Plant Pathol. J. 22(3) : 203-210 (2006)

The Plant Pathology Journal

Mini-Review

Regulation of Fumonisin Biosynthesis in *Fusarium verticillioides*-Maize System

Uma Shankar Sagaram, Mike Kolomiets and Won-Bo Shim*

Department of Plant Pathology and Microbiology, Program for the Biology of Filamentous Fungi, Texas A&M University, College Station, TX 77843-2132, USA

(Received on July 1, 2006)

Fumonisins are a group of mycotoxins produced by a pathogen *Fusarium verticillioides* in infected maize kernels. Consumption of fumonisin-contaminated maize has been implicated in a number of animal and human illnesses, including esophageal cancer and neural tube defects. Since the initial discovery, chemistry, toxicology, and biology of fumonisins as well as the maize-*Fusarium* pathosystem have been extensively studied. Furthermore, in the past decade, significant progress has been made in terms of understanding the molecular biology of toxin biosynthetic genes. However, there is a critical gap in our understanding of the regulatory mechanisms involved in fumonisin biosynthesis. Here, we review and discuss our current knowledge about the molecular mechanisms by which fumonisin biosynthesis is regulated in *F. verticillioides*. In addition, we discuss the impact of maize kernel environment, particularly sugar and lipid molecules, on fumonisin biosynthesis.

Keywords : fumonisin biosynthesis, *Fusarium verticillioides*, lipids, mycotoxins, oxylipins

Fusarium verticillioides (Sacc.) Nirenberg (teleomorph *Gibberella moniliformis* Wineland; formerly known as *F. moniliforme*) is a fungal pathogen of maize causing ear rots and stalk rots worldwide (Munkvold and Desjardins, 1997). More importantly, *F. verticillioides* produces a group of mycotoxins called fumonisins when the fungus colonizes maize and maize-based products. Fumonisin B_1 (FB_1), the major fumonisin found in nature, can cause detrimental health effects when consumed by animals and humans (Gelderblom et al., 1988; Nelson et al., 1993; Marasas, 2001). Fumonisin contamination of maize has been perceived as a problem in the Midwest, however, recent reports describing the link between fumonisin-contaminated maize and prevalent incidents of neural tube defects (NTD) near the Texas-Mexico border suggest otherwise (Missmer et al., 2006). Since the discovery of fumonisins in 1988, scientists have invested substantial efforts to understand the chemistry, toxicology, and biology of fumonisins as well as the maize-*Fusarium* pathosystem. Several excellent reviews are available on these topics (ApSimson, 2001; Merrill et al., 2001; Munkvold and Desjardins, 1997; Nelson et al., 1993), thus these topics will not be discussed in detail in this review.

Our knowledge of fumonisins biosynthesis has greatly increased over the past decade. Fumonisins, a group of polyketide-derived secondary metabolites, are synthesized by a cluster of genes designated as the *FUM* gene cluster (Proctor et al., 1999; Proctor et al., 2003). To date, the *FUM* gene cluster, which is approximately 42-kb in length, is known to harbor 22 open reading frames (ORFs) - 15 of which are known to be co-regulated. *In silico* analysis suggests that eleven of the 15 co-regulated genes encode secondary metabolite biosynthesis genes, e.g., monooxygenases, dehydrogenases, and fatty acyl-CoA synthetases. The *FUM* gene cluster also contains genes encoding putative transporters and proteins involved in self-protection (Proctor et al., 2003). Molecular genetic characterization of *FUM1*, a polyketide synthase, *FUM6*, a cytochrome P450 monoxygenase, and *FUM8*, an aminotransferase, demonstrated their critical roles in FB_1 biosynthesis; disruption of *FUM1*, *FUM6*, and *FUM8* resulted in significant reduction in FB_1 production (Proctor et al., 1999; Seo et al., 2001). Butchko et al. (2003) provided the first biochemical evidence directly linking a *FUM* gene (*FUM13*) to a specific reaction during fumonisin biosynthesis. The Fum13 protein, which has similarity to short-chain dehydrogenases/reductases, was found to catalyze the reduction of the C-3 carbonyl of the fumonisin backbone to a hydroxyl group. Similarly, *FUM3* encodes a 2-ketoglutarate dependent dioxygenase and catalyzes the hydroxylation of carbon-5 of the fumonisin backbone (Butchko et al., 2003; Ding et al., 2004). Bojja et al. (2004) have also elucidated two of the early steps in fumonisin biosynthesis. Utilizing disruption mutants, the authors demonstrated that the enzyme encoded by *FUM8* catalyzes the condensation of alanine, which is followed by the oxidation of the backbone at carbons 14 and 15 by the P450 monooxygenase encoded by *FUM6*.

