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Chapter

Fusarium graminearum Species Complex and Trichothecene Genotype

Jianhua Wang, Zhiyong Zhao, Xianli Yang, Junhua Yang, Andong Gong, Jingya Zhang, Lei Chen and Changyan Zhou

Abstract

The fungal phytopathogen in *Fusarium* species can cause Fusarium head blight of wheat, barley, oats, and other small cereal grain crops worldwide. Most importantly, these fungi can produce different kinds of mycoxins, and they are harmful to humans and animal health. FAO reported that approximately 25% of the world's grains were contaminated by mycotoxins annually. This chapter will focus on several topics as below: (1) composition of *Fusarium graminearum* species complex; (2) genotype determination of *Fusarium graminearum* species complex strains from different hosts and their population structure changes; (3) genetic approaches to genotype determination in type B-trichothecene producing *Fusaria* fungi; and (4) some newly identified trichothecene mycotoxins, their toxicity, and distribution of the producers.

Keywords: *Fusarium graminearum* species complex, trichothecene, Fusarium mycotoxin, trichothecene genotype

1. Introduction

The fungal phytopathogen in *Fusarium graminearum* species complex (FGSC) are the primary etiological agent of Fusarium head blight (FHB) of wheat, barley, oats, and other small cereal grain crops worldwide. Besides, the Gibberella ear rot (GER) caused by FGSC and the related species *F. verticillioides* is one of the most devastating diseases on maize. FHB and GER are economically devastating plant disease that greatly limits grain yield and quality. Warm and humid weather conditions at the flowering stage are conducive to disease development. During the 1990s, economic losses in cereals (wheat and barley) caused by *Fusarium* were estimated at close to US $3 billion (US $2.5 billion in wheat and US $400 million in barley) and US $520 million (US $220 million in wheat and US $300 million in barley) in the United States and Canada, respectively [1]. It was reported that due to the changes in climatic conditions and in agricultural practices, outbreaks of FHB have occurred more frequent and serious in China. From 2008 to 2015, serious yield loss of wheat caused by FHB was occurred in more than 5 million ha each year.

In addition, infested grain is often contaminated with Fusarium toxins which are harmful to human and animal health and pose a serious threat to food or feed safety. FHB and GER are among the most destructive and economically important
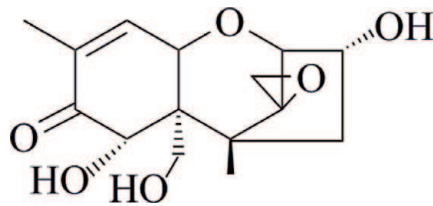
Figure 1.
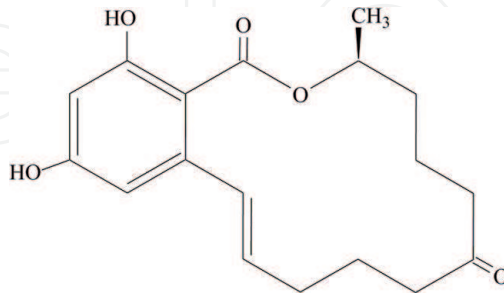*Chemical structure of Deoxynivalenol (DON).*

Figure 2.
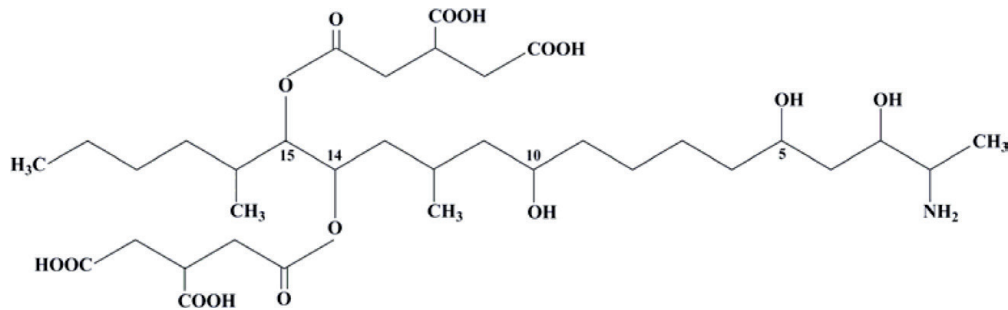*Chemical structure of Zearalenone (ZEN).*

Figure 3.
Chemical structure of Fumonisin B$_1$ (FB$_1$).

diseases through the world. A survey made by the journal *Molecular Plant Pathology* from the international community, and resulted in the generation of a top 10 fungal plant pathogen list with FGSC in fourth place [2].

