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Review

Gene recognition and role of foodomics in mycotoxin control: A review

Upali Samarajeewa

Department of Food Science and Technology, University of Peradeniya, Peradeniya 20400, Sri Lanka; smrjee@gmail.com

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Abstract: Since recognition of toxic and carcinogenic aflatoxins in Brazilian groundnut meal in 1960, much research has been done to prevent and detoxify aflatoxins in foods and feeds, identifying a variety of methods. The research has expanded to other mycotoxins. The biotic and abiotic factors favoring mycotoxin contaminations have been understood through experiments under laboratory conditions and analysis of field data. However, many gaps remain in the knowledge on mycotoxin control at the molecular level that may be useful in addressing mycotoxigenic hazards. Recognition of responsible genes in hosts and fungi and omics methods applying genomics, transcriptomics, proteomics, and metabolomics to understand mycotoxin biosynthesis at the molecular level may open new avenues to interact with plant-fungi-bacteria cross-talks, apply regulatory mechanisms in biosynthesis, and explore checks and controls addressing abiotic and biotic factors favoring mycotoxin biosynthesis. The new knowledge is expected to generate probable molecular biological mechanisms to eliminate mycotoxin biosynthesis on foods. The current level of omics knowledge requires application of research to achieve deeper understanding, aiming at new methods for mycotoxin controls and applying next-generation technologies. This review examines the current knowledge on the biosynthesis of aflatoxins, fusarium toxins, and patulin in foods and host-fungi interactions at a molecular level.

Keywords: omics; biosynthesis; prevention; quantitative traits loci; cross-talks; molecular biology

1. Introduction

Foodomics addresses the application of omics technologies in food science and nutrition to improve consumer well-being. Ensuring food safety from mycotoxins is a continuing effort exploring new mechanisms to prevent or get rid of mycotoxins from already contaminated foods and feeds. Protection of foods and feeds from mycotoxins requires minimizing contaminations at crop production and product storage by controlling biotic and abiotic factors favoring toxin formation. Mycotoxins play a significant role in fungal cells in protecting the cells and the hosts on which they grow from other invading competitors of fungi and bacteria. While mycotoxins play an antimicrobial role against the competing microorganisms, they also may be signaling the hosts to produce antimicrobials against other microbial invaders. This study reviews the existing molecular-level interventions that may be used or modified for use to prevent contamination of maize, peanuts, and grains by toxic and carcinogenic *Aspergillus* and *Fusarium* metabolites with an emphasis on mycotoxins.

More than 300 potentially toxic metabolites from fungi have been recorded since hepatocarcinogenic aflatoxins were detected first in Brazilian groundnut meal in 1960 [1]. Of the 300 metabolites, although less than 4% are of immediate concern to food safety, considering the potentially U-shaped (instead of linear) dose-dependence of endocrine disruptors such as mycotoxins, dose dependency studies may significantly

change the proportion of the latter percentage. Complete control of the mycotoxigenic fungi and their activities in foods continues to be a challenge. The emerging knowledge envisages working on methods to minimize mycotoxin contaminations through an interlinked series of activities along the crop production-processing chain [2,3]. Among the crops more vulnerable to mycotoxin contamination, peanuts (groundnuts) and maize (corn) stand high as major food crops globally.

2. Omic studies

Omics techniques examine the DNA, RNA, proteins, and metabolites generated and engaged in cellular activities in various tissues and their responses to ecosystems. Omics techniques are used to uncover genetic information and protein expression patterns associated with phenotypes of mycotoxigenic fungi interacting with host plants.

Of the mycotoxins, aflatoxins, ochratoxins, and patulin are produced by the fungi through the polyketide synthetase pathway, initiated by reactions between acetyl CoA with malonyl CoA leading to a series of gene-controlled biosynthetic steps [4]. Recognizing the genes controlling enzymic activity during the biosynthesis of mycotoxins forms an important approach in mycotoxin control. Mycotoxin biosynthesis is influenced by the nutritional status of the host, temperature, water stress, pH, and constituents in the host resisting fungal growth and toxin production [5]. The above factors affect the enzymatic controls in the fungi, which may be understood through omic applications. Omic studies also may help to understand changes in the virulence of fungi and mycotoxin biosynthesis in response to inherent and environmental factors.

Omic studies include genomics to understand molecular genetics in fungi, transcriptomic studies to understand the role of RNA in transferring signals from genes to proteins to engage in biosynthesis, proteomic studies to recognize structural and functional proteins guiding mycotoxin biosynthesis, and profiling of primary and secondary metabolites to recognize outcomes of gene expressions occurring through transcriptomic and proteomic actions [6]. Genomic studies have shown that *Aspergillus flavus* adapted to live under a wide range of environments [7].

2.1. Genomics and traits associated with mycotoxin production

A genome consists of all genetic materials of an organism required to maintain life. The genome carries specific instructions for the organism to build and maintain its functions. Genomic technology could be used in sequencing information on the DNA of the mycotoxigenic fungi at the cellular level. Genomics, in a broad sense, refers to the mapping, analyzing, and sequencing of the genes present in the chromosomes. Mapping of functional genes opens the avenue to recognize the enzymatic reactions producing mycotoxins. The location of the genes in the chromosomes and the application of genome techniques to understand the traits to resist mycotoxin production in host plants help in the molecular biological control of mycotoxins. Resistance may take the form of suppressing fungal infections or preventing mycotoxin production depending on the mechanisms triggered by the host. Genomic studies can provide information on structural, functional, and comparative

activities guided by the genes in cells. The cellular functions associated with genes require understanding of the functions of all proteins in the gene. Functional genomics helps to understand interactions between hosts and mycotoxigenic fungi and possible interactions to change mycotoxin biosynthesis. This approach is useful in addressing pre-harvest control of mycotoxin contamination of crops.

Traits linked to mycotoxin control in hosts

Identification of the traits in food crops resisting mycotoxin production and recognition of genes linked to the traits is the first molecular approach applied in mycotoxin control. The aflatoxin-resistant traits were identified from the existing cultivars of grains and peanuts or were transmitted by transgenic techniques to crops such as cottonseed, where no naturally resistant traits are recognized. The genes associated with the traits responsible for suppressing aflatoxin production in the resistant maize varieties have been identified. Details on the gene expressions in different tissues and at different developmental stages of the plants are continuously studied [5,8].

The step prior to identifying genomic regions is the effort to identify Quantitative Trait Loci (QTL) in the chromosomes, statistically linking phenotypic data with genotypic data associated with mycotoxin biosynthesis. A QTL represents a DNA region associated with a phenotypic trait of interest within the chromosome. Recognizing the genomic regions and the locations of genes in the chromosomes with the capacity to control aflatoxin biosynthesis attempted to eliminate aflatoxin production on food commodities. Several QTLs on peanuts and maize responsible for resisting aflatoxin biosynthesis by fungi have been mapped [9]. Of them, two QTLs with origins on 4 QTLs have been examined further. The two QTLs showed resistance to the production of aflatoxin B1 and aflatoxin B2, respectively. The traits were reported to interact additively, providing a combined resistance to the production of both aflatoxins. Several QTLs responsible for protecting maize and peanuts against aflatoxins through different mechanisms and their associations with the respective genomes are summarized following initial observations [9]. In a different study on peanuts, among seven QTLs identified for resisting aflatoxin B1 production, 2 major consistent QTLs and 5 minor QTLs have been recognized from the peanut breed lines [10]. The same study identified 4 major QTLs and 1 minor QTL against aflatoxin B2 production. The major QTLs showed additive resistance against aflatoxin B1 and B2 biosynthesis. This opened new investigations on functional interactions among the QTLs and their locational relationships in the genome. Liao et al. [11] identified two DNA markers closely linked to resistance to aflatoxin biosynthesis from peanuts. On introduction of the markers to peanut cultivars, reduction of aflatoxin biosynthesis was reported. One of the markers has been converted to a SCAR marker for more efficient breeding applications. It would form an important preventive approach.