While our knowledge of the fumonisin biosynthesis gene cluster is nearly complete, there is a critical gap in our

*Corresponding author.
Phone) +1 979 458 2190, FAX) +1 979 845 6483
E-mail) wbshim@tamu.edu

understanding of the regulatory mechanisms involved in fumonisin biosynthesis. A decade of research has shown that there are multiple factors, environmental as well as genetic, that play a role in this complex biological process. Significantly, the regulation mechanism governing fumonisin biosynthesis in *F. verticillioides* may have several distinct attributes quite different from what is known in other filamentous fungi (Yu and Keller, 2005). In this review, we will discuss our current understanding of how fumonisin biosynthesis is regulated in *F. verticillioides*. We also describe recent functional genomics approaches that are helping scientists to better understand this intriguing regulatory mechanism in *F. verticillioides*-maize system.

Regulation of FB_1 biosynthesis in *F. verticillioides*

Effect of ambient environmental factors on FB_1 production. A variety of environmental factors, such as host specificity, moisture content, temperature, maize kernel environment, and nutritional condition, are known to significantly influence fumonisin production. A couple of environmental factors known to impact FB_1 production are humidity and temperature. A positive correlation between late season rainfall and *F. verticillioides* infection severity has been reported (Kommedahl and Windels, 1981; Munkvold and Desjardins, 1997). Fumonisin production increased with an increase in water activity (a_W) at all tested temperatures between 15-30°C (Samapundo et al., 2005). A marginal effect of temperature on fumonisin was observed at high a_W (optimal for growth), whereas more drastic effect was observed at low a_W. Thus, these studies suggest that relative water activity in maize kernels has more direct effect on fumonisin production whereas the effect of temperature seems to be dependent on a_W (Marín et al., 1999; Samapundo et al., 2005).

Nitrogen limitation, ambient pH, and carbon nutrient specificity are other important factors known to impact FB_1 production in *F. verticillioides* (Bluhm and Woloshuk, 2005; Shim and Woloshuk, 1999). A positive correlation between nitrogen limitation and FB_1 biosynthesis was described in *F. verticillioides* and *F. proliferatum* (Keller et al., 1997; Shim and Woloshuk, 1999). Studies also indicated that carbon and phosphate contents did not have a repressive effect on FB_1 production. It was also determined that the FB_1 production was repressed under higher concentrations of nitrogen (ammonium phosphate, glycine, or glutamate) in the culture media, suggesting that FB_1 biosynthesis is under nitrogen repression (Shim and Woloshuk, 1999). In addition to nitrogen, Keller et al. (1997) showed that acidic pH (3.0-4.0) under well-aerated conditions enhanced FB_1 biosynthesis in *F. proliferatum*. In a later study, it was determined that acidic pH is critical for FB_1 production which prompted the investigation of *F. verticillioides* pH regulator gene and its role in FB_1 biosynthesis (Shim and Woloshuk, 2001; Flaherty et al., 2003).