Up to now, more than 70 *Fusarium* species have been identified within the *Fusaria* genus. FGSC, *F. verticillioides*, *F. culmorum*, *F. oxysporum*, *F. solani*, *F. proliferatum*, *F. poae*, *F. equiseti*, and *F. fujikuroi* are the most commonly isolated species worldwide on wheat, maize and other plants. The most important thing is that, many different kinds of mycotoxins can be produced by these molds, such as deoxynivalenol (DON, **Figure 1**), zearalenone (ZEN, **Figure 2**), and fumonisin B$_1$ (FB$_1$, **Figure 3**) are the most prevalent Fusarium mycotoxins in cereal grains and they are very important in food and feed safety. It is clear now that one mold species may produce many different kinds of mycotoxins, and the same mycotoxin may be produced by several species. For example, FGSC can produce trichothecene and zearalenone, while trichothecene can be produced by FGSC, *F. culmorum*, *F. poae*, and *F. equiseti*. This chapter mainly focused on the FGSC and summarized the genetic methods used for trichothecene genotype determination of the strains.

2. Composition and identification of FGSC strains

Prior to 2000, due to the failure of morphological species recognition to accurately assess species limits for the FGSC, the species complex were considered a single

cosmopolitan species. Applying the genealogical concordance phylogenetic species recognition (GCPSR), FGSC was first divided into seven phylogenetic lineages in 2000 [3]. Phylogenetic analyses of multilocus genotyping (MLGT) of DNA sequences from portions of 13 housing keeping genes, combined with GCPSR and molecular marker technologies, it revealed that this morphospecies comprises at least 16 biogeographically structured, phylogenetically distinct species. After that the species designation *Fusarium graminearum* have been *sensu stricto* in some conditions. Up to now, 15 of the 16 species have been formally described, including *F. acaciae-mearnsii*, *F. aethiopicum*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum sensu stricto*, *F. louisianense*, *F. meridionale*, *F. mesoamericanum*, *F. nepalense*, *F. ussurianum*, *F. vorosii*, and one additional species was informally recognized based on genealogical exclusivity and conidial morphology on SNA [4].

Proper species identification is critical to research aimed at improving disease and mycoxins control programs. However, it is difficult to discriminate the FGSC strains accurately by morphological characters. A partial region of the translation elongation factor 1 alpha gene (*TEF-1α*) was widely used for molecular identification of *Fusarium* genus. Some specific databases were created for *Fusarium* DNA sequence alignment analysis. For example, similarity searches of the obtained sequences can be performed with the Pairwise DNA alignments network service of the Fusarium MLST database (http://www.westerdijkinstitute.nl/fusarium/), Basic Local Alignment Search Tool (BLAST) network service of the Fusarium ID database (http://www.fusariumdb.org/index.php), and NCBI nucleotide database.

3. Mycotoxins produced by FGSC

In addition to yield reduction, the FGSC fungi are also of concern because they can produce different kinds of mycotoxins, e.g. zearalenone (**Figure 2**) and trichothecenes (**Figures 4** and **5**) in infested grains. Mycotoxin contamination can occur in both unprocessed and processed grains, representing a risk for human and animal health. Deleterious health effects caused by different mycotoxins include nephropathy, infertility, cancer or death [5].

Up to now, more than 200 trichothecenes have been identified [6]. Due to the chemical structure diverse, trichothecenes are divided into four types, namely type A (have a single bond at carbon atom 8, C-8), e.g. T-2 toxin (**Figure 4**), type B (have a keto at C-8), type C (have an epoxide at C-7, 8), and type D (have a macrocyclic ring between C-4 and C-15). All trichothecenes share a common tricyclic 12, 13-epoxytrichothec-9-ene, and they are derived from the isoprenoid intermediate farnesyl pyrophosphate via a series of biochemical reactions in *Fusarium*.

