain samples. In this study, species-specific PCR assay was used for the detection and identification of nine *Fusarium* species in pure mycelial cultures and grain samples.

MATERIALS AND METHODS

Grain samples – The wheat samples were composites of producer deliveries from 39 crop districts across western Canada. The other grain samples were from individual producers.

Mycological analysis – 200 seeds from each grain sample were surface disinfected and placed onto potato dextrose agar (PDA) for 5 days and analyzed according to Clear and Patrick (2000).

Fungal cultures – For each culture in this study, initial fungal isolations were made by transferring mycelia growing from infected cereal seed (or for three isolates of *F. pseudograminearum*, from straw) to a petri plate containing PDA. After 5 to 10 days of growth at room temperature and under UV light, a spore suspension was prepared and spread onto a fresh PDA plate and incubated for 18 h at room temperature. A single germinating conidium was then removed from the PDA plate, transferred to an SNA plate (Nirenberg 1981), and after 7 days growth stored in a plastic bag at 4°C for up to one year.

DNA extraction – For DNA extraction, mycelia from the SNA plates were transferred to PDA plates and incubated in the dark at room temperature for 7 to 10 days. The mycelia were then harvested and freeze-dried prior to DNA extraction. DNA was also extracted from fresh mycelial cultures without freeze drying. For cereal samples, a 20g sample was ground in a coffee grinder and then a subsample of 0.2g was

taken for DNA extraction. Sodium dodecyl sulphate (SDS) based buffer was used for DNA extraction.

Polymerase chain reaction – PCR for single species detection was performed in 96-well plates containing 25 µL of a reaction mixture consisting of 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH8.3), 0.2 mM of each of the four dNTPs, 0.25 µM of each oligonucleotide primer (Table 1), 1.5 µL DNA solution and 0.15 units of *Taq* DNA polymerase (Applied Biosystems, Foster, CA). AccuPrime™ *Taq* DNA polymerase (Invitrogen, Burlington, CA) was used for multiplex PCR according to the manufacturer's recommendations. DNA amplification was performed in an MJ Research PTC-200 Thermal Cycler using an initial 3.0 min denaturation at 95°C; and then 38 cycles of 30 s at 9°C, 20 s at 62°C, and 45 s at 72°C, followed by a final extension of 5 min at 72°C. Annealing temperatures of 56°C and 57°C were used for *F. acuminatum* and *F. pseudograminearum* PCR reactions, respectively.

RESULTS AND DISCUSSION

PCR detection of Fusarium species – Twelve *F. acuminatum*, nine *F. avenaceum*, seven *F. crookwellense*, 12 *F. culmorum*, 11 *F. equiseti*, 77 *F. graminearum* (72 isolates from Canada and 5 isolates from Australia), 10 *F. poae*, 23 *F. pseudograminearum* (five of them from Australia) and 10 *F. sporotrichioides* isolates were correctly identified using species-specific PCR (data not shown). The primers for *F. acuminatum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae*, *F. pseudograminearum* and *F. sporotrichioides* are specific as there was no cross reaction when each specific primer was used to amplify DNA from the other *Fusarium* species. One of the primer sets for *F. avenaceum* (FaF/FaR) also amplified DNA fragments from *F. acuminatum*. The other primer set (J1AF/R) was specific and did not amplify DNA from the other eight *Fusarium* species.

A multiplex PCR reaction was developed for the simultaneous detection of the three most important mycotoxin producing *Fusarium* species (*F. culmorum*, *F. graminearum* and *F. sporotrichioides*).

Detection of *Fusarium* species in cereal grains – Most grain samples were infected with several *Fusarium* species. A comparison between the results for PCR and whole seed plating for six *Fusarium* species is shown in Table 2. *Fusarium avenaceum* was the most often detected species by both PCR (using FaF/FaR primer) and whole seed agar plating (Table 2). The *Fusarium avenaceum* specific primer (J1AF/R) was less sensitive than FaF/FaR primer (amplifies both *F. avenaceum* and *F. acuminatum*) in amplification of infected grain samples. Four of the six species were detected in more samples by PCR than by whole seed plating, with only *F. poae* and *F. sporotrichioides* being detected slightly more often by whole seed plating than by PCR (Table 2). Three species, *F. crookwellense*, *F. acuminatum* and *F. pseudograminearum*, were not included in Table 2. For *F. crookwellense*, only one sample of barley from New Brunswick revealed a positive reaction with PCR and whole seed agar plating. *Fusarium acuminatum* was detected in several grain samples when assayed with whole seed agar plating. However, only those samples that had a relatively high frequency of seed infection by *Fusarium acuminatum* (~^{3}5%) were positive with the PCR assay (data not shown). *Fusarium pseudograminearum* was not detected in any samples by either PCR (85 samples) or whole seed agar plating (82 samples), reflecting the rare occurrence of this species infecting cereal seed in western Canada.

Out of 474 comparisons (using *F. avenaceum*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae*, and *F. sporotrichioides*) between PCR and whole seed agar plating results, a discrepancy occurred 83 times (Table 2). Fifty-four times a *Fusarium* species-specific PCR amplification was obtained even though the target species was not observed during whole seed agar plating, whereas in 29 comparisons the reverse was true (Table 2). In all samples where a discrepancy was noted the level of the target species in the plating results was very low. Most importantly, *F. graminearum* was detected more often by PCR (50 of 85 samples) than by whole seed agar plating (36 of 82 samples), and the PCR-based method was able to accurately distinguish between *F. graminearum* and *F. pseudograminearum*. Failure of whole seed

agar plating to detect species that PCR detected may be due to the removal of fungal material during surface disinfestations, failure to detect the Fusaria due to competition on the plate, or lack of viability of the target species. Differences between PCR and whole seed agar plating results may also be due to variation in the two sampling techniques and to low levels of the target species on the grain. In this trial, whole seed agar plating was done on 200 individual kernels; whereas 0.2g subsample from 20g ground seed was used for DNA extraction and PCR analyses.

In summary, species-specific PCR assay was successfully used for the identification of nine *Fusarium* species. The PCR assay was also used for the detection of *Fusarium* species in several types of cereal grain. The PCR assay used in this study can be used for routine detection and identification of *Fusarium* species in mycelial cultures and grain samples in Canada.

ACKNOWLEDGEMENTS

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Table 1. List of primer sequences, expected DNA fragment length and sources of primers.

