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Regional differences in the composition of Fusarium Head Blight pathogens and mycotoxins associated with wheat in Mexico

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ABSTRACT

Fusarium Head Blight (FHB) is a destructive disease of small grain cereals and a major food safety concern. Epidemics result in substantial yield losses, reduction in crop quality, and contamination of grains with trichothecenes and other mycotoxins. A number of different fusaria can cause FHB, and there are significant regional differences in the occurrence and prevalence of FHB pathogen species and their associated mycotoxins. Information on FHB pathogen and mycotoxin diversity in Mexico has been extremely limited, but is needed to improve disease and mycotoxin control efforts. To address this, we used a combination of DNA sequence-based methods and in-vitro toxin analyses to characterize FHB isolates collected from symptomatic wheat in Mexico during the 2013 and 2014 growing seasons. Among 116 *Fusarium* isolates, we identified five species complexes including nine named *Fusarium* species and 30 isolates representing unnamed or potentially novel species. Significant regional differences ($P < 0.001$) in pathogen composition were observed, with *F. boothii* accounting for > 90% of isolates from the Mixteca region in southern Mexico, whereas *F. avenaceum* and related members of the *F. tricinctum* species complex (FTSC) accounted for nearly 75% of isolates from the Highlands region in Central Mexico. *F. graminearum*, which is the dominant FHB pathogen in other parts of North America, was not present among the isolates from Mexico. *F. boothii* isolates had the 15-acetyldeoxynivalenol toxin type, and some of the minor FHB species produced trichothecenes, such as nivalenol, T-2 toxin and diacetoxyscirpenol. None of the FTSC isolates tested was able to produce trichothecenes, but many produced chlamydosporol and enniatin B.

1. Introduction

Fusarium Head Blight (FHB) is a destructive disease of wheat and other small grain cereals worldwide. Epidemics result in significant yield losses, reduction in crop quality and contamination of grain with trichothecenes and other mycotoxins that can compromise food safety and animal health (Goswami and Kistler, 2004). *Fusarium graminearum* is the principal FHB pathogen of wheat in many regions, although other members of the *F. graminearum* species complex (FGSC) are locally predominant and may have an adaptive advantage on some hosts (Boutigny et al., 2011; Kuhnem et al., 2016; Lee et al., 2009; O'Donnell et al., 2004). The FGSC is a component of the broader *F. sambucinum* species complex (FSAMSC), which includes additional FHB pathogens such as *F. cerealis* and *F. poae* (O'Donnell et al., 2013). However, numerous *Fusarium* species from other species complexes defined by O'Donnell et al. (2013), including *F. avenaceum* from the *F. tricinctum* species complex (FTSC), can be important contributors to FHB of wheat (Gräfenhan et al., 2013).

FHB pathogens within the FGSC typically produce one of three strain-specific profiles (chemotypes) of B-trichothecene mycotoxins. These chemotypes are distinguished by production of (i) nivalenol and acetylated derivatives (NIV chemotype), (ii) 3-acetyldeoxynivalenol (3ADON chemotype) or (iii) 15-acetyldeoxynivalenol (15ADON chemotype) (Miller et al., 1991). Although these toxins are structurally similar, B-trichothecene chemotype diversity has been maintained by natural selection through multiple speciation events and appears to

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have important consequences for pathogen fitness (Ward et al., 2002). B-trichothecenes are common contaminates of wheat, and regulatory or advisory limits for these toxins, particularly deoxynivalenol (DON), have been established in many countries. However, other *Fusarium* species can produce A-trichothecenes such as T-2 toxin and diacetoxyscirpenol (DAS) that are highly toxic but less frequent contaminants of wheat. In addition, a small percentage of *F. graminearum* isolates from the United States and Canada were found to produce a novel A-trichothecene, NX-2 (Kelly et al., 2015; Liang et al., 2014; Varga et al., 2015). Given this diversity, understanding the regional composition of FHB pathogen species and their trichothecene toxin potential is critical to promoting effective disease and mycotoxin control programs. For example, the discovery of *F. graminearum* and *F. asiaticum* isolates with the NIV chemotype in Louisiana (Gale et al., 2011) highlighted the need for mycotoxin monitoring programs capable of detecting NIV contaminated cereals.

In Mexico, FHB was observed for the first time in Jalisco during the 1977 growing season. In 1994, the International Maize and Wheat Improvement Center (CIMMYT) recorded severe FHB outbreaks in some states of the North-Central and Highland regions in Mexico. A particularly severe outbreak in Los Altos and Sierra del Tigre, Jalisco caused losses of up to 90% of the wheat crop, depending on the wheat variety and local climatological conditions (Ireta and Gilchrist, 1994). However, information on *Fusarium* species and mycotoxin diversity associated with FHB of wheat in Mexico is limited. Osman et al. (2016) identified 15ADON as the dominant chemotype among 388 FHB isolates from wheat, but this study did not include species identifications within the FGSC. Malihipour et al. (2012) conducted species and chemotype analyses of FHB isolates from Mexico, but this was limited to 15 isolates. The aim of the current study was to improve understanding of FHB pathogen composition and toxin potential in Mexico by characterizing the prevalence of *Fusarium* species and mycotoxins among 116 FHB isolates from collections obtained during the 2013 and 2014 growing seasons.