In parallel studies, genomic regions responsible for the resistance of maize to Fusarium-Ear-Rot (FER) and contamination of the kernels by fumonisin have been mapped [12]. The quantitative trait loci (QTL) in maize responsible for different expressions against FER were identified [13]. In the resistant inbreds of maize against *Fusarium verticillioides*, upregulating of the host genes responsible for secondary metabolism, production of antifungal compounds causing increased resistance, and

genes related to cell wall biosynthesis and flavonoid biosynthesis were recognized. Tyrosine, flavanols, flavones, and anthocyanins in maize appear to be generated through upregulation of biosynthetic pathways in maize, causing resistance to FER and fumonisin production [14]. The observations on regulating the biochemical pathways indicate the adjustments by the host through differentially expressed genes to resist the infections by *Fusarium* and the production of fumonisin [12]. The up-regulating of flavonoid-producing gene expressions and down-regulating of starch and fatty acid biosynthesis gene expressions by the resistant varieties of maize in response to mycotoxigenic fungi indicates a growth-defense trade-off. Either use of the regulatory mechanisms beneficially or manipulating them to create resistance to fungal growth is a useful approach in mitigating fumonisin production. The observations suggest the availability of new genomic mechanisms to be explored to prevent fumonisin biosynthesis in maize. However, effects of overproduction of flavanols in hosts and their probable antinutritional effects on humans need to be addressed. The effects on nutritional aspects of foods may be positive or negative. Responses of mycotoxigenic fungi to defense mechanisms of hosts may also result in deviations or interruptions in mycotoxin biosynthetic pathways midway, accumulating new toxic entities.

Examining the ongoing studies on the QTLs of maize resistant to fumonisin, Santiago et al. [15] postulate that the selected loci provide minor effects scattered in all chromosome bins, making it difficult to achieve added resistance at the required level. Although candidate genes have been proposed for high-resolution QTL, only one gene creating resistance to FER has been cloned. The gene is *ZmAuxRP1*. Comparison of transcriptomes between resistant and susceptible inbred maize bulks 10 days after inoculation with *F. verticillioides* has revealed 364 differentially expressed candidate genes for the QTL [12]. More research leading to cloning based on a much wider gene pool, to be identified, is needed to achieve success in the genomic approach for developing maize cultivars resistant to FER and fumonisin contamination.

The chromosomal fragments responsible for traits of maize causing resistance towards Aspergillus-Ear-Rot and for aflatoxin production were reported to be different, while traits with the same chromosomal region affecting both fungal growth and toxin production had also been reported. Identification of QTLs in peanuts and related applications appears to be easy compared to applications to tree nuts such as almonds and pistachios. The relationships have also been established for genetically triggered resistance to Head-Blight producing Deoxynivalenol (DON) in wheat. Genomic technology carries the potential to mitigate mycotoxin accumulation commencing at the crop production stage by selecting the traits in hosts based on genetic information [16]. The authors also review the success of genetic engineering in introducing resistance to aflatoxin biosynthesis in cottonseed. In maize, wheat, and peanuts, the responsible resistant genes could be identified, whereas the potential to identify resistant genes leading to desirable traits appears low with the cottonseed. Creating resistance to mycotoxigenic fungi in cottonseed needs a transgenic approach.

The genes responsible for producing aflatoxins, each step of the biosynthetic pathway, control mechanisms at each step, the metabolites produced, and transcriptomics have been a subject of several publications and reviews discussing the

details [6,17]. Gene expressions regulating biosynthesis of aflatoxins appear to occur at multiple levels and by multiple regulatory mechanisms. The expressions are controlled by mixed influences from biotic, abiotic, and genetic factors. The complexities associated with regulations of biochemical pathways compel scientists to investigate further. It requires exploring proteomics and transcriptomics of mycotoxin biosynthesis deeply to understand how the regulatory mechanisms operate and change.

Identification of the genome and the QTLs carrying the genes forms an important basis to work on resistance to mycotoxin biosynthesis in crops. The contributions to resistance recognized in traits form a step towards advanced molecular biological studies to work out effective mechanisms to prevent mycotoxin contaminations.

2.2. Proteomics in regulating biosynthesis of mycotoxins

Proteomic studies penetrate deep into genomic studies, providing an opportunity for intense understanding of the control of mycotoxin biosynthesis at the molecular level. Proteomics examines the structural and functional features of proteins in cells, triggering biochemical reactions. Proteins are produced by transcription of information from the genome. While the genome is constant in an organism, the proteome differs in response to biotic and abiotic factors [7]. Proteomics provides the opportunity to understand the mycotoxigenic nature of fungi responding to biotic and abiotic factors. The proteomic approach also provides an opportunity to recognize new proteins in the host that may generate anti-mycotoxigenic properties. Thus, the expression of functional proteins in signaling and regulating biosynthesis of mycotoxins may be used to develop preventive mechanisms.

Proteomic studies on three types of *A. flavus* isolates with high, moderate, and no aflatoxin-producing capability have shown the presence of 220 proteins, differentially expressing, and carrying varying capacities to direct aflatoxin biosynthesis [18]. These functional proteins trigger a series of cascade reactions producing aflatoxins. Proteomic studies have identified resistance-associated proteins (RAP) in peanuts active against aflatoxin biosynthesis [19]. Further research in the same area has resulted in the identification of more proteins in the host plants interacting with aflatoxin biosynthesis [9].

In examining the resistance of peanuts and maize to *A. flavus* colonization, the genes and the proteins contributing to resistance by the host plants have been elucidated. The genes carry pathogenesis-related proteins *PR-10*, *PR-10.1*, *14-kDa trypsin inhibitor*, *chitinase*, *zeamatin*, and *B1,3-glucanase* [20]. Additionally, there are stress-responsive functional proteins, catalase, superoxide dismutase, glycoxalase I, and glutathione-S-transferase in the hosts interfering with aflatoxin biosynthesis by fungi. Reactive oxygen species (ROS) are suggested to enter crosstalk between the host cells and *Aspergillus* species, resulting in the formation of oxylipins having the capacity to regulate the growth of Aspergilli and aflatoxin biosynthesis [20]. There is also evidence of host-induced ROS stimulating aflatoxin production. The contradictions on aflatoxin biosynthesis arising with the above observations demand intense research on the role of ROS at different concentrations and in varying environmental situations.

A new insight into the peanut-pathogen interaction is reported with the identification of 18 genes in peanuts encoding pathogenicity-related protein *PR10*, 1-aminocyclopropane-1-carboxylate oxidase (ACO1), MAPK kinase, STK, PRRs, cytochrome P450, SNARE protein SYP121, pectin esterase, phosphatidylinositol transfer protein, and PPR protein responsible for resistance against *A. flavus* [21]. Understanding the molecular mechanisms in the host plants resisting fungal growth or aflatoxin biosynthesis and expanding research based on the above findings may provide long-lasting solutions in aflatoxin control in foods and feeds.

The outcomes to be generated by the genomic and proteomic interactions depend on the way the host tissues initiate responses to the fungal invasions and the way the responses succeed. The signaling in the host tissues against the invading fungi appears to be conducted by calcium compounds and ROS working together. The biochemical interactions between host tissues and fungi appear to get more complicated with the environmental stresses caused by low soil nutrient levels, droughts, and hot & dry climates on host plants enhancing mycotoxin biosynthesis. Environmental stresses are beyond human control, making infection control challenging.

Maize is known to carry proteins resisting the growth of A. flavus. Some of the entities responsible for the resistance are zeamatin and germination-induced ribosome inactivating proteins (RIP) in maize. Cleveland et al. [16] have identified the possible components, such as quinones and products derived from peroxidase activity, for their resistance to aflatoxin biosynthesis. The preventive mechanisms are based on the intracellular signaling cascades operating in the hosts and the production of a variety of chemicals resisting fungal infections by the host.

Protecting crops against mycotoxigenic fungi through proteomic approaches is a useful step applicable in mitigating the food safety hazards during crop production. Identification of the resistant varieties of crops based on the biochemical criteria following proteomics addresses only a part of the infection control approach. The behavior of crops in the field to the environmental moisture stresses and moisture availability needs to be linked with the emerging biochemical knowledge to minimize mycotoxin biosynthesis.