Primer name	Target	Sequence (5' – 3')	Size (bp)	Source*
FAC F	F. acuminatum	GGGATATCGGGCCTCA	600	A
FAC R		GGGATATCGGCAAGATCG		
FaF	F. avenaceum	CAAGCATTGTCGCCACTCTC	920	B
FaR		GTTTGGCTCTACCGGGACTG		
J1AF	F. avenaceum	GCTAATTCTTAACTTACTAGGGGCC	220	C
J1AR		CTGTAATAGGTTATTTACATGGGCG		
CroA F	F. crookwellense	CTCAGTGTCCACCGCGTTGCGTAG	842	D
CroA R		CTCAGTGTCCCAATCAAATAGTCC		
FC01F	F. culmorum	ATGGTGAACTCGTCGTGGC	570	E
FC01R		CCCTTCTTACGCCAATCTCG		
FEF1	F. equiseti	CATACCTATACGTTGCCTCG	400	F
FER1		TTACCAGTAACGAGGTGTATG		
FG11F	F. graminearum	CTCCGGATATGTTGCGTCAA	450	E
FG11R		GGTAGGTATCCGACATGGCAA		
FP82F	F. poae	CAAGCAAACAGGCTCTTCACC	220	G
FP82R		TGTTCCACCTCAGTGACAGGTT		
AF330109CF	F. sporotrichioides	AAAAGCCCAAATTGCTGATG	332	H
AF330109CR		TGGCATGTTCATTGTCACCT		
FP1-1	F. pseudograminearum	CGGGGTAGTTTCACATTTCYG	523	I
FP1-2		GAGAATGTGATGASGACAATA		

*Sources of primers – A = Williams et al., 2002; B = Doohan et al., 1998; C = Turner et al., 1998; D = Yoder et al., 1998; E = Nicholson et al., 1998; F = Mishra et al., 2003; G = Parry and Nicholson 1996; H = Genbank AF330109 (primer designed by authors); I = Aoki and O'Donnel, 1999.

Table 2. Summary of Fusarium detection in grain samples using PCR and whole seed agar plating.

Fusarium species	Crop	PCR # +ve	PCR #-ve	PCR TTL	Whole seed # +ve	Whole seed #-ve	Whole seed TTL	PCR + & WS -	PCR - WS +	Similarity %
F. avenaceum[a]	CWRS	37	1	38	32	6	38	4	0	34/38 (89.5)
	CWAD	26	0	26	23	3	26	3	0	23/26 (88.5)
	Barley	8	2	10	9	1	10	0	1	9/10 (90.0)
	Oat	3	1	4	3	1	4	0	0	4/4 (100.0)
	Corn[b]	0	6	6	0	3	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)
F. culmorum	CWRS	5	33	38	4	34	38	5	4	29/38 (76.3)
	CWAD	11	15	26	10	16	26	3	2	21/26 (80.8)
	Barley	0	10	10	1	9	10	0	1	9/10 (90.0)
	Oat	1	3	4	1	3	4	1	0	3/4 (75.0)
	Corn	0	6	6	0	3	-	-	-	-
	Rye	0	1	1	0	1	1	0	0	1/1 (100.0)
F. equiseti	CWRS	20	18	38	13	25	38	12	5	21/38 (55.3)
	CWAD	21	5	26	19	7	26	4	2	20/26 (76.9)
	Barley	8	2	10	7	3	10	3	1	6/10 (60.0)
	Oat	1	3	4	1	3	4	0	0	4/4 (100.0)
	Corn	0	6	6	1	2	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)
F. graminearum	CWRS	21	17	38	14	24	38	8	1	29/38 (76.3)
	CWAD	13	13	26	10	16	26	4	1	21/26 (80.7)
	Barley	9	1	10	9	1	10	0	0	10/10 (100.0)
	Oat	3	1	4	2	2	4	1	0	3/4 (75.0)
	Corn	3	3	6	0	3	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)
F. poae	CWRS	14	24	38	18	20	38	0	4	34/38 (89.5)
	CWAD	9	17	26	9	17	26	1	1	24/26 (92.3)
	Barley	6	4	10	9	1	10	0	3	7/10 (70.0)
	Oat	2	2	4	3	1	4	0	1	3/4 (75.0)
	Corn	0	6	6	0	3	-	-	-	-
	Rye	0	1	1	0	1	1	0	0	1/1 (100.0)
F. sporotrichioides	CWRS	20	18	38	21	17	38	3	0	35/38 (92.1)
	CWAD	15	11	26	19	7	26	2	2	22/26 (84.6)
	Barley	9	1	10	9	1	10	0	0	10/10 (100.0)
	Oat	3	1	4	3	1	4	0	0	4/4 (100.0)
	Corn	0	6	6	0	3	-	-	-	-
	Rye	1	0	1	1	0	1	0	0	1/1 (100.0)

[a] FaF/FaR primer set was used for the PCR assay. [b]Three corn samples were received ground, and thus whole seed agar plating was carried out for only three of the six corn samples. WS = whole seed agar plating method. Two hundred seeds were used for whole seed agar plating assay, whereas 0.2g from 20g ground sample was used for DNA extraction and PCR assay.

IMPLICATION OF DISRUPTION OF THE FUM12 GENE ON THE FUMONISINS PRODUCTION BY *FUSARIUM VERTICILLIOIDES*

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ABSTRACT

Fumonisins are polyketide-derived mycotoxins produced by *Fusarium* species of the *Gibberella fujikuroi* complex. Wild type strains of the fungus produce predominantly four B-series fumonisins, designated FB1, FB2, FB3 and FB4. Recently, a cluster of 15 putative fumonisin biosynthetic genes (*FUM*) was described in *F. verticillioides*. We have now conducted a functional analysis of the *FUM12* gene that is predicted to encode a cytochrome P-450 monooxygenase. Therefore, we generated *FUM12* disrupted mutants (*FUM12*) of the wild-type *F.verticillioides* strain MUCL 43478 (M-3125). HPLC analyses revealed that the FB1 and FB3 production of *FUM12-* mutants was reduced by over 98% and was at least doubled for FB2 compared to the progenitor strain. These results indicate that the *FUM12* protein catalyses the hydroxylation of the C-10 of the fumonisin backbone. As the phenotype of the mutants is identical to that of previously described mutants with defective alleles at the meiotically defined *fum2* locus, it appears that *FUM12* and *fum2* locus are the same gene which is now renamed *FUM2*.

IDENTIFICATION OF GENES EXPRESSED BY *FUSARIUM GRAMINEARUM* DURING INFECTION OF WHEAT

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ABSTRACT

Our initial studies have shown that the *Fusarium graminearum* species complex is comprised of strains belonging to at least nine biogeographically structured cryptic species that may differ significantly in aggressiveness on wheat and mycotoxin production. To study this host-pathogen interaction at a genomic level and identify fungal genes expressed during initial infection (48 hours after inoculation), cDNA libraries were created by suppression subtractive hybridization. One such library was constructed using RNA isolated from wheat heads inoculated with a highly aggressive strain (tester) or with water (driver). The ESTs sequenced from this library could be assembled into 182 contigs and 630 singletons. Of these, 349 ESTs were determined to be of fungal origin according to their matches to the *F. graminearum* genome sequence. These sequences were compared with ESTs from libraries created using the *F. graminearum* grown under various culture conditions and the whole genome sequences of *Magnaporthe grisea* and *Neurospora crassa*. Putative functions of genes corresponding to the fungal ESTs obtained from this library were predicted based on comparisons with sequences from publicly available databases. Interestingly, nearly 56% of the fungal ESTs were mitochondria related. Also, a significant number of ESTs with no known homologs in currently available databases were observed. These are believed to be new open reading frames specific to *F. graminearum*. Additionally, candidate genes potentially involved in pathogenicity were identified e.g. those corresponding to genes coding for an ABC transporter, amino acid permease, polyketide synthase, histidine kinase and the regulatory gene alcR. Some of these have been selected for targeted deletion mutagenesis using gene replacement. Analysis of these sequences and the methods used for successful gene replacement in *F. graminearum* will be presented.

