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Chemotypes and geographic distribution of the *Fusarium graminearum* species complex

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ABSTRACT

The *Fusarium graminearum* species complex (FGSC) consists of phylogenetically distinct pathogenic species. Isolates from various regions display genetic variety worldwide. Three type B trichothecene chemotypes have been identified within the FGSC: nivalenol, 3-deoxynivalenol and 15-deoxynivalenol. The variations in morphological, genetic and virulence traits of FGSC fungi can be attributed mainly to their geographic boundaries. The geographic range of host plants, type of farming system and weather conditions also influence the prevalence of FGSC taxa. The geographic distribution of FGSC members may reflect not only their chemotype but also adaptive traits. While 15-acetyl-deoxynivalenol (15-ADON) chemotype is prevalent in most of Europe, the 3-acetyl-deoxynivalenol (3-ADON) chemotype has achieved greater prevalence in parts of North America. The Asian species *F. asiaticum* has spread into new territories. Isolates of *F. asiaticum* have been identified in North America and Europe, and the species has recently been reported to be infecting cereal crops in South America. The occurrence of numerous members of the FGSC in those regions and the introduction of *F. asiaticum* into new areas raise significant food safety concerns and indicate the need for monitoring mycotoxin concentrations in harvested grain.

ABBREVIATIONS

15-ADON	15-acetyl-deoxynivalenol
3-ADON	3-acetyl-deoxynivalenol
AFLP	amplified fragment length polymorphism
DON	deoxynivalenol
FGSC	*Fusarium graminearum* species complex
FHB	Fusarium head blight
GC-MS	gas chromatography-mass spectrometry
GCPSR	genealogical concordance phylogenetic species recognition
HPLC	high-performance liquid chromatography
MLGT	multilocus genotyping
NIV	nivalenol
PCR	polymerase chain reaction

RAPD	random amplification of polymorphic DNA
RFLP	restriction fragment length polymorphism
SCAR	sequence characterized amplified region
SNP	random single nucleotide polymorphism
SSCP	single stranded confirmation polymorphism
VNTR	variable number tandem repeat
ZEA	zearalenon (F-2 toxin)

INTRODUCTION

Members of the genus *Fusarium* are one of the major groups of pathogens that generate very high losses in global agriculture. *F. graminearum* is one of the most dangerous and widespread species of pathogenic fungi capable of producing mycotoxins. The pathogen's toxicity is associated with high variations in virulence traits, the spread of isolates with specific attributes into new regions, the ability to produce three types of mycotoxins as well as fungicide resistance (Chen and Zhou 2009; Yli-Mattila 2010; Yli-Mattila et al. 2009; Spolti et al. 2014; Zhang et al. 2012). Significant genetic and morphological diversity of FGSC members across geographic regions has prompted researchers to establish the *Fusarium graminearum* species complex (FGSC or Fg complex) of species (lineages) identified in phylogenetic analyses (Feng 2007; O'Donnell et al. 2000, 2004; Zhang et al. 2012).

F. graminearum causes massive losses in global production of small grain cereals, corn and other, often unrelated, host plants. FGSC members have varied morphological traits, and their key diagnostic features are distinctly elongated, straight or sickle-shaped macroconidia and round chlamydospores that often form chains (McMullen et al. 1997; Suchorzyńska and Misiewicz 2009). Differences in the organization of macroconidia were noted between species of the FGSC (Aoki et al. 2012; O'Donnell et al. 2004).

To date, 16 species have been identified within the FGSC. Most of them are confined to specific geographic regions, but migration between regions stimulates the flow of genes to new populations, and contributes to the emergence of more virulent FGSC taxa that had not been previously encountered in a given part of the world (Carter et al. 2002; Yli-Mattila 2010).

CHEMOTYPES OF FGSC MEMBERS

The mycotoxins produced by *Fusarium graminearum* are characterized by different degrees of toxicity, which are determined by their chemical structure. Chemical compounds were classified and chemotypes were identified based on the toxic potential of metabolites produced by FGSC members. *F. graminearum* produces type B trichothecenes (8-ketotrichothecenes), 3-acetyldeoxynivalenol (3-ADON, chemotype IA) and 15-acetyldeoxynivalenol (15-ADON, chemotype IB), which are derivatives of deoxynivalenol (DON), and nivalenol (NIV, chemotype II) (Figure 1). An analysis of *F. graminearum* based on its chemotypes revealed that NIV is much more toxic for humans and animals than DON, whereas DON has higher phytotoxic potential. FGSC isolates are capable of producing both DON and NIV. When one of the genes responsible for DON or NIV expression becomes silent, only one toxic substance is secreted (Amarasinghe et al. 2011; Feng 2007; Panthi 2012; Tőth et al. 2005; Yli-Mattila 2010).

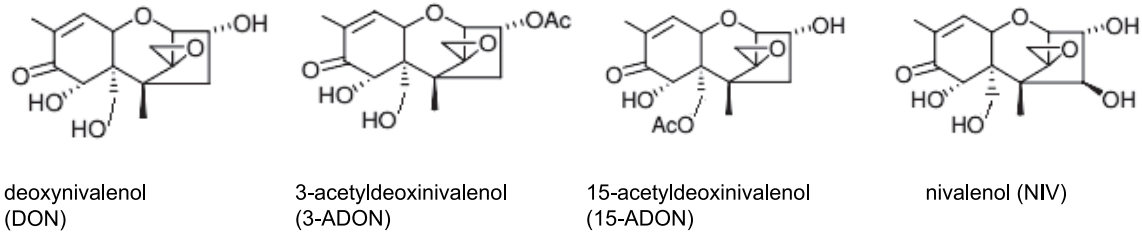

Figure 1. Chemical structure of type B trichothecenes produced by *Fusarium graminearum* (based on McCormick et al. 2011).

The expression profile of trichothecene mycotoxins is highly complex in *Fusarium* species. The *Tri* cluster consists of the following genes: *Tri3* encoding C-15 acetyltransferase, *Tri4* encoding cytochrome P450 oxygenase for hydroxylation at C-2, *Tri5* encoding trichodiene synthase, *Tri6* regulatory gene, *Tri7* encoding C-4 acetyltransferase, *Tri8* encoding C-3 esterase, *Tri9* gene with unknown function, *Tri10* regulatory gene, *Tri11* gene encoding cytochrome P450 oxygenase for hydroxylation at C-15, *Tri12* trichothecene efflux pump, Tri13 encoding C-4 hydroxylase and *Tri14* gene with unknown function. The *Tri1* gene encoding cytochrome P450 oxygenase and the *Tri101* gene encoding 3-*O*-acetyltransferase have also been identified in the NIV chemotype outside the FGSC (Brown et al. 2004; Kimura et al. 2007; Rep and Kistler 2010; Ward et al. 2002) (Figure 2).

