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# *Fusarium graminearum* Trichothecene Mycotoxins: Biosynthesis, Regulation, and Management

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## Keywords

*Fusarium graminearum*, deoxynivalenol, DON, toxisome, regulator, signal transduction, epigenetic regulation, management

## Abstract

Fusarium head blight (FHB) of small grain cereals caused by *Fusarium graminearum* and other *Fusarium* species is an economically important plant disease worldwide. *Fusarium* infections not only result in severe yield losses but also contaminate grain with various mycotoxins, especially deoxynivalenol (DON). With the complete genome sequencing of *F. graminearum*, tremendous progress has been made during the past two decades toward understanding the basis for DON biosynthesis and its regulation. Here, we summarize the current understanding of DON biosynthesis and the effect of regulators, signal transduction pathways, and epigenetic modifications on DON production and the expression of biosynthetic *TRI* genes. In addition, strategies for controlling FHB and DON contamination are reviewed. Further studies on these biosynthetic and regulatory systems will provide useful knowledge for developing novel management strategies to prevent FHB incidence and mycotoxin accumulation in cereals.

## INTRODUCTION

*Fusarium graminearum*, a member of the *F. graminearum* species complex (FGSC), is the most prevalent and aggressive pathogen of Fusarium head blight (FHB) in cereal crops worldwide, especially in wheat (139). Severe FHB epidemics have occurred at a minimum of every fourth or fifth year in FHB-prone wheat-growing regions of the world (32). FHB epidemics have been even more frequent (every one to two years) in China since 2010. The increase in FHB incidence may be attributable to global warming, reduced tillage, crop rotation techniques, and/or the practice of returning straw to the field. The average yearly occurrence of FHB in China affects more than 4.5 million hectares, approximately 20% of the total planted area of wheat, and has caused annual yield losses of more than 3.41 million tons between 2000 and 2018 (**Supplemental Figure 1**). In the United States, yield losses as a result of FHB are estimated to be US$3 billion between the early 1990s and 2008 (118). Severe FHB outbreaks also frequently occurred in the main wheat-growing regions of South America. For example, FHB resulted in yield losses of up to 70% in Argentina in 2012 and caused losses ranging from 11.6% to 39.8% between 2000 and 2010 in southern Brazil (103, 112).

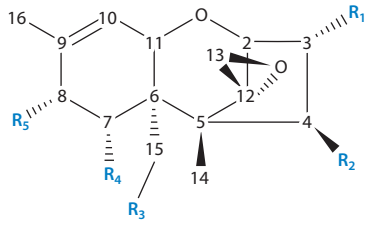
Supplemental Material >

In addition to enormous yield losses, FHB pathogens have serious potential impacts on human and animal health via the contamination of wheat, barley, and maize with various mycotoxins (8). Two groups of mycotoxins commonly associated with *F. graminearum* are zearalenone (ZEA) and trichothecenes, including the type B trichothecenes (TCT-B) deoxynivalenol (DON), its 3-acetylated (3-ADON) or 15-acetylated derivatives (15-ADON), and nivalenol (NIV) (31, 87). DON is the most frequently detected mycotoxin in cereal grains worldwide, with a 59% average incidence rate, ranging from 50% in Asia to 76% in Africa, but tends to occur at higher concentrations in Europe and Asia (67). Furthermore, DON had a higher occurrence in feedstuffs, with a 72%–79% incidence rate in corn samples sourced from North America and Central Europe, and 17%–47% in corn samples from South America and Southern Europe. The average contamination levels in raw materials and compound feeds varied from 794 to 1,304 mg/kg (123). DON inhibits protein synthesis by binding to the ribosome and causes emetic effects, anorexia, and immune dysregulation as well as growth, reproductive, and teratogenic effects in mammals (107). To minimize human and animal exposure to DON, regulatory organizations have established maximum permissible levels for DON in cereals and their products in many countries (9, 49).

On the basis of the economic importance of FHB and trichothecene toxicity, *F. graminearum* was listed as one of the top 10 fungal plant pathogens (25), and its mechanisms for pathogenicity and regulation of trichothecene biosynthesis have been extensively investigated during the past three decades. During this time, the availability of the complete genomic sequence of *F. graminearum* and the development of molecular genetic tools for the fungus have led to a better understanding of the comprehensive regulation of trichothecene mycotoxins. This review elucidates our current knowledge of the biosynthetic circuitry as well as genetic and epigenetic regulation for trichothecene synthesis in *F. graminearum*. In addition, approaches for controlling DON contamination are also discussed.

## TRICHOTHECENE BIOSYNTHETIC PATHWAY AND DEOXYNIVALENOL TOXISOMES

Trichothecenes are a large family of sesquiterpenoid secondary metabolites (SMs) produced by *Fusarium* species and other fungal genera. Trichothecenes are defined by their heterocyclic structure, which includes a 9,10-double bond and a 12,13-epoxide (**Figure 1**). To date, more than 200 trichothecenes have been described and are divided into four types (A–D). The type B trichothecenes, characterized by a C-8 keto group, are produced by many different *Fusarium* species,




Trichothecene

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| DON | –OH | –H | –OH | –OH | =O |
| 3-ADON | –OAc | –H | –OH | –OH | =O |
| 15-ADON | –OH | –H | –OAc | –OH | =O |
| NIV | –OH | –OH | –OH | –OH | =O |
| 4-ANIV | –OH | –OAc | –OH | –OH | =O |
| NX-2 | –OAc | –H | –OH | –OH | –H |
| NX-3 | –OH | –H | –OH | –OH | –H |
| NX-4 | –OH | –H | –OAc | –OH | –H |

**Figure 1**

Structures of trichothecenes produced by *Fusarium graminearum*. Structures of type A and B trichothecenes produced by *F. graminearum*, including deoxynivalenol (DON), nivalenol (NIV), and their acetylated derivatives (ADON), as well as of the new trichothecene mycotoxins NX-2, NX-3, and NX-4.

especially *F. graminearum*, and include the important trichothecenes DON, NIV, and acetylated derivatives thereof (3, 87). More recently, a new type A trichothecene called NX-2 and its derivatives were identified in *F. graminearum* (131) (**Figure 1**). These new mycotoxins show similar potency to inhibit protein synthesis to that of DON (60, 78, 132).

## Trichothecene Biosynthesis Pathway

The biosynthetic enzymes required for trichothecene production are encoded by 15 *TRI* genes, which are located at three different loci on different chromosomes in *F. graminearum*: a 12-gene core *TRI* cluster, two genes at the *TRI1-TRI16* locus, and the single-gene *TRI101* locus (3, 34). The gene *TRI5* (formerly designated *TOX5*) encodes trichodiene synthase, which cyclizes farnesyl pyrophosphate (FPP) to trichodiene (TDN), the first step in trichothecene biosynthesis (44). TDN is then converted to calonectrin (CAL) by nine reactions sequentially catalyzed by Tri4, Tri101, Tri11, and Tri3 (3). *TRI4* encodes a key multifunctional CYP58 family cytochrome P450 monooxygenase for four consecutive oxygenation steps in trichothecene biosynthesis, converting TDN to isotrichotriol (83, 127). The enzymes Tri101 (C-3 acetyltransferase) (85), Tri11 (C-15 hydroxylase) (1), and Tri3 (15-O-acetyltransferase) (86) catalyze in sequence to produce CAL from isotrichotriol. All these reaction steps catalyzing FPP to CAL are shared among *Fusarium* species that produce type A trichothecenes (T-2 toxin and NX-2) and type B trichothecenes (NIV and DON) (**Supplemental Figure 2**). In DON producers, CAL is hydroxylated at both the C-7 and C-8 positions by cytochrome P450 monooxygenase Tri1 and deacetylated by Tri8, leading to the formation of either 3-ADON or 15-ADON followed by DON (16, 82). Two alternative pathways for 4-ANIV and NIV biosynthesis exist for *F. graminearum* NIV producers, either the Tri13-Tri7-Tri1-Tri8 pathway using the CAL as a substrate or the Tri13-Tri7-Tri8 pathway using the 3,15-acetyl DON (3,15-diADON) as the initial substrate (3, 87). For more information on trichothecene biosynthesis pathways, please refer to previous excellent reviews (e.g., 3, 110). It is worth noting that allelic variants of Tri1 are responsible for an important structural difference in Type A and Type B trichothecenes produced by *Fusarium sporotrichioides* and *F. graminearum*, respectively. The FsTri1 enzyme catalyzes hydroxylation of trichothecenes at C-8 only and thus produces trichothecenes without a C-7 hydroxyl, such as T-2 toxin. However, FgTri1 enzyme catalyzes hydroxylation at both C-7 and C-8 in *F. graminearum* DON and NIV producers (3, 84).