Genes associated with fumonisin biosynthesis regulation. Notably, unlike other fungal secondary metabolite gene clusters, the *FUM* cluster does not contain a pathway-specific regulatory gene (Brown et al., 1996; Kennedy et al., 1999; Proctor et al., 2003; Woloshuk et al., 1994). Rather, fumonisin biosynthesis is regulated by several genes not linked to the *FUM* cluster. To date, the genes that are known to regulate FB_1 biosynthesis are *FCC1*, *FCK1*, *PAC1*, *ZFR1*, and *GBP1*. Mutational analysis of these genes demonstrated that a functional copy of these genes are necessary for proper production of FB_1 in *F. verticillioides*. Detailed information on these genes is presented in Table1. Other than *FCC1* and *FCK1*, no definitive epistatic relationship between these regulatory genes has been demonstrated, thus it is likely that these genes operate independently (Fig. 1). However, it is clear that additional regulatory genes, yet to be identified, are associated with fumonisin biosynthesis in *F. verticillioides*.

Functional genomic approaches to identify putative fumonisin regulators in *F. verticillioides*

***F. verticillioides* cDNA libraries, Microarrays, and genome sequence.** Genomics have facilitated our efforts to identify

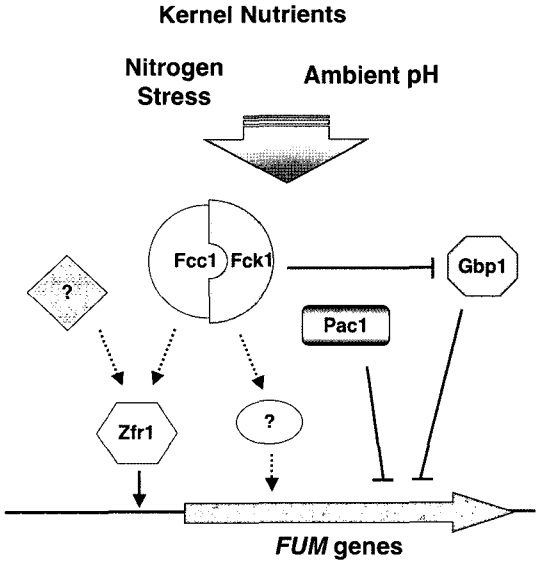

Fig. 1. Schematic description of physiological and molecular mechanisms of fumonisin regulation in maize-*F. verticillioides* system. The physiological conditions (kernel nutrients, nitrogen stress, and pH) impact expression of genes that are associated with transcriptional activation and repression of *FUM* genes. The dotted arrows indicate putative pathways that are not fully characterized to date.

genes associated with fumonisin regulation. Shim and Woloshuk (2001) took advantage of subtractive suppressive hybridization (SSH) technique to identify differentially expressed genes in the wild-type and *fcc1* mutant strain. As described in Table 1, *fcc1* strain is blocked in FB_1 biosynthesis when grown on maize kernels. The SSH cDNA fragments randomly selected from each strain, which averaged 500 bp in length, were sequenced and putative functions were designated based on sequence homology. Wild-type SSH cDNAs were of particular interest since these cDNAs are hypothesized as genes positively associated with fumonisin biosynthesis. The fact that cDNAs corresponding to the *FUM* genes, e.g., *FUM1* and *FUM6* through *FUM14*, were identified in the wild-type library strongly support this hypothesis. Significantly, *ZFR1*, positive regulator of FB_1 biosynthesis, was originally identified in the wild-type EST library (Flaherty and Woloshuk, 2004).

Subsequently, Woloshuk and colleagues utilized microarray technology to further verify putative genes associated with fumonisin biosynthesis (Pirtillä et al., 2004). A total of 716 spots of cDNA from the SSH EST libraries described earlier were spotted on the microarray. The sequence data of the SSH cDNAs and microarrays can be accessed at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). While this microarray represented a small number of genes by comparison to whole-genome microarrays, these cDNAs were obtained from two strains having contrasting FB_1-production phenotypes when grown on corn kernels. The analysis compared genotype (wild type or *fcc1* mutant), pH, and genotype × pH interactions and resulted in isolation of genes that are expressed concomitantly with fumonisin production. The study identified 19 genes displaying expression profile similar to the *FUM* genes (Pirtillä et al., 2004). Thus far, Woloshuk and colleagues have tested expression of 12 of these genes by quantitative real-time PCR (qPCR) during growth on corn kernels and found that their expression was higher in the wild-type strain than the *fcc1* mutant (Woloshuk, unpublished data).