DEVELOPMENT OF AN AFFYMETRIX GENECHIP MICROARRAY USING THE GEN-AU / MIPS *FUSARIUM GRAMINEARUM* GENOME DATABASE

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ABSTRACT

Shortly after public release of the genome sequence of the plant pathogenic fungus *Fusarium graminearum* by the Broad Institute, automated draft gene calls were processed at MIPS and also at the Broad Institute. For both predicted gene sets, a variety of bioinformatics methods were applied at MIPS using the PEDANT system. Manual inspection of the calls using different gene prediction programs and EST sequences were also conducted to examine underpredicted genes and verify predicted coding regions. In order to reduce mis-designed probe sets, manual inspection of genes, at least the most interesting targets, is desirable before the design of an Affymetrix GeneChip microarray design.

With the help of the *Fusarium* community we manually processed ~860 entries; 408 of the calls were altered or added as completely new calls. To integrate all different calls as well as the results of the applied bioinformatics methods, the *F. graminearum* Genome Database was created (http://mips.gsf.de/genre/proj/fusarium/). However, only 6.1 % of the putative 14,000 *Fusarium* genes were manually processed.

During the manual gene modeling and correction procedure it appeared that the MIPS draft gene call set performed significantly better than the Broad set. Therefore we produced a combined gene call set for the Affymetrix GeneChip design with the order of preference "manually processed new calls" > "MIPS draft set" > "Broad set". To reduce the number of ~26,000 gene calls, the ORF sequences were truncated to 500 bases towards the 3' end and all redundant call names were added to the preferred ones as an alias. This approach resulted in a set of 16,926 calls. The set of full length ORF sequences and an additional 611 EST- and rRNA-sequences were submitted to Affymetrix for initial computation of probe sets. After 3 rounds of chip design proposals, the sets were approved for mask design. First chip experiments for validation are on the way.

FUNCTIONAL GENOMICS OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

We are conducting gene profiling experiments using *Fusarium graminearum* ~4.5K uniEST cDNA microarrays and targeted gene disruptions to identify and functionally test candidate genes suspected to be involved in *F. graminearum* (*Fg*) pathogenicity. Fourteen *F. graminearum* cDNA libraries have been constructed from fungal cultures grown under a variety of conditions to generate a collection of ~10,000 expressed sequence tags (ESTs) which group into ~5500 contigs/singletons. A 4.5K unigene *F. graminearum* cDNA microarray has been printed in-house and array hybridization experiments are underway to explore metabolite biosynthesis and genes induced upon plant contact. Initial array hybridizations have compared expression profiles of *Fg* grown in liquid culture under conditions of trichothecene production over a 12-day period versus *Fg* in log phase. Metabolite analysis (HPLC/NMR) of ethyl acetate extracts of these liquid cultures has indicated increasing production of 15A-DON over the growth period as well as the presence of a second metabolite, butenolide.

Functional analysis of candidate genes through gene disruption or modification is the focus of a collaborative project between the USDA and Agriculture & Agri-Food Canada. For example, an analysis of *Fg* ESTs revealed a hotspot of gene expression from a putative novel biosynthetic gene cluster. Eight consecutive predicted genes are represented by a total of 51 *Fg* ESTs isolated from trichothecene-producing culture conditions. In addition, five of the genes are represented by six ESTs originating from *Fg*-infected wheat or barley libraries, suggesting these genes are expressed *in planta.* Northern analysis conducted on seven of the eight genes showed coordinated induction of gene expression, beginning at 4 days post induction and peaking at approximately 10 days post induction in liquid culture. Expression analysis of the eighth gene is in progress. Two of these genes have been initially targeted for gene disruption.

TYPE-A TRICHOTHECENE PRODUCTION BY *FUSARIUM* IN A PHYLOGENETIC CONTEXT

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ABSTRACT

Most Fusaria causing wheat and barley head scab produce the type-B trichothecene deoxynivalenol and other 8-keto trichothecenes such as 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol. However, some fungi found on head-blighted wheat are known to produce the more toxic type-A trichothecenes such as diacetoxyscirpenol. Our objective is to fully elucidate species and species boundaries within the trichothecene-producing group of *Fusarium* and to determine the trichothecene mycotoxin profile of all species within the group. We analyzed a set of 57 suspected type-A producers from the Penn State Fusarium Research Center collection and the NRRL collection at Peoria using molecular phylogenetics and HPLC-MS for trichothecenes. For HPLC–MS analysis, cultures were grown on autoclaved rice grains and subjected to analysis for ten trichothecenes: T-2 tetraol, nivalenol, deoxynivalenol, fusarenon-X, neosolaniol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, diacetoxyscirpenol, HT-2, and T-2 toxin. Peak identities were determined using mass spectral analysis and comparison to standards. DNA sequences were generated from seven nuclear genes, EF1-a translation elongation factor, phosphate permease, 28S rDNA, *Tri101, Tri4, Tri5* and Bð-tubulin and subjected to phylogenetic analysis using maximum parsimony as the optimality criterion. Trichothecene-producers resolved to four well-supported clades. Most type-B trichothecene producers associated with head scab fell into a single clade, termed the *Gibberella zeae* complex, which is derived from within the others. Our initial observations indicate that there are species within each of the three type-A producer clades that produce type-B trichothecenes exclusively or in addition to type-A compounds. Species capable of producing both type-A and type-B are found in two of three type-A clades. Previous reports have noted this phenomenon only for *F. kyushuense* and recently for *F. poae*. We will report our ongoing work to define species and mycotoxigenic potential within the trichothecene-producing Fusaria.

THE ALIGNMENT BETWEEN PHYSICAL AND GENETIC MAPS OF *GIBBERELLA ZEAE*

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ABSTRACT

Jurgenson et al. (2002) previously published a genetic map of *Gibberella zeae* (*Fusarium graminearum*) based on a cross between Kansas strain Z-3639 (lineage 7) and Japanese strain R-5470 (lineage 6). The genetic map was based on 1048 AFLP markers and consisted of nine linkage groups. We aligned the genetic map with the first assembly of the genomic sequence of strain PH-1 (lineage 7) that was released by The Broad Institute (Cambridge, MA). We used 7 sequenced structural genes and 130 sequenced AFLP markers from all nine linkage groups (LG) of the genetic map. One hundred and fifteen markers were associated with nine supercontigs (SC) of the genomic sequence. LG1, LG7, LG8 and LG9 aligned with SC2 and SC5; LG2 aligned with SC3, SC8 and SC9; LG 3 aligned with SC4 and SC6; and LG4, LG5 and LG6 aligned with SC1 and SC7. Approximately 99% of the sequence was anchored to the genetic map, indicating the high quality of the sequence assembly and the relative completeness and validity of the genetic map. The alignments grouped the linkage groups and supercontigs into four sets, suggesting that there are four chromosomes in this fungus.