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Nevertheless, recent studies demonstrated that the FgTri1 allele in *F. graminearum* NX-2 strains (found so far in Canada and the northern United States only) catalyzes hydroxylation solely at the C-7 position and produces NX-2 and derivatives thereof (60, 78, 131) (**Supplemental Figure 2**).

## Subcellular Location of Deoxynivalenol Biosynthesis Enzymes and Transporters

Fungal SMs are often associated with bioactive properties, including antimicrobial activity, and therefore may play an important role in defense against biotic stresses and for antagonistic competition within their living niches. To achieve these functional outcomes, the SM-producing fungi must develop mechanisms to avoid injuring themselves by attacking the same cellular targets (59). Alternatively, fungi must sequester potential toxicants by compartmentalizing the SM biosynthetic pathways within organelles and/or by timely SM cellular export. The cellular localization of DON biosynthesis and the mechanisms by which DON is exported by *F. graminearum* are just being discovered.

DON can be initially detected after 24 h of growth in trichothecene biosynthesis induction (TBI) medium. The levels of DON increase dramatically between 48 and 72 h and then increase slowly after 72 h and reach the stationary phase (125). The timing of accumulation of DON in TBI medium correlates with highly induced *TRI* gene transcription (**Supplemental Table 1**) and translation. During DON biosynthesis, the morphology of hyphae dramatically changes and forms bulbous subapical structures after 40-h incubation in TBI (51, 58). The protein accumulation pattern of Tri1-GFP (green fluorescent protein) as an indicator of DON biosynthetic enzymes was assessed using confocal laser scanning microscopy and western blots. Tri1-GFP was visualized after 36 h of incubation in TBI medium, reached peak levels at 48 h, and decreased after 72 h (125). Menke and coworkers (89) first demonstrated cellular localization of two cytochrome P450 oxygenases (Tri4 and Tri1) involved in early and late steps in trichothecene biosynthesis in *F. graminearum* under *TRI*-inducing conditions. These two enzymes were localized to spherical organelles, called toxisomes, approximately 3 to 4 μm in diameter that were presumed to be the site of trichothecene biosynthesis. Meanwhile, Menke and coworkers found that GFP-tagged hydroxymethylglutaryl (HMG) CoA reductase Hmr1 also localized to the periphery of toxisomes and colocalized with Tri4-RFP (red fluorescent protein) in toxin-induced cells. Because the Hmr1 homolog has been shown to accumulate at the perinuclear or peripheral endoplasmic reticulum (ER) in yeast (66), the authors speculated that toxisomes may have developed from reorganized ER responsive to trichothecene induction. Through the use of fluorescent protein-tagged *TRI* enzymes, ER protein Sec22, and ER-Tracker staining, combined with proteomics and transmission electron microscopy (TEM), it was discovered that the toxisome is stacks of ER cisternae arranged as organized smooth ER (OSER) (12). These OSER were perinuclear or within the peripheral ER. When perinuclear, they appear spherical or crescent shaped, as originally described by Menke et al. (89) (**Figure 2*a*,*b***). Additionally, OSER occur in the peripheral ER, where they appear ovoid and lack nuclei (**Figure 2*b***). Regardless of location, the toxisome OSER have a lumen width of ~10–20 nm and capture layers of cytoplasmic space ~10 nm in width in vitro and in planta (12). The source of the mechanical force for ER remodeling and how the ER is reorganized into toxisomes are still under investigation. Tang and colleagues recently found that the class I myosin of *F. graminearum*, FgMyo1, interacts with Tri1 and actin and participates in toxisome formation. Inhibition of FgMyo1 by the small molecule phenamacril and the actin polymerization disruptor latrunculin A or deletion of the actin-associated proteins FgPrk1 and FgEnd3 leads to marked reduction in toxisome formation (125, 145). In eukaryotic cells, myosins binding with filamentous actin are able to produce physical force by hydrolyzing ATP, therefore converting chemical energy into mechanical force (42, 43). Therefore, we presumed that the FgMyo1–actin

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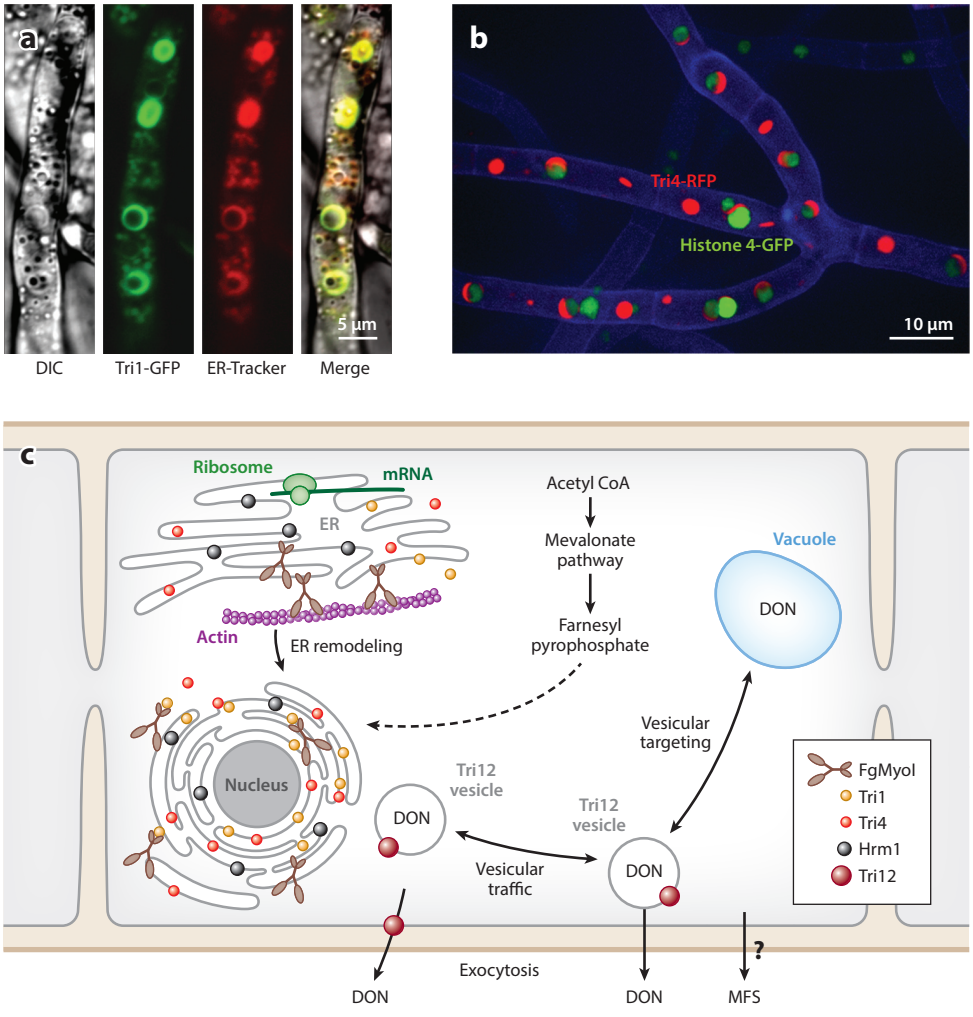