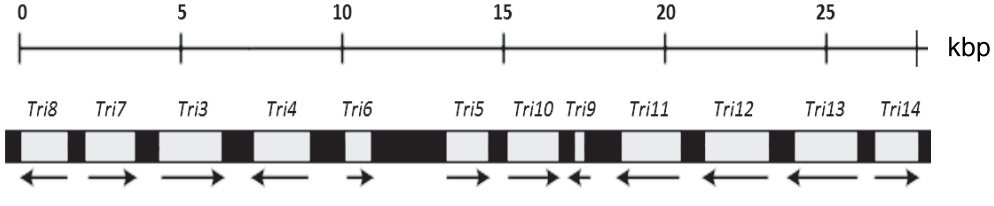

Figure 2. Organization of the cluster of genes responsible for trichothecene production in FGSC (based on: Kimura et al. 2007; Ward et al. 2002).

Molecular techniques emerged as the most popular and reliable methods of identifying FGSC members and *F. graminearum* chemotypes. Due to differences in virulence levels and limited knowledge about the geographic distribution and of correlations between FGSC chemotypes, several molecular markers are used for describing the species' ability to produce trichothecenes (Table 1). Unlike in the NIV chemotype, the Tri13 gene has a functional defect in the DON chemotype, and it is believed that *Tri13* is the main subunit determining the toxin-producing potential of trichothecene-secreting fungi. For this reason, the *Tri13* sequence is one of the most popular diagnostic markers. 3-ADON, 15-ADON and NIV chemotypes can be detected in a single PCR assay based on differences in their product size (Chandler et al. 2003; Lee et al. 2002; Wang et al. 2008) (Figure 3).

Table 1. Selected methods applied in analyses of the *Fusarium graminearum* species complex (FGSC).

Method/objective	Basis	Result	Reference
***F. graminearum*-specific PCR marker**	Species-specific primers: Fg16F: 5'-CTCCGGATATGTTGCGTCAA-3' Fg16R: 5'-GGTAGGTATCCGACATGGCAA-3'	Amplification product of 400-500 bp in length	Nicholson et al. (1998)
PCR primers differentiating 3-ADON, 15-ADON and NIV chemotypes based on the *Tri3* sequence	Identification of the 3-ADON chemotype; primers: Tri303F: 5'-GATGGCCGCAAGTGGA-3' Tri303R: 5'-GCCGGACTGCCCTATTG-3'	Amplification product of 583 bp in length	Jennings et al. (2004)
	Identification of the 15-ADON chemotype; primers: Tri315F: 5'-CTCGCTGAAGTTGGACGTAA-3' Tri315R: 5'- GTCTATGCTCTCAACGGACAAC-3'	Amplification product of 863 bp in length	
	Identification of the NIV chemotype; primers: Tri3NIVF: 5'-GGACGTGASTACTCTTGGCAA-3' Tri3NIVR: 5'-CCCAGRGCCTCTAAGAARGGB-3'	Amplification product of 549 bp in length	
Multiplex-PCR primers differentiating 3-ADON, 15-ADON and NIV chemotypes based on the *Tri3* sequence; one PCR assay with four primers	Identification of the 3-ADON chemotype; primers: 3D3A: 5'-CGCATTGGCTAACACATG-3'	Amplification product of 243 bp in length	Ward et al. (2002)
	Identification of the 15-ADON chemotype; primers: 3D15A: 5'-ACTGACCCAAGCTGCCATC-3'	Amplification product of 610 bp in length	
	Identification of the NIV chemotype; primers: 3NA: 5'-GTGCACAGAATATACGAGC-3'	Amplification product of 840 bp in length	
	Common primer for 3D3A, 3D15A, 3NA: 3CON: 5'-TGGCAAAGACTGGTTCAC-3'	Amplification product of 243 bp, 610 bp or 840 bp in length	
Multiplex-PCR primers differentiating 3-ADON, 15-ADON and NIV chemotypes based on the Tri12 sequence; one PCR assay with four primers	Identification of the 3-ADON chemotype; primers: 12–3F: 5'-CTTTGGCAAGCCCGTGCA-3'	Amplification product of 840 bp in length	Ward et al. (2002)
	Identification of the 15-ADON chemotype; primers: 12–15F: 5'-TACAGCGGTCGCAACTTC-3'	Amplification product of 670 bp in length	
	Identification of the NIV chemotype; primers: 12NF: 5'-TCTCCTCGTTGTATCTGG-3'	Amplification product of 410 bp in length	
	Common primer for 12–3F, 12–15F, 12NF: 12CON: 5'-CATGAGCATGGTGATGTC-3'	Amplification product of 410, 670 or 840 bp in length	

Method/objective	Basis	Result	Reference
qPCR primers differentiating 3-ADON, 15-ADON and NIV chemotypes based on the *Tri12* sequence	Identification of the 3-ADON chemotype; primers: 3ADONf : 5'-AACATGATCGGTGAGGTATCGA-3' 3ADONr: 5'-CCATGGCGCTGGGAGTT-3'	60 bp	Nielsen et al. (2012)
	Identification of the 15-ADON chemotype; primers: 15ADONfwd: 5'-GTTTCGATATTCATTGGAAAGCTAC-3' 15ADONrev: 5'-CAAATAAGTATCGTCTGAAATTGGAAA-3'	57 bp	
	Identification of the NIV chemotype; primers: Nimf: 5'-GCCCATATTCGCGACAATGT-3' NIVr: 5'-GGCGAACTGATGAGTAACAAAACC-3'	77 bp	
PCR primers differentiating DON and NIV chemotypes based on the Tri13 sequence	Tri13F: 5'-CATCATGAGACTTGTKCRAGTTTGGG-3' Tri13DONR: 5'-GCTAGATCGATTGTTGCATTGAG-3'	Amplification product of 282 bp in length	Chandler et al. (2003)
	Identification of the NIV chemotype; primers: Tri13NIVF: 5'-CCAAATCCGAAAACCGCAG-3' Tri13R: 5'-TTGAAAGCTCCAATGTCGTG-3'	Amplification product of 312 bp in length	
GCPSR/genomic loci sequences for differentiating FGSC and defining species limits	α-tubulin (α-TUB) gene	Sequence of 1686 bp in length	O'Donnell et al. (2000, 2004, 2007)
	β-tubulin (β-TUB) gene	Sequence of 1337 bp in length	
	translation elongation factor 1α (EF-1α) gene	Sequence of 648 bp in length	
	histone H3 (HIS) gene	Sequence of 449 bp in length	
	complete conjugated sequence of four mating type genes: MAT1-1-1, MAT1-1-2, MAT1-1-3, MAT1-2-1	Sequence of 6592 bp in length	
	complete sequence of three genes: ammonia ligase (URA), - (Tri101) and phosphate permease (PHO)	Sequence of 4124 bp in length	
	reductase (RED) gene	Sequence of 1273 bp in length	
	GCPSR analysis based on the above gene sequences	Sequence of 16109 bp in length	
GC-MS/ chromatographic detection and quantitative analysis of DON and NIV	Separation in two capillary columns (12m·0.2mm·33μm), program temperature: start 235°C, 5 min; heating 10°C·min^{-1} to 295°C; end 295°C, 10 min.	Detection of product ions	Tóth et al. (2005)