More recently, scientists at the Mycotoxin Research Unit, USDA-ARS (Northern Regional Research Laboratory) reported generating over 87,000 ESTs from *F. verticillioides* (Brown et al., 2005). This collection of ESTs was generated from nine different fungal cDNA libraries and represents over 11,000 unique sequences. Analysis of this extensive collection of ESTs, which is estimated to represent over 80% of the expressed genes in the fungus, revealed candidate genes that are likely to regulate fumonisin biosynthesis and *F. verticillioides*-maize interactions. The EST database is publicly available at The Institute for Genomic Research (TIGR) and can be accessed at TIGR *F. verticillioides* Gene Index website (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=f_verticill). Collaborative research is in progress at USDA-ARS, Purdue University, and Texas A&M University to further characterize the functional role of these putative regulatory genes.

Another significant development that will impact fumonisin research in coming years is the public release of *F. verticillioides* genome sequence. Currently, *F. verticillioides* genome assembly with 4x sequence coverage is available at Broad Institute of Harvard and MIT (http://www.broad.mit.edu). The current assembly contains 61 supercontigs, and the genome size of *F. verticillioides* is estimated as 46 Mb. Additional 4X sequencing is currently in progress with

Table 1. Genes associated with fumonisin B_1 regulation in *F. verticillioides*

Gene Name	Gene Family	FB_1 production in mutants	Other Mutant Phenotype	Regulatory mode	Reference
FCC1	C-type Cyclin	No FB_1 when grown on maize kernel. Leaky FB_1 production in acidic defined media	Severe reduction in conidiation	Positive	Shim and Woloshuk, 2001
FCK1	Cyclin-dependent kinase	Significant reduction in FB_1 production	Severe reduction in growth and conidiation	Positive Physically interacts with Fck1	Bluhm et al., 2006
PAC1	*PACC* group of pH regulatory genes	Higher FB_1 when grown on acidic conditions and on maize kernels	Severely impaired growth at alkaline pH	Negative	Flaherty et al., 2003
ZFR1	Zn(II)2Cys6 binuclear cluster	Severe reduction in FB1 production	No observable effect on growth and development	Positive	Flaherty and Woloshuk, 2004
GBP1	Developmentally regulated monomeric G protein	Increased FB_1 production	No observable effect on growth and development	Negative	Sagaram et al., 2006

the support from US Department of Agriculture and US National Science Foundation. It is expected that annotated genome assembly will be released to the public in the fall of 2007.

Proteomics. While transcriptional profiling is a more frequently selected functional genomics strategy for mining genes involved in certain biological processes, it is important not to overlook its limitations. The change in mRNA level of a certain gene can be transient while influencing down-stream gene regulation and ultimately cellular function. On the other hand, mRNA abundance may not be indicative of a gene's regulatory or metabolic potential. In some instances, pathway regulations occur at the post-transcriptional level, and therefore activity of the final gene product, the protein, may provide better evaluation of a gene's functional role.

With the advancements in protein mass spectrometry technology that can perform high-throughput analyses of protein spots isolated from 2D protein gels, researchers are utilizing proteomics to investigate fumonisin regulatory pathways in *F. verticillioides*. One potential use of proteomic technology is to analyze the proteome that is altered due to environemental factors or a single gene mutation. For example, we show 2D gels of wild-type *F. verticilliodes* grown in ammonium-limiting culture and ammonium-supplemented culture in Fig. 2. As discussed earlier in this review, nitrogen limitation is one of the important factors in triggering fumonisin biosynthesis in *F. verticillioides*. The differentially expressed protein spots can be excised and sequenced, and subsequently this information can be used to compare the correlation between gene transcription and translation. Furthermore, characterization of gene that encodes the respective protein can provide insight into fumonisin regulatory signaling pathway.