2.3. Transcriptomics in controlling biosynthesis of mycotoxins

The gene expressions guide biochemical reactions at the cellular level. The genetic information stored in the DNA is translated through RNA by transcription to synthesize the structural and functional proteins engaged in mycotoxin biosynthesis. The transcriptomic profile helps to understand how the information in the gene is transferred dynamically, in regulating the synthesis of structural and functional proteins in real time, and in modifying the enzymatic proteins causing changes in mycotoxin biosynthesis in response to biotic and abiotic factors. Transcriptomic studies on aflatoxins, ochratoxins, and patulin have revealed cross-talks and the influence of abiotic factors on mycotoxin biosynthesis [22]. Biosynthesis of mycotoxins in the fungi may be independently initiated by the fungal genome or get adjusted and triggered by cross-talks between the host plant and mycotoxigenic fungi. Controlling the biosynthetic pathways either to suppress mycotoxin production by fungi or to enhance the resistance of the host forms the scientific basis for mycotoxin

control. However, interruptions on the biosynthetic pathway at midpoints may result in the accumulation of other metabolites not identified as mycotoxins but possessing a certain degree of toxicity.

A fungus may exist in different forms with varying qualitative and quantitative capacities to produce mycotoxins. This is evident with the capacities shown by *A. flavus* and *A. parasiticus* cultivars in producing only specific types of aflatoxins, type B or types B and G, and different concentrations of aflatoxin B1, B2, G1, and G2. Similar differences in producing fumonisin B1, B2, or B3 and trichothecenes A and B groups are known. The differences may originate from varying degrees of expressions of the fungal genes governing biosynthesis of the respective mycotoxins. The prevention of mycotoxin biosynthesis may require complete inactivation of the gene expressions or suppressing one or a few of the biosynthesis steps. Environmental factors may add to the varying mycotoxin-producing capacities among the fungi, positively or negatively. Environmental factors may also influence the enzymatic activities or gene expressions associated with certain steps in biosynthesis.

2.3.1. Biosynthesis of aflatoxins

Biosynthesis of aflatoxins by *Aspergillus* species occurs through 18 enzymatic steps, with 25 genes having the regulating enzymes in a cluster [23,24]. Of them, the regulatory genes from *aflA* to *aflQ*, in alphabetical order, are engaged in controlling the enzymes responsible for stepwise conversion of fatty acids to aflatoxins. One or several of these genes may be expressing at each step of aflatoxin biosynthesis. Two other transcription activator genes *afl*R and *afl*S regulate the genes in the aflatoxin biosynthesis pathway. Theoretically it would be possible to manipulate any of the genes *aflA* to *aflQ* to influence biosynthesis of aflatoxins in *A. flavus.* The regulatory gene *aflR* isolated from *Aspergillus* is reported to generate a product AflR. The product AflR possesses the capacity to regulate structural gene *aflP* in the aflatoxin biosynthetic pathway. In *A. parasiticus,* the induction patterns of *aflR* mRNA and AflR are reported to be regulating *aflP* expression in the aflatoxin biosynthetic pathway. In non-aflatoxigenic *Aspergillus* species too induction of *aflR* mRNA and AflR occurs, but the structural gene *aflP* does not seem to express. Expression of the product AflR in *Aspergillus* species is down regulated by low availability of carbon, nitrogen and zinc, and the environmental factors such as non-optimal temperatures [25,26]. The authors have shown that nitrates down regulate expression of product AflR, suppressing aflatoxin biosynthesis. The cluster also carries several genes whose role is not clearly understood or assigned. Inadequate knowledge on behavior of certain genes continues to be a limitation understanding aflatoxin biosynthesis fully.

The environmental and nutritional influence on the production of aflatoxins in the plant cells is well known. It is confirmed that the regulatory genes *aflR* and *aflS,* and structural gene *aflD* in the transcriptional pathway, response to environmental temperatures, regulating aflatoxin biosynthesis [24]. The regulatory gene *aflS* functions as an enhancer for the gene *aflR*. While the ratio of the genes *aflR* : *aflS* regulates the aflatoxin biosynthesis, *aflD* gene is reported to be influenced by the environmental temperature and water activity. The *aflR* : *aflS* ratio appears to be a crucial factor in deciding aflatoxin biosynthesis under stressed conditions. Increased upregulation of *aflS* is reported to increase aflatoxin biosynthesis under stressed

conditions [27,28]. Relative expressions of the genes *aflD* or *aflM* to a*flR* and *aflS* are reported to be affected by water activity and temperature of the hosts [29]. The authors have observed that the expression of *aflS* remains consistent, indicating its key regulatory capacity in aflatoxin biosynthesis. The above observations together with the host-fungi interactions suggested by Fountain et al. [20] highlight the interactive roles between the toxigenic Aspergilli and the environmental factors. The absence of *aflD* and *aflP* genes in *A. flavus* was reported to restrict aflatoxin biosynthesis, although infection occurs in certain maize varieties [30]. The authors suggest the possibility of metabolic interactions to prevent aflatoxin biosynthesis by deleting the genes *afl*D, *afl*P or *afl*R. The latter (*aflR*) regulates other genes. Metabolic interactions by deleting *afl*D, *afl*P or *afl*R would block reductase enzymes, O-methyltransferase A enzymes, and transcription activation respectively in the aflatoxin biosynthetic pathway.

In a study on the role of selenium on gene expression, namely *afl*R, *afl*S, their ratio, and *afl*D, the antioxidant selenium was found to strengthen the competitive activities of atoxic *A. flavus*. The authors predict an advantage in fortifying soil with selenium to down-regulate the aflatoxin genes by manipulating the antioxidative mechanisms in *A. flavus*. However, authors also observed up-regulation of biosynthesis of aflatoxin B1 and G1, if the crops already having active aflatoxigenic fungi are exposed to selenium [31]. The time of applying or the presence of selenium in soil appears critical in regulating biosynthesis of aflatoxins. The findings open a new area where micronutrient levels in the soil or fertilizer may be used cautiously, to gain pre-harvest benefits of suppressing aflatoxin biosynthesis and enhancing growth of atoxic *A. flavus*, providing increased competitiveness. The observation on selenium opens a key area to examine soil micronutrient status as an approach to bring about pre-harvest protection through inhibiting aflatoxin biosynthesis during crop growth. There are no studies on the roles of other micronutrients in soil or in plants on aflatoxin biosynthesis.

The ability of four species of bacteria to inhibit expressions of genes *aflD* and *aflR* in nutrient agar at water activities of 0.94 and 0.98 is reported by Labeed et al. [32]. While this observation suggests a probable mechanism in control of aflatoxin biosynthesis under pre-harvest conditions, the results did not confirm consistent inhibition of aflatoxin biosynthesis. The difficulties observed in the field on the use of bacteria as biocontrol agents are associated with the inconsistency of the bacterial agents to inhibit toxin production, counteracting the molecular mechanisms in the fungi.

Correlation of temperature and water activity with expression of regulatory genes for aflatoxin biosynthesis *afl*R and *afl*S, is doubted by Mannaa and Kim [33]. They postulated that the effects vary with strain of *A. flavus*. Medina et al. [34] suggests interactions among 4 aflatoxin biosynthesis genes together with regulatory and transcription activators in responding to abiotic factors. A deep understanding of the environment linked regulatory activities is needed before applying concepts of gene expressions to regulate aflatoxin biosynthesis under varying situations. There may be other combinations of factors such as nutrients at growth phase of crops and variations of environmental conditions, not examined at omics level so far. The molecular biological activities conducive to fungal colonization appears to differ from those

enhancing aflatoxin biosynthesis on growing crops. The information on the effects of temperature and water activity on aflatoxin accumulation during post-harvest storage is quite different with contaminations during the growth phases of crops.

Among the functional proteins associated in aflatoxin biosynthesis by *A. flavus*, two bZip transcription factors of relevance are reported. They are designated as global regulators *AflatfA* and *AflatfB*, mediating the aflatoxin B1 biosynthesis responding to other stresses. The two proteins have been shown to play multiple roles including influencing aflatoxin biosynthesis, causing variations in conidia production by aspergilli, and responding to stress by hydrogen peroxide, when examined through deletion of one or both transcription factors. It is of particular interest to note the reduction of aflatoxin B1 biosynthesis of mutated *A. flavus* strains in the presence of *AflatA* and increase of aflatoxin B1 biosynthesis in mutated strains with *AflatB*, when conducted in laboratory cultures of peanut and maize extracts [35]. In the study, aflatoxin biosynthesis was compared with the unmuted wild type *A. flavus* as controls. A similar approach by examining functional proteins has been taken in controlling the biosynthesis of *Fusarium* toxins. Research on transcription factors may produce added information useful in reducing mycotoxin biosynthesis by fungi [36].