New methods have recently been developed for determining the virulence of various *F. graminearum* populations. The most noteworthy techniques involve combined analyses of SNPs of seven *Tri* genes (*Tri1*, *Tri5*, *Tri6*, *Tri10* and *Tri14*) and the incorporation of two genes (*MetAP1* and *Erf2*) responsible for pathogen growth in the analytical process, which has significantly improved the determination and estimation of linkage disequilibrium (LD) (Talas et al. 2012). Researchers are also investigating the zearalenone (ZEA)-producing activity of *F. graminearum*. Real-time quantitative PCR assay targeting the *PKS13* gene responsible for ZEA synthesis is combined with HPLC to determine the virulence of ZEA-producing *F. graminearum* strains (Atoui et al. 2011).

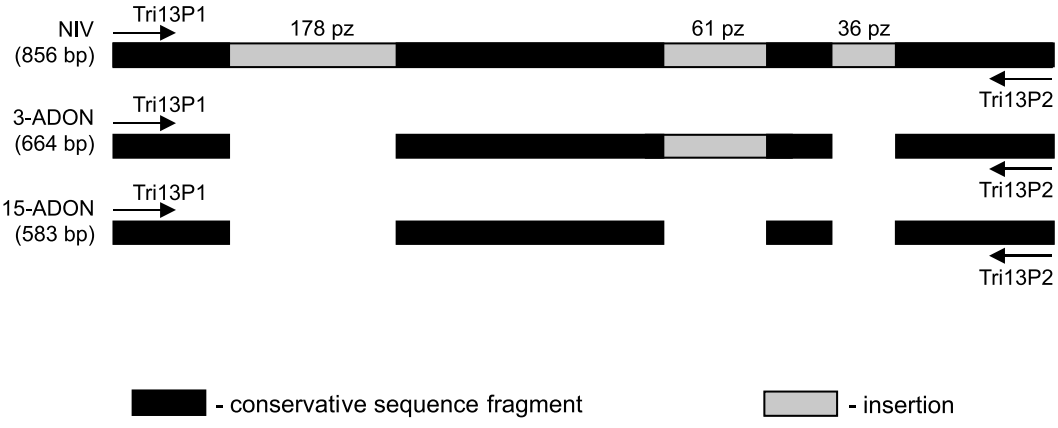

Figure 3. Diagram of the *Tri13* gene encoding NIV, 3-ADON and 15-ADON chemotypes on the example of *Fusarium graminearum* isolates from China and Canada (based on: Amarasinghe et al. 2011; Wang et al. 2008).

RESEARCH METHODS IN FGSC ANALYSIS

The use of molecular markers improves the identification of FGSC members and the determination of genetic differences between isolates. Carter et al. (2002), Qu (2002) and Qu et al. (2008a) used molecular techniques, such as AFLP, SCAR, SSCP and PCR with species-specific primers, to classify the analyzed isolates into FGSC species and differentiate between the populations inhabiting China, Europe and North America.

Restriction fragment length polymorphism (RFLP) is a technique that uses restriction enzymes, which are enzymes that cut genomic DNA or amplified fragments in specific sites. The location of specific sites differs in polymorphic individuals, which leads to the production of genetic material fragments of different sizes. In a study of *F. graminearum*, RFLP enabled the determination of differences between morphologically similar FGSC taxa and *F. pseudograminearum*, which had been previously classified as a single species (Benyon et al. 2000).

Patterns characteristic of selected species and strains within the FGSC were observed with the use of the randomly amplified polymorphic DNA (RAPD) method, which relies on the amplification of short random primers and electrophoresis of the resulting products (Narayanasamy 2008). The above technique supported the determination of genotypic variations between *F. graminearum* populations in Canada, the United States (Ouellet and Seifert 1993), China (Liu et al. 2002), Europe (Tóth et al. 2005) and Brazil (Busso et al. 2007).

Species-specific primers are used to identify distinct taxa, and in some cases, they support the differentiation of closely related species. SCAR Fg16F/R primers were used to distinguish *F. asiaticum* isolates from the remaining FGSC members. The primers demonstrated cross-reactivity with *F. meridionale*. Cross-reactivity was eliminated when the obtained fragments were amplified in single-strand conformation polymorphism (SSCP) analysis. Polymorphism between the sequences of haplotypes 1A and 1B in *F. graminearum* was observed at positions 412 (T-A) and 348 (C-T) (Qu 2002; Qu et al. 2008a, 2008b).

Amplified fragment length polymorphism (AFLP) is a novel DNA fingerprinting technique that poses an alternative to RFLP (Mueller and Wolfenbarger 1999). The use of the AFLP method in FGSC analyses supported the determination of differences between isolates (Abd-Elsalam et al. 2002; Qu et al. 2008a, 2008b; Sivaramakrishnan et al. 2002). AFLP genotyping revealed significant differences between Australian isolates (Akinsanmi et al. 2006) and the polymorphic character of *F. graminearum* isolates from Europe, the United States and Nepal (Qu et al. 2008a).