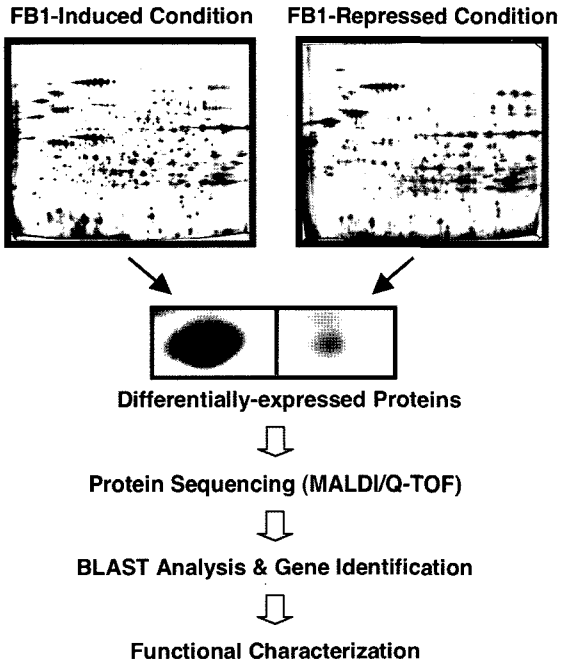

Fig. 2. Schematic description of proteomic approaches to identify and characterize genes associated with fumonisin regulation in *F. verticillioides*. *F. verticillioides* was grown in two different culture conditions - one FB_1-inducing and the other FB_1-repressing - and the total protein samples were extracted and subjected to 2D electrophoresis (shown here). The differentially expressed proteins can be identified via sequencing, and the corresponding *F. verticillioides* gene can be characterized to verify its role in fumonisin regulation.

Maize physiology and genomics associated with FB_1 biosynthesis

Maize kernel microenvironment. One of the complicated issues in fumonisin research yet to be understood is the interaction between maize kernel and *F. verticillioides* that ultimately influences fumonisin biosynthesis. It is clear from published reports that maize kernel microenvironment plays a critical role in fumonisins biosynthesis in maize (Bluhm and Woloshuk, 2005; Shim et al., 2003; Warfield and Gilchrist, 1999). Warfield and Gilchrist (1999) observed a developmental stage-dependent relationship between FB_1 production and maize kernels. Their study showed that later developmental stages - dough (R4) and dent (R5) - produced higher levels of FB_1 while the earlier stages produced lower levels indicating that the maize kernel stage influences FB_1 production. The researchers suggested that the effect on toxin production could be due to substrate composition change as well as moisture content. Shim et al. (2003) examined FB_1 production in two components of the corn kernel, namely the germ tissues and the degermed kernel. Growth of *F. verticillioides* was similar in colonized germ tissue and the degermed kernels, but FB_1 production was at least five-times higher in degermed corn kernels than in germ tissue. Expression of the *FUM1*, as measured by a GUS assay and northern blot analysis, followed the same pattern as FB_1 production. Also correlated with FB_1 was a concomitant drop in pH of the colonized degermed kernels. A time course experiment showed that degermed kernels inoculated with *F. verticillioides* became acidified over time (from pH 6.4 to 4.7 after 10 days of incubation), while colonized germ tissue became alkaline over the same period (from pH 6.5 to 8.5). Since conditions of acidic pH are conducive to FB_1 production and alkaline pH is repressive (Shim and Woloshuk, 2001), the observed correlation between the acidification of degermed kernels and the increase in FB_1 provides one explanation for the observed differences in FB_1 levels.