Host induced silencing of gene expression (HIGS) of aflatoxigenic fungi are carried out in maize. In the experiments the expression of the enzyme *afl*C (polyketide synthase) in the aflatoxin biosynthetic pathway of *A. parasiticus* isolated from maize, was silenced by interfering through RNAi cassette [37]. The authors were able to suppress the biosynthesis of aflatoxins in selected maize lines by silencing *aflC*. The transgenic technique however carries the theoretical risk of silencing gene expressions outside the target, resulting in unintended phenotypes of the host species, which needs to be guarded against. The unintended phenotypes may pose new potential risks to consumers due to production of new or modified toxins arising from application of omics technology. The molecular biological approach in silencing the genes and enzymes in the mycotoxin biosynthesis pathways using RNA, is reported by McDonald et al. [36] too.

Another approach for HIGS to prevent aflatoxin biosynthesis in crops, is through introduction of new genes. *MsDef1* and *MtDef4.*2 genes from two plant species, *Medicago sativa* and *M. truncatula* respectively, were transferred to peanuts to strengthen the inherent resistant mechanisms in the peanuts, by introducing antimicrobial peptides synthesized by the introduced genes. The newly inserted genes *MsDef1* and *MtDef4.2*, carrying antifungal plant defensins, resulted in overexpressing the resistant trait in peanuts. Overexpressing of the defensins through HIGS in peanuts, silenced the genes *aflM* and *aflP* in the aflatoxin biosynthetic pathway responsible for producing AFLM and AFLP proteins in the cytoplasm [38]. The dual effect of strengthened inherent system in the host, and functional inhibition of the expression of *aflM* and *aflP* genes in the pathogens at molecular level, have effectively suppressed aflatoxin biosynthesis on peanuts. The modified defensive mechanism may carry the advantage of providing protection against aflatoxigenic fungi even after peanut harvest. The role of host mediated proteins, and a variety of resistance factors associated with maize and peanuts providing host-pathogen interactions through molecular mechanisms against aflatoxigenic fungi, were reviewed by Soni et al. [9]. The gene-

initiated interactions occurring during aflatoxin biosynthesis are presented graphically in **Figure 1**.

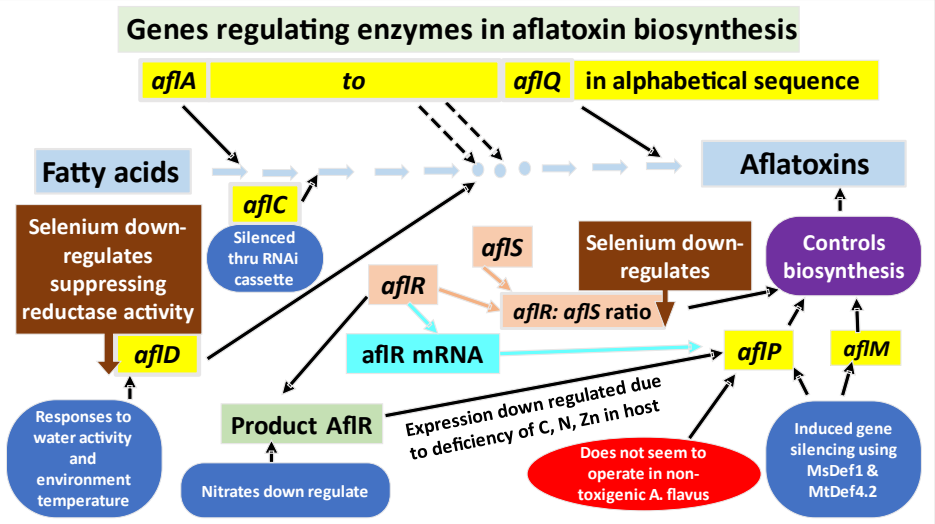

Figure 1. Gene expressions recognized in biosynthesis of aflatoxins that may be manipulated for pre-harvest control of aflatoxin biosynthesis.

Figure 1 represents a combination of events reported by different research groups on aflatoxin biosynthesis. The advanced regulatory mechanisms in aflatoxin biosynthesis are described in detail by Loi et al. [39]. The complexity of interactions among the genes, the effects of the nutritional status of the hosts, and the influence of the environment, affecting aflatoxin biosynthesis, are clear in **Figure 1**. The figure also shows that there are genes whose behavior in relation to exogenous factors is not fully understood, though their association with enzymes in the biosynthetic pathway is established. The aflatoxin control would finally succeed only after exploring the expression of all genes yet to be understood in relation to influences.

Factors affecting gene expressions during aflatoxin biosynthesis

Microorganisms respond to environmental and other stresses by using alternate or adjusted biosynthetic pathways [17]. Responses to stresses in mycotoxin biosynthesis are established in studies examining effects of temperature and water activity in the contamination of food crops and stored products [33,40]. Roles of carbon sources, nitrogen sources, pH, oxidative stresses, and plant metabolites are indicated as vital factors in several studies [17]. Transcriptomics is useful in identifying the molecular processes in cells responding to stresses at gene expression [41]. Understanding gene expressions in relation to stress provides methods to enhance the expressions to control mycotoxin biosynthesis by fungi. **Figure 1** also indicates biotic and abiotic factors affecting aflatoxin biosynthesis through interactions with *AflR* and *AflS* regulatory genes individually, and by altering their regulatory balance. After examining relative expressions of 10 key genes (*aflF*, *aflD*, *aflE*, *aflO*, *aflP*, *aflQ*, *aflX*, *aflR* and *aflS*) during aflatoxin biosynthesis at varying temperatures and water activities in the laboratory, the relative expressions of structural genes *aflD* and *aflM* to regulatory genes *aflR* and *aflS* were reported to be significant [29]. The authors have also observed relative consistency of expression of the gene *aflS*. The observations

suggest that expression of *aflR* is influenced by the abiotic factors to a higher extent, influencing the balanced effect associated with the ratio of genes *aflR: aflS* in the situation studied. The influence on *aflR* may be occurring through modulation of *aflS* by transcription on pathway genes associated with *aflR* in the same way as suggested by Yu [17].

In addition to the cross-talks between hosts and aflatoxigenic fungi, co expressions of host and fungal genes during biosynthesis of mycotoxins have also been reported [42]. During interactions between *Zea mays* and *A. flavus*, formation of interspecies sub-networks was observed by the authors. While the gene *aflR* is known to regulate aflatoxin biosynthesis, direct role of the gene *aflS* is still less evident. In early research, *AflS* was identified to be working together with *aflR* as a coregulator. During formation of interspecies sub-networks, *aflS* appears to be coregulating along with *Z. mays* genes engaged in producing reactive oxygen species (ROS). The observations indicate the action of mycotoxigenic fungi is linked to biochemical activities of hosts, rather than simple utilization of host as a nutrient resource for the fungi. The sub-networks indicate triggering of mechanisms by *A. flavus* in response to resistance developed by host, through molecular level interactions for survival within the host, and at the expense of the host, though mycotoxins provide protection to the host from bacteria through its antibacterial potential.

There is continuing evidence of cross-talk between fungi and hosts at the molecular level guiding the biosynthesis of aflatoxins. The evidence is of significance in working out mechanisms to control aflatoxin biosynthesis commercially. However, use of this information requires establishing the patterns and consistency of cross-talks to overcome the influence of the environment in mycotoxin biosynthesis.

The understanding of gene expressions leading to aflatoxin biosynthesis throws light on what may be happening and what may be controllable. The limitations arise in the application of the findings due to high variations arising from combinations of several factors operating together, such as drought resistance, response to the environment, etc., on one hand, and the responses of the crops arising from the varying inherent physical and chemical characteristics within the crops on the other hand. The challenge is to establish consistent control mechanisms through regulation of gene expressions discussed above and probable host interactions. Continued research and application of next-generation omics may help in clearing the knowledge gaps on aflatoxin control.

2.3.2. Biosynthesis of ochratoxins

Ochratoxins are produced by several species of Aspergilli and Penicillium, suggesting probable multi-mechanisms linked to genetic variations. Ochratoxin biosynthesis occurs through polyketide synthesis pathway. Several other pathways for ochratoxin biosynthesis have been proposed by different scientists [43–45]. In exploring ochratoxin biosynthesis in several fungal species, five common genes *otaA, otaB, otaC, otaD, and a transcription factor otaR1* were reported [46]. The role of each gene has been established through inactivating the individual genes and following the outcome. A consistent pathway for biosynthesis of ochratoxin has been reported by the same authors.

