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Resistance to *Aspergillus flavus* in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives

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ABSTRACT

The colonization of maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) by the fungal pathogen *Aspergillus flavus* results in the contamination of kernels with carcinogenic mycotoxins known as aflatoxins leading to economic losses and potential health threats to humans. The regulation of aflatoxin biosynthesis in various *Aspergillus* spp. has been extensively studied, and has been shown to be related to oxidative stress responses. Given that environmental stresses such as drought and heat stress result in the accumulation of reactive oxygen species (ROS) within host plant tissues, host-derived ROS may play an important role in cross-kingdom communication between host plants and *A. flavus*. Recent technological