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J. E. Jurgenson, R. L. Bowden, K. A. Zeller, J. F. Leslie, N. J. Alexander and R. D. Plattner. 2002. A genetic map of *Gibberella zeae* (*Fusarium graminearum*). Genetics 160: 1451-1450.

MOLECULAR AND PHENOTYPIC DIVERSITY WITHIN AND AMONG POPULATIONS OF *FUSARIUM GRAMINEARUM*

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ABSTRACT

We aimed for the analysis of genetic variation in populations of *Fusarium graminearum* collected on a world-wide, regional, and local basis and within a crossing progeny by molecular markers (RAPD, AFLP) and phenotypic traits. The traits were: Aggressiveness on a susceptible host, deoxynivalenol [DON] and nivalenol [NIV] production, and host colonization. The amount of genetic variation is an indicator for the evolutionary potential of a pathogen and provides an important clue for risk analysis of host resistance. Analysis of the world-wide collection showed that practically all isolates were mycotoxin producers, mostly of the DON (22/26), but some of the NIV (4/26) chemotype. NIV producer were from Germany, Italy, and Brazil and generally lower aggressive. AFLP profiles of 38 isolates of the world collection showed a high level of molecular diversity (mean GS 0.21). No grouping with respect to geographical origin or host species occurred. On a regional basis (southwestern Germany) also a significant ($P<0.01$) genetic variation for aggressiveness was found, isolates displayed a similar genotypic range than the world collection. Similarly, molecular diversity was high even on the local scale of two small (about 10.000 m^2) wheat fields amounting to 46 to 64% of the maximal genotypic diversity (G_{max}). In total, 70 isolates yielded 53 multi-locus haplotypes within the largest German population. Allele frequencies ranged from 0.01 and 0.97 in this local population compared to 0.04 to 0.92 in the world collection. A hierarchical analysis of diversity for RAPD bands and aggressiveness showed that 84 and 54% of total variance, respectively, was already found within sampling sites of about 1 m^2. Within single-field populations from Hungary and Canada a lower, but still considerable genotypic variation (27-28% of G_{max}) was detected. Crossing of two medium aggressive isolates from Europe (Germany, Hungary) resulted in a progeny of 155 isolates displaying a significant ($P<0.01$) and large segregation variance for aggressiveness, host colonization, and DON production. Moreover, significant transgression directed towards higher aggressiveness, colonization and DON production was found consistently across three environments. This illustrates that different alleles for these traits were present in both parents that were obviously combined to a higher performance. The large molecular and phenotypic diversity of *F. graminearum* even on small spatial scales might be most likely caused by a large population size of the pathogen and regular recombination with some extent of outcrossing. This enlarges allele diversity, but favourable genotypes can still be maintained by asexual propagation. The frequent alternation between saprophytic and parasitic life cycle might additionally support a high degree of polymorphism within populations. In conclusion, the results reflect a high evolutionary potential of *F. graminearum* that might allow a non-specific adaptation to host resistance, especially when highly effective resistance sources are used on large acreages.

VARIABILITY AND STRUCTURE OF THE IGS REGION IN THE *GIBBERELLA FUJIKUROI* SPECIES COMPLEX

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ABSTRACT

The intergenic spacer of the rDNA units is located between 28S and 18S genes of the nuclear rDNA in filamentous fungi with a length of about 2,5 kb in species of *Fusarium*. This region shows a high variability which is widely used to analyse variability at intraspecific level or among closely related species. Variability is due to nucleotide substitutions and to differences in length, which are attributed to duplications or deletions during crossing-over events. However, IGS region also contains a number of relevant conserved motifs such as those motifs controlling rDNA expression. We report the results of the comparative analysis of the primary structure of the IGS region to unravel the presence of repetitions and/or functional motifs in the *Gibberella fujikuroi* species complex and closely related species. This complex is especially relevant because it includes cosmopolitan pathogens of important agricultural plant species and they are important source of mycotoxins. Several short (14 nt) and long (120 nt) repetitions have been identified which showed variability in the number and position, in the case of the short repeats, causing the occurrence of characteristic specific patterns and differences in length which may be accompanying the speciation in this complex. These patterns could be explained by recombination events reducing or increasing the number of repetitions and its relative position.

ACKNOWLEDGEMENT

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MATING TYPES DETERMINATION IN *FUSARIUM PHYLLOPHILUM* AND *FUSARIUM RAMIGENUM*

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ABSTRACT

F. phyllophilum and *F. ramigenum* are two species newly described within the *Gibberella fujikuroi* complex (Nirenberg and O'Donnell, 1998). In previous studies, it was demonstrated that they were fumonisin producers (Fotso *et al.*, 2002; Van Hove *et al.*, 2002). As only fragmentary data concerning their sexual stage exist, we decided to focus our study i) on their mating identification, ii) on the search of their sexual stage, and iii) on their phylogenetic relationships within the complex.

Therefore, the primers Gfmat1a and Gfmat1b, previously developed by Steenkamp *et al.* (2000) for identification of the mating type in the *Gibberella fujikuroi* complex, were used to characterize the isolates. Interestingly, two *F. ramigenum* strains (MUCL 43904 and 7612) presented two different mating sequences (*MAT-1* and *MAT-2*). Furthermore, two *F. phyllophilum* strains (MUCL 239 and 43905) were identified as *MAT-1* strains, while two *F. phyllophilum*-like strains (MUCL 27661 and 44479) were identified as *MAT-2* strains. Phylogenetic analyses clearly separated *F. ramigenum* and *F. phyllophilum* from the other sequenced species, particularly those for which the sexual stage has already been described (mating population A to I). The analyses also demonstrated the close relationships of the two species with *F. verticillioides.*

Diallele crosses were tested on six different media, and under different light conditions. Besides the development of perithecia in compatible *F. verticillioides* crosses used as control, development of mature perithecia was observed in *F. ramigenum* crosses, what is the first report of the sexual form for this species. On basis of integration of the various results (morphological, molecular and crosses), we propose to create a new mating type population J and its teleomorphic stage *Gibberella ramigena*. Additionnal data are expected for *F. phyllophilum*.