Examining the effects of ochratoxin on yeast growth through transcriptomic analysis, deregulation of developmental genes in yeasts by ochratoxin has been speculated [47]. The authors also observed that citrinin, produced by the same fungal species along with ochratoxin, leads to oxidative stresses in yeasts. The observations provide a clue to the role of citrinin in creating pathogenic reactions in humans and animals by gene interactions through transcriptional reactions. The two molecular biological observations indicate the multiple effects of mycotoxins leading to pathogenic reactions on hosts, plants, or animals.

2.3.3. Biosynthesis of fusarium toxins

Biochemical pathways producing *Fusarium* toxins are complex, as there are more than 17 fungal species producing 4 groups of trichothecenes A, B, C & D and other mycotoxins. The genes engaged in trichothecene biosynthesis consist of a cluster of *Tri*-genes [48]. The cluster carries two transcriptional regulators controlling the genes.

Transcriptomic studies have revealed roles of genes *Tri1* to *Tri16* and *Tri101* in biosynthesis of trichothecene mycotoxins [49]. Of the genes, *Tri1* and *Tri16* appear to play a key role in biosynthesis of trichothecenes as a group, with the other *Tri*-genes deciding on the identity of the final trichothecene derivative. The gene *Tri5* is reported to encode the first step of producing trichodiene in the biosynthesis pathway. Ten other genes consisting of 2 regulatory genes, 7 pathway genes and 1 transporter gene, functional in the *Tri5* gene cluster have been identified. The pathway genes *Tri1*, *Tri16* and *Tri101* described earlier are reported to express enzymatic reactions outside the *Tri5* gene cluster, operating independently [50].

Variations in the expression of *Tri*-genes associated with different species of *Fusarium* and under varying environmental conditions, with pH playing a dominant role, are documented [48]. Some of the *Tri*-genes appear to influence biosynthesis based on the constituents in host, namely carbohydrates and amino acids. Acidic conditions are reported to reduce the biosynthesis of trichothecenes. Antioxidants phenolic acids and ferulic acid in grains appear to inhibit trichothecene biosynthesis. Multiple genes appear to engage in several biosynthetic pathways to produce the trichothecenes A, B, C and D. The presence of several biosynthetic pathways suggests the need of multiple approaches to down-regulate trichothecene biosynthesis. With the evolution of *Fusarium*, varying gene relationships, gains, losses, functional changes, and trans-species polymorphism appears to occur qualitatively and quantitatively modifying transcriptomics in trichothecene biosynthesis [51]. These evolutionary changes make it more difficult to understand the molecular basis for preventing trichothecene biosynthesis. However, the current observations on behavioral dependence of a few controlling *Tri*-genes on external factors, suggest the possibility of identifying anti-mycotoxigenic compounds, which may be used to suppress the gene expressions associated with the biosynthesis of *Fusarium* toxins.

The transcriptomics associated with mycotoxin biosynthesis in fusarium also may adapt to climatic conditions in different geographical zones or other factors producing different chemotypes of the same mycotoxin. Such variations were observed with the biosynthesis of deoxynivalenol (DON). In comparing the performance of *Fusarium graminearum* isolates from Southern Europe producing 15-acetyl deoxynivalenol (15-ADON chemotype) with that from Northern Europe and

Southern Russia producing 3-acetyl deoxynivalenol (3-ADON chemotype) in wheat and oat by artificial infections, the 15-ADON type was found to be dominant in wheat. However, no clear difference between production of the two chemotypes were observed in oats [52]. Examining behavior of cultures from several ecological regions on biosynthesis of deoxynivalenols (3-ADON, 15-ADON, DON) in relation to expression of the three genes *Tri101*, *Tri3* and *Tri8,* the gene *Tri8* was found to be signaling the three different biosynthetic pathways [53]. The authors recognize *Tri8* as the primary gene determining the difference in 3-ADON and 15-ADON biosynthesis with changing sequences. *Tri8* may be a more useful gene to be manipulated for controlling biosynthesis.

Different models for biosynthetic pathways and genes responsible for trichothecene production are suggested [50,54]. The findings suggest co-regulation among the genes in the *Tri*-gene cluster and by genes outside the cluster, making it difficult to understand the total picture on biosynthesis of different fusarium toxins. With the current level of knowledge, working on preventive approaches for Fusarium mycotoxins continues to be challenging. Additionally, the host-pathogen interactions could depend on the capacity of the host to generate resistance to changing gene expressions in the pathogen associated with environmental factors, and the metabolites of the biosynthetic pathway. The observations in this study need to be expanded to different geographic situations and different crops to understand different biosynthetic pathways resulting from the host-pathogen relationships (cross-talks) to work on effective prevention of *Fusarium* toxins.

Biosynthesis of zearalenone by Fusarium species occurs through a different pathway. Zearalenone (previously described as F-2 Toxin) is produced by several *Fusarium* species, including *Fusarium graminearum*, the most predominant species producing trichothecenes. Zearalenone biosynthesis cluster consists of 15 polyketide synthases (PKS) as revealed by genome sequencing. Of the 15 synthases, only 8 have been identified to be responsible for Zearalenone biosynthesis. Of the 8 genes, *PKS4* and *PKS13* and two adjacent genes, *ZEB1* and *ZEB2* are reported to be the cluster vital for biosynthesis of zearalenone [55,56]. The gene *PKS4* initiates synthesis by a condensation reaction between acetyl-CoA and Malonyl-CoA. Gene *PKS13* continues to add more malonyl-CoA molecules. Of the two genes, *PKS4* or its encoded proteins or products appear to stimulate *PKS13* [57]. Both genes *PKS4* and *PKS13* were observed to be essential for zearalenone biosynthesis. The gene *ZEB1* is engaged in the final step of biosynthesis, converting zearalenol to zearalenone. *ZEB2* is a transcription factor carrying basic leucine zipper (bZIP) autoregulating biosynthesis. The biosynthetic pathway for zearalenone is less complex than for trichothecenes. Interrupting biosynthesis through *ZEB2* appears more feasible. Down-regulation of *PKS4* and *PKS13* are also options to regulate zearalenone biosynthesis. The detailed biosynthetic pathway for zearalenone is still to be worked out.

2.3.4. Biosynthesis of patulin

Biosynthesis of patulin occurs through the polyketide pathway in the same way as aflatoxins, but the synthesis is simpler. Biosynthesis is by a cluster of 15 genes identified as *PePatA* to *PePat0* [58], of which 4 genes, *PatK*, *PatN*, *PatH*, and *PatI* encoding four steps in patulin biosynthesis, are identified. Biosynthesis is carried out

by 11 enzymes [59]. Much research is needed to elucidate patulin biosynthesis completely.

The above findings highlight the effects of gene expressions and transcriptomic variations on the biosynthesis of different mycotoxins. The genes responsible appear to be between 15 and 25. Working on transcriptomics may carry potential to provide solutions to prevent mycotoxin biosynthesis in food crops, though it may take several years of research to generate adequacy and consistency of solutions. Translating the information generated on gene functions into practical control strategies and converting them into metabolic engineering to manipulate biosynthesis in mycotoxins is a challenge to the scientists. Continued research on transcriptomics is needed to strengthen the current knowledge on the role of biotic and abiotic factors in governing mycotoxin production. The expansion of knowledge in transcriptomics may lead to the identification of nutrients in hosts and storage conditions that may be manipulated to control mycotoxin production at post-harvest stages of foods and feeds.

2.4. Metabolomic interactions to prevent mycotoxin biosynthesis

Metabolomics addresses the small molecules produced during primary and secondary metabolism in the cells. Mycotoxins are metabolic products. The metabolites play physiological, ecological, and protective roles in cells. The metabolites reflect the influence of the biotic and abiotic factors in depleting or overexpressing genes in biosynthesis. Metabolomics is useful to understand the outcomes of interactions between host plants and toxigenic fungi. They also indicate changing genome functions. The genomic, proteomic, and transcriptomic studies have contributed much to understanding the host-fungi interactions during the biosynthesis of mycotoxins. The metabolites of host biosynthesis carry the potential to resist infection by fungi at critical concentrations.