Detection of point mutations, random single nucleotide polymorphism (SNP) mutations that occur at one point, is also a useful diagnostic method. More than 10,000 SNPs were identified in a comparison of *F. graminearum* s. s. strains PH-1 and GZ3639. The highest accumulation of point mutations was observed in those regions which enable pathogens to quickly adapt to changes in the environment and the host plant: telomers and genomic loci of the protein secretion system, amino acid transports, cytochrome P450 and sites responsible for infectiing plants (Cuomo et al. 2007). The use of SNP-specific primers facilitates the identification of different FGSC species and trichothecene-producing chemotypes (*Tri* primer kits) (Yang et al. 2008). The primers designed for *Tri13* and *Tri7* genes are used to distinguish DON and NIV chemotypes (Burlakoti et al. 2011; Chandler et al. 2003; Kimura et al. 2007; Lee et al. 2002; Scoz et al. 2009; Waalwijk et al. 2003), whereas the pair of primers designed for the *Tri3* gene (Tri303F/R and Tri315F/R) support the differentiation of individuals producing 3-ADON and 15-ADON (Jennings et al. 2004; Zhang et al. 2007).

Variable number tandem repeat (VNTR) markers pose an alternative to RFLP markers. VNTRs provide detailed information about polymorphic sites in FGSC. The cost of analyses relying on VNTR markers is very high, and in several studies of *F. graminearum* s. s., this approach had to be abandoned and replaced with bioinformatic tools (Suga et al. 2004; Zhang et al. 2012) and genome sequencing (Cuomo et al. 2007).

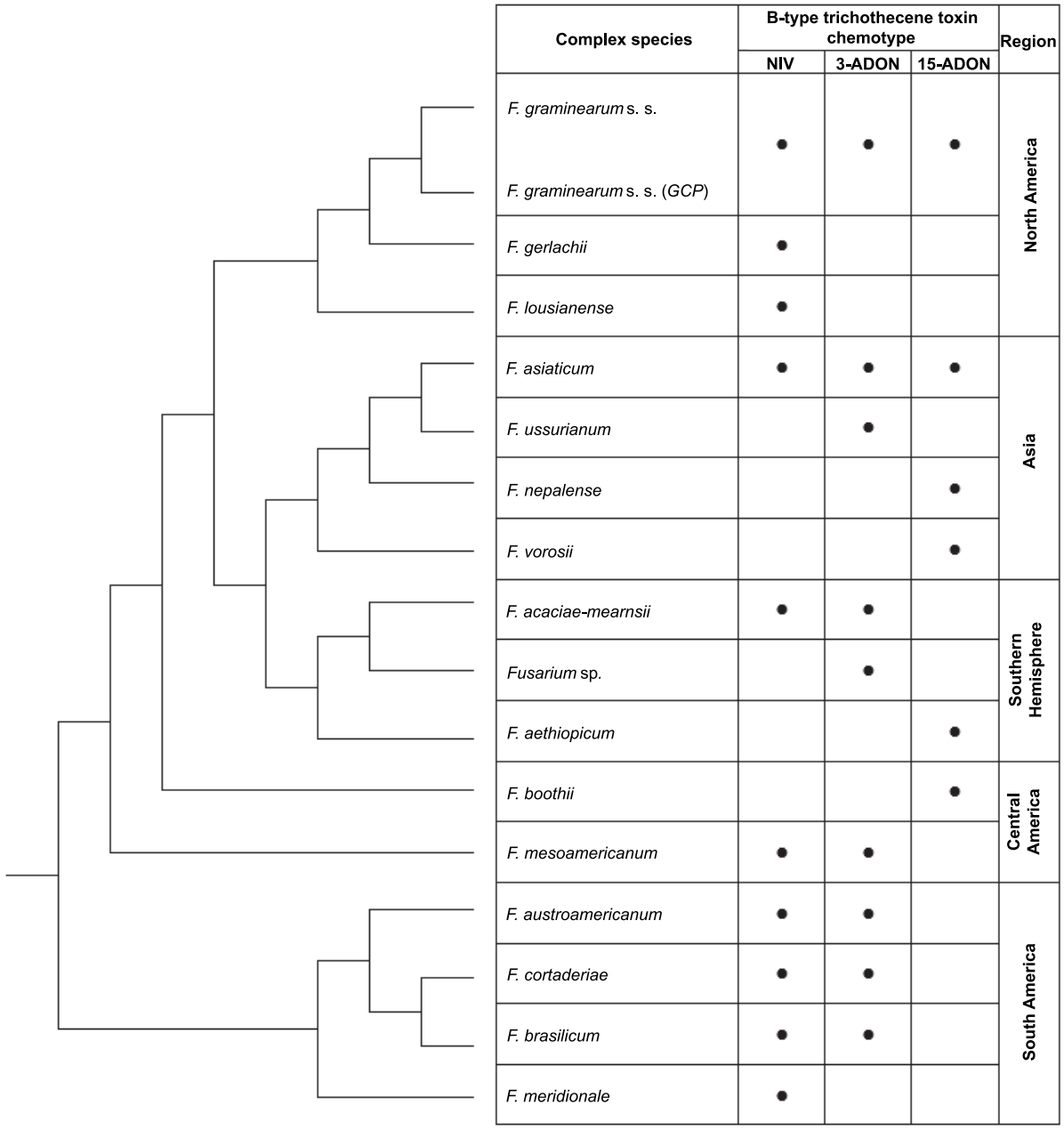

Figure 4. Similarities and geographic ranges of *Fusarium graminearum* species complex (FGSC) members, with a specification of mycotoxins produced by each species (based on: Aoki et al. 2012; O'Donnell et al. 2004).

Diagnostic loci in phylogenetic analyses of *F. graminearum* and other fungi are known as genealogical concordance phylogenetic species recognition (GCPSR) loci (Table 1, Figure 4) and multilocus genotyping assay (MLGT) loci. Both methods are based on evolutionary mechanisms, and a simultaneous analysis of multiple sequences (loci) in the GCPSR approach supports the determination of similarities and boundaries between fungal species. The MLGT method relies on an analysis of SNPs, and it generates a marker database that can be used to track changes within a population, taxon migration and mycotoxin distribution within a species. In phylogenetic analyses of the genus *Fusarium*, the following sequences facilitate the identification of the origin and similarity between species: EF-1α (elongation factor 1-α), α-TUB (α-tubulin), β-TUB (β-tubulin), CAM (calmodulin), RPB (larger subunit of polymerase RNA II), MAT locus (mating-type, responsible for sexual reproduction in fungi), TRI101(3-*O*-acetyltransferase), RED (reductase), URA (UTP-ammonia ligase), PHO (phosphate permease), histone H3 and ITS-28S (ribosomal internal transcribed spacer). FGSC species were identified by analyzing multiple GCPSR loci based on the sequences of the following genes: α-tubulin (α-TUB), β-tubulin (β-TUB), elongation factor 1α (EF-1α), histone H3 (HIS), four mating-type (MAT) genes, ammonia ligase (URA), 3-O-acetyltransferase (Tri101), phosphate permease (PHO) and reductase (RED) (Table 1). *F. graminearum* comprises at least 16 phylogenetically distinct species (Figure 4), and it is genetically most similar to species that produce type B trichothecenes: *F. culmorum*, *F. cerealis*, *F. lunulosporum* and *F. pseudograminearum* (Aoki et al. 2012; O'Donnell et al. 2000, 2004, 2007; Starkey et al. 2007; Waalwijik et al. 2004; Wang et al. 2008, 2011).