Among these mycotoxins, type B trichothecenes (**Figure 5**) are the most common detected in cereal grains and their related products. They are distinguished from type A by the presence of a keto function at C-8, and include deoxynivalenol (DON) and its acetylated forms 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), as well as nivalenol (NIV) and its acetylated form 4-acetylnivalenol (4-ANIV). DON is characterized by the absence of a hydroxyl
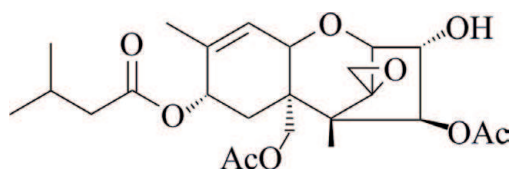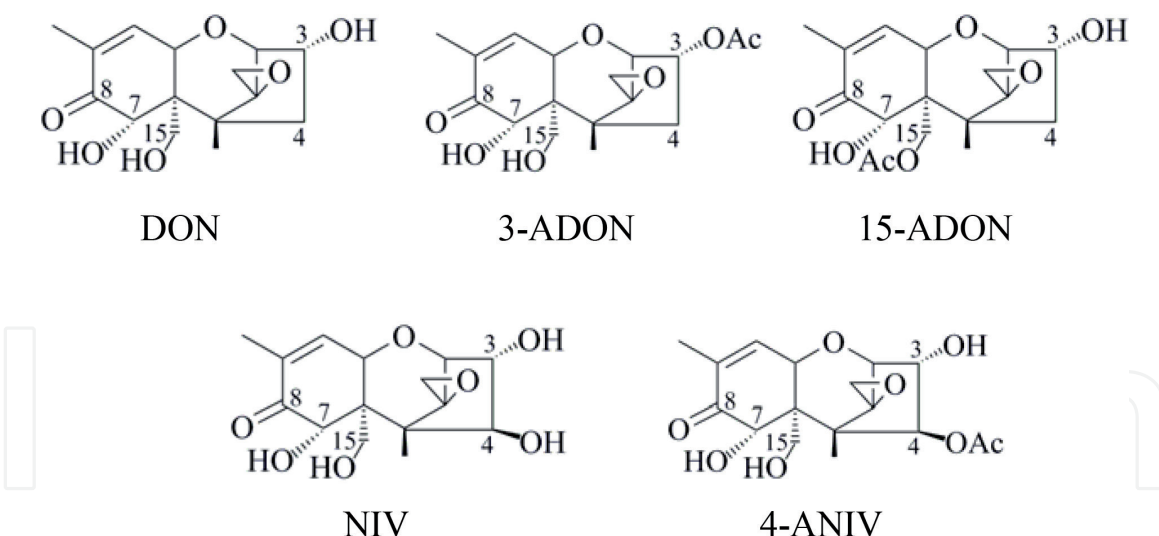

Figure 4.
Chemical structure of T-2 toxin.

Figure 5.
Chemical structures of deoxynivalenol (DON), 3-acetyl deoxynivalenol (3-ADON), 15-acetyl deoxynivalenol (15-ADON), nivalenol (NIV), and 4-acetyl nivalenol (4-ANIV).

function at C-4, whereas NIV is characterized by the presence of a hydroxyl function at C-4. 3-ADON and 15-ADON are the acetylated forms of DON at C-3 and C-15, respectively. Meanwhile, NIV and 4-ANIV can be differed by the absence (NIV) and presence (4-ANIV) of an acetyl function at C-4 (**Figure 5**).

Type B trichothecenes are of the greatest concern in wheat and barley-growing regions worldwide, because they can represent a major threat to food and feed safety. These toxins are potent inhibitors of protein synthesis and are responsible for neurologic, gastrointestinal, immune function and other disorders. Although type B trichothecenes differ only slightly from each other in terms of the pattern and position of acetylation or hydroxylation, these changes can greatly affect the toxicity and the activity of these chemical compounds [7]. For example, DON is associated with feed refusal, vomiting and suppressed immune functions, and NIV is more toxic to humans and domestic animals than is DON. Determination of these trichothecene variations are important because the toxicity of DON and NIV may vary according to the eukaryotic organism affected. Minervini et al. [8] found that NIV was approximately four times more toxic than DON to human cells. Conversely, DON is 10 to 24 times more toxic to plant cells than NIV [9].

Type B trichothecenes are mainly produced by FGSC. Due to the ability of FGSC strains that producing different kinds of trichothecenes, three strain-specific trichothecene genotypes (chemotypes) were identified in FGSC: the 3-ADON genotype produces DON and 3-ADON, the 15-ADON genotype produces DON and 15-ADON, and the NIV genotype produces NIV and its acetylated derivatives 4-ANIV [10].

4. Mycotoxins act as virulence on certain hosts

Evidence is presented to show that trichothecene toxins act as virulence factors on certain hosts. Strains carrying a disrupted trichodiene synthase gene *Tri5* do not produce trichothecenes or their biosynthetic intermediates [11]. Disruption of the trichodiene synthase which catalyzes the first step in trichothecene biosynthesis in a 15-ADON producer GZ3639 exhibited reduced virulence on seedlings and heads of wheat, but wild-type virulence on seedlings of maize. The results indicated that trichothecene production contributes to the virulence of FGSC on wheat [12]. Eudes et al. [13] confirmed earlier findings that trichothecenes are a principal determinant

of *F. graminearum* aggressiveness on wheat cultivars. More recently, Maier et al. [14] investigated the involvement of trichothecenes in the virulence of the pathogen by disrupting the *Tri5* gene encoding the first committed enzyme trichodiene synthase in FGSC strains with 3-ADON, 15-ADON, and NIV genotype, respectively. The results demonstrated that disruption mutants can cause disease symptoms on the inoculated spikelet but the symptoms cannot spread into other spikelets on wheat. And on maize, mutants derived from the NIV genotype strain caused less disease than their progenitor strain, while no significant difference compared to the wild-type strains were observed on barley [14]. Trichothecenes are therefore also potent phyto-toxins and act as virulence factors of pathogenic fungi thus facilitate tissue coloniza-tion on sensitive host plants, e.g. of DON produced by *F. graminearum* in wheat [15].