In the host plants, the lipoxygenases respond to fungal infections by producing oxylipins from the cellular lipids. The maize genome encodes 13 isoforms of lipoxygenases having a defense role against fungi. Recent research based on *in-silico* analysis has identified the responsible lipoxygenases and conceptualized the biochemical pathways capable of producing specific lipoxygenases, capable of acting against *A. flavus* and *Fusarium verticillioides* infections in maize [60]. The study has identified the genes responsible for inducing the enzymes to form oxylipins. The oxylipin-based control of infections was observed to be highest in roots and lowest in seeds in maize, with shoots showing intermediate capacity. Enhancing the infection control mechanism in the seeds through metabolites may provide an approach to protect maize seeds pre-harvest, extending to field storage. From a different point of view, the production of the specific lipoxygenases also may mark the beginning of susceptibility to infections by the host tissues. These anti- and pro-mycotoxigenic responses by different lipoxygenases need to be understood well and controllable before embarking on using lipoxygenases for mycotoxin control. There is much research interest in this aspect currently.

Fusarium solani causing root rot in maize affects plant growth and yields. Rashad et al. [61] demonstrated 80% growth inhibition of *F. solani in vitro* by atoxic *A. flavus*. The atoxic *A. flavus* caused a 73% reduction in Fusarium-Root-Rot. Among the 17

secondary metabolites of atoxic *A. flavus* identified, several fatty acids, phenols, and phthalates exhibiting anti-fusarium activity are notable. The interaction with atoxic *A. flavus* has resulted in increased concentrations of antioxidant enzymes and phenols in the maize roots. Field evaluations and commercialization of the findings, either as biocontrol microbial cultures or effective mixtures of the antagonistic compounds, need to be further explored. Though the observation does not imply mycotoxin control directly, it carries the potential to use atoxic *A. flavus* to check the growth of mycotoxigenic *Fusarium* species.

During T-2 toxin biosynthesis by *Fusarium oxysporum*, the amino acids aspartic acid, methionine, isoleucine, serine, phenylalanine, and cysteine are observed to inhibit mycelial growth but promote T-2 toxin synthesis, operating through the TORC1 signaling pathway [62]. Of the amino acids, cysteine has been more effective in inhibiting mycelial growth of *F. oxysporum* through the Gtr2/Tap42 pathway and promoting mycotoxin T2 biosynthesis through the Gtr1/Tap42 pathway. The interaction may bring a hazard created by low visible production of fungal mycelium, but high T-2 concentrations on amino acid-rich foods, due to mediation by cysteine in the host.

Understanding the behavior of host-fungi interactions as consistent molecular biological phenomena would pave the way to apply them beneficially. The findings would provide advantages in pre-harvest agronomic applications, especially in biocontrol, by selection of resistant crop varieties and using soil micronutrients to regulate gene expressions in biosynthetic pathways. It may lead to novel approaches based on the omics knowledge in pre-harvest mycotoxin control.

Omics tools are also used to recognize the toxicodynamic of the exposure of humans and animals to combined mycotoxins using micro-RNA techniques, addressing health implications [63]. Aflatoxin M1, though produced in minor quantities along with aflatoxin B1 during fungal biosynthesis [64], is highly converted from aflatoxin B1 to aflatoxin M1 in animal cells, finally appearing in milk [65]. The miRNA technique may provide evidence to understand aflatoxin M1 production during animal metabolism. The authors recognize similarities through miRNA findings between mechanisms of toxicity in animals and defenses in plants associated with signaling mechanisms in ochratoxin biosynthesis. This opens new avenues to think of signaling mechanisms related to other mycotoxins. Combined effects of mycotoxins on plants and the pathogenic role of masked mycotoxins are already recorded [66,67]. The molecular basis of generating masked mycotoxins and the possibility of using the principle to biosynthesize non-toxic masked mycotoxin derivatives by host interactions may open new avenues for mycotoxin control. However, the production of masked mycotoxins carries the risk of them entering the human body as non-toxic entities and releasing the parent mycotoxins, affecting the consumers. Integration of mi-RNA findings with other diagnostic food safety protocols may pave new ways to mitigate mycotoxin hazards.

3. Conclusions and suggestions

Omics research enables a deep understanding of biological processes leading to mycotoxin biosynthesis through gene expressions, metabolite production, and

responses of biosynthesis to biotic and abiotic factors, enabling identification of mechanisms to control mycotoxins. Foodomics has revealed a wealth of information on host-fungus interactions and responses of mycotoxin biosynthesis to biotic and abiotic factors at the molecular level. The expression of genes responsible for mycotoxin biosynthesis occurs through multiple regulatory mechanisms operating at several levels. Multiple regulatory mechanisms make understanding biosynthesis and effective mycotoxin control challenging. Biological systems naturally contain inherent mechanisms to overcome stresses, making manipulating mycotoxin biosynthesis difficult, even at the molecular level. The current knowledge of constituents in the genes and their functionalities needs further examination using next-generation sequencing technologies to work on mycotoxin control mechanisms [68]. An aspect that has not been adequately addressed includes new toxicities that may arise due to the interruption of biochemical pathways by omics applications.

There is emerging evidence on cross-talks between mycotoxigenic fungi and the hosts beneficial in identifying approaches to control mycotoxins. In a competitive environment among fungi and bacteria to use the host for their survival, cross-talks between different types of fungi and with competing bacteria are noted [69]. Perhaps cross-talks between aflatoxigenic fungi and *Aspergillus oryzae* (which are quite close to each other genetically, being in the same genus), may create an environment for *A. oryzae* to co-exist in a mixed culture and ferment foods. In a different approach, mycotoxins may be engaged in eliminating bacteria, which are competing for the same ecological niche, having environmental limitations such as temperature, moisture, and relative humidity, both on contaminated crops and cultivated soils. Omics studies on benefiting from such cross-talks may provide new knowledge to apply mycotoxin control.

The future lies in combined knowledge on functional genomics, transcriptomics, proteomics, and metabolomics handled through bioinformatics using databases in working towards mycotoxin control.

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References

1. Allcroft R, Carnaghan RBA, Sargeant K. et al. A toxic factor in Brazilian groundnut meal. Veterinary Record; 1961; 73: 428-429.
2. Samarajeewa U. Agronomic Prevention of Pre-Harvest Mycotoxin Contamination in Foods: A Review. Food Science and Nutrition. 2024; 10(4): 1-8. doi: 10.24966/fsn-1076/100192
3. Samarajeewa U, Sen AC, Cohen MD, et al. Detoxification of Aflatoxins in Foods and Feeds by Physical and Chemical Methods. Journal of Food Protection. 1990; 53(6): 489-501. doi: 10.4315/0362-028x-53.6.489
4. Chang PK, Yabe K, Yu J. The Aspergillus parasiticus estA -Encoded Esterase Converts Versiconal Hemiacetal Acetate to Versiconal and Versiconol Acetate to Versiconol in Aflatoxin Biosynthesis. Applied and Environmental Microbiology. 2004; 70(6): 3593-3599. doi: 10.1128/aem.70.6.3593-3599.2004
5. Cary JW, Rajasekaran K, Brown RL, et al. Developing Resistance to Aflatoxin in Maize and Cottonseed. Toxins. 2011; 3(6): 678-696. doi: 10.3390/toxins3060678