FGSC taxa are characterized by different levels of genetic diversity due to geographic boundaries, which often leads to an absence of panmictic populations. This type of genetic, chemotypic and morphological variation is manifested at the endemic (continental) and intercontinental level with different intensity (Gale et al. 2002; O'Donnell et al. 2000; Zeller et al. 2004).

CONTINENTAL OCCURRENCE OF THE FGSC

Europe

Europe demonstrates a predominance of *F. graminearum* s. s. with various chemotypes, where the 3-ADON chemotype dominates in the northern parts of the continent, reaching north-western Russia and Scandinavia. A prevalence of the 15-ADON chemotype is noted in the remaining parts of Europe, which is the major FGSC species in the British Isles, Germany and Austria. *F. graminearum* s. s. infects corn, cereals, grasses and ferns (Aoki et al. 2012; Schwabe 1839; Yli-Mattila 2010; Yli-Mattila et al. 2009).

Carter et al. (2002) identified two *F. asiaticum* haplotypes in a small grouping of European isolates. Their results indicate that the species had spread from Asia to other regions of the world. Qu et al. (2008a) did not identify *F. asiaticum* in the European population and noted that the European population of *F. graminearum* was dominated by the 15-ADON chemotype, with a small share of individuals capable of producing 3-ADON and NIV.

Szécsi et al. (2005) investigated 42 Hungarian isolates from cereals, grasses and air. Trichothecenes were identified by gas chromatography-mass spectrometry. The authors demonstrated that the Hungarian population of *F. graminearum* was dominated by the DON chemotype, which was noted in 39 isolates. In that group, 34 isolates were capable of producing both 15-ADON and 3-ADON. One isolate produced DON and NIV. In the analyzed isolates, DON secretion levels varied between 46 to 6840mg·kg^{-1}.

In a study of 26 Hungarian isolates and 3 Austrian isolates colonizing wheat, Tóth et al. (2005) observed that the Central European population of *F. graminearum* belonged to chemotype I (DON). A detailed GC-MS and HPLC analysis revealed a predominance of isolates producing 15-ADON over those secreting 3-ADON, and they were classified into subgroup B of chemotype I. IGS and RAPD analyses demonstrated that all investigated isolates were characterized by high virulence levels and they supported the differentiation of 17 and 16 haplotypes. The results of both analyses were combined to differentiate 27 out of 29 analyzed isolates.

Stępień et al. (2008) studied the populations of three *Fusarium* species to demonstrate that *F. graminearum* had increased its share of the population in southern Poland between 1998 and 2006 and that the abundance of *F. culmorum* had decreased. The authors relied on microscopic and molecular methods to identify fungi isolated from wheat. They used species-specific primers such as Fc01 *F. culmorum*, UBC85 and Fg16N *F. graminearum* and CroA *F cerealis* (synonym: *F. crookwellense*). The SCAR-PCR assay of 41 *F. graminearum* isolates revealed the dominance of the 15-ADON chemotype (31 isolates), including 5 isolates which were also capable of producing NIV (Nicholson et al. 1998; Schilling et al. 1996; Yoder and Christianson 1998).

Pasquali et al. (2010) analyzed the geographic distribution of 313 *F. graminearum* isolates from wheat in 2007 and 2008 from 17 sites in Luxembourg using the chemotype-specific primers developed by Ward et al. (2002) and the LC-MS/MS assay. Their results indicate that 94.2% of the population in the studied region belonged to the 15-ADON chemotype and only 5.8% to the NIV chemotype.

Four distinct geographic groups were identified in 12 populations of *F. graminearum* isolates sampled from 11 sites in Germany in 2006-2009. The chemotype composition of 338 *F. graminearum* s. s. isolates was analyzed to reveal that 92% produced 15-ADON, 6.8% produced 3-ADON, and only 1.2% produced NIV. SSR analysis identified a total of 300 different genotypes in

a population, pointing to high levels of genetic diversity between isolates. Intrapopulation variation was high at 71.2% and it significantly exceeded interpopulation differences estimated at 28.8%. The rate of gene flow between populations was also relatively high (Nm=0.76-3.16). The variations between German isolates showed no regional correlation (Talas et al. 2011).

Nielsen et al. (2012) analyzed differences in the expression of the *Tri1* sequence in Danish isolates of species colonizing cereal grain. The number of *F. graminearum* and *F. culmorum* isolates obtained from wheat and barley in 1957-2000 was very small and representative mainly of the 3-ADON chemotype. The 15-ADON chemotype was predominant in *F. graminearum* and *F. culmorum* populations isolated from wheat and triticale in 2003-2007, whereas all three *F. graminearum* chemotypes were identified in barley.

North and central America

Ouellet and Seifert (1993) determined low levels of genetic diversity between Canadian isolates by RAPD-PCR. Further analyses of Canadian populations revealed a 14-fold increase in the frequency of the 3-ADON chemotype between 1998-2004, which superseded the previously prevalent 15-ADON chemotype (Ward et al. 2008).

An analysis of *F. graminearum* populations in North America revealed the presence of both genetically diverse and genetically homogeneous populations, which could be attributed to the pathogens' geographic range (Bowden et al. 2000). Zeller et al. (2003) relied on the AFLP method to observe a high level of similarity (D<99%) between *F. graminearum* s. s. populations in Kansas and North Dakota. Further research demonstrated high levels of similarity to *F. graminearum* s. s. and high homogeneity among populations identified in the United States. Minor genetic differences are indicative of frequent exchange of genetic material between subpopulations from very distant regions (Zeller et al. 2004).

A GCPSR analysis (Table 1) of 2100 isolates from the United States supported the identification of *F. gerlachii*, a species that is closely related to the American population of *F. graminearum* s. s. discovered in the Gulf Coast. The analyzed isolates produced only NIV, and they were discovered on the giant reed (*Arundo donax* L.) and common wheat (*Triticum aestivum* L.). The presence of *F. graminearum* s. s. (*F. graminearum* GCP) genetically distinct from members of the species in other North American regions was also noted on the coast of the Gulf of Mexico (Starkey et al. 2007).