Host preference was identified among the FGSC on wheat, maize, barley, and rice in certain regions. Several studies suggested that maize played a significant role for the presence of the NIV genotype for FGSC. NIV producers were found to be more aggressive toward maize compared to DON producers [16]. This can be due to the fact that NIV is a virulence factor useful for maize colonization [14], and there-fore the plant probably represents an ecological niche for hosting the NIV genotype strains. A high proportion of NIV producers of FGSC on maize were observed in China by our group based on a collection from 59 districts in 19 provinces through-out China, and NIV producers preferentially to maize [17].

5. Distribution and population changes of FGSC

The species composition and genotype prevalence of FGSC vary widely in different regions, which reflecting the level of risk factors in feed/food safety. Investigations on *Fusarium* species isolated from wheat, barley, and maize crops have been reported in the last two decades. Dynamic changes of species composi-tion and chemotype proportion have been found in different agricultural ecosys-tems worldwide. Prior to 2000, strains from the United States and Canada were almost exclusively 15-ADON producers, while they have been increasingly replaced by the 3-ADON producers in some major wheat-growing regions, e.g. the frequency of the 3-ADON genotype in western Canada increased more than 14-fold between 1998 and 2004 [18]. Also Schmale et al. [19] analyzed the trichothecene genotypes of *Gibberella zeae* collected from winter wheat fields in the eastern parts of the US. They revealed an increasing gradient in 3-ADON distribution from south to north and closer to Canada. In some regions, the *F. graminearum* 15-ADON chemotype is being replaced by the 3-ADON chemotype. The epidemiology data indicated that 3-ADON chemotype dominates in northern Europe, while 15-ADON chemotype dominates in North America, central Europe and southern Russia and some parts of Asia.

The composition of FGSC population appears to be host and location dependent. The results by Zhang et al. [20] and Shen et al. [21] indicated that *F. asiaticum* was the predominated in wheat. Among the 97 FGSC assayed from rice (30 strains), maize (33 strains), and wheat (34 strains) by Qiu and Shi [22], 73 strains were identified as *F. asiaticum* and 63 of them were collected from rice or wheat. The remaining 24 strains belonged to *F. graminearum sensu stricto* and 23 of them were isolated from maize, only 1 strain was collected from wheat. Similarly, FGSC strains were isolated from GER samples in South Korea with *F. graminearum sensu stricto* to be the dominant species which account for 75% of the FGSC [23], while *F. asiaticum* was the dominant species (78.5%) on Korean rice and followed by *F. graminearum sensu stricto* [24].

The distribution of FGSC may correlate with annual temperature. Qu et al. [25] reported that temperature affected the geographic distribution of *F. graminearum*

sensu stricto and *F. asiaticum* on wheat spikes in China. A comprehensive study on FGSC from wheat was conducted by Zhang et al. [20]. They found that the geographic distribution of FGSC associated with the annual average temperature. The cooler temperatures (annual average temperature ≤15°C) appear to favor *F. graminearum sensu stricto*, while the warmer regions (annual average temperature ≥15°C) appear to favor *F. asiaticum*. A hypothesis was made that the distribution of FGSC members are climate dependent [20].

F. graminearum sensu stricto with the 15-ADON genotype and *F. asiaticum* with either the NIV or the 3-ADON genotype were the dominant causal agents on wheat, and the two species dominated the northern and southern regions of China, respectively, which is consistent with earlier studies [20, 26, 27].

However, more recently the study by Zhang et al. [28] indicated that temperature may not be the only factor in the distribution of FGSC and that other, yet unknown factors affected their distribution. To explain genotype distribution in different geographic areas, hypotheses based on grain seed shipment, international trade, long-distance spore transportation, and environmental favorable conditions were proposed.

6. FGSC fitness vary

Phylogenetic analyses of trichothecene gene cluster demonstrated that genotype polymorphism is trans-specific and have been maintained by balancing selection on the ancestral pathogens, and genotype differences may have a significant impact on pathogen fitness [29].

The FGSC strains with different genotype showed different fitness to the ecological environment, such as the hosts, temperature, rotation, and so on. 3-ADON producer was more aggressive than 15-ADON population in susceptible wheat, and also the 3-ADON isolates exhibit a higher DON production than the 15-ADON isolates. Similar conclusions were made by Zhang et al. [28] that *F. asiaticum* strains with 3-ADON chemotype revealed significant advantages over the strains that produce NIV in pathogenicity, growth rate, trichothecene accumulation, etc. Their data also indicated that the growth of rice may be a key factor for the presence of *F. asiaticum* [28]. Liu et al. [30] compared the fitness of three chemotype *Fusarium* strains, and found that 15ADON producers had the advantage in perithecia formation and ascospore release, whereas more DON were produced by the 3-ADON chemotypes. Qiu and Shi [22] estimated the effect of rice or maize as former crops on mycotoxin accumulation in wheat grains, and they concluded that rice-wheat rotation favors DON accumulation.