6. Bhatnagar D, Rajasekaran K, Gilbert M, et al. Advances in molecular and genomic research to safeguard food and feed supply from aflatoxin contamination. World Mycotoxin Journal. 2018; 11(1): 47-72. doi: 10.3920/wmj2017.2283
7. Bhatnagar D, Rajasekaran K, Payne G, et al. The "omics" tools: genomics, proteomics, metabolomics and their potential for solving the aflatoxin contamination problem. World Mycotoxin Journal. 2008; 1(1): 3-12. doi: 10.3920/wmj2008.x001
8. Chen ZY, Rajasekaran K, Brown RL, et al. Discovery and confirmation of genes/proteins associated with maize aflatoxin resistance. World Mycotoxin Journal. 2015; 8(2): 211-224. doi: 10.3920/wmj2014.1732
9. Soni P, Gangurde SS, Ortega-Beltran A, et al. Functional Biology and Molecular Mechanisms of Host-Pathogen Interactions for Aflatoxin Contamination in Groundnut (Arachis hypogaea L.) and Maize (Zea mays L.). Frontiers in Microbiology. 2020; 11. doi: 10.3389/fmicb.2020.00227
10. Yu B, Huai D, Huang L, et al. Identification of genomic regions and diagnostic markers for resistance to aflatoxin contamination in peanut (Arachis hypogaea L.). BMC Genetics. 2019; 20(1). doi: 10.1186/s12863-019-0734-z
11. Liao B, Zhuang W, Tang R, et al. Peanut Aflatoxin and Genomics Research in China: Progress and Perspectives. Peanut Science. 2009; 36(1): 21-28. doi: 10.3146/at07-004.1
12. Cao A, de la Fuente M, Gesteiro N, et al. Genomics and Pathways Involved in Maize Resistance to Fusarium Ear Rot and Kernel Contamination With Fumonisins. Frontiers in Plant Science. 2022; 13. doi: 10.3389/fpls.2022.866478
13. Xu Q, Xu F, Qin D, et al. Molecular Mapping of QTLs Conferring Fusarium Head Blight Resistance in Chinese Wheat Cultivar Jingzhou 66. Plants. 2020; 9(8): 1021. doi: 10.3390/plants9081021
14. Ciasca B, Lanubile A, Marocco A, et al. Application of an Integrated and Open Source Workflow for LC-HRMS Plant Metabolomics Studies. Case-Control Study: Metabolic Changes of Maize in Response to Fusarium verticillioides Infection. Frontiers in Plant Science. 2020; 11. doi: 10.3389/fpls.2020.00664
15. Santiago R, Cao A, Malvar RA, et al. Genomics of Maize Resistance to Fusarium Ear Rot and Fumonisin Contamination. Toxins. 2020; 12(7): 431. doi: 10.3390/toxins12070431
16. Cleveland TE, Dowd PF, Desjardins AE, et al. United States Department of Agriculture—Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. Pest Management Science. 2003; 59(6-7): 629-642. doi: 10.1002/ps.724
17. Yu J. Current Understanding on Aflatoxin Biosynthesis and Future Perspective in Reducing Aflatoxin Contamination. Toxins. 2012; 4(11): 1024-1057. doi: 10.3390/toxins4111024
18. Pandey MK, Kumar R, Pandey AK, et al. Mitigating Aflatoxin Contamination in Groundnut through A Combination of Genetic Resistance and Post-Harvest Management Practices. Toxins. 2019; 11(6): 315. doi: 10.3390/toxins11060315
19. Wang T, Zhang E, Chen X, et al. Identification of seed proteins associated with resistance to pre-harvested aflatoxin contamination in peanut (Arachis hypogaea L). BMC Plant Biology. 2010; 10(1). doi: 10.1186/1471-2229-10-267
20. Fountain JC, Khera P, Yang L, et al. Resistance to Aspergillus flavus in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives. The Crop Journal. 2015; 3(3): 229-237. doi: 10.1016/j.cj.2015.02.003
21. Cui M, Han S, Wang D, et al. Gene Co-expression Network Analysis of the Comparative Transcriptome Identifies Hub Genes Associated With Resistance to Aspergillus flavus L. in Cultivated Peanut (Arachis hypogaea L.). Frontiers in Plant Science. 2022; 13. doi: 10.3389/fpls.2022.899177
22. Eshelli M, Qader MM, Jambi EJ, et al. Current Status and Future Opportunities of Omics Tools in Mycotoxin Research. Toxins. 2018; 10(11): 433. doi: 10.3390/toxins10110433
23. Bhatnagar D, Ehrlich KC, Cleveland TE. Molecular genetic analysis and regulation of aflatoxin biosynthesis. Applied Microbiology and Biotechnology. 2003; 61(2): 83-93. doi: 10.1007/s00253-002-1199-x
24. Yu J, Chang PK, Ehrlich KC, et al. Clustered Pathway Genes in Aflatoxin Biosynthesis. Applied and Environmental Microbiology. 2004; 70(3): 1253-1262. doi: 10.1128/aem.70.3.1253-1262.2004
25. Liu BH, Chu FS. Regulation ofaflRand Its Product, AflR, Associated with Aflatoxin Biosynthesis. Applied and Environmental Microbiology. 1998; 64(10): 3718-3723. doi: 10.1128/aem.64.10.3718-3723.1998
26. Natarajan S, Balachandar D, Senthil N, et al. Interaction of water activity and temperature on growth, gene expression, and aflatoxin B1 production in Aspergillus flavus on Indian senna (Cassia angustifolia Vahl.). International Journal of Food Microbiology. 2022; 361: 109457. doi: 10.1016/j.ijfoodmicro.2021.109457
27. Schmidt-Heydt M, Abdel-Hadi A, Magan N, et al. Complex regulation of the aflatoxin biosynthesis gene cluster of Aspergillus flavus in relation to various combinations of water activity and temperature. International Journal of Food Microbiology. 2009; 135(3): 231-237. doi: 10.1016/j.ijfoodmicro.2009.07.026

28. Schmidt-Heydt M, Rüfer CE, Abdel-Hadi A, et al. The production of aflatoxin B1 or G1 by Aspergillus parasiticus at various combinations of temperature and water activity is related to the ratio of aflS to aflR expression. Mycotoxin Research. 2010; 26(4): 241-246. doi: 10.1007/s12550-010-0062-7
29. Abdel-Hadi A, Schmidt-Heydt M, Parra R, et al. A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by Aspergillus flavus. Journal of The Royal Society Interface. 2011; 9(69): 757-767. doi: 10.1098/rsif.2011.0482
30. Yusuf HO, Olu J, Ajenifujah-Solebo SO, et al. Identification and Sequencing of aflP (omt) and aflD (Nor-1) Genes from Aspergillus flavus Species isolated from Maize (Zea mays L.), Obtained Across Abuja, Nigeria. Journal of Biochemistry and Molecular Biology. 2022; 1(1): 10-19. doi: 10.36108/jbmb/2202.10.0120
31. Katati B, Kovacs S, Njapau H, et al. Aflatoxigenic Aspergillus Modulates Aflatoxin-B1 Levels through an Antioxidative Mechanism. Journal of Fungi. 2023; 9(6): 690. doi: 10.3390/jof9060690
32. Al-Saad LA, Al-Badran AI, Al-Jumayli SA, et al. Impact of bacterial biocontrol agents on aflatoxin biosynthetic genes, aflD and aflR expression, and phenotypic aflatoxin B1 production by Aspergillus flavus under different environmental and nutritional regimes. International Journal of Food Microbiology. 2016; 217: 123-129. doi: 10.1016/j.ijfoodmicro.2015.10.016
33. Mannaa M, Kim KD. Influence of Temperature and Water Activity on Deleterious Fungi and Mycotoxin Production during Grain Storage. Mycobiology. 2017; 45(4): 240-254. doi: 10.5941/myco.2017.45.4.240
34. Medina Á, Rodríguez A, Sultan Y, et al. Climate change factors andAspergillus flavus: effects on gene expression, growth and aflatoxin production. World Mycotoxin Journal. 2015; 8(2): 171-180. doi: 10.3920/wmj2014.1726
35. Wang X, Zha W, Yao B, et al. Genetic Interaction of Global Regulators AflatfA and AflatfB Mediating Development, Stress Response and Aflatoxins B1 Production in Aspergillus flavus. Toxins. 2022; 14(12): 857. doi: 10.3390/toxins14120857
36. McDonald T, Brown D, Keller NP, et al. RNA silencing of mycotoxin production in Aspergillus and Fusarium species. Molecular Plant Microbe Interactions. 2005; 18(6): 539-545. DOI: 10.1094/ MPMI -18-0539.
37. Thakare D, Zhang J, Wing RA, et al. Aflatoxin-free transgenic maize using host-induced gene silencing. Science Advances. 2017; 3(3). doi: 10.1126/sciadv.1602382
38. Sharma KK, Pothana A, Prasad K, et al. Peanuts that keep aflatoxin at bay: a threshold that matters. Plant Biotechnology Journal. 2017; 16(5): 1024-1033. doi: 10.1111/pbi.12846
39. Loi M, Logrieco AF, Pusztahelyi T, et al. Advanced mycotoxin control and decontamination techniques in view of an increased aflatoxin risk in Europe due to climate change. Frontiers in Microbiology. 2023; 13. doi: 10.3389/fmicb.2022.1085891
40. Bernáldez V, Córdoba JJ, Magan N, et al. The influence of ecophysiological factors on growth, aflR gene expression and aflatoxin B1 production by a type strain of Aspergillus flavus. LWT-Food Science and Technology. 2017; 83: 283-291. doi: 10.1016/j.lwt.2017.05.030
41. Caceres I, Al Khoury A, El Khoury R, et al. Aflatoxin Biosynthesis and Genetic Regulation: A Review. Toxins. 2020; 12(3): 150. doi: 10.3390/toxins12030150
42. Musungu BM, Bhatnagar D, Brown RL, et al. A Network Approach of Gene Co-expression in the Zea mays/Aspergillus flavus Pathosystem to Map Host/Pathogen Interaction Pathways. Frontiers in Genetics. 2016; 7. doi: 10.3389/fgene.2016.00206
43. Huff WE, Hamilton PB. Mycotoxins – Their Biosynthesis in Fungi: Ochratoxins – Metabolites of Combined Pathways. Journal of Food Protection. 1979; 42(10): 815-820. doi: 10.4315/0362-028x-42.10.815
44. Harris JP, Mantle PG. Biosynthesis of ochratoxins by Aspergillus ochraceus. Phytochemistry. 2001; 58:709-716. doi: 10.1016/S0031-9422(01)00316-8
45. Gallo A, Bruno KS, Solfrizzo M, et al. New Insight into the Ochratoxin A Biosynthetic Pathway through Deletion of a Nonribosomal Peptide Synthetase Gene in Aspergillus carbonarius. Applied and Environmental Microbiology. 2012; 78(23): 8208-8218. doi: 10.1128/aem.02508-12
46. Wang Y, Wang L, Wu F, et al. A Consensus Ochratoxin A Biosynthetic Pathway: Insights from the Genome Sequence of Aspergillus ochraceus and a Comparative Genomic Analysis. Drake HL, ed. Applied and Environmental Microbiology. 2018; 84(19). doi: 10.1128/aem.01009-18
47. Vanacloig-Pedros E, Proft M, Pascual-Ahuir A. Different Toxicity Mechanisms for Citrinin and Ochratoxin A Revealed by Transcriptomic Analysis in Yeast. Toxins. 2016; 8(10): 273. doi: 10.3390/toxins8100273