**Figure 2**

Toxisome features and the compartmentalization model for biosynthesis of trichothecenes in *Fusarium graminearum*. (*a*) Colocalization of Tri1-GFP (green fluorescent protein) and the endoplasmic reticulum (ER) tracker in the mycelia of *F. graminearum*. After growth in trichothecene biosynthesis induction (TBI) medium for 48 h, hyphae of ΔTri1::Tri1-GFP were stained with ER-Tracker red and examined for GFP and ER-Tracker signals. (*b*) Fluorescent patterns of dual-label Tri4-RFP (red fluorescent protein; *red*) and histone 4-GFP (*green*) in the mycelia of *F. graminearum*. After growth in TBI for 48 h, hyphae of PH-1::Tri4-RFP+H4-GFP were examined. Photo provided by Dr. Marike Boenisch. (*c*) A proposed model of toxisome formation under the deoxynivalenol (DON) induction conditions in *F. graminearum*. Panel *c* adapted from Tang et al. (125). Abbreviations: DIC, differential interference contrast; MFS, major facilitator superfamily.

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cytoskeleton system provides mechanical force for toxisome construction. Taken together, several lines of evidence suggest that trichothecene biosynthetic enzymes localize to OSER toxisomes formed by way of the FgMyo1–actin system.

How fungi transport SMs between cellular compartments and export them out of cells are still largely unknown. Gene clusters for SM biosynthesis typically encode predicted transporters, especially major facilitator superfamily (MFS) transporters (20). These membrane-spanning

proteins may promote traffic of the product of the biosynthetic pathway between organelles or participate in their export (63). *TRI12* located in the trichothecene biosynthetic core gene cluster encodes a predicted 14-membrane-spanning domain MFS transport, which is associated with trichothecene accumulation and resistance in *F. graminearum* (88). Deletion of *TRI12* results in a significant reduction of trichothecene biosynthesis and growth in *TRI*-inducing media in both *F. sporotrichioides* and *F. graminearum*, suggesting that *TRI12* may play a role in *Fusarium* spp. self-protection against trichothecenes. Coheterologous expression of the *F. sporotrichioides TRI12* and *TRI3* genes in yeast accumulated much higher levels of the acetylated product calonectrin than those observed for strains carrying *TRI3* alone (2). This suggests that Tri12 may facilitate transport of trichothecene metabolites across a membrane barrier and confer toxin resistance. Further studies demonstrate that GFP-tagged Tri12 localizes to the plasma membrane, vacuole, and small (~1 μm) motile vesicles in *F. graminearum* under trichothecene induction conditions (89). These vesicles interact with toxisomes and may accumulate or transfer trichothecenes to the vacuole or export trichothecenes by exocytosis. In addition to Tri12, 15 other MFS transporters are also suggested to be involved in DON efflux in *F. graminearum* based on the RNA-seq analysis, although their exact functions on trichothecene export need to be investigated (135).

Therefore, the processes of trichothecene biosynthesis and its traffic and export may occur in different cellular compartments. We propose a model (**Figure 2*c***): (*a*) The ER is remodeled to form toxisomes by way of the energy generated by the FgMyo1-actin cytoskeleton system. (*b*) Trichothecene synthetic enzymes are integral membrane proteins of toxisomes. Toxisomes may sequester trichothecene products and intermediates, thus protecting the cell from their toxic activity and promoting pathway efficiency. (*c*) The motile vesicles containing Tri12 may accumulate trichothecenes and transport them to the vacuole for storage or the plasma membrane for export via exocytosis. Other MFS transporters may also contribute to this export process. However, in the future, it will be necessary to directly verify that trichothecenes and their intermediates exist in toxisomes, vacuoles, and vesicles.

## REGULATORY MECHANISMS OF TRICHOTHECENE BIOSYNTHESIS IN FUSARIA

Regulation of fungal SM biosynthesis is a complex process that operates on different regulatory levels, including pathway-specific and global regulators, signal transduction pathways, and epigenetic modifications. During the past two decades, the regulation of trichothecene biosynthesis has been extensively investigated. The following section introduces these regulatory mechanisms and highlights their impact on DON biosynthesis in *F. graminearum*.

### Specific Regulators of the Trichothecene Pathway

Generally, fungal SM biosynthesis gene clusters encode cluster-specific transcription factors that directly regulate the expression of biosynthetic genes. In the *TRI* gene cluster, *TRI6* and *TRI10* have been identified as positive regulatory genes for trichothecene biosynthesis in *F. graminearum* and *F. sporotrichioides*. *TRI6*, encoding a $Cys_2$-$His_2$-type transcription factor, positively regulates the expression of *TRI* genes through binding the DNA sequence motif TNAGGCCT in their promoters (119, 124). Targeted disruption of *TRI6* in *Fusarium* species significantly reduced the expression of biosynthetic enzyme-encoding genes and totally abolished the DON and T2-toxin biosynthesis (119). In addition to *TRI* genes, microarray and chromatin immunoprecipitation followed by Illumina sequencing (ChIP-Seq) revealed that Tri6 is a global transcription factor, which regulates more than 200 additional targets involved in carbohydrate metabolism and signal

transduction as well as ABC transporters and others. Furthermore, Tri6 autoregulates its own expression under nutrient-rich conditions, depending on the GTGA repeats in its own promoter (98, 119).

*TRI10* encodes a protein without any known functional domains but does share low levels of identity (30%) with some fungal transcription factors (124). It was also reported as an essential regulatory gene in trichothecene biosynthesis. In *F. sporotrichioides*, *TRI10* was suggested to act upstream of *TRI6*. Disruption of *TRI10* abolished T-2 toxin production and drastically decreased the expression of all *TRI* genes, including *TRI6* (106, 124). Unlike in *F. sporotrichioides*, *TRI6* expression levels were not significantly reduced in the Δ*TRI10* mutant in *F. graminearum* PH-1, although deletion of *TRI10* significantly reduced DON production. Instead, Tri6 negatively regulates *TRI10* expression in this species (119).

## Global Regulators of the Trichothecene Pathway

In addition to pathway-specific regulators, fungal SM biosynthetic gene clusters also may be positively or negatively controlled by global regulators. Currently, a few such global regulators and their corresponding cues have been identified, mainly the regulators of response to carbon and nitrogen sources, ambient light, and pH.

## Nutrient Sources on Trichothecene Production via CreA and AreA

Carbon sources are one of the critical factors for fungal SM biosynthesis. The key regulator of carbon metabolism is the $Cys_2His_2$ zinc finger transcription factor CreA/Cre1, which represses transcription of target genes by binding to the 5′-SYGGRG-3′ specific consensus sequence in the promoters of genes in response to glucose (116). The effects of carbon sources on the production of trichothecenes were examined in *F. graminearum*. It has been shown that sucrose better induces trichothecene production than glucose (57, 146). However, increasing amounts of glucose combined with sucrose did not affect DON production, suggesting that the glucose repressor CreA homolog may not be a key regulating mechanism for DON biosynthesis (57). The CreA homolog has been identified as FgCreA (locus: FGSG_09715) in *F. graminearum*. The deletion mutant ΔFgCreA is dramatically reduced in mycelial growth (~90%) and virulence in planta. Interestingly, sequence analysis indicated that 10 *TRI* genes, including *TRI6*, contain the typical CreA DNA binding sites (117). However, it is not yet known whether FgCreA regulates expression of *TRI* genes and trichothecene production.