Changes in DON chemotypes distribution were reported for FGSC from Canada, USA, and Northern Europe. Recently, Nicolli et al. [31] assessed a range of fitness-related traits (perithecia formation, mycelial growth, sporulation and germination, pathogenicity, and sensitivity to tebuconazole) with 30 strains representatives of 3ADON-, 15ADON-, and NIV-producers. The pathogenicity assay results indicated that strains with the DON chemotypes were generally more aggressive than the NIV ones [31].

Phenotypic analyses indicated that *F. asiaticum* with a 3-ADON genotype revealed significant advantages over *F. asiaticum* that produce NIV in pathogenicity, growth rate, and trichothecene mycotoxin accumulation. It shall be noted that a biased gene flow from 3-ADON to NIV producers was identified in *F. asiaticum* from wheat in China [28].

FGSC from wheat-maize rotation regions on wheat spikes and maize stalks in Henan province, China, was determined by Hao et al. [32], and significant

differences were found in the frequencies of *F. graminearum sensu stricto* and *F. asiaticum* species within the hosts with *F. graminearum sensu stricto* to be the dominant. Genotype analysis revealed that 15-ADON producers represented 92.7 and 98.5% of isolates from wheat and maize, respectively. The three genotypes may affect species distribution or population ecology because these mycotoxins are differing in toxicity and bioactivity [7, 29, 33].

7. Genetic genotype determination of FGSC

Traditionally, chemotyping of FGSC strains has been carried out using gas chromatography/mass spectroscopy. This method can be time-consuming and expensive. The genome sequences of several FGSC strains have been published. The trichothecene core gene cluster nucleotide sequences of many strains representatives 3-ADON, 15-ADON, and NIV genotypes have also been deposited in the GenBank. The availability of this information makes it possible to reveal the structural features and allowed selection of several primer sets used successfully in PCR experiments for the molecular characterization of the various chemotypes. Molecular genetic assays allow for high throughput screening of large numbers of field isolates.

Lee et al. [34] sequenced the gene cluster for trichothecene biosynthesis from a 15-ADON producer (strain H-11) and a NIV producer (strain 88-1), and sequence polymorphisms within the *Tri7* open reading frame was found between the two strains. Alignment analysis suggesting that the *Tri7* gene of H-11 carried several mutations and an insertion compared to the *Tri7* gene from 88-1, and based on the sequence difference a PCR-based diagnostic method for differentiating DON and NIV producers by polyacrylamide gel electrophoresis was developed.

Lee et al. [35] subsequently sequenced the *Tri13* homolog from DON (strain H-11) and NIV producers (strain 88-1) and found that the gene differs drastically between the two producers, suggesting that the *Tri13* gene could be used for genetic genotype distinction for DON and NIV producers [35, 36]. They further confirmed the roles of the *Tri7* and *Tri13* genes in trichothecene production, and the results suggested that both the *Tri7* and *Tri13* genes are nonfunctional in DON producers [35].

The PCR assays to *Tri7* and *Tri13* genes developed by Lee et al. [34, 35] allowed clear differentiation between DON and NIV genotypes. However, they could not be used to further classify the DON-producing isolates to 3-ADON or 15-ADON producer. Ward et al. [29] examined a 19-kb region of the trichothecene gene cluster that sequenced in 39 strains representing 3-ADON, 15-ADON, and NIV genotypes. They found that Tri-cluster haplotypes group according to genotype rather than by species indicated that 3-ADON, 15-ADON, and NIV genotypes each have a single evolutionary origin. Reciprocally monophyletic groups, corresponding to each of 3-ADON, 15-ADON, and NIV genotypes, were strongly supported in *Tri3*, *Tri11*, and *Tri12* genes trees. Two sets of primers specific to the individual genotypes were designed from *Tri3* and *Tri12* genes. The genotype-specific PCR tests developed by Ward et al. [29] provide a rapid and direct genetic method for distinguishing among 3-ADON, 15-ADON, and NIV producer, this is the first report differentiated these three genotype strains by a PCR method.

The work by Lee et al. [34, 35] and Brown et al. [37] indicated that the genes *Tri13* and *Tri7* from trichothecene biosynthetic cluster are responsible for conversion of DON to NIV (*Tri13* gene) and the *Tri7* gene product modifies NIV by acetylation of C-4 atom hydroxyl to produce 4-ANIV. Based on these results sets of positive-negative PCR assays to *Tri7* and *Tri13* genes for trichothecene determination of FGSC were developed by Chandler et al. [38], and the assays can accurately indicate a DON or NIV genotype in FGSC, *F. culmorum* and *F. cerealis*. The assays

were successfully used to screen isolates from different countries and the genotype-specific assays were able to detect and characterize a wider range of species and haplotypes than previous methods.

