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Analysis of the *Fusarium graminearum* species complex from wheat, barley and maize in South Africa provides evidence of species-specific differences in host preference

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ABSTRACT

Species identity and trichothecene toxin potential of 560 members of the *Fusarium graminearum* species complex (FGSC) collected from diseased wheat, barley and maize in South Africa was determined using a microsphere-based multilocus genotyping assay. Although three trichothecene types (3-ADON, 15-ADON and NIV) were represented among these isolates, strains with the 15-ADON type predominated on all three hosts. A significant difference, however, was identified in the composition of FGSC pathogens associated with Gibberella ear rot (GER) of maize as compared to Fusarium head blight (FHB) of wheat or barley ($P < 0.001$). *F. graminearum* accounted for more than 85% of the FGSC isolates associated with FHB of wheat and barley ($N = 425$), and was also the dominant species among isolates from maize roots ($N = 35$). However, with the exception of a single isolate identified as an interspecific hybrid between *Fusarium boothii* and *F. graminearum*, GER of maize ($N = 100$) was exclusively associated with *F. boothii*. The predominance of *F. graminearum* among FHB isolates, and the near exclusivity of *F. boothiii* among GER isolates, was observed across all cultivars, collection dates, and provinces sampled. Because these results suggest a difference in host preference among species of the FGSC, we hypothesize that *F. graminearum* may be less well adapted to infect maize ears than other members of the FGSC.

1. Introduction

Members of the *Fusarium graminearum* species complex (FGSC) infect wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and other cereal crops across the world, causing diseases such as Fusarium head blight (FHB) and Gibberella ear rot (GER). These diseases significantly reduce grain yield and quality. In addition, FHB and GER are significant food safety problems because infected grain is often contaminated with trichothecene mycotoxins that inhibit protein synthesis and can produce a variety of health problems in humans and other animals (Bennett and Klich, 2003; Pestka, 2010). Trichothecenes also are phytotoxic and act as virulence factors on sensitive plant hosts (Jansen et al., 2005; Proctor et al., 1995). In South Africa, GER has been reported on maize (Marasas et al., 1979) and FHB on irrigated wheat (Marasas et al., 1988; Scott et al., 1988). With the substantial increase in wheat, barley and maize production areas overlapping in recent years, FHB and GER have become important diseases, particularly in irrigated fields. As is the case in much of the world, mycotoxin contamination of maize and wheat is of great concern in South Africa because these cereals constitute the major staple food of South Africans and because they are used extensively as animal feed. Moreover, trichothecene contamination of barley is of concern to the malting and beer brewing industries of South Africa.

To date, 13 species have been identified and formally described within the FGSC (O'Donnell et al., 2000, 2004, 2008; Starkey et al., 2007; Yli-Mattila et al., 2009), with *F. graminearum sensu stricto* being the species most commonly associated with FHB worldwide. In addition, a putatively new species within the FGSC was recently identified from Nepal (Chandler et al., 2003; Desjardins and Proctor, 2011). FGSC species produce type B trichothecenes (Goswami and Kistler, 2005; O'Donnell et al., 2008; Starkey et al., 2007; Yli-Mattila et al., 2009) that include deoxynivalenol (DON) and its acetylated forms 3-acetyl-deoxynivalenol

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(3-ADON) and 15-acetyl-deoxynivalenol (15-ADON), as well as nivalenol (NIV) and its acetylated form 4-acetylnivalenol or fusarenone X (FX). Among the type B trichothecene-producing *Fusarium* species, three strain-specific chemotypes have been identified: the NIV chemotype produces NIV and its acetylated derivatives, the 3-ADON chemotype produces DON and 3-ADON, and the 15-ADON chemotype produces DON and 15-ADON (Miller et al., 1991). Although a slight difference exists between these compounds in the pattern of hydroxylation and acetylation, the biological activities of these compounds can be different, indicating that chemotype differences may have important health consequences (Kimura et al., 1998).

In recent years, numerous studies have been conducted to assess species and trichothecene chemotype diversity among FGSC isolates infecting wheat, barley and maize around the world, including North America (Ward et al., 2008), South America (Ramirez et al., 2006; Scoz et al., 2009), Europe (Láday et al., 2004; Tóth et al., 2005; Yli-Mattila et al., 2009), Asia (Chandler et al., 2003; Desjardins and Proctor, 2011; Suga et al., 2008; Yang et al., 2008; Yli-Mattila et al., 2009), and New Zealand (Monds et al., 2005). However, very little information is available on FGSC diversity in Africa, with limited surveys having been conducted in Ethiopia (O'Donnell et al., 2008) and Kenya (Wagacha et al., 2010). Moreover, few studies worldwide have investigated the diversity of the FGSC recovered from maize, with most of the research focusing on wheat and barley. In this context, the objective of this study was to determine the species identity and trichothecene toxin potential of FGSC isolates collected from wheat, barley and maize from South Africa using a recently described microsphere-based multilocus genotyping assay (MLGT) (Ward et al., 2008). Such information is critical for developing management strategies for FHB and GER in South Africa, since these crops are often produced in rotation on the same fields, and is also needed to improve understanding of the distribution and significance of FGSC diversity worldwide.