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VARIATION AMONG ISOLATES OF *FUSARIUM GRAMINEARUM* ASSOCIATED WITH FUSARIUM HEAD SCAB IN INDIA

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ABSTRACT

Head scab of wheat caused by *Fusarium spp.* is characterized by bleaching of the wheat spike, shriveled kernels and accumulation of mycotoxins which may cause various ailments in humans and animals. Understanding the variability of the fungal population associated with head scab could improve disease control strategies. The main aim was to investigate the genotypic and pathogenic variability within *F. graminearum* in relation to geographic origin. In the present study, *F. graminearum* were isolated from diseased spikes sampled from naturally infected wheat from Punjab, Tamil Nadu and high Himalayas of Himachal Pradesh during 2000-2002. Inter and intra species specific aggressiveness assessed could also be noticed on the basis of head scab rating on different varieties of wheat. *F. graminearum* isolates of Dalang Maidan and Wellington were significantly more aggressive than Ludhiana and Gurdaspur isolates of *F. graminearum*. Fusarium head scab ratings were more on varieties Sonalika, HD 29 and PBW 222, irrespective of the isolate used. Randomly amplified polymorphic DNA (RAPD) was used to study genetic variation in natural pathogen populations of F. graminearum (15 isolates). A screening of sixty one 10-mer oligonucleotide RAPD primers (OPAA 1-20, OPAC 1-20, OPAD 1-20, OPV 14) revealed 19 RAPD primers to yield informative (polymorph), strong and reproducible DNA amplicons (bands) by PCR. The number and size of the amplified fragments varied with different primers. The amplification products were in the range of 300 bp to 1.2 Kb. Maximum number of bands (11) were scored with primer OPAD 12 followed by 10 bands with OPAA 12. The genetically most similar isolates belonged to Punjab viz., G 31 and L 23 (92.65 %) while the Lahaul valley isolate (D 3) and the Wellington isolate (W 3) were found genetically most dissimilar (47.79 %). Cluster analysis of band sharing coefficients separated isolates of *F. graminearum* into four clusters. This study has shown that there is a considerable pathogenic and genotypic variability among *F. graminearum* isolates obtained from infected wheat earheads from different geographic regions of India.

VARIABILITY OF *FUSARIUM CULMORUM* – AGENTS OF HEAD BLIGHT

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OBJECTIVES

Variability of Fusarium culmorum to pathogenic and toxic will study.

Strains of *F. culmorum* will characterize on 5 rye cultivars.

INTRODUCTION

Scab or Fusarium head blight (FHB) is a serious disease in many cereal crops Russia. This disease is the most dangerous to the winter rye. FHB can significantly reduce rye grain yield and quality (Novozhilov K.V., Levitin M.M., 1990; Monastirskij O.A. 2000; G. Gang et al., 1998; Ivaschenko V.G. et al, 2004).

The mycotoxins produced by *Fusarium* spp. in infected grains are harmful to animals and human health (Bilai V.I., 1955; Bamburg et all, 1968; Mirocha et all, 1976; Ivaschenko V.G. et al, 2004; Levitin M.M., 1994; S. L. Walker et al., 2001).

The selection of winter rye cultivars immune to FHB considers the very perspective. But it is impossible without study pathogenic and toxic qualities of frequent fungus.

Fusarium culmorum (W.G.Sm.) Sacc. is one of frequent pathogens of scab in Moscow region.

MATERIALS AND METHODS

Genotypes *F. culmorum* with various morphological - cultural types ("C") of colonies were received on potato agar plates at 24°C during 2 weeks.

With the purpose of pathogenic and toxic qualities sterile seeds were inoculated with conidial suspensions and culture filtrates of the 4 genotypes *F. culmorum*. Inoculated and control (treated by adding 5 ml sterile water) seeds were allowed to continue germination for 3 days, on filter paper with low moisture level (25 seeds/Petri dish). In fours reiteration samples were evaluated for each experiment. For each genotype, records of following parameters are taken:- the germination's percent of seeds; - length of root of seeds.

Accounting information of control taken for 100 %.

RESULTS AND DISCUSSION

Genotypes *F. culmorum* with various MCT significantly differed pathogenic and toxic quality. The analysis of variance has showed significant influence of factors (cultivars and strains) on percent of seed germination of winter rye (fig. 1, 2). Each bar represents the mean of experiments with ten replications for cultivar-strain combinations averaged across 4 strains *Fusarium culmorum* or 5 winter rye cultivars with the purpose of revealing their influence on percent of seed germination. Cultivars or strains with a letter in common above the bar do not differ significantly according to Tukey's multiple test (a= 0.05)*.

The strain MCT IV significantly differed (P<0.05) from strains "C" I, "C" III and "C" V according to Fisher's criterion. Strain MCT IV showed the greatest pathogenic and toxic quality. It was reduced percent of seed germination of winter rye on 50 % in comparison with control. Differences between strains *F. culmorum w*ith MKT I, III and V were inessential.

They were reduced percent of seed germination of seeds on 61 % on average.

Distinctions in percent of seed germination on cultivars were significant (P<0.05). The least susceptible to cultural suspension and culture filtrates were cultures of rye Purga and Falenskaja 4 – percent of seed germination was 67 % in comparison with control. Genotype of rye Vjatka 2 was the most susceptible to studied strains. Percent of seed germination was 50 % in average.

Influence of cultivar-strain interactions was also statistically significant (P<0.05), separate distinctions between values of average percent of seed germination have been insignificant and marked with identical letters above the bars (fig. 3, 4). Each bar represents the mean of experiments with ten replications for cultivar-strain combinations averaged across 4 strains *Fusarium culmorum* or 5 winter rye cultivars with the purpose of revealing their influence on percent of seed germination. Cultivars or strains with a letter in common above the bar do not differ significantly according to Tukey's multiple test (a = 0.05)*.

The analysis of variance has showed significant influence of factors (cultivars and strains) on length of root of seeds of winter rye. The strains MCT IV and MCT I significantly differed (P<0.05) from strains "C" III and "C" V according to Fisher's criterion (fig. 5, 6). These strains were characterized high pathogenic qualities. Strain MCT V was characterized less pathogenic qualities to length of root. Strain MCT IV was characterized high toxic qualities to length of root, and strain MCT I was characterized less toxic qualities.

Distinctions in length of root of seeds of winter rye were significant (P<0.05). The least susceptible to cultural suspension were culture of rye Falenskaja 4 – length of root was 66 % in comparison with control. The least susceptible to culture filtrates were culture of rye Falenskaja 4, Purga and Bezenchukskaja 88. The most susceptible to culture filtrates were culture of rye Snezhana.

So *F. culmorum* showed high variability pathogenic and toxic qualities. It is necessary to note at the selection of resistance cultivars.

