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Determination of the trichothecene mycotoxin chemotypes and associated geographical distribution and phylogenetic species of the *Fusarium graminearum* clade from China

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ABSTRACT

A large number of isolates from the *Fusarium graminearum* clade representing all regions in China with a known history of *Fusarium* head blight (FHB) epidemics in wheat were assayed using PCR to ascertain their trichothecene mycotoxin chemotypes and associated phylogenetic species and geographical distribution. Of the 299 isolates assayed, 231 are from *F. asiaticum* species lineage 6, which produce deoxynivalenol and 3-acetyldeoxynivalenol (3-AcDON); deoxynivalenol and 15-acetyldeoxynivalenol (15-AcDON); and nivalenol and 4-acetylnivalenol (NIV) mycotoxins, with 3-AcDON being the predominant chemotype. Ninety-five percent of this species originated from the warmer regions where the annual average temperatures were above 15 °C, based on the climate data of 30 y during 1970–1999. However, 68 isolates within *F. graminearum* species lineage 7 consisted only of 15-AcDON producers, 59 % of which were from the cooler regions where the annual average temperatures were 15 °C or lower. Identification of a new subpopulation of 15-AcDON producers revealed a molecular distinction between *F. graminearum* and *F. asiaticum* that produce 15-AcDON. An 11-bp repeat is present in *F. graminearum* within their *Tri7* gene sequences but is absent in *F. asiaticum*, which could be directly used for differentiating the two phylogenetic species of the *F. graminearum* clade.

Introduction

Fusarium head blight (FHB) or scab of wheat and other small cereal grains caused by the *Fusarium graminearum* clade is an economically devastating disease worldwide (Windels 2000). FHB takes place both in field and during storage, producing mycotoxins in mouldy corn and wheat that are detrimental to human and animal health. Recently, FHB has reached epidemic proportions in Europe and North America, resulting in huge losses in crop revenues due to reduced yields and mycotoxin contamination of stored grains (Windels 2000). In China, epidemics of FHB occur frequently in the middle and downstream regions of the Yangtze River and in the Heilongjiang province in the northeastern region (Chen *et al.* 2000). With the changes of global climates FHB has gradually spread to the northwestern regions, covering more than ten provinces that are mainly agricultural areas in China (Chen *et al.* 2000). *Fusarium* mycotoxins are among the main fungal mycotoxin contaminations in food and livestock (Bai 1997), and some human diseases, such as Kashi neck diseases and

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oesophageal cancer, have been epidemiologically associated with consumption of trichothecences (Chen et al. 2000).

Type B trichothecenes (8-ketotrichothecenes) are the principal toxins produced by the *F. graminearum* clade. Based on the production and chemical structures of different 8-ketotrichothecenes, three trichothecene mycotoxin chemotypes were identified within the type B-trichothecene-producing *F. graminearum* clade: (1) nivalenol and 4-acetylnivalenol (NIV chemotype), (2) deoxynivalenol and 3-acetyldeoxynivalenol (3-AcDON chemotype), and (3) deoxynivalenol and 15-acetyldeoxynivalenol (15-AcDON chemotype) (Miller et al. 1991; Ward et al. 2002). The chemotypes appear to differ in geographical distribution, with both DON and NIV chemotypes reported in several countries of Africa, Asia, and Europe (Miller et al. 1991; Jennings et al. 2004b) but only the DON chemotype was reported in North America (Mirocha et al. 1989). Earlier studies by chemical analysis focused on DON detections of *F. graminearum*, and a recent study has revealed the presence of both DON and NIV chemotypes in China (Li et al. 2005). However, the acetylated derivatives of the *Fusarium* chemotypes and their associations with phylogenetic species and geographical distribution are yet to be investigated.