Subsequent research revealed an intensified inflow of other FGSC. Ward et al. (2008) observed that isolates introduced to North America belonged to more virulent chemotypes, mainly of *F. asiaticum*. Qu et al. (2008a) observed a similar trend in Europe, and argued that geographic dispersion is important when considering the distribution of more virulent species of the FGSC.

Puri and Zohng (2010) analyzed *F. graminearum* populations isolated in 1980-2000 and 2008 in North Dakota. In the "new" population, the number of isolates producing 3-ADON had increased 15-fold to reach 44%; whereas in the "old" population, the 15-ADON chemotype (93%) predominated. In an analysis of FHB-susceptible and resistant wheat cultivars, isolates producing 3-ADON caused severe disease and produced more DON than 15-ADON chemotype isolated in susceptible varieties of wheat, whereas no differences in virulence or DON production were noted between the above chemotypes in FHB resistant wheat varieties. Virulence levels, DON production and the number of spores formed on solid growth media were also higher for 3-ADON chemotypes. The use of AFLP and VNTR markers revealed clear genetic differences between the "old" and "new" populations. The cited authors observed that 3-ADON, a more virulent chemotype, had been gradually outcompeting the 15-ADON chemotype for several years. The new, more virulent populations of the pathogenic fungus have created a demand for more resistant cereal varieties. The most virulent 3-ADON isolates should be used for evaluation of wheat germplasm resistance to FHB.

Gale et al. (2011) investigated an American population of *F. graminearum* of 534 isolates from 12 US states. 237 isolates were genotyped by multiplex PCR with chemotype-specific primers to identify pathogens capable of producing 15-ADON, 3-ADON and NIV. The results were compared with a population of 297 isolates from 11 Midwestern states. The NIV chemotype of *F. graminearum* s. s. predominated in Louisiana (79%). The newly discovered population in Louisiana differed significantly from the 3-ADON chemotype of *F. graminearum* s. s. characteristic of the Mexican Gulf, whereas in the Midwestern population, 15-DON chemotype fungal isolates predominated. Forty one isolates of the NIV chemotype of *F. asiaticum* were also identified in Louisiana. Greenhouse tests revealed that DON-producing isolates secreted four times more mycotoxins than NIV chemotype strains. In a study of fungal populations from Louisiana, Sarver et al. (2011) detected a new species, *F. louisianense*, using the GCPRS assay with MLGT markers.

High levels of genetic diversity (H=0.8) and genotypic diversity were noted in an analysis of two fungal populations isolated from barley in 1997-2000 (115 isolates) and 2008 (147 isolates) with the use of 10 markers. In those populations, linkage disequilibrium (LD<0.2) and genetic diversity (Fst~0.01) were low. Subpopulations of *F. graminearum* s. s. were also similar in the studied region (Burlakoti et al. 2011).

The presence of *F. mesoamericanum* was noted in Honduras and the United States. This species was isolated from bananas and Boston ivy (*Parthenocissus tricuspidata* [Siebold & Zucc.], Planch) and produced 3-ADON and NIV (Aoki et al. 2012).

South America

Cereal stands in South America are generally colonized by *F. graminearum* s. s., but the structure of those pathogenic populations has not been intensively studied. *F. meridionale* and *F. graminearum* s. s. isolates were reported in southern Brazil. In an analysis of 82 isolates, 6 were NIV chemotype isolates of *F. meridionale*, and the remaining isolates were capable of producing both DON and 15-ADON and were classified as belonging to *F. graminearum* s. s. (Scoz et al. 2009). However, Busso et al. (2007) analyzed the Brazilian population of *F. graminearum* by RAPD-PCR to reveal low levels of genetic diversity between isolates. Similar results were observed by Ouellet and Seifert (1993) in a study of a Canadian population.

O'Donnell et al. (2004) and Aoki et al. (2012) observed expansion of *F. boothii* in central America and Africa. Its presence has also been reported in Nepal, Korea and the United States. The species is characterized by high morphological similarity to *F. graminearum* s. s., but it produces spores identical to those of *F. meridionale*, a corn-infecting taxon.

Astolfi et al. (2012) analyzed 140 isolates from 3 populations in southern Brazil. *Tri3* and *Tri12* gene fragments were amplified by multiplex PCR with Fg16F/R species-specific primers (Table 1). Genetic diversity was investigated in 103 *F. graminearum* s. s. isolates with the use of the AFLP method. A predominance of isolates producing 15-ADON was noted in 3 populations. NIV-producing isolates had a significant share (18%) of only one population, and their percentage in the remaining two populations was estimated at only 2%. Isolates producing 15-ADON were classified as belonging to *F. graminearum* s. s., and isolates producing NIV as belonging to *F. meridionale*. Most isolates had different haplotypes (genotypic diversity was determined at 98%), pointing to a significant influence of genetic recombination. According to the cited authors, genetic exchange in the studied populations occurred over long periods of time. They also observed that Brasilian FGSC genotypes differed from the FGSC genotypes encountered in most parts of Asia, Europe and North America.

Sampietro et al. (2012) evaluated chemotype differences between 112 isolates from the FGSC responsible for corn infections in northwestern Argentina using multiplex PCR and chemical methods. According to the PCR procedure, 22.3% of isolates were *F. boothii*, which were classified as DON or 15-ADON chemotypes. Although PCR indicated that the remaining 77.7% of isolates were *F. meridionale* that belonged to chemotype NIV, chemical analysis showed that they were also capable of producing other trichothecenes: 41.4% of the isolates were capable of producing DON in addition to NIV, and a further 8.1% produced more DON than NIV. From this the authors drew two conclusions. First, that the isolates of *F. meridionale* that produce DON and NIV could be new chemotypes originating from chemotypes that only produced NIV. Second, that sequencing these genes alone is not an effective way to determine chemotypes.

Umpiérrez-Failache et al. (2013) studied FGSC populations in Uruguay. In the group of 151 isolates responsible for FHB in wheat, *F. graminearum* of the 15-ADON type accounted for 86%. The presence of other members of the FGSC, such as *F. asiaticum*, *F. brasilicum*, *F. cortaderiae* and *F. austroamericanum*, was reported in six of the eight regions investigated in the study. *F. graminearum* isolates with the 15-ADON type were most prevalent (95%) in the western provinces of Uruguay, whereas *F. asiaticum* predominated (43%) in new wheat growing areas along Uruguay's eastern border with Brazil. All NIV-producing FGSC isolates accounted for 61% of all isolates collected in the studied region. *F. graminearum* isolates of the 15-ADON type were more virulent than 3-ADON and NIV isolates, but 15-ADON isolates were more sensitive to tebuconazole than NIV-producing species. The results of the cited study indicate that the diversity of pathogens responsible for FHB in Uruguay and the presence of the 3-ADON, 15-ADON and NIV chemotypes pose significant threats to food safety. Therefore, the levels of mycotoxin contamination in grain should be closely monitored.