The type and quantity of the available nitrogen source in the growth media have special effects on not only fungal growth and development but also the biosynthesis of their SMs. Two main regulators of nitrogen metabolism, AreA and AreB, have been identified and their functions on SM formation are well studied in ascomycetes. Both regulators belong to the GATA family transcriptional factor, which recognizes and binds to the consensus DNA sequence 5′-HGATAR-3′ (128). Orthologs of AreA, including AreA-Gf in *Fusarium fujikuroi* (129), Fnr1 in *Fusarium oxysporum* (27), and AreA in *Fusarium verticillioides* (61), have been identified in different *Fusarium* species and are critical for SM production. Deletion mutants of AreA orthologs significantly reduce the expression of structural genes involved in the biosynthesis of gibberellin in *F. fujikuroi* and fumonisin B1 in *F. verticillioides* (61, 96). In *F. graminearum*, AreA accumulates in nuclei and regulates the expression of *TRI* genes and subsequent DON production. AreA interacts with the regulator Tri10 in coimmunoprecipitation assays and is probably related to its role in regulating *TRI* gene expression. Furthermore, multiple AreA binding sites exist in the promoter regions of *TRI6*, *TRI10*, and other *TRI* genes, suggesting that AreA may directly regulate the expression

of some *TRI* genes (45). The second nitrogen-dependent transcriptional factor AreB physically interacts with AreA, which can act as both a positive and negative regulator. AreB not only regulates certain genes together with AreA, e.g., the gibberellin biosynthetic gene, but also has specific targets, such as genes for apicidin F and fusaric acid biosynthesis in *F. fujikuroi* (108). The ORF FGSG_16452 is predicted to encode the AreB ortholog in the genome of *F. graminearum*, but much less is known about its role in SM biosynthesis in *F. graminearum*.

## Light Regulates Trichothecene Production via the Velvet Complex

Light has been known to influence the expression of fungal SMs through the fungal-specific regulator velvet complex VelB/VeA/LaeA. The impact of the velvet complex in regulating SMs is well studied in *Aspergillus flavus* (7). The velvet complex and its impact on secondary metabolism have also been studied in *Fusarium*. Disruption of this complex almost totally abolished biosynthesis of gibberellins, fumonisin, fusarins, and fusaric acid in *F. fujikuroi* (100, 137). The ΔFgVeA or ΔFgVe1 deletion mutants of *F. graminearum* exhibit reduced aerial hyphal formation as well as reduced aurofusarin and DON biosynthesis (53, 94). The ΔFgVelB mutant shows the identical phenotypes of ΔFgVeA (55). FgLaeA localizes to both the nucleus and cytoplasm under various growth conditions. Deletion of ΔFgLaeA results in a marked reduction in expression of seven *TRI* genes, abolishes 15A-DON accumulation, and leads to a 30-fold reduction of ZEA. In addition, overexpression of FgLaeA produces an 11-fold increase in the amounts of 15A-DON and other SMs, such as butenolide and culmorin (62). Furthermore, all three mutants of the velvet complex show attenuated virulence toward wheat heads (53, 55, 62, 137).

Although the functions of the velvet complex on SM biosynthesis have been widely examined in various filamentous fungi, the molecular mechanisms regulated by the complex are still largely unknown. It has been suggested that LaeA regulates gene transcription via epigenetic modification of the chromatin structure because it contains a methyltransferase domain (13). In addition, the ΔlaeA mutant showed increased heterochromatin protein A (HepA) occupancy and repressive histone 3 lysine 9 trimethylation (H3K9me3) in genes of the sterigmatocystin cluster in *Aspergillus nidulans* (114). However, a substrate directly methylated by LaeA has not yet been identified. More recently, overexpression of the histone acetyltransferase gene *HAT1* in *F. fujikuroi* results in full restoration of gibberellin biosynthesis in the ΔFflaeA background, suggesting LaeA may also interplay with histone acetylation (100). It will be worthwhile to investigate whether FgLaeA regulates trichothecene biosynthesis by way of epigenetic modification in *F. graminearum*.

## pH Regulates Trichothecene B Production via PacC

Acidic pH is essential for promoting *TRI* gene transcription and trichothecene production in *F. graminearum* in vitro. Acidification of the extracellular pH occurs during fungal growth in tested mycotoxin induction media, which coincides with the initial induction of *TRI* gene transcription and accumulation of trichothecenes (35, 91). Neither *TRI* gene expression nor trichothecene accumulation is detected when culture media are maintained at a neutral or alkaline pH. In addition, *TRI* gene transcription and mycotoxin accumulation are restored when the fungal mycelia are transferred from a neutral to an acidic medium (91).

PacC is the key transcription factor for pH regulation in filamentous fungi. The protein contains three $Cys_2His_2$ zinc fingers and recognizes the DNA core consensus sequence 5′-GCCARG-3′. The pH signal transduction and regulatory system has been well studied in *A. nidulans* (4). In *F. graminearum*, the PacC homolog (FgPac1) represses *TRI* transcription and negatively regulates the trichothecene production. The deletion mutant ΔFgPac1 exhibits a growth defect phenotype

under neutral and alkaline pH, as well as earlier *TRI* gene induction and trichothecene (DON+15A-DON) accumulation under acidic pH. Constitutively expressing the active form FgPaC1$^{c}$ (truncated FgPac1, 1–491 amino acids) strongly represses *TRI* transcription and mycotoxin accumulation both in an acidic toxin-inducing medium and in planta (92). A total of 14 PacC binding motifs are found in the core *TRI* gene cluster, including the regulator genes *TRI6* and *TRI10*, indicating FgPac1 may regulate *TRI* gene expression by directly binding to their promoters (93).

## Signal Transduction Pathways Regulate Deoxynivalenol Biosynthesis

Organisms quickly respond to extracellular stimuli and regulate gene expression through multiple signal transduction pathways to adapt to changing environmental conditions. In fungi, conserved signaling routes control fundamental aspects of growth, development, and SM formation. Several signal transduction pathways in trichothecene biosynthesis have been studied in *F. graminearum*, including target of rapamycin (TOR), mitogen-activated protein kinase (MAPK) cascades, and cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA). These pathways cross talk with each other and tightly regulate mycotoxin biosynthesis.

## Target of Rapamycin Signaling Pathway

TOR is a signaling pathway conserved from yeast to human that connects environmental cues (e.g., nutrients and growth factors) to developmental and metabolic processes (36). The core components of TOR and their signaling events have been extensively investigated in *Saccharomyces cerevisiae*, where Tor kinase interacts with other proteins to form two distinct high-molecular-mass complexes known as TORC1 and TORC2 (136). Two direct effectors, phosphatase Tap42 and kinase Sch9, have been identified as downstream of TORC1 and regulate ribosome biosynthesis, nutrient availability, aging, and other biological processes in *S. cerevisiae* (134).