Recently, studies conducted by Bluhm and Woloshuk

(2005) provided further understanding on how the fungal colonization of maize kernels affects FB_1 production. It was demonstrated that kernel development stage, and not the fungal growth, is critical for FB_1 biosynthesis. Subsequently, it was hypothesized that the difference in starch content in the kernels - the dent stage has highest starch content (70%) followed by dough (58%), milk (20%) and blister (12%) - is the key reason for varied FB_1 production at different kernel stages. At ten days after fungal inoculation, high level of FB_1 occurred only in dent stage kernels whereas trace FB_1 level was observed in other stages. In agreement with this set of data, quantitative realtime-PCR (qPCR) analysis indicated that kernel development affected transcription of genes involved in fumonisin biosynthesis, nitrogen repression, and starch metabolism. Worth noting is the low expression level of *AREA*, a nitrogen metabolism regulating transcription factor in colonized blister kernels. These data suggest that the blister kernel environment represses *FUM* gene expression that is then derepressed as the kernels mature. Also, expression of *AMY1* (α-amylase) and ATPase (a putative H^+ ATPases) is elevated in the colonized dough and dent kernels, correlating with starch accumulation and the decrease in pH, respectively.

These observations led Bluhm and Woloshuk (2005) to investigate the role of starch in regulation of FB_1 biosynthesis in maize kernels. Four maize mutants with varied starch compositions, i.e., *shrunken-2* (sh2), *sugary-1* (su1), *waxy-1* (*wx1*), and *amylose extender-1* (*ae1*), along with wild-type maize line (5322) were challenged with *F. verticillioides*. One striking discovery from this study was that a very low level of FB_1 was detected in *ae1* (less than 10% of FB1 level observed in 5322) that has low starch content. Significantly, *ae1* was the only maize variety in which the pH increased after *F. verticillioides* colonization. Furthermore, it was demonstrated that α-amylase activity is necessary for growth, FB_1 production, and kernel acidification in degermed-tissue of mature 5322 kernels. Strikingly, cultures provided with amylopectin or dextrin (a product of amylopectin hydrolysis) as sole carbon source produced significantly more FB_1 than ones with other carbon sources, suggesting that these carbon sources induce FB_1 biosynthesis in *F. verticillioides* (Bluhm and Woloshuk, 2005).

Lipid-mediated signaling between mycotoxigenic fungi and maize: a role in fumonisin regulation? Accumulating evidence strongly suggest that small molecules that mediate host-pathogen communication are lipid-derived secondary metabolites which are produced by both plant hosts and their fungal pathogens (Burrow et al., 1997; Feng et al., 2005; Tsitsigiannis and Keller, 2006). Furthermore, this lipid-based cross-kingdom signaling is proposed to govern the outcomes of plant pathogen interactions that result either in successful pathogenesis including invasive fungal growth and production of spores and mycotoxins or in the activation of effective defense response of the host plant (Burow et al., 2000; Wilson et al., 2001). One particular class of the lipids that only recently has begun receiving considerable attention as signals in plant-pathogen communication is oxylipins. In diverse eukaryotic organisms, oxylipins comprise a large family of oxygenated long chain fatty acid-derived molecules most of which are produced by the addition of oxygen to the fatty acid skeleton by a variety of oxygenases including lipoxygenases (LOXs), cytochrome P450s, cyclooxygenases and linoleic acid dioxygenases (LDS) (Feussner and Wasternack, 2002; Noverr et al., 2003).

Use of an array of molecular, biochemical, and genetic approaches provided new insights into the functional significance of fungal oxylipins. These new lines of evidence suggest that fungal oxylipins appear to have a dual role as (1) potent regulators of spore development and secondary metabolism including biosynthesis of mycotoxins (Champe et al., 1987; Tsitsigiannis et al., 2005; Tsitsigiannis and Keller, 2006); and (2) as signals in communication with their hosts (Calvo et al., 1999; Noverr et al., 2003; Wilson et al., 2001). The *psi* factors, prime examples of fungal oxylipins, are produced from the fatty acids and are structurally similar to plant oxylipins, particularly the LOX products 9*S*-HPODE (9*S*-hydroperoxy-10*E*,12*Z*-octadecadienoic acid) and 13*S*-HPODE (13*S*-hydroperoxy-9*Z*,11*E*-octadecadienoic acid). It was hypothesized that plant seed oxylipins would have a sporogenic effect on *Aspergillus* species, and indeed the study showed that plant-derived linoleic acid and hydroperoxy linoleic acids induced precocious and increased conidial development in several *Aspergillus* species (Calvo et al., 1999). Lower concentrations of linoleic acid and 9S-HPODE stimulated sexual spore development rather than conidial development in several *Aspergillus* species. Sporogenic effects of fatty acids and oxylipins has been also reported in several other fungi (Kock et al., 2001; Noverr and Huffnagle, 2004).