Asia

The presence of *F. asiaticum*, a member of the FGSC, was confirmed in south Asia. The species colonizes barley, wheat, corn, rice and oats. It is widespread in China, Japan, Korea and Nepal, but it was also reported in the United States and Brazil. Other species of the FGSC identified in south Asia were *F. nepalense*, *F. boothii*, *F. graminearum*, *F. meridionale*, *F. ussurianum* and *F. vorosii* (Aoki et al. 2012).

A study of isolates from north-western Europe, the United States and Nepal revealed three groups of *Fusarium*. Two groups were identified as characteristic of Nepal, and one as typical of Europe and the United States (Carter et al. 2002). *F. graminearum* isolates from regions of China with different climates were similar, although their virulence varied (Liu et al. 2002).

Carter et al. (2002), Qu (2002) and Qu et al. (2008b) studied *F. graminearum* populations in China, Europe and North America with the use of species-specific AFLP, SCAR, SSCP and PCR markers to demonstrate six different genotypes. Haplotypes 1 and 5 were observed in China. Haplotype 5 was classified as belonging to *F. asiaticum*. Haplotype 1 was divided into subgroups: 1A, 2A, and 3A. Haplotype 1A was prevalent in China. Three haplotypes of *F. asiaticum* (3, 4 and 5) were identified in Nepal, and two haplotypes were observed in a small group of isolates characteristic of Europe (1A and 6) and the United States (1A and 1B) (Carter et al. 2002).

Gale et al. (2002) analyzed *F. graminearum* populations from four wheat fields in the Chinese province of Zhejiang and observed that all isolates belonged to one population of *F. asiaticum*. The RFLP analysis identified 65 haplotypes with low levels of genetic diversity (H=0.306-0.364) and high rates of gene flow.

SCAR Fg16F/R primers of 410 bp for *F. graminearum* were used to distinguish *F. graminearum* s. s. isolates from *F. asiaticum* isolates in China (Qu et al. 2008a), but the *F. asiaticum* amplification product was identical to that of *F. meridionale* and had a length of 497 bp (Carter et al. 2002). The SSCP analysis of the fragment amplified by Fg16F/R in *F. graminearum* s. s., *F. asiaticum* and *F. meridionale* revealed 3 haplotypes of *F. graminearum* s. s. (1A, 1B and 1C). The amplified region did not show polymorphism in the other 2 species. *F. graminearum* isolates from China and Europe belonged to haplotype 1A, and American isolates belonged to haplotypes 1B and 1C. *F. meridionale* isolates were classified into haplotype 2, and *F. asiaticum* isolates, to haplotypes 3-6. Nearly all isolates produced 3-ADON or 15-ADON, except isolates from Nepal and NIV-producing isolates from China, Japan and France (Qu et al. 2008a, b).

Fusarium asiaticum and *F. graminearum* s. s. were observed in South Korea, where *F. asiaticum* predominated in the western part, and *F. graminearum* s. s., in the eastern part of the country. *F. asiaticum* is probably more virulent in rice plants in Korean production systems. A field experiment with rice host plants revealed that *F. graminearum* s. s. had been gradually replaced by *F. asiaticum* (Lee et al. 2004, 2009).

A study of *F. asiaticum* from 5 Korean populations revealed that 81% of isolates had different genotypes, and the percentage of AFLP markers in gene sequences was 36%. Nearly 17% of polymorphic isolates were observed more than once. Genetic diversity was estimated to be in the range of 32 to 56 (Lee et al. 2009).

In a study of FGSC taxa isolated from wheat grain, Zhang et al. (2012) emphasized the significance of natural selection, which contributed to the spread of strains that were more virulent and resistant to fungicides. Based on MLGT and VNTR analyses, the studied populations were divided into three groups: *F. graminearum* producing 15-acetyldeoxynivalenol (15-ADON), *F. asiaticum* producing nivalenol (NIV) and *F. asiaticum* producing 3-acetyldeoxynivalenol (3-ADON). The groups were characterized by high genetic diversity ($p \geq 0.8$, Bayesian method, $Fst = 1.703\text{-}0.984$) and a low number of effective migrants ($Nm = 0.337\text{-}0.272$). Further genotypic analyses confirmed sympatric speciation of *Fusarium* species in every group. A tendency towards gene flow from the group of virulent, 3-DON-producing individuals in eastern China to NIV-producing individuals in western China was observed. A phenotypic analysis revealed that the 3-DON-producing group had higher levels of pathogenicity, more complex conidial morphology, higher proliferation rates and higher pesticide resistance than the NIV-producing group.

A VNTR analysis of 1106 isolates from China's eastern, central and western provinces demonstrated the presence of subgroups in *F. asiaticum* (Zhang et al. 2010). A VNTR analysis of 488 *F. asiaticum* isolated from barley grown along the Yangtze river revealed significant variations within the population ($P < 0.001$) and a low rate of gene flow ($Nm = 1.210$) (Zhang et al. 2012).

Desjardins and Proctor (2011) studied 251 isolates obtained from corn plants in Nepal. Analyses of the MAT1-1-3 conjugation sequence and the *Tri13* marker demonstrated similarities between the investigated isolates and *F. asiaticum*, *F. meridionale* and *F. boothii*. A new species was also identified in Nepal. Corn was infected mainly (80%) by *F. graminearum* of the NIV chemotype with low virulence. In a study of Nepalese isolates analyzed by the MLGT method and GCPRS, Sarver et al. (2011) confirmed the presence of a new species, *F. nepalense*, in the investigated region.

Suga et al. (2008) analyzed 298 isolates and identified two FGSC species in Japan. A phylogenetic analysis and a PCR-RFLP assay with species-specific primers revealed that the first species, *F. graminearum* s. s., was predominant in northern Japan, and the second species, *F. asiaticum*, in southern Japan, whereas both species were distributed equally in the region of Thoku in the north-eastern part of the Honsiu island. The investigated population was dominated by *F. asiaticum* (70%). Multiplex PCR demonstrated that the isolates present in the *F. graminearum* s. s. population were capable of producing 15-ADON or 3-ADON, whereas all isolates from the *F. asiaticum* population belonged to the NIV chemotype. The geographic distribution of both species could be attributed to the fact that *F. graminearum* s. s. was more resistant to low temperatures in northern Japan and other factors such as humidity and the agricultural production system. The cited study also revealed a low rate of gene flow between the FGSC species.