By comparing the published sequences for *Tri13* gene from known DON- and NIV- producers, Waalwijk et al. [39] designed a primer pair to discriminate the two genotypes which generated a 234 bp fragment in DON-producers and a fragment of 415 bp in NIV-producers. The *Tri13* primer pair was capable and robust to determine the genotype of strains from *F. culmorum*.

Based on information reported and deposited by Ward et al. [29], three primer sets were designed to the *Tri3* gene by Jennings et al. [40] to allow further differentiation of the DON genotype into either 3-ADON or 15-ADON. Each isolate produces a PCR product with only one of these primer sets but not the other two from *F. culmorum* and FGSC strains [40, 41].

Li et al. [42] found that the intergenic sequences between *Tri5* and *Tri6* genes appear to be mycotoxin genotype-specific, and based on the sequence length polymorphism a generic PCR assay was developed to detect a 300 bp fragment of DON-genotype strains and a 360 bp fragment of NIV-genotypes from FGSC.

Based on the sequences of FGSC described by Lee et al. [34] and Ward et al. [29], a series of PCR assays have been designed to *Tri3* and *Tri7* by Quarta et al. [43], in order to permit specific detection of 3-ADON, 15-ADON, and NIV genotypes, respectively. These primers were subjected to a multiplex PCR assay for the identification of the different genotypes of *Fusarium* strains combined with the primer pair derived from the *Tri5* gene by Bakan et al. [44]. The multiplex PCR was validated on FGSC, *F. cerealis*, *F. culmorum* strains from different European countries, and successfully used to identify the genotype of the *Fusarium* strain contaminating wheat kernels [43, 45].

The possibility to distinguish by a singleplex PCR 3-ADON, 15-ADON, and NIV genotypes was not yet resolved until very recently. Wang et al. [46] developed a *Tri13* based PCR assay and successfully identified the 3-ADON, 15-ADON, and NIV genotypes in FGSC from Asia, Europe, and America. Using the primer pair, specific amplification products of 644, 583, and 859 bp were obtained from isolates producing 3-ADON, 15-ADON, and NIV, respectively. All three types of PCR fragments had different molecular sizes with a smallest difference of 61 bp can be directly differentiated on an agarose gel. The method should be more reliable than other PCR-based assays that show the absence or presence of a PCR fragment since these assays may generate false-negative results. This is a rapid, reliable and cost-effective method for the determination of 3-ADON, 15-ADON, and NIV genotype strains in FGSC.

Recently Suzuki et al. [47] reported a multiplex PCR assay for simultaneous identification of the species and trichothecene genotypes for *F. graminearum sensu stricto* and *F. asiaticum* based on *Tri3* and *Tri6* genes. This approach proved successful for Japanese strains [47].

An alternative method based on *Tri11* polymorphism was developed by Zhang et al. [48] to differentiate 3-ADON, 15-ADON, and NIV genotypes of FGSC strains. Similarly, we presented another multiplex assay based on the single nucleotide polymorphism of *Tri11* gene between strains of different genotype [49]. The assay was also validated on plant material.

Recent work by Kulik [50] and Nielsen et al. [51] to detect and quantify FGSC genotypes in plants/grains were developed based on TaqMan probe set and SYBR green method with *Tri12* gene, respectively.

Due to the toxicological differences between DON and NIV, it is important to monitor the population and determine the chemotypes of strains present in any given geographic region. Mycotoxin producing capability of a certain strain could be established both through biochemical and molecular techniques.

Target gene	Primers	Sequences (5′ to 3′)	Fragment size (bp)	Chemotypes	References
Tri3	3CON	TGGCAAAGACTGGTTCAC	243	3-ADON	Ward et al. [29]
	3D3A	CGCATTGGCTAACACATG			
	3CON	TGGCAAAGACTGGTTCAC	610	15-ADON	
	3D15A	ACTGACCCAAGCTGCCATC			
	3CON	TGGCAAAGACTGGTTCAC	840	NIV	
	3NA	GTGCACAGAATATACGAGC			
	Tri303F	GATGGCCGCAAGTGGA	586	3-ADON	Jennings et al. [40, 41]
	Tri303R	GCCGGACTGCCCTATTG			
	Tri315F	CTCGCTGAAGTTGGAC GTAA	864	15-ADON	
	Tri315R	GTCTATGCTCTCAACG GACAAC			
	Tri3NivF	GGACGTGA(CG)TACT CTTGGCAA	549	NIV	
	Tri3NivR	CCCAG(AG)GCCTCTA AGAA(AG)GGB			
	Tri3F971	CATCATACTCGC TCTGCTG	708	15-ADON	Quarta et al. [43]
	Tri3R1679	TT(AG)TAGTTTGCATC ATT(AG)TAG			
	Tri3F1325	GCATTGGCTAACACATGA	354	3-ADON	
	Tri3R1679	TT(AG)TAGTTTGCA TCATT(AG)TAG			
	3D15AF	AACTGACCCAAGCTG CCATC	420	15-ADON (*F. asiaticum* and *F. graminearum ss*)	Suzuki et al. [47]