For analysis of the mycotoxins and their derivatives, chemical methods such as capillary gas chromatography and hplc are commonly applied (Langseth & Rundberget 1998), and quick and simple screening methods such as ELISAs have also been used (Dietrich et al. 1995). However, these methods are rather labour-intensive, expensive, and require sophisticated instrumentation and skilled operators. Molecular characterization of trichothecene mycotoxin biosynthesis pathways has brought the development of new methods for the fast analysis of the mycotoxins *via* PCR with specific primers, which have been widely used due to their advantages over the conventional methods (Bakan et al. 2002; Chandler et al. 2003; Gale et al. 2003; Jennings et al. 2004a,b; Li et al. 2005).

Our specific objectives in this study were (1) to determine the mycotoxin chemotypes of the *F. graminearum* clade from China using a series of PCR assays, (2) to reveal association of the chemotypes with their phylogenetic species and geographical distribution, and (3) to find a molecular distinction between two *Fusarium* phylogenetic species that produce 15-AcDON.

Materials and methods

Isolates of Fusarium *strains*

Diseased wheat spikes were taken in 1999 from fields that were separated by at least 3 km, and the numbers of the collected wheat ears with clear FHB symptoms were proportional to the acreage of wheat infected by the disease in the regions. These collections represent samples from all the areas in the Yangtze River valleys and other regions in China with known history of FHB epidemics in wheat, covering 12 provinces in China. Strains were obtained by single spore isolation from the diseased wheat spikes and identified by the methods described previously (Booth 1971; Nelson et al. 1984). In total 299 isolates of *F. graminearum* were used in this study and detailed information of the strains are listed in Table 1. All strains used in the study have been deposited in the Molecular Biotechnology Laboratory, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China. These materials are available for public use on request.

Mycelial DNA extraction

All the *Fusarium* strains were grown on sterile glass–membrane paper over potato-dextrose agar at 23 °C for one week. The mycelia were harvested and ground to a fine powder in the presence of liquid nitrogen. The total genomic DNA was extracted as described by Nicholson et al. (1997).

Identification of DON and NIV chemotypes

A pair of generic primers ToxP1/P2 derived from the intergenic sequences between *Tri5* and *Tri6* genes was synthesized and used for the identification of DON and NIV chemotypes of *Fusarium graminearum*, generating a 300 bp fragment specific for DON producers and a 360 bp fragment from NIV chemotypes, respectively (Li et al. 2005). To verify the mycotoxin chemotypes identified by the ToxP1/P2 primers, another pair of primers GzTri7f1/r1, based on the *Tri7* sequence, was used to differentiate DON and NIV chemotypes. This set of primers produced PCR products ranging in size from 173–327 bp for DON chemotypes or a product of 161 bp for NIV producers (Lee et al. 2001).

Identification of 3-AcDON- and 15-AcDON chemotypes

Two sets of primers, Tri303F/R and Tri315F/R, developed based on the *Tri3* gene sequences, were used to further differentiate the DON chemotypes of *Fusarium graminearum* into 3-AcDON- or 15-AcDON chemotypes. *Fusarium* DON-producing isolates produce either a 3-AcDON-specific product of 586 bp with the primers Tri303F/R or a 15-AcDON specific fragment of 864 bp with the primers Tri315F/R (Jennings et al. 2004a). NIV-producing strains in *F. graminearum* do not produce any fragments with both primer sets.

To validate the chemotypes identified above, a pair of primers designed based on *Tri3* and *Tri8* intergenic sequences, MinusTri7F/R, were used to amplify a specific 483 bp fragment from *Fusarium* 3-AcDON-producing isolates only, which lack the entire *Tri7* gene and flanking regions (Ward et al. 2002; Chandler et al. 2003; Kimura et al. 2003).

SCAR analysis

All the *Fusarium* strains were subjected to SCAR (sequence characterised amplified region) analysis with a pair of primers Fg16F/R (Carter et al. 2000). A 410 bp DNA fragment specific for SCAR group I and a 497 bp fragment for SCAR group V were generated, respectively.

Analysis of lineage and phylogenetic species

Portions of three phylogenetically informative genes (*Tri101*, *reductase* and *histone H3*) from 30 strains of different chemotypes and originations were amplified and sequenced as described previously (O'Donnell et al. 2000, 2004). Among these strains, 20 strains are from phylogenetic species *Fusarium*