2. Materials and methods

2.1. Isolates

A total of 560 South African isolates tentatively identified as members of the FGSC were selected for this study (Supplemental Table 1). These included 277 isolates from FHB-infected heads of five different wheat cultivars collected from all wheat-growing areas (six provinces, 23 localities) in 2008 and 2009 (Fig. 1a). In addition, 100 isolates were recovered from GER-infected maize ears of nine different cultivars collected from all maize-growing areas (five provinces, 19 localities) in 2008 and 2010 (Fig. 1b). An additional 35 isolates were obtained from maize roots collected from one cultivar at one locality in the KwaZulu-Natal Province in 2006; those 35 isolates were provided by Dr. Sandra Lamprecht (Plant Protection Research Institute, South Africa). Finally, 148 isolates were obtained from FHB-contaminated barley heads collected in 2008 and 2009 from two different cultivars at eight localities in the Northern Cape Province of South Africa, where barley cultivation under irrigation is most common. Kernels were surface-disinfected by washing them in 70% ethanol for 1.5 min and then in 2% sodium hypochlorite for 3 min. The kernels were then extensively rinsed with sterile distilled water and placed on potato dextrose agar (PDA) plates containing 40 mg l^{-1} streptomycin. Plates were incubated for 5 days at 25 °C. *Fusarium* colonies were sub-cultured on new PDA plates using a single spore technique (Leslie and Summerell, 2006) and subsequently stored in 15% glycerol at −80 °C at the Department of Plant Pathology, Stellenbosch University, South Africa.

2.2. Multilocus genotyping assay (MLGT)

After isolates were grown on PDA plates for 5 days, genomic DNA was extracted from harvested aerial mycelia using the Wizard® SV Genomic DNA purification System Kit (Promega, South Africa). Isolates were subjected to a multilocus genotyping assay (MLGT) for simultaneous determination of species identity and prediction of trichothecene chemotype as previously described (Ward et al., 2008). Briefly, multiplex amplifications were performed targeting six genes. The loci targeted in this assay include housekeeping genes and genes responsible for trichothecene biosynthesis. PCR products were subjected to allele-specific primer extension (ASPE) reactions in multiplex reactions containing 45 probes, with each species and B-trichothecene chemotype targeted by probes in two different genes. Probes are described by O'Donnell et al. (2008), Ward et al. (2008), and Yli-Mattila et al. (2009). ASPE probes were designed with a unique 5′ sequence tag specific to individual sets of Luminex (Luminex Corporation, Austin, TX, USA) xMAP fluorescent polystyrene microspheres. Biotinylated extension products from ASPE reactions were sorted by hybridization to polystyrene microsphere sets coated with antitag sequences specific to the unique sequence tags appended to each of the extension probes. Individual microsphere sets also were labeled with a specific mix of fluorescent dyes creating a unique spectral address that enabled extension products from different probes to be evaluated individually. Hybridization and detection were performed using a Luminex 100 flow cytometer (Luminex Corporation). Significance of differences in species composition was assessed via chi-square or Fisher exact probability tests.

2.3. Sequence analysis

One isolate (M-0014), collected from a maize ear in Gauteng Province in 2008, that could not be typed to species based on MLGT analysis was further characterized by sequencing portions of the translation elongation factor 1α (*TEF-1*) gene, the trichothecene 3-*O*-acetyltransferase (*TRI101*) gene, a putative reductase gene (RED), and the mating-type locus (MAT). Sequencing reactions were carried out according to the method of Platt et al. (2007). Sequencing reaction products were purified using BigDye XTerminator (Applied Biosystems, Foster City, CA, USA) and analyzed with an ABI 3730 DNA Analyzer (Applied Biosystems). DNA sequences were edited and aligned with Sequencher (version 4.10, Gene Codes, Ann Arbor, MI). The resulting DNA sequences were compared to previously published sequences (O'Donnell et al., 2000, 2004, 2008; Starkey et al., 2007; Ward et al., 2002; Yli-Mattila et al., 2009) deposited in the NCBI database using the Basic Local Alignment Search Tool (BLAST) and were also analyzed phylogenetically by the neighbor-joining method with the Kimura two-parameter model of molecular evolution implemented in MEGA (version 4.0; Tamura et al., 2007). Support for individual nodes was assessed by bootstrap analysis with 1000 replications.

3. Results

3.1. Species diversity

Six of the 13 previously described species within the FGSC were recovered in our survey of FHB and GER in South Africa. These included *F. graminearum*, *Fusarium boothii*, *Fusarium meridionale*, *Fusarium cortaderiae*, *Fusarium acaciae-mearnsii*, and *Fusarium brasilicum* (Table 1). Among the isolates collected from wheat ($N = 277$), the vast majority were identified as *F. graminearum* (85.2%). However, *F. boothii* (8.3%), *F. meridionale* (3.6%), *F. acaciae-mearnsii* (1.4%), *F. cortaderiae* (1.1%), and *F. brasilicum* (0.4%)