The function of the TOR pathway in filamentous fungal SM biosynthesis was first reported in *F. fujikuroi*. TOR regulates the expression of gibberellins and bikaverin biosynthesis genes via AreA-mediated nitrogen metabolite repression in *F. fujikuroi* (126). In *F. graminearum*, we have identified several key components of the TOR pathway and investigated their role in trichothecene biosynthesis. The ortholog TOR kinase FgTor (FGSG_08133), FgTap42 (FGSG_09800), and Pph21/Pph22/Pph3 ortholog FgPp2A (FGSG_09815) are essential for the fungal growth. Deletion of FgPpg1 (FGSG_05281) leads to trace amounts of DON biosynthesis on sterilized wheat kernels, which is partially dependent on FgAreA regulation, similar to the role of *F. fujikuroi* TOR in SM biosynthesis (141). However, DON production by a ΔFgSit4 (FGSG_01464) mutant is not significantly different from that of the wild type. Two PP2A activators (PTPAs), FgRrd1 and FgRrd2, control FgPP2A activity through FgTap42-mediated physical interaction with FgPP2A. Both ΔFgRrd1 and ΔFgRrd2 mutants are dramatically reduced in DON production during the infection process on wheat heads (77). The formation of DON toxisomes coincides with increased lipid droplets (LDs) during DON biosynthesis in vitro and in planta. Furthermore, inactivation of TOR by treatment with rapamycin results in the dramatic induction of LD biogenesis in the mycelium of *F. graminearum*, similar to LD accumulation in the mycelium grown in DON-inducing conditions (12, 141). These observations imply that TOR may also regulate DON production via LD biogenesis in this fungus. Recently, combining genetic, cytological, and phenotypic analyses, we reveal that TOR regulates LD biogenesis through the FgPah1-Nem1-Spo7 cascade located downstream of FgTap42 (74a). Besides the FgTap42-PP2A branch, the second branch of the FgSch9 cascade is also involved in

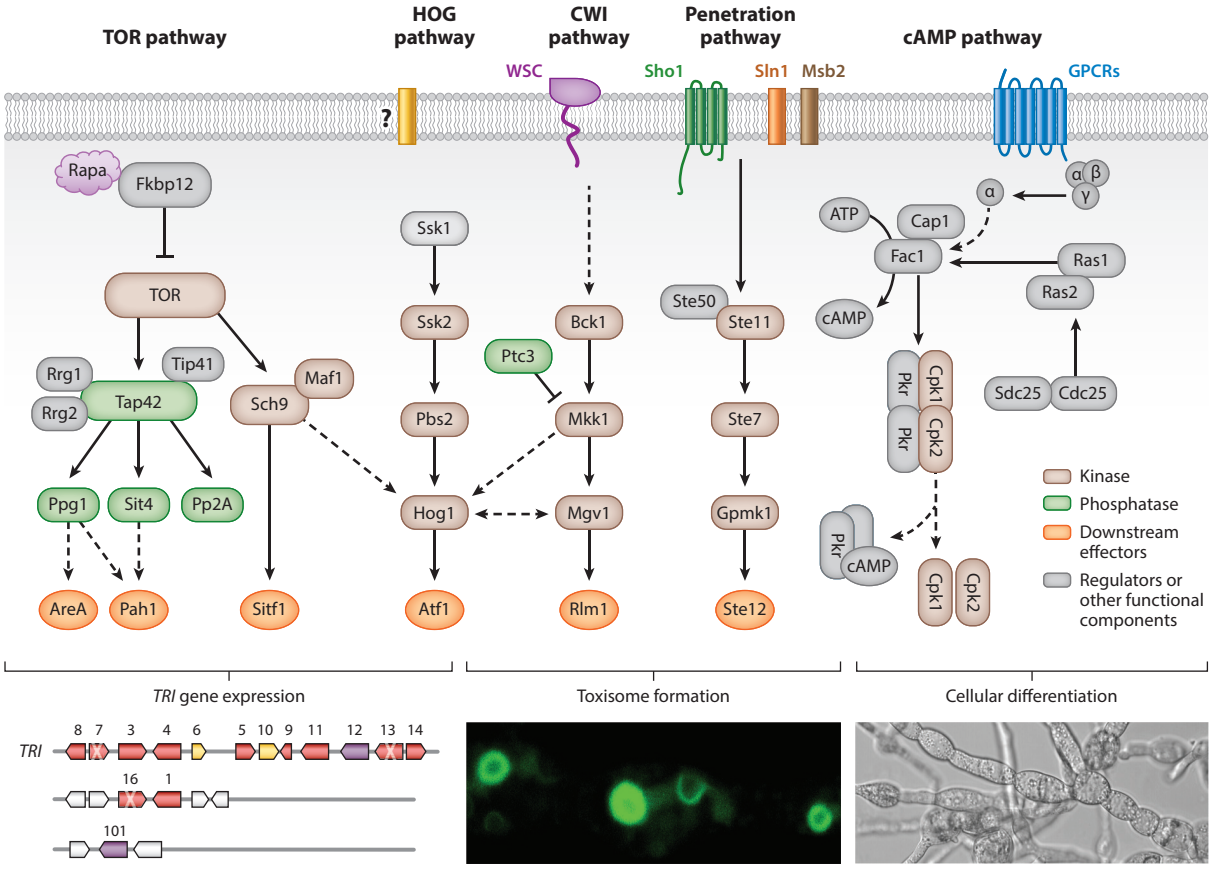


**Figure 3**

Representation of major signaling pathways in *Fusarium graminearum* and their connections to deoxynivalenol (DON) production. Transmembrane proteins WSC, Sho1, Sln1, Msb2, and GPCR (G protein–coupled receptor) and other unidentified receptors recognize extracellular stimuli to activate the TOR (target of rapamycin), three MAPK (mitogen-activated protein kinase), and cAMP (cyclic adenosine monophosphate) signal pathways for *TRI* gene expression, toxisome formation, cellular differentiation and final DON production. Colors indicate different roles of major components involved in above pathways: brown, kinase; green, phosphatase; orange, downstream effectors, including transcription factors AreA, Sitf1, Atf1, Rlm1, and Ste12, and phosphatidate phosphatase Pah1; gray, regulators or other functional components. Solid lines indicate direct interaction; dashed lines show interactions that are not well understood or are likely to be indirect. Activation is depicted by arrowheads and repression by T-bars. Abbreviations: CWI, cell wall integrity; HOG, high osmolarity glycerol; Rapa, rapamycin.

DON biosynthesis mainly via its interacting protein, FgMaf1 (39). Collectively, TOR regulates trichothecene biosynthesis by way of at least three downstream signal transduction circuitries in *F. graminearum*, including TOR-Tap42-AreA, TOR-Tap42-Pah1, and TOR-Sch9-Maf1 (**Figure 3**).

## Mitogen-Activated Protein Kinase Signaling Pathway

MAPK cascades are characterized by a three-tiered module comprising a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAPK, which are activated by phosphorylation at the well-conserved threonine-x-tyrosine (TXY) motif within the activation loop

(17). Signals are received by receptors or sensors at the membranes and transduced through small GTPases to MAPKs that activate each other by phosphorylation. The last level of MAPK then migrates into the nucleus and activates downstream effectors such as transcription factors that regulate biological processes for environmental adaptation. As in other eukaryotic organisms, MAPK cascades are conserved in fungi and regulate responses to environmental stimuli (115). In general, the fungal MAPK signal transduction pathway is divided into three major cascades: the cell wall integrity (CWI) signaling pathway, which is responsible for cell wall biosynthesis and repair; the high osmolarity glycerol (HOG) pathway, which responds to osmotic and oxidative stress; and the mating/filamentation pathway, which usually coordinates pseudohyphal formation, sexual crossing, and virulence of pathogenic fungi (52). These MAPK cascades interplay with each other and are also important for fungal SM formation. Key kinases of all three MAPK cascades have been functionally analyzed in *F. graminearum*, and their roles in trichothecene biosynthesis have been examined.

In *F. graminearum*, the MAPKs in the CWI signaling pathway consist of FgBck1 (FGSG_06326), FgMkk1 (FGSG_07295), and FgMgv1 (FGSG_10313). Mycelia of ΔFgMgv1 show weakened cell walls and are hypersensitive to cell wall–degrading enzymes (46). The ΔFgMgv1 mutant also had significantly reduced trichothecene accumulation in inoculated wheat heads. Two weeks after inoculation, less than 1.5 ppm DON was found in samples inoculated with the ΔFgMgv1 mutant compared to >300 ppm DON produced by the wild-type strain. The mutant was also attenuated in virulence toward wheat (46). Consistent with the finding for FgMgv1, FgMkk1 is also involved in the regulation of hyphal growth, DON biosynthesis, and virulence and increases the sensitivity to cell wall–damaging agents (144). In addition, ΔFgBck1 also exhibited reduced DON production and virulence (133). Type 2C protein phosphatases (PP2Cs) have been reported to regulate the CWI pathway upstream of MAPK in *S. cerevisiae* (72). We searched the genome for orthologs of PP2Cs and found seven putative *PP2C* genes in *F. graminearum*. Phenotype analysis of mutants for each gene indicates that FgPtc3 (FGSG_10239) negatively regulates transcription of *FgMKK1* and *FgMGV1*. Consistent with this finding, ΔFgPtc3 increases resistance toward cell wall–damaging agents (56). Furthermore, the MADS-box transcription factor FgRlm1 directly interacts with FgMgv1 and is considered a downstream effector of the CWI pathway. The ΔFgRlm1 mutant demonstrates identical phenotypes for the cell wall, DON biosynthesis, and virulence to its upstream kinase mutants (144).