Target gene	Primers	Sequences (5' to 3')	Fragment size (bp)	Chemotypes	References
	3D15AR	CTTCTGTCCCTTCG AACGGA			
Tri5- Tri6 intergenic	ToxP1	GCCGTGGGG(AG)TAA AAGTCAAA	300	DON	Li et al. [42]
region	ToxP2	TGACAAGTCCGGTC GCACTAGCA	360	NIV	
Tri6	6A3AF	CCAAGACTT(GT)GTT (AC)CCCGAA	1100	DON (*F. asiaticum*)	Suzuki et al. [47]
	6A3AR	GCAATCTTTAGAGTG CCGAC			
	6G3AF	T(AG)TCCCATCCCAT CAAGGCT	330	DON (*F. graminearum ss*)	
	6G3AR	AACAAGTGGTTCTT CGGAGT			
	6CNF	CAAGCAAATGCCC GTATCCC	660	NIV (*F. asiaticum*)	
	6ANR	CGCAACAATATCA ATGGCTGTGCTA			
Tri7	GzTri7/f1	GGCTTTACGACTC CTCAACAATGG	173–327	15-ADON	Lee et al. [34]
	GzTri7/r1	AGAGCCCTGCGAA AG(CT)ACTGGTGC	161	NIV	
	Tri7F	TGCGTGGCAATATC TTCTTCTA	458–535	DON	Chandler et al. [38]
	Tri7R	TGTGGAAGCCGCAGA	436	NIV	
	Tri7F	TGCGTGGCAATAT CTTCTTCTA	381–445	DON	

Target gene	Primers	Sequences (5′ to 3′)	Fragment size (bp)	Chemotypes	References
	Tri7DON	GTGCTAATATTGT GCTAATATTGTGC			
	Tri7F	TGCGTGGCAATAT CTTCTTCTA	465	NIV	
	Tri7NIV	GGTTCAAGTAAC GTTCGACAATAG			
	MinusTri7F	TGGATGAATGAC TTGAGTTGACA	483	3-ADON	
	MinusTri7R	AAAGCCTTCATT CACAGCC			
	Tri7F340	ATCGTGTACAAG GTTTAC G	625	NIV	Quarta et al. [43]
	Tri7R965	TTCAAGTAACGT TCGACAAT			
Tri11	3D11	GCAAGTCTGGC GAGGCC	342	3-ADON	Zhang et al. [48]
	11R	TCAAAGGCCAG AGCAACCC			
	15D11	AAGTATGGTCC AGTTGTCCGTATT	424	15-ADON	
	11R	TCAAAGGCCAG AGCAACCC			
	N11	CTTGTCAGGCGG CACAGTAG	643	NIV	
	11R	TCAAAGGCCAGA GCAACCC			

Target gene	Primers	Sequences (5′ to 3′)	Fragment size (bp)	Chemotypes	References
	Tri11-CON	GACTGCTCATGG AGACGCTG	334	3-ADON	Wang et al. [49]
	Tri11-3AcDON	TCCTCATGCTCG GTGGACTCG			
	Tri11-CON	GACTGCTCATGG AGACGCTG	279	15-ADON	
	Tri11-15AcDON	TGGTCCAGTTG TCCGTATT			
	Tri11-CON	GACTGCTCATG GAGACGCTG	497	NIV	
	Tri11-NIV	GTAGGTTCCAT TGCTTGTTC			
Tri12	12CON	CATGAGCATGG TGATGTC	410	3-ADON	Ward et al. [29]
	12-3F	CTTTGGCAAGC CCGTGCA			
	12CON	CATGAGCATGG TGATGTC	670	15-ADON	
	12-15F	TACAGCGGTCG CAACTTC			
	12CON	CATGAGCATGG TGATGTC	840	NIV	
	12NF	TCTCCTCGTTG TATCTGG			
Tri13	GzTri13/p1	AATACTA(CA)AAG(CT) CTAG(GT)ACGACGC	470	DON	Kim et al. [36]