48. Merhej J, Richard-Forget F, Barreau C. Regulation of trichothecene biosynthesis in Fusarium: recent advances and new insights. Applied Microbiology and Biotechnology. 2011; 91(3): 519-528. doi: 10.1007/s00253-011-3397-x
49. Wang J, Zhang M, Yang J, et al. Type A Trichothecene Metabolic Profile Differentiation, Mechanisms, Biosynthetic Pathways, and Evolution in Fusarium Species—A Mini Review. Toxins. 2023; 15(7): 446. doi: 10.3390/toxins15070446
50. Kimura M, Tokai T, Takahashi-Ando N, et al. Molecular and Genetic Studies of FusariumTrichothecene Biosynthesis: Pathways, Genes, and Evolution. Bioscience, Biotechnology, and Biochemistry. 2007; 71(9): 2105-2123. doi: 10.1271/bbb.70183
51. Proctor RH, McCormick SP, Alexander NJ, et al. Evidence that a secondary metabolic biosynthetic gene cluster has grown by gene relocation during evolution of the filamentous fungus Fusarium. Molecular Microbiology. 2009; 74(5): 1128-1142. doi: 10.1111/j.1365-2958.2009.06927.x
52. Yli-Mattila T, Hussien T, Abbas A. Comparison of biomass and deoxynivalenol production of northern European and southern European Fusarium graminearum isolates in the infection of wheat and oat grains. Journal of Plant Pathology. 2022; 104(4): 1465-1474. doi: 10.1007/s42161-022-01233-9
53. Alexander NJ, McCormick SP, Waalwijk C, et al. The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in Fusarium. Fungal Genetics and Biology. 2011; 48(5): 485-495. doi: 10.1016/j.fgb.2011.01.003
54. Seong K, Pasquali M, Zhou X, et al. Global gene regulation by Fusarium transcription factors Tri6 and Tri10 reveals adaptations for toxin biosynthesis. Molecular Microbiology. 2009; 72(2): 354-367. doi: 10.1111/j.1365-2958.2009.06649.x
55. Kim JE, Son H, Lee YW. Biosynthetic mechanism and regulation of zearalenone in Fusarium graminearum. Japanese Society of Mycotoxicology and Mycotoxins. 2018; 68(1): 1-6. doi: 10.2520/myco.68-1-2
56. Mahato DK, Devi S, Pandhi S, et al. Occurrence, impact on agriculture, human health, and management strategies of zearalenone in food and feed: A review. Toxins. 2021; 13: 92. doi: 10.3390/ toxins13020092
57. Lysøe E, Klemsdal SS, Bone KR, et al. The PKS4 Gene of Fusarium graminearum Is Essential for Zearalenone Production. Applied and Environmental Microbiology. 2006; 72(6): 3924-3932. doi: 10.1128/aem.00963-05
58. Li B, Zong Y, Du Z, et al. Genomic Characterization Reveals Insights Into Patulin Biosynthesis and Pathogenicity in Penicillium Species. Molecular Plant-Microbe Interactions®. 2015; 28(6): 635-647. doi: 10.1094/mpmi-12-14-0398-fi
59. Snini SP, Tadrist S, Laffitte J, et al. The gene PatG involved in the biosynthesis pathway of patulin, a food-borne mycotoxin, encodes a 6-methylsalicylic acid decarboxylase. International Journal of Food Microbiology. 2014; 171: 77-83. doi: 10.1016/j.ijfoodmicro.2013.11.020
60. Guche MD, Pilati S, Trenti F, et al. Functional Study of Lipoxygenase-Mediated Resistance against Fusarium verticillioides and Aspergillus flavus Infection in Maize. International Journal of Molecular Sciences. 2022; 23(18): 10894. doi: 10.3390/ijms231810894
61. Rashad YM, Abdalla SA, Shehata AS. Aspergillus flavus YRB2 from Thymelaea hirsuta (L.) Endl., a non-aflatoxigenic endophyte with ability to overexpress defense-related genes against Fusarium root rot of maize. BMC Microbiology. 2022; 22(1). doi: 10.1186/s12866-022-02651-6
62. Qiu M, Deng Y, Deng Q, et al. Cysteine Inhibits the Growth of Fusarium oxysporum and Promotes T-2 Toxin Synthesis through the Gtr/Tap42 Pathway. Microbiology Spectrum. 2022; 10(6). doi: 10.1128/spectrum.03682-22
63. Murtaza B, Li X, Nawaz MY, et al. Toxicodynamic of combined mycotoxins: MicroRNAs and acute-phase proteins as diagnostic biomarkers. Comprehensive Reviews in Food Science and Food Safety. 2024; 23(3). doi: 10.1111/1541-4337.13338
64. Huang JH, and Hsieh DP. Comparative study of aflatoxins M1 and B1 production in solid-state and shaking liquid cultures. In: Proceedings of the National Science Council Republic China; 1988.
65. Garrido NS, Iha MH, Santos Ortolani MR, et al. Occurrence of aflatoxins M1and M2in milk commercialized in Ribeirão Preto-SP, Brazil. Food Additives and Contaminants. 2003; 20(1): 70-73. doi: 10.1080/0265203021000035371
66. Cortinovis C, Caloni F, Schreiber NB, et al. Effects of fumonisin B1 alone and combined with deoxynivalenol or zearalenone on porcine granulosa cell proliferation and steroid production. Theriogenology. 2014; 81(8): 1042-1049. doi: 10.1016/j.theriogenology.2014.01.027
67. Berthiller F, Crews C, Dall'Asta C, et al. Masked mycotoxins: A review. Molecular Nutrition & Food Research. 2012; 57(1): 165-186. doi: 10.1002/mnfr.201100764
68. Zhang J, Chiodini R, Badr A, et al. The impact of next-generation sequencing on genomics. Journal of Genetics and Genomics. 2011; 38(3): 95-109. doi: 10.1016/j.jgg.2011.02.003

69. Venkatesh N, Keller NP. Mycotoxins in Conversation With Bacteria and Fungi. Frontiers in Microbiology. 2019; 10. doi: 10.3389/fmicb.2019.00403