The FgSte11-Ste7-Gpmk1 signaling cascade, encoded by the loci FGSG_05484, FGSG_09903, and FGSG_06385, respectively, functions similarly to the yeast Fus3/Kss1 mating/filamentation pathway. Deletion mutants of this cascade lead to decreases in the expression of *TRI* genes and reduced DON production (37, 48, 133). MAPK mutants also abolish the penetration structure formation in planta and result in the attenuated virulence, which largely depends on the downstream transcription factor FgSte12. FgGpmk1 regulates the subcellular localization of FgSte12 and, subsequently, controls the secretion of cell wall–degrading enzymes. Disruption of *FgSte12* caused the nonpathogenic phenotype similar to that of ΔFgGpmk1 on wheat heads, but it did not affect DON production (38). This result indicates that other unidentified downstream effectors are responsible for DON biosynthesis in this MAPK signaling cascade.

In *F. graminearum*, the HOG pathway is activated by osmotic and oxidative stresses via the osmosensor histidine kinase FgOs1 (FGSG_16781), histidine phosphotransferase HPT1p (FGSG_04363), and FgRrg1(FGSG_08948), which is the osmolarity two-component response regulator Ssk1 ortholog in *S. cerevisiae*. Stress signals lead to enhanced levels of dephospho-FgRrg1 and sequential phosphorylation of the MAPKKKs Ssk2 (FGSG_00408), MAPKK FgPbs2 (FGSG_0869), and MAPK FgHog1 (FGSG_09612) (54, 99, 101, 102, 130, 148) (**Figure 3**). Downstream targets of FgHog1 presumably include MAPK-dependent protein kinases as well

as the transcription factors; however, to date, only the ATF/CREB transcription factor FgAtf1 has been characterized as the interactor of FgHog1 in the nucleus under osmotic stress (130). The HOG cascade orchestrates numerous physiological functions in *F. graminearum* by regulating hyphal growth, branching, and virulence as well as hyperosmotic and oxidative stress responses. Furthermore, it is also involved in the trichothecene biosynthesis (54, 99, 101, 102, 130, 148). Ochiai et al. (101) showed that production of trichothecenes was markedly suppressed by NaCl without a significant effect on fungal growth. In rice grain or wheat kernel cultures and in planta, all three HOG MAPK gene mutants are reduced in DON and NIV production in different *F. graminearum* strains (99, 101, 148). Disruption of the upstream response regulators FgOs1 and FgRrg1 and response factor FgAtf1 also caused marked reduction of DON production, virulence, and expression of *TRI* genes (54, 101, 130). In addition, the HOG pathway can be activated by the deletion of the bZIP family TF Yap1 Fgap1, which enhances DON production in the fungus (97).

Cross talk among these MAPK pathways has been reported in several filamentous fungi, including *F. graminearum*, particularly between the osmoregulation and cell wall integrity pathways (52, 115). In ΔFgMKK1, phosphorylation of FgMgv1 is totally abolished, and the phosphorylated FgHog1 is also partially reduced. Consequently, the mutant is increased in sensitivity to osmotic and oxidative stresses (144). The HOG pathway is also involved in responses to cell wall stresses (99, 148). These MAPK cascades may synergistically regulate trichothecene biosynthesis upon reception of a stimulus by their cognate receptors. However, few upstream receptors of these three MAPK cascades have been identified in this fungus (52). The yeast osmosensor Sho1 ortholog FgSho1 physically interacts with the MAPK module FgSte50-Ste11-Ste7 and regulates the FgGpmk1 signaling pathway, whereas FgSln1 has an additional function with FgSho1 (37). The Msb2 ortholog is also involved in the FgGpmk1 cascade for DON biosynthesis and virulence in this fungus (Y. Chen, unpublished data). Wsc1, Wsc2, and Wsc3 orthologs are predicted as the sensors of the CWI pathway in *F. graminearum*, although their function remains to be experimentally confirmed.

## Cyclic Adenosine Monophosphate–Protein Kinase A Signaling Pathway

The cAMP-PKA signaling pathway is well studied in yeast and plays an important role in the regulation of cell growth, morphogenesis, metabolism, and stress resistance in response to environmental stimuli (28). This signaling pathway is highly conserved and is critical for growth, development, and virulence as well as SM biosynthesis in filamentous pathogenic fungi (68). The central components of the cAMP pathway have been identified and their functions in DON biosynthesis also have been determined in *F. graminearum* (40) (**Figure 3**). The intracellular cAMP level is elevated when mycelia are grown in DON-inducing conditions, and exogenous cAMP or the PDE inhibitor IBMX stimulates *TRI* gene expression and subsequently results in increased DON production in the wild type (51). Mutants of the PKA catalytic subunit gene *CPK1* or the adenylate cyclase gene *FAC1* significantly reduces virulence and production of DON in cultures or infected plant tissues (15, 47). In addition, deletion of *FgCAP1*, which encodes the adenylate cyclase-associated protein (CAP), also reduces the intracellular cAMP levels and PKA activity, DON production, and *TRI* gene expression, which can be suppressed by exogenous cAMP, indicating a PKA-dependent regulation of DON biosynthesis by FgCap1 (140). In contrast, deletion of *PDE2* and *PKR* increases DON production (51, 73). Yu et al. (142) genetically analyzed three putative Gα subunits upstream of adenylate cyclase, GzGpa1, GzGpa2 and GzGpa3, and one Gβ subunit, GzGpb1, and found deletion of GzGpa1 and GzGpb1 enhanced DON production, suggesting that both Gα GzGpa1 and Gβ GzGpb1 negatively control mycotoxin production. Two Ras GTPase orthologs (Ras1 and Ras2) exist in the genome of *F. graminearum*. Disruption of Ras2

did not affect the intracellular level of cAMP or DON biosynthesis in planta, despite attenuating virulence through the Gpmk1 cascade and regulating the secreted lipase Fgl1 (11). This suggests that the essential protein Ras1, but not Ras2, may play a critical role in the cAMP signaling pathway in *F. graminearum*.

## Epigenetic Regulation

Chromatin remodeling changes the assembly and disassembly of nucleosomes and tightly controls gene transcription in eukaryotic cells. Covalent post-translational modifications (PTMs) of histones, including methylation, acetylation, and phosphorylation, are considered critical for the regulation of chromatin structure and function. In fungi, the clustered SM genes have been found to be regulated by proteins and histone marks involved in chromatin remodeling. For instance, HepA (heterochromatin protein 1), ClrD (H3 lysine 9, H3K9 methyltransferase), CclA (H3 lysine 4, H3K4 methyltransferase), HdaA (histone deacetylase), and GcnE (histone acetyltransferase) have been reported to mediate remodeling of SM clusters in *Aspergillus* (33, 80).