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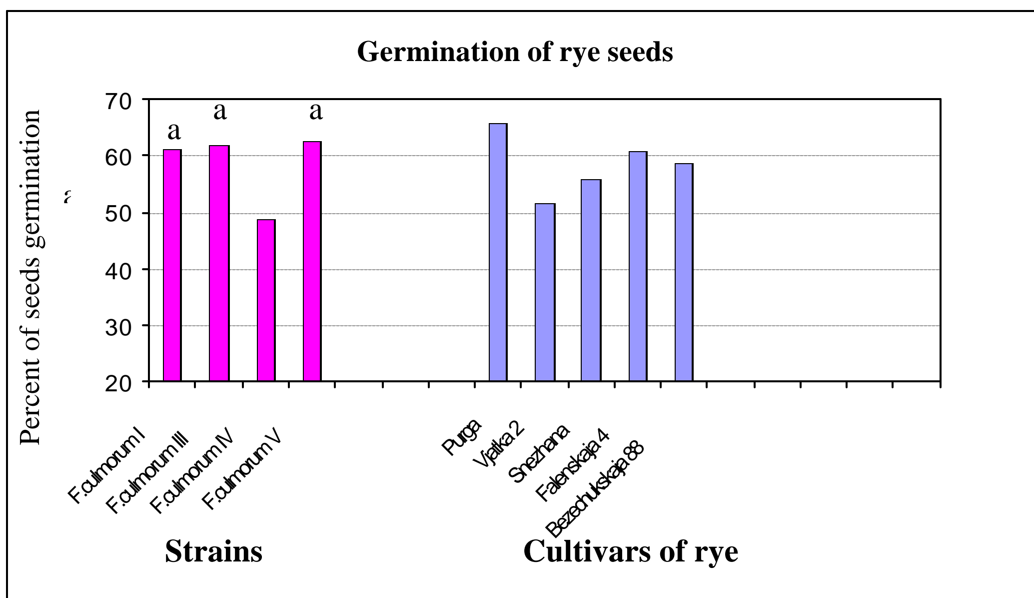

Figure 1. Mean percent of seed germination of five winter rye cultivars inoculated with conidial suspension of four strains *Fusarium culmorum* with various morphological - cultural types (? C?) of colonies (I, III, IV and V).

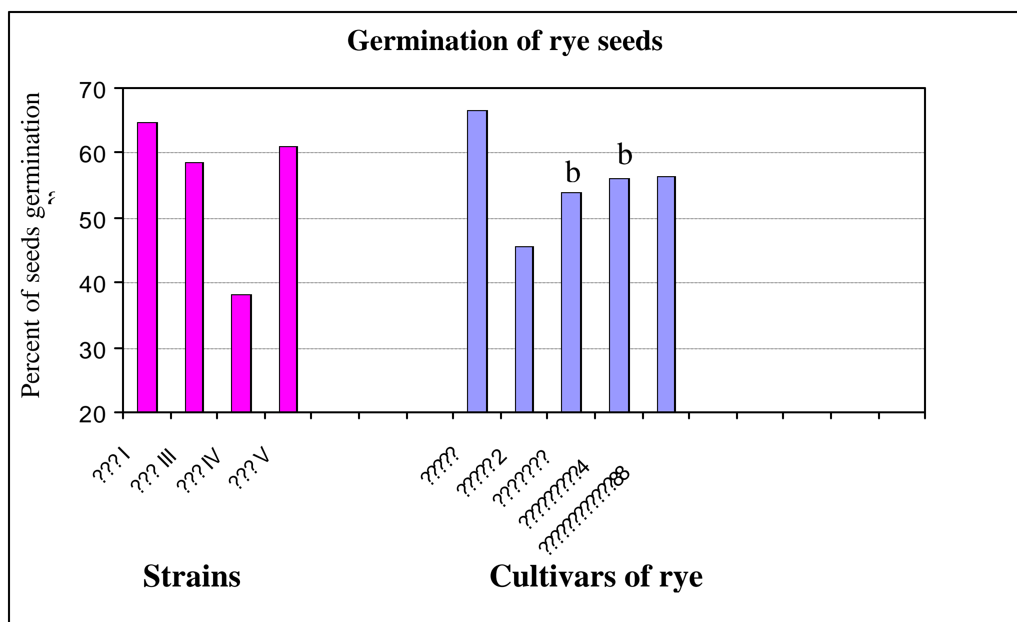

Figure 2. Mean percent of seed germination of five winter rye cultivars inoculated with culture filtrates of four strains *Fusarium culmorum* with various ? C? of colonies (I, III, IV and V).

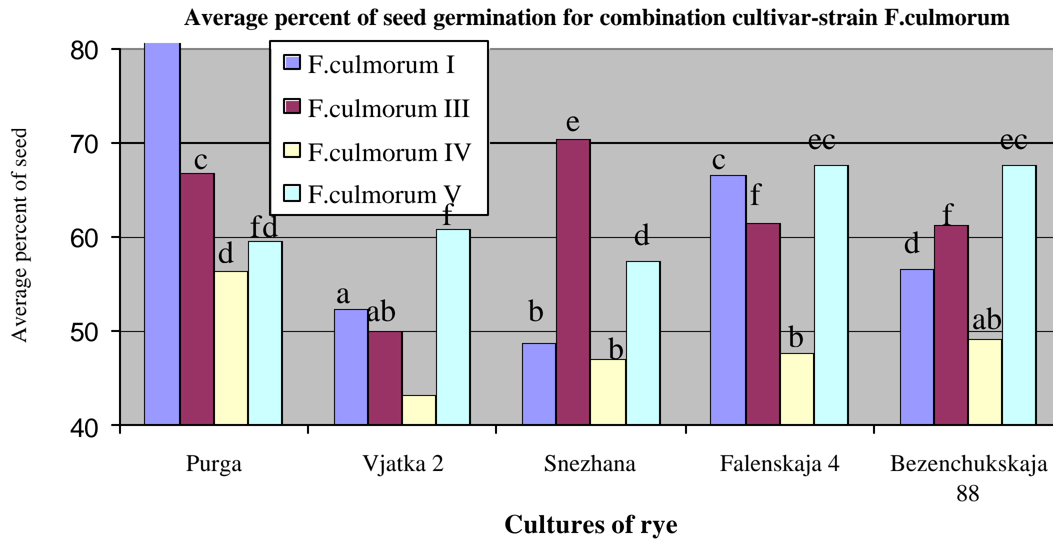

Figure3. Mean percent of seed germination of five winter rye cultivars inoculated with conidial suspension of four strains *Fusarium culmorum* with various ? C? of colonies (I, III, IV and V*)*.