A predominance of 3-ADON and 15-ADON chemotypes was reported in the Middle East and Russia, mostly *F. ussurianum*, which produces 3-ADON and *F. vorosii* that secretes 15-ADON. *F. asiaticum*, a widely occurring species in China, was not identified in Russia (Yli-Mattila 2010; Yli-Mattila et al. 2009).

Abedi-Tizaki and Sabbagh (2013) studied 100 isolates of *F. graminearum* recovered from northern Iran (Golestan province). Species-specific Fg16 primers were used to identify members of the FGSC. Chemotypes were identified by amplifying the sequence of the *Tri3* gene. In the studied population NIV chemotype was dominated (72 isolates), whereas 18 isolates produced 15-ADON, and 10 isolates produced 3-ADON.

Australasia

In Australia, Fusarium head blight is caused by *Fusarium graminearum* and *F. pseudograminearum*. The following FGSC members have been identified in Australia: *F. cortaderiae* (that colonizes pampas grass (*Cortaderia selloana* [Schult. & Schult.] Asch. & Graebn.), corn, cloves, barley, wheat and is widespread in soil), *F. meridionale* (that infects black wattle), and *F. meridionale* (that colonizes corn and barley stems) (Aoki and O'Donnell 1999; O'Donnell et

al. 2004). High levels of genetic diversity were reported from AFLP analysis of isolates from a different region of Australia. Genomic differences between those isolates were observed even in a single field, and 56 out of 59 analyzed isolates had distinct haplotypes (Akinsanmi et al. 2006).

Monds et al. (2005) identified *F. graminearum* s. s. and *F. cortaderiae* isolates in New Zealand. *F. graminearum* s. s. isolates produced NIV or 15-ADON, whereas all *F. cortaderiae* isolates belonged strictly to the NIV chemotype.

Africa

In Africa, Maina et al. (2009) studied soil-borne *Fusarium* communities in Kenya. They reported that *F. graminearum* consituted 5.9% of those populations; *F. oxysporum* constituted 37.9%; *F. solani*, 10.0%; and *F. sporotrichioides*, 7.2%. In the southern parts of Africa, the most widespread members of the FGSC are the species characteristic of Australasia as well as *F. boothii* that colonizes corn, *F. graminearum* s. s. and *F. aethiopicum* that infect wheat, and the soil-borne isolate of *Fusarium* sp. NRRL 34461 (O'Donnell et al. 2004, 2008; Schwabe 1839).

O'Donnell et al. (2008) studied 31 isolates from Ethiopia. A new species of *F. aethiopicum* producing 15-ADON was identified with the use of the MLST method. A phylogenetic analysis revealed that *F. aethiopicum* is genetically most similar to *F. acaciae-mearnsii*.

Boutigny et al. (2011) relied on the MLGT technique and chemotype identification to analyze 560 FGSC isolates obtained from cereals in South Africa. The investigated population was composed of all chemotypes with a clear predominance of 15-ADON. In the group of 277 wheat isolates, 93.1% produced 15-ADON, 6.1% produced NIV, and 0.7% produced 3-ADON. In the group of 148 barley isolates, 99.3% produced 15-ADON, and 0.7% produced 3-ADON. All 100 isolates infecting the above-ground parts of corn plants were 15-ADON chemotypes. Of the 35 isolates from corn roots, 86% produced 15-ADON, and 14% produced NIV. FGSC isolates colonizing wheat comprised *F. graminearum* s. s. (85.2%), *F. boothii* (8.3%), *F. meridionale* (3.6%), *F. cortaderiae* (1.1%), *F. acaciae-mearnsii* (1.4%) and *F. brasilicum* (0.4%). The barley-infecting isolates were *F. graminearum* (87.2%) and *F. boothii* (12.8%). Those infecting above-ground parts of corn plants were only *F. boothii*, whereas those isolated from corn roots were *F. graminearum* (74%), *F. boothii* (12%) and *F. meridionale* (14%).

SUMMARY AND CONCLUSIONS

The *Fusarium graminearum* species complex was previously classified as a single species. Advanced phylogenetic analyses based on 12 GCPSR sequences revealed the complex structure of this group of plant pathogens and the presence of 16 phylogenetic species. FGSC species cannot be reliably identified based on their morphological attributes alone. The genealogical division of FGSC members into clades demonstrates that similarities are closely correlated with the geographic range of a phylogenetic species. Phylogenetic species differ in their virulence and the quantity and type of produced mycotoxins.

The variations in the pathogenicity and mycotoxin production levels of FGSC species colonizing different regions makes species identification and pathogen elimination difficult, and it contributes to intermingling between newly introduced phylogenetic species. Advanced monitoring methods are required to counteract the spread of species into new regions around the globe, as well as the emergence of new diseases and infections of new plant species. The virulence of pathogenic isolates and the geographic distribution of host plants can be determined based on analyses of 8-ketotrichothecene mycotoxins, molecular markers, genotyping and polymorphism detection. This may help to control pathogen populations and minimize economic losses.

FGSC isolates of the 15-ADON type are widespread across continents. They are highly virulent towards cereal crops, but usually less resistant to plant protection agents than NIV-producing species. Strains with the 15-ADON type dominate in Europe, where they are much more common than the 3-ADON chemotype. The reverse trend can be observed in North America, where the more virulent 3-ADON chemotype is isolated more frequently, particularly in the northern regions. Such differences in chemotype virulence between continents suggest that many members of the FGSC can adapt to local environmental conditions. Species such as *F. ussurianum* (3-ADON) and *F. vorossi* (15-ADON), reported from the Russian Far East, are well adapted to cold and temperate climates, whereas *F. acaciae-mearnsii* (3-ADON/NIV) and *F. boothii* (15-ADON) are found in areas characterized by high temperatures.

Fusarium asiaticum, which is the predominant causal agent of FHB in Asia, has also been detected in the North America and Europe, and it has recently been reported in South America. *Fusarium asiaticum*, which occurs mainly in warm regions and is known for its ability to infect rice, can also spread to wheat plants, thus posing the risk of grain contamination with NIV, a highly toxic trichothecene.

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