Chromatin-mediated regulation is also important for trichothecene biosynthesis in *F. graminearum*. In addition to LaeA described above, heterochromatin, histone methylation, and acetylation also epigenetically contribute to DON production. Deletion of Hep1, an ortholog of *Aspergillus* HepA, leads to strongly decreased transcription of *TRI5* and *TRI6* and results in DON reduction (113). Among all 45 SM clusters, 35 are enriched with the trimethylated histone H3 lysine 27 (H3K27me3), which is associated with silenced gene expression. Furthermore, deletion of the gene encoding the putative H3K27 methyltransferase (Kmt6) causes constitutive expression of genes for mycotoxins, pigments, and other SMs, including the DON biosynthetic genes *TRI3*, *TRI4*, and *TRI5* and regulator gene *TRI10* (21). In contrast, elimination of the histone modification associated with gene activation [H3 lysine 4 (H3K4) methylation] by disruption of the H3K4 methyltransferase gene *FgSET1* abolishes DON production. ChIP-qPCR results indicate that mono- and trimethylated H3K4 are enriched in the promoter region of *TRI6*, and methylation of H3K4 positively regulates transcription of *TRI6* (76).

To date, histone acetyltransferases Gcn5, Sas3, and Elp3 have been implicated in a regulatory role in DON induction (18, 65, 69). Gcn5, as part of the multisubunit complex SAGA/ADA, is responsible for the acetylation of H3K9, H3K18, and H3K27, and thereby for the subsequent activation of the *TRI* gene cluster in DON-inducing conditions (18, 65). Consistent with that, the deletion mutants, ΔFgSPT7 and ΔFgADA3, of the other components of the SAGA/ADA complex also eliminated DON production. In addition, expression of *TRI* genes is regulated by Sas3 and Elp3, which are responsible for H3K4 and H3K14 acetylation, respectively (65, 69). Interestingly, deletion of the histone deacetylase gene *HDF1* also results in a significant reduction in virulence and DON production (74). It seems to imply that the *TRI* gene cluster may be precisely regulated by histone acetylation, whereby both hyperacetylation and deacetylation repress *TRI* gene transcription. In summary, chromatin-based regulation involving both methylation and acetylation of histones is an important step for the activation of *TRI* gene cluster. Discovering the roles of other epigenetic modifications, including DNA methylation, PTMs of histones, incorporation of histone variants, chromatin remodelers, and even noncoding RNAs (ncRNAs), and their cross-talk mechanisms in DON biosynthesis remains an important goal. Eukaryotic cells must integrate multiple signals at the cellular level to adapt to the surrounding environment (5). Extracellular signals such as nutrient sources, pH, and light as described above are transmitted to the nucleus via various signaling pathways where they directly or indirectly induce transcription of DON biosynthesis genes. This may occur via induction or modification of sequence-specific transcription factors that may serve to integrate various signals, at least in part, and induce

epigenetic states. Thus, it would be interesting to elucidate the cross talk between signaling pathways and epigenetic modifications in regulating DON biosynthesis.

### Other Genes Are Involved in Deoxynivalenol Biosynthesis

Taking advantage of the high homologous recombination efficiency in *F. graminearum* for site-directed mutagenesis, systematic characterization of all putative kinases (133), phosphatases (143), transcription factors (121), peroxidases (70), cytochrome P450 monooxygenases (120), and autophagy-related proteins (79) has been reported, and thus the roles of these genes in DON production have been empirically determined. A survey of these genes and others from available publications identifies a total of 252 genes directly or indirectly affecting DON biosynthesis (**Supplemental Table 2**). Therefore, the regulation of DON biosynthesis is a complex network needing further elucidation.

Supplemental Material >

## ENVIRONMENTAL FACTORS AFFECT DEOXYNIVALENOL PRODUCTION

Environmental cues trigger the multiple signal transduction pathways described above and thereby regulate DON biosynthesis. In addition to the above-described nutrients, pH, and light, various biotic agents have been reported to influence *TRI* gene expression and DON production.

Signals generated during the host plant–*F. graminearum* interaction play an important role in triggering DON biosynthesis. During infection, higher concentrations of $H_2O_2$, an inducer of DON production, are observed in infection cushions relative to runner hyphae of *F. graminearum* on host plants (90, 109). In addition, polyamine and phenolic acids produced in wheat in response to fungal infection are able to increase DON production (14). Furthermore, microbes that coexist with *F. graminearum* on wheat heads may also affect DON biosynthesis and pathogen virulence. For example, the biocontrol agent (BCA) *Pseudomonas piscium* ZJU60 from the wheat head microbiome is able to suppress DON production by altering histone acetylation (18). Nevertheless, the interaction mechanisms of the pathogen and other microbes and their subsequent influence on DON biosynthesis in the plant-associated microbiome under natural conditions are currently poorly understood and require further investigation.

## MANAGEMENT OF DEOXYNIVALENOL CONTAMINATION

DON contamination in infected grains is closely linked to the severity of FHB disease in the field. The best way to manage DON contamination is to control FHB in the field during crop cultivation. Several reviews have summarized the available strategies for managing FHB, including host resistance, fungicide application, biological control, and cultural practice. These approaches must be integrated to achieve relatively high efficacy.

### Host Resistance

Deploying disease-resistant cultivars is considered the most economical and durable method for controlling FHB. However, most wheat cultivars are susceptible or moderately susceptible to the *F. graminearum* species complex, and highly resistant genotypes are not yet available (6). FHB resistance has been mainly classified as type I (resistance to the initial infection), type II (resistance to spreading within spike and kernel), or type III (resistance to accumulation of toxins) (95). These FHB resistance traits in wheat are controlled by numerous quantitative trait loci (QTLs). To date,

more than 250 QTLs for FHB resistance have been described from various wheat genotypes (50). Among them, Fhb1 (syn. Qfhs.ndsu-3BS) derived from Chinese cultivar Sumai 3 on chromosome 3BS is the most important QTL and has been reported to provide a moderately high level of resistance against various isolates and species of *Fusarium*. More recently, through the use of mutation analysis, gene silencing, and transgenic overexpression, a pore-forming toxin-like (PFT) gene at Fhb1 has been identified that confers FHB resistance (111). The transfer of Fhb1 into *Triticum durum* represents a significant step forward for the enhancement of FHB resistance (141). With the increasing effort to breed for resistance using traditional and molecular techniques and more native or exotic sources of resistance identified in FHB-prone regions of the world, more moderately resistant cultivars are becoming available to producers.

Transgenic germplasms could provide new additional sources for FHB control. Constitutive overexpression of plant genes encoding defense signaling pathway–related proteins, cell wall–degrading enzyme inhibitors, or detoxification proteins results in greatly enhanced resistance to *F. graminearum*. For instance, transgenic wheat lines expressing *Arabidopsis thaliana* Npr1, polygalacturonase inhibitor or UDP-glycosyltransferase showed increased resistance to FHB (30, 81, 138). Silencing of pathogen genes by interfering RNAs derived from engineered plant genomes is referred to as host-induced gene silencing (HIGS). HIGS has emerged as a novel transgenic approach to generate FHB-resistant wheat germplasms. HIGS transgenic lines targeting cytochrome P450 lanosterol 14α-demethylase (*CYP51*) or chitin synthase 3b (*CHS3b*) significantly reduce FHB disease and DON contamination under greenhouse and field conditions, suggesting that HIGS may have great potential for engineering FHB-resistant wheat cultivars in the future (19, 64).

## Chemical Fungicides

Currently, application of chemical fungicides is still one main approach for controlling FHB in many regions of the world because of the lack of effective disease-resistant cultivars. Demethylation inhibitor (DMI) class fungicides, especially tebuconazole, metconazole, and prothioconazole, block fungal sterol biosynthesis and are the most widely used and effective fungicides for the suppression of FHB and reduction of mycotoxin accumulation (104). Another older fungicide, the benzimidazole carbendazim, is also effective against FHB, with approximately 70% disease reduction (75). Fungicides in the quinone inhibitor class have been shown to increase DON levels in grain (29) and therefore are not recommended for FHB and DON control. In China, a novel fungicide, phenamacril, targeting myosin I in combination with tebuconazole is also widely used for FHB control (145). The novel compound not only results in the suppression of FHB but also reduces DON production effectively by disrupting the toxisome formation (125). More recently, a highly effective succinate dehydrogenase inhibitor (SDHI), pydiflumetofen, has been registered in Argentina and the United States for management of FHB.