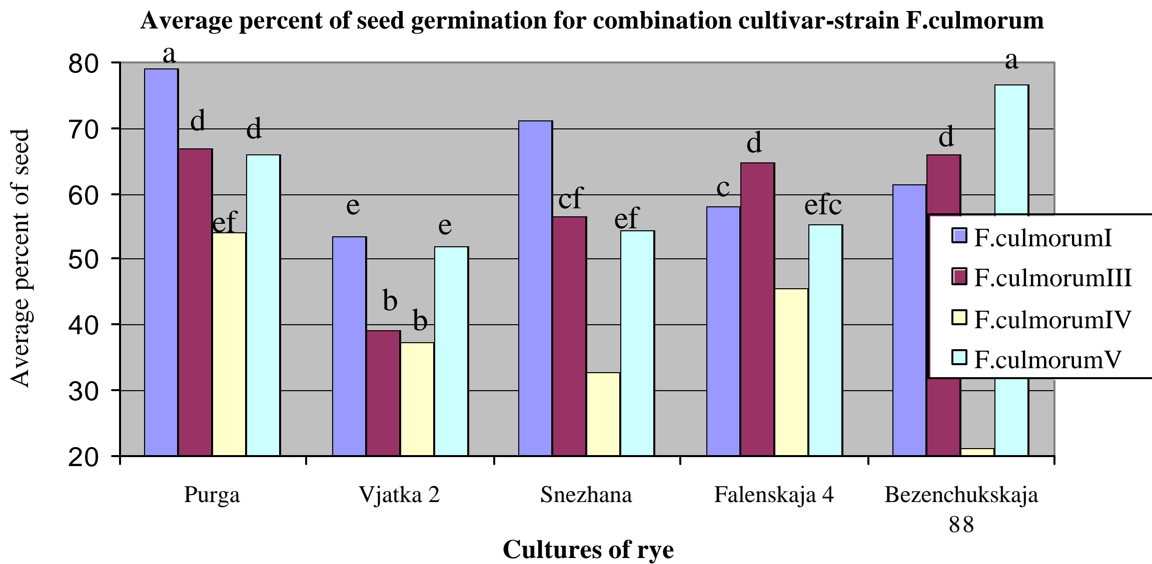

Figure 4. Mean percent of seed germination of five winter rye cultivars inoculated with culture filtrates of four strains *Fusarium culmorum* with various ? C? of colonies (I, III, IV and V*)*.

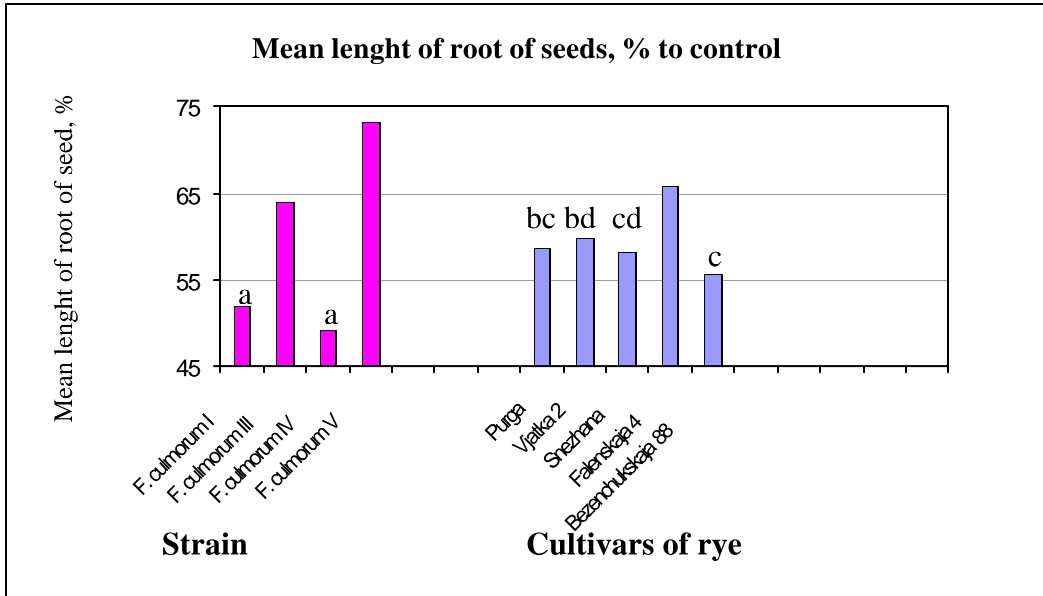

Figure 5. Mean length of root of seeds of five winter rye cultivars inoculated with conidial suspension of four strains *Fusarium culmorum* with various morphological - cultural types (? C?) of colonies (I, III, IV and V*).*

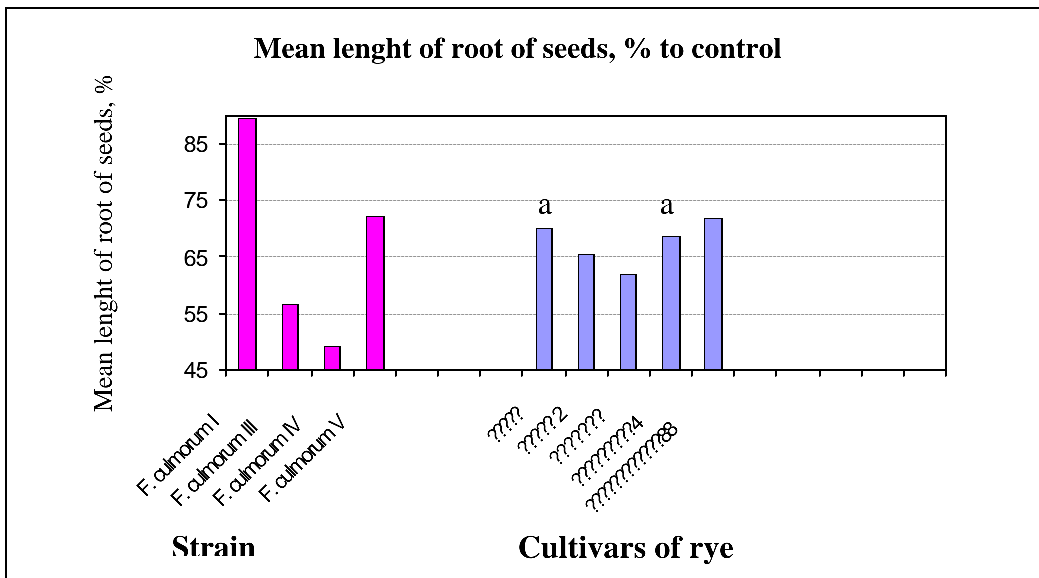

Figure 6. Mean length of root of seeds of five winter rye cultivars inoculated with culture filtrates of four strains *Fusarium culmorum* with various morphological - cultural types (? C?) of colonies (I, III, IV and V*).*