2. Materials and methods

2.1. Sampling

Spikes of spring wheat with symptoms of FHB were collected from a total of 53 municipalities and 77 fields in Mexico, collecting 10 FHB symptomatic spikes in each field. The 10 spikes from each field were combined into a single sample for analysis. Samples were grouped into three regions distinguished by temperature and precipitation: (i) the Mixteca region (Oaxaca State) in southern Mexico has a warm and humid climate, where the average annual temperature is 25 °C and average annual precipitation is 1550 mm; (ii) the Bajio region in central Mexico, specifically southwest of Guanajuato has a temperate climate with an average temperature of 18 °C and 650 mm of annual precipitation on average; and (iii) the Highlands region, including Tlaxcala, Puebla and Mexico states where the average temperatures are 14, 17.5 and 14.7 °C, respectively, with dry and hot summers and cold winters (Fig. 1). Sampling in the Highlands was conducted in 2013 and 2014. Sampling in Bajio and Mixteca was limited to 2014. The typical sowing and harvesting dates for Mixteca, Bajio and Highlands regions were June–September, December–March and May–August, respectively. The average annual precipitation in these Highlands states are 1270 mm, 720 mm and 900 mm, respectively. Climatic conditions near the heading dates for wheat were different among the regions. In Mixteca, the incidence of rain was higher than the Highlands and Bajio. Fifteen fields were sampled from 5 municipalities in Mixteca, 14 fields were sampled from seven municipalities in Bajio, and 48 fields were sampled from 40 municipalities in the Highlands.

2.2. Fungal isolation

Fusarium sp. were isolated using the freezing blotter test (Warham et al., 1997). For each of the 77 fields that were sampled, kernels from ten FHB symptomatic spikes were pooled and 60 randomly selected kernels were surface sterilized for 3 min in 3% sodium hypochlorite and rinsed three times with autoclaved distilled water. Kernels were placed in germination boxes with wet filter paper and incubated for six days at 20–25 °C with a 12:12 h light:dark photoperiod. Isolated colonies, macro- and microscopically identified as *Fusarium* spp., were transferred to water-agar and sub-cultured onto potato dextrose agar (PDA) using a single spore or hyphal tip culture (Leslie and Summerell, 2006). Cultures were stored in glycerol (15%, v/v in water) at −80 °C. Subcultures of each isolate are held by the U. S. Department of Agriculture, Peoria, IL.

2.3. Multilocus genotyping and DNA sequencing

Total genomic DNA was extracted from 6- to 8-day old cultures grown on V8 using ZR Fugal/Bacterial DNA MiniPrep™ (Zymo Research) kits according to manufacturer instructions. Species identification and determination of trichothecene type was performed by multilocus genotyping (MLGT) using a Luminex 100 flow cytometer as previously described (Sarver et al., 2011; Ward et al., 2008). The MLGT assay was designed to simultaneously determine the species identity and trichothecene genotype of isolates from the 16 described species within the FGSC as well as five related species within FSAMSC lineage 1 (FSAMSC-1, also referred to as the B clade) (Kelly et al., 2016).

Isolates that could not be identified by MLGT were analyzed using partial sequences of the translation elongation factor 1α (*TEF1*) gene generated with primers EF-1 (5′-ATGGGTAAGGAGGACAAGAC-3′) and EF-2 (5′-GGAAGTACCAGTGATCATG-3′) (O'Donnell et al., 1998). Amplification reactions were carried out in 25 μL volume and included 50 mM $MgSO_4$, 2 mM of each deoxynucleoside triphosphate, 0.6 mM each primer, 1 U of Platinum Taq High Fidelity polymerase (Invitrogen) and 50 ng of genomic DNA. PCR conditions were: initial denaturation for 90 s at 96 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 53 °C, 45 s at 68 °C. PCR products were assessed by agarose gel electrophoresis and were purified with Millipore MultiScreen–PCR_{96} filter plates (Millipore). DNA sequencing was performed using Big Dye Terminator Sequencing kit v3.1 (Applied Biosystems) and analyzed with an ABI 3730 DNA Analyzer (Applied Biosystems). The sequences were edited and aligned manually using Sequencher (version 4.10, Gene Codes). Sequence similarity searches were performed with the Basic Local Alignment Search Tool (BLAST) network service of the Fusarium ID database (http://isolate.fusariumdb.org/blast.php) (Geiser et al., 2004; Park et al., 2011) and the National Center for Biotechnology Information (NCBI) nonredundant nucleotide database. Fisher's exact test was used to evaluate regional differences in pathogen composition.

2.4. Phylogenetic analyses

Isolates from the FTSC were subjected to phylogenetic analyses in order to further evaluate species identity and relationships among strains. In addition to *TEF1* sequences, we collected partial sequences of the RNA polymerase II second largest subunit (*RPB2*) gene from each of the FTSC isolates using primers 5F2 (5′GGGGWGAYCAGAAGAAGGC3′) and 11ar (5′GCRTGGATCTTRTCRTCSACC3′). Amplification reactions were carried out in 25 μL volumes and included 50 mM $MgSO_4$, 2.5 mM of each deoxynucleoside triphosphate, 0.01 mM each primer, 1 U of Platinum Taq High Fidelity polymerase (Invitrogen) and 40 ng of genomic DNA. PCR was performed with an initial denaturation at 96 °C for 90 s, followed by 35 cycles of 94 °C for 30 s, 54 °C for 35 s, and 68 °C for 2 min. Purification and DNA sequencing reactions were conducted as described above.

Two sets of phylogenetic analyses were undertaken. In the first set