Target gene	Primers	Sequences (5′ to 3′)	Fragment size (bp)	Chemotypes	References
	GzTri13/p2	GTG(AG)T(AG)TCCCA GGATCTGCGTGTC	760	NIV	
	Tri13F	TACGTGAAACAT TGTTGGC	234	DON	Waalwijk et al. [39]
	Tri13R	GGTGTCCCAGGA TCTGCG	415	NIV	
	Tri13F	CATCATGAGACTTGT (GT)C(AG)AGTTTGGG	282	DON	Chandler et al. [38]
	Tri13DONR	GCTAGATCGATT GTTGCATTGAG			
	Tri13NIVF	CCAAATCCGAA AACCGCAG	312	NIV	
	Tri13R	TTGAAAGCTCC AATGTCGTG			
	Tri13F	CATCATGAGACTTGT (GT)C(AG)AGTTTGGG	799	DON	
	Tri13R	TTGAAAGCTCC AATGTCGTG	1075	NIV	
	Tri13P1	CTC(CG)ACCGCATC GAAGA(CG)TCTC	583	15-ADON	Wang et al. [46]
	Tri13P2	GAA(CG)GTCGCA (AG)GACCTTGTTTC	644	3-ADON	
			859	NIV	

Table 1.
Primers designed for genetic genotyping of FGSC so far.

The biochemical approach involves the incubation and extraction of mycotoxins, the methods being complicated and time consuming. The molecular techniques are based on detection of specific gene by using specific primers. All these molecular methods developed for genotype analysis are based on nucleotide diversity of trichothecene synthesis genes. Chemotype characterization has been extensively used to characterize FGSC for their toxigenic potential [52]. The information about the genetic genotyping methods developed so far, such as targeted gene, primer name, primer sequence, and amplification fragment sizes are summarized in **Table 1**.

More effective and accuracy genetic methods are needed. We are doing genomic sequencing of FGSC strains with different trichothecene genotypes, and we believe some new molecular genetic methods will be developed based on the genomic data.

8. Newly identified trichothecene mycotoxins

In addition to the well characterized fungal mycotoxins, plant-derived mycotoxin metabolites, masked mycotoxins, have emerged as important co-contaminants in cereals [53, 54]. The most commonly detected masked mycotoxin conjugates are β-linked glucose-conjugates of trichothecenes, such as DON-3-glucoside (**Figure 6**). The possible hydrolysis of masked mycotoxins back to their toxic parents during mammalian digestion raises great concerns. Recently, a new series of type-A trichothecene, NX-toxins (**Figure 7**), produced by FGSC were characterized [5]. In vitro translation assays indicated that NX-3 can inhibit protein biosynthesis to almost the same extent as DON [5]. Comprehensive work on intestinal hydrolysis, absorption, metabolism, and toxicity of newly characterized mycotoxins need to be determined (**Figure 7**).
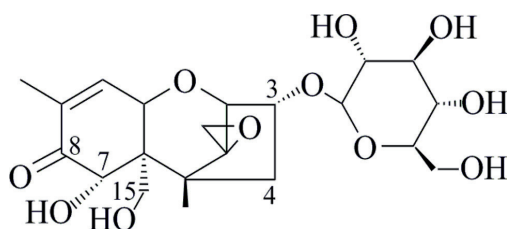

Figure 6.
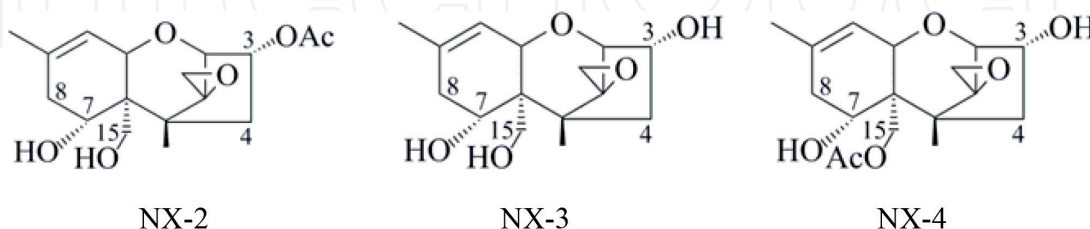*Chemical structure of deoxynivalenol-3-glucoside (D3G).*

NX-2   NX-3   NX-4

Figure 7.
Chemical structures of NX-2, NX-3, and NX-4.

9. Conclusion

The knowledge about the mycotoxins chemotypes could contribute to a better management of fungal infections and breeding of resistance, in order to obtain grains of better quality. The results will also contribute to improve our understanding of the ecology and epidemiology of FGSC members, which may be of value for

improving models for assessing the risk or epidemics and mycotoxin production. Genetic genotyping has been proved to be a useful tool for predicting trichothecene type produced by FGSC, and future work on the more effective tools for genotype determination is needed. The discovery of novel toxic metabolites belonging to trichothecenes, such as NX-toxins is also suggesting that the prevalence, distribution, and genetic diversity of FGSC require continuous monitoring. Further research on the biosynthesis molecular mechanism of trichothecene, especially the novel mycotoxins is needed.

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