MYCOTOXIN PRODUCTION AND LINEAGE DISTRIBUTION IN CENTRAL EUROPEAN ISOLATES OF THE *FUSARIUM GRAMINEARUM* CLADE

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ABSTRACT

Fusarium head blight caused mainly by *Fusarium graminearum* and *F. culmorum* is the most important disease of wheat in Central Europe. Contamination of wheat by the mycotoxins produced by these and other species is the most serious effect of FHB, since these mycotoxins are harmful to both humans and animals. We examined the mycotoxin producing abilities, aggressiveness and molecular variability of *Fusarium graminearum* isolates using different techniques. Altogether 26 Hungarian, three Austrian isolates and representatives of eight species identified in the *F. graminearum* clade were involved in this study. Mycotoxin producing abilities of the isolates were tested by GC-MS and HPLC. The mycotoxins tested included type B trichothecenes (deoxynivalenol, 3- and 15-acetyl-deoxynivalenol, nivalenol, 4-acetyl-nivalenol (fusarenone X)) and zearalenone. All but one of the isolates produced zearalenone. The Central-European isolates were found to belong to chemotype I (producing deoxynivalenol). Most isolates produced both 15- and 3-acetyl-DON, but the majority of them produced more 15-acetyl-deoxynivalenol than 3-acetyl-deoxynivalenol suggesting that they belong to chemotype 1b. Huge differences were observed among DON producing abilities of the isolates (54-16.000 mg kg^{-1}). All *F. graminearum* isolates were found to be highly pathogenic in *in vitro* aggressiveness tests. During previous studies, most Central-European isolates were found to belong to lineage 7 characteristic to the Northern hemisphere, with the exception of one Hungarian isolate based on RAPD and IGS-RFLP data, and two other Hungarian isolates based on mitochondrial DNA RFLP analysis. We carried out sequence analysis of a putative reductase gene to ascertain the taxonomic position of these isolates. Sequence data confirmed that these isolates do not belong to the *F. graminearum* species, but represent isolates of the species *F. boothii* and a so far undescribed species closely related to *F. asiaticum*. The presence of mating type gene homologs have also been studied in the Central European isolates. All isolates carried both mating type idiomorphs in accordance with previous findings. Further work is in progress to compare the pathogenicity of the isolates belonging to different lineages in field tests.

USING GENOMICS TO UNDERSTAND THE LIFE CYCLE OF *GIBBERELLA ZEAE*

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ABSTRACT

In the last two years, the genome of *Gibberella zeae* has been sequenced and genome-based microarray chips have become available. These tremendous resources will provide an opportunity to study the life cycle of this important plant pathogen at a level not previously within reach. Over the last several years, we have characterized the development of perithecia *in vitro*, and more recently, we have elucidated the process of colonization of wheat tissue which leads to perithecium production. The latter studies have revealed the formation of vascular occlusions which prevent mycelia from colonizing the stem tissue. We have also been investigating the mechanism of forcible spore discharge in ascospore-producing fungi. For over 100 years, the working hypothesis has been that turgor pressure drives ascospore ejection. For the first time, we have shown that components of the ascus fluid are crucial to generating turgor within the ascus. The components include mannitol and ions. We are in the process of elucidating the role of each in discharge. In addition, we have identified a DNA binding protein that may be involved in controlling mechanism of ascospore discharge. These findings shed light on the environmental factors that influence spore discharge in the field.

How can we use genomics to extend these studies? We have begun to elucidate the gene expression shifts that accompany sexual development *in vitro* using a limited EST-based microarray. Genes showing highest expression level at earlier development stages were mainly those related to metabolism and cell type differentiation, while genes showed highest expression level at later development stages were mainly those related to cellular transport. We will be able to extend this survey to all of the genes in the genome with the new genome chip, and pinpoint those important for each developmental stage. We can identify the genes regulated by the DNA binding protein controlling discharge of spores, and thereby uncover the process whereby environmental factors are translated into spore release.

We have already used the genomic sequence to understand the production of mycotoxins. During host colonization, the fungus produces mycotoxins, including deoxynivalenol, zearalenone and aurofusarin, which make the grain unfit for human and animal consumption. Zearalenone and aurofusarin belong to the family of compounds called *polyketides*. Polyketides are produced by Polyketide Synthases (PKS) using acetyl or malonyl precursors. We used the recently released genomic sequence to identify all the PKS genes in the genome. We then disrupted each gene individually and analyzed the mutants phenotypically. We were able to assign function to five of 15 identified PKS genes. We continue to explore their role in the life cycle of this important pathogen.

These studies provide new targets for control of this devastating pathogen. Elucidation of the developmental processes underlying the disease cycle and the response of the pathogen to the environment is an important step towards developing integrated control strategies. This research provides information about infection pathways and serves as a basis for these and future investigations into the genetics of host-pathogen interactions.

GECCO: A BIOINFORMATICS TOOL FOR COMPARATIVE ANALYSIS OF FUNGAL GENOMES

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ABSTRACT

Decisive steps in the interactions between hosts and pathogens occur at the onset of infection. During these stages the fungal biomass is still very limited and the majority of mRNAs will originate from the host. To get a better understanding of the genes involved in the infection process we generated cDNA libraries of infected plants. In order to avoid sequencing large numbers of ESTs, before obtaining fungal genes, we have developed a procedure that specifically enriches for fungal sequences during the early (and later stages) of infection. This allowed us to generate large datasets containing pathogen genes involved in the early stages of pathogenesis in various pathosystems. Together with the huge amounts of data available through several genome-sequencing efforts (Whitehead, Sanger, TIGR, JGI/DOE, Genescope) there is a great need for bioinformatics tools to mine and compare these large datasets. A bioinformatics platform has been developed that performs automated analysis of sequence datasets and allows for fast and robust comparison of different databases. Among 1724 *F. graminearum* in *planta* unigenes, we identified three ABC transporters that were not present in the annotated genome of *Magnaporthe grisea*, two of which were also absent in the *Neurospora crassa* genome. Among 4452 *in planta* unigenes from *Mycosphaerella graminicola*, we identified 41 unigenes involved in signal transduction, four of which were not previously identified in *N. crassa.*

AN EVOLUTIONARY FRAMEWORK FOR TACKLING FUSARIUM HEAD BLIGHT; SPECIES RECOGNITION, TOXIN EVOLUTION, AND BIOGEOGRAPHY OF THE *FUSARIUM GRAMINEARUM* SPECIES COMPLEX

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ABSTRACT

Effective disease control programs that minimize the threat of FHB to the producers, processors, and consumers of wheat and barley require a detailed understanding of pathogen diversity. Although the primary etiological agent of FHB, *Fusarium graminearum,* has been regarded as a single, panmictic species worldwide, we previously used genealogical concordance phylogenetic species recognition (GCPSR) to demonstrate that this morphospecies actually consists of at least nine phylogenetically distinct and biogeographically structured species (the *Fg* complex).

Here we report the identification of several additional species within the *Fg* complex, including a novel species isolated from wheat within the U.S. In addition to the extraordinary species diversity that we have discovered, we have also demonstrated that the virulence-associated trichothecene mycotoxin genes are under a novel form of balancing selection, which may have important consequences for the fitness and aggressiveness of FHB pathogens on particular hosts or in particular environments. It appears that only a fraction of FHB species/chemotype diversity is currently represented within North America. Therefore, the introduction of novel FHB pathogens or chemotypes via global trade in agricultural products has the potential to exacerbate the FHB problem in the U.S. Using a unique multi-locus DNA sequence database (11 nuclear genes, 13.6 kb of DNA sequence) we have developed a high-throughput single tube assay for the simultaneous identification of all known B-trichothecene FHB species and chemotypes in order to improve disease surveillance efforts and to facilitate a greater understanding of the ecology, epidemiology, and population dynamics of these FHB pathogens.