The timing of fungicide application is crucial for the control of FHB. To be effective, fungicide application is usually timed at the anthesis growth stage or up to six days after anthesis (24) because susceptibility to FHB infection is highest at this stage. Another issue for chemical control of FHB in practice is fungicide resistance. After long-term intensive application of fungicides, *F. graminearum* strains resistant to azoles and carbendazim have been detected in fields (75, 122). For example, during the past decade, the percentage of carbendazim-resistant populations in field samples from Jiangsu province and Anhui province in China has dramatically increased, reaching 41% and 11%, respectively, in these main wheat-producing areas (**Supplemental Figure 3**). What is worse is that the carbendazim-resistant strains are able to produce more mycotoxin during

Supplemental Material >

infection (147). Therefore, development of new fungicides for FHB control and DON management is urgently needed.

### Biological Control

Biological control is defined as an environmentally friendly strategy using living microorganisms or their derivatives to reduce a targeted pathogen. Utilization of BCAs alone or as part of an integrated management program is an alternative approach for the control of FHB and DON. Biocontrol of FHB has been intensively studied, and many BCAs have been isolated from various environmental conditions. A comprehensive overview of biocontrol strategies for fighting FHB has been well summarized by Legrand et al. (71). Successful reduction of FHB incidence and severity in the field has already been achieved by several bacterial strains, mainly including *Bacillus* spp., *Pseudomonas* spp., *Lysobacter enzymogenes*, and *Streptomyces* spp., and fungal BCAs, including *Cryptococcus* spp., *Trichoderma* spp., *Clonostachys rosea*, and *Aureobasidium pullulans*. These BCAs can be applied directly to spikes to slow disease progression or applied to straw residues to suppress production of perithecia. The main modes of action of the BCAs include parasitism, production of antifungal metabolites, competition for space and nutrients, and induction of host plant resistance. Despite more than 70 years of intensive research and some promising results, few BCAs are currently available on the market. Lack of commercialized BCAs for FHB may be because of their unstable biocontrol efficacy in field conditions, strict storage and transport conditions for BCAs, unidentified biocontrol mechanisms, and the complexity of registration. Overcoming these challenges will promote BCA usage for managing FHB and DON contamination in the future.

### Cultural Practices

Tillage to bury host crop residues and crop rotation with nonhosts has been found to reduce FHB intensity and DON accumulation in infected grain. *F. graminearum* is able to survive on wheat, maize, barley, and rice stubble or residues and on wild grasses in the forms of mycelia, conidia, or perithecia. Tillage buries host crop residues and can be effective in reducing the initial inoculum for subsequent infection cycles as well as reducing both DON and pathogen levels in the soil (105). The no-tillage practice, which leaves crop residues unburied, clearly increases DON contamination in wheat grain (10). Crop rotation with nonhost species may also reduce FHB intensity and DON accumulation (26). In addition, irrigation management to prevent excessive moisture in irrigated wheat fields and an early sowing date to limit the load of inoculum can also significantly lower the risk of FHB development and DON accumulation in wheat (22). In summary, the individual options may reduce the FHB incidence and DON levels, but an integrated management program would be more efficient. Therefore, producers are encouraged to integrate as many management approaches as possible in FHB-prone regions.

## CONCLUSION AND PERSPECTIVE

In the past two decades, the trichothecene biosynthetic pathway has been extensively characterized by using precursor feeding and gene knockout approaches. However, to date, the overall regulation of Tri genes remains poorly understood. Although *F. graminearum* has one of the best characterized fungal genomes, all gene regulation studies to date have suffered from a lack of comprehensive gene annotation. Of the 14,143 protein-coding genes predicted for *F. graminearum*, more than 40% have no known function and are annotated as hypothetical proteins. This is problematic for DON and SM regulatory studies because hypothetical protein gene expression is often enriched during times of increased DON or SM accumulation such as during plant infection.

Regulation of secondary metabolism in most fungi has been shown to be controlled by various regulatory systems in response to the external environment. In *Fusarium*, a number of transcriptional regulators and several important signaling pathways (e.g., VeA, TOR, MAPK, cAMP-PKA, and Pac signaling pathways) have been identified to regulate trichothecene biosynthesis in response to extracellular conditions. However, a complete understanding of the regulatory mechanism is far from being achieved.

Initial studies of gene regulatory networks have been promising. Using *F. graminearum* microarray data from diverse experimental conditions, regulatory modules were identified that correspond to gene networks enriched for distinct gene ontology (GO) categories with predicted regulators (transcription factors and signaling proteins), also enriched with target genes having shared GO assignments (41). Remarkably, these networks also revealed the compartmental nature of the *F. graminearum* genome between core genes (shared with most other fungi) and lineage-specific (LS) genes (found only in *F. graminearum* or closely related fungi). Core genes are significantly associated with core regulators, whereas LS genes are associated with LS regulators. As core and LS genes are found in different regions of the genome (23), regulatory networks may also reflect differences in epigenetic control and their distinct evolutionary paths. Future studies should focus on functional validation to increase the resolution of network relationships among signaling pathways and the transcription regulators and to understand DON synthesis under novel environmental conditions such as fungicide treatment or microbial interactions. These details will be helpful for establishing reliable control strategies against mycotoxin contamination.

Epigenetics now appears at the forefront for understanding the regulation of gene expression in response to the external environment in eukaryotes. Epigenetic modifications include DNA methylation, histone modification, and RNA-associated silencing by small noncoding RNAs. A growing body of recent evidence suggests that these epigenetic mechanisms are critical for fungal growth, reproduction, and pathogenesis. It is noteworthy that many genes involved in fungal secondary metabolism are arranged in a clustered format that is easily impacted by epigenetic modifications. As described previously, histone acetylation and methylation play important roles in regulating expression of *Tri* genes. However, studies highlighting the role of many other epigenetic factors in the modulation of trichothecene and other mycotoxin biosyntheses are lacking. More recently, a few studies have suggested that different fungal species may make use of different epigenetic-based regulatory systems to control secondary metabolism. Therefore, the identification of specific and conserved epigenetic modifications in diverse *Fusarium* species can offer real opportunities to broaden our knowledge concerning the regulation of trichothecene and SM biosynthesis.

Control of FHB disease and mycotoxin accumulation at the field level is still not assured. Evidently, control of FHB will be possible only through integration of resistance breeding, chemical and biological controls, and optimal agronomic practices. The generation of resistant varieties remains the best way to reduce field disease levels. Nevertheless, through efforts spanning several decades, it is clear that the development of completely resistant lines that preclude the need for fungicide usage in FHB management still remains elusive. FHB resistance is not simply determined by a small number of discrete QTLs/genes with large effect. It remains a challenge to breed highly resistant cultivars containing numerous minor genes for resistance as well as all the determinants necessary for acceptable agronomic and grain quality traits. Current fungicides used against FHB are generally ergosterol DMIs. However, usage of these fungicides is complicated by the fact that they are effective only during a narrow time during wheat flowering. In addition, chemical control of FHB faces a major problem because of the development of fungicide resistance. More seriously, in some cases, applying ineffective doses of some fungicides could trigger fungi to produce more trichothecene mycotoxins. As such, there is a great need for more effective

antimycotoxinogenic molecules to address the significant threat of FHB and mycotoxin contamination. Theoretically, commercialization of biological control agents will increase the options available for integrated management of FHB. The challenge, however, lies in devising practical control methods that can deliver potent formulations combining high efficacy, long shelf life, and easy application. Therefore, considerable efforts are needed to improve on current approaches and develop future strategies for more effective management of FHB and mycotoxins.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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