In addition to the sporogenic effect, there is evidence suggesting that lipid metabolism governs the outcome of plant-pathogen interactions. One example came from the functional characterization of *NhL1*, an extra-cellular lipase-encoding gene that is induced during infection in the pea pathogen *F. solani f. sp. pisi* (Eddine et al., 2001). In addition, two secreted triglyceride lipase-encoding genes *FGL1* and *LIP1* were recently characterized in *F. graminearum* (Voigt et al., 2005; Feng et al., 2005). The gene expression of both lipases was induced *in planta* at the time of infection and in minimum medium with wheat germ oil. Disruption of *FGL1* led to reduction in extracellular lipolytic activity in culture and to reduced virulence to

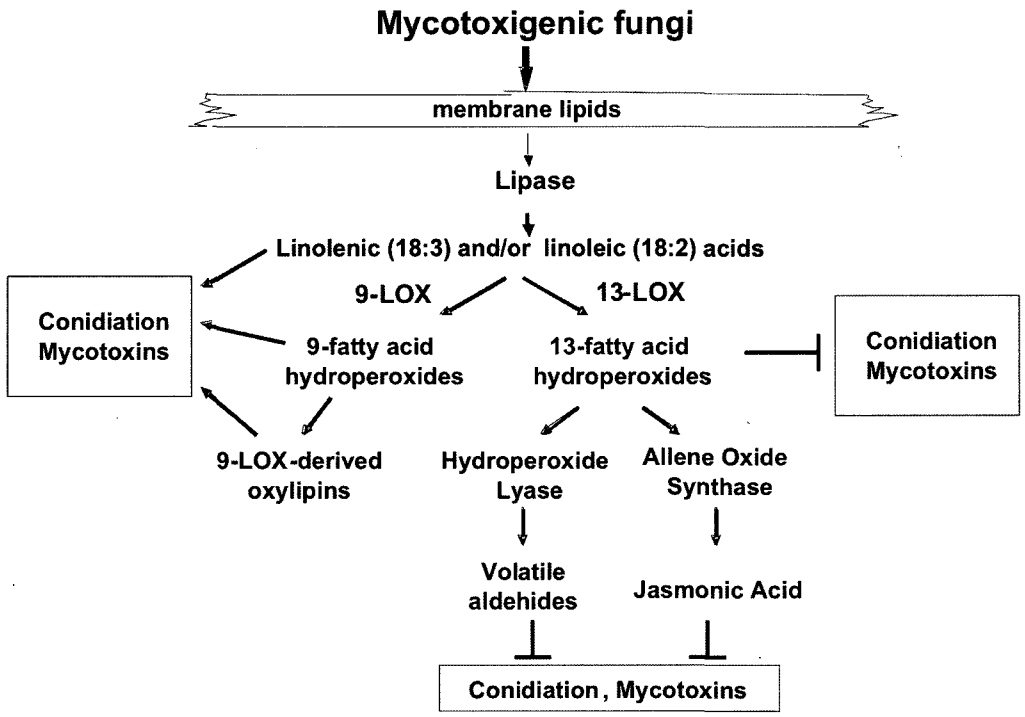

Fig. 3. Proposed role of plant-derived oxylipins in the regulation of conidia and mycotoxin production by mycotoxigenic fungi (a working model). Plant oxylipins are produced by the enzymatic action of lipoxygenases. There are two groups of lipoxygenases 9- and13-lipoxygenases that are classified so based on the carbon position at which fatty acid oxygenation occurs. 9-LOX-derived oxylipins are implicated as signals inducing production of conidia and mycotoxins, whereas 13-LOX derivatives inhibit these processes.

maize and wheat (Voigt et al., 2005). Our analysis of the available genomic and cDNA databases revealed that *F. verticillioides* contain an ortholog of the *FGL1* gene and it is reasonable to speculate that this gene may contribute to *F. verticillioides* virulence on maize.

Another proposed function of fungi- or plant-derived oxylipins is to regulate mycotoxin biosynthesis. There is direct evidence that fungal infection induces production of polyunsaturated fatty acids and their LOX derivatives in maize kernels that activate or repress fungal development and mycotoxin production (Burow et al., 1997; Burrow et al., 2000). Accumulation of oxylipin compounds is known to have diverse physiological functions in plants. Importantly, distinct LOX isoforms, referred to as 9-LOXs or 13-LOXs, preferentially catalyze different enzymatic reactions. For instance, while LOX natural substrates, linoleic and linolenic acids, have been demonstrated to activate *Aspergillus* conidiation and sclerotial development (Calvo et al., 1999), their metabolic derivatives may induce or suppress mycotoxin production depending on whether they are catalyzed by the 9- or 13-LOXs (Fig. 3). Specifically, LOX activity and accumulation of the 13-LOX-derived volatile compounds correlated strongly with inhibition of aflatoxin production (Zeringue et al., 1996). Moreover, some of these volatile products such as hexanal inhibit both fungal growth and aflatoxin production in vitro (Wright et al., 2000). Importantly, 13-hydroperoxy fatty acids and jasmonic acid are potent inhibitors of aflatoxin biosynthesis (Burow et al., 1997; Goodrich-Tanrikulu et al., 1995). Collectively, 13-LOXs contribute to resistance by producing compounds that inhibit both aflatoxin biosynthesis and fungal growth.

In contrast, 9-LOX-derived fatty acid hydroperoxides are known to induce aflatoxin biosynthesis in *Aspergillus* species (Burow et al., 1997; Wilson et al., 2001). Interestingly, at least two maize 9-LOX genes *csaap92* and *chssh76* were induced in kernels infected with *F. verticillioides* (Wilson et al., 2001). This finding suggested that fumonisin biosynthesis may be positively regulated by 9-LOX products (Fig. 3). Based on these studies, it is hypothesized that the 9-LOX pathways are utilized by mycotoxigenic fungi, including *F. verticillioides*, to induce biosynthesis of mycotoxins and that the 9-LOX genes are mycotoxin susceptibility factors in corn plants as depicted in Fig. 3. Current research is focused on functional characterization of the 9-LOX genes in maize, particularly their role in maize-*F. verticillioides* interaction, fungal conidiation, and fumonisin production.

Conclusion

Accumulating evidence suggests that regulatory mechanism involved in fumonisin biosynthesis is complex. It has been

well documented that multiple environmental and genetic factors play a role in the regulation, and what is more intriguing is that recent data demonstrate the interaction between maize kernel and *F. verticillioides* is also a key factor influencing the toxin biosynthesis. The future challenge the investigators are facing is to identify additional physiological and molecular factors that are associated with fumonisin regulation and to ultimately link these to better understand the regulatory network. Technical advancements, particularly genomics and proteomics technologies, are expected to facilitate the discovery of key genes and regulators in *F. verticillioides* and maize. These new discoveries will lead to a better understanding of the molecular mechanisms that govern fumonisin biosynthesis in *F. verticillioides* as it grows on maize kernels, thus enhancing the prospects for control.

Acknowledgements

We thank Dr. Clint Magill (Texas A&M University) for careful reading of this manuscript. This work was supported in part by Texas A&M University/Texas Agricultural Experiment Station (to WB Shim), Texas Corn Producers Board and USDA-ARS Mycotoxin Workshop Program (to MV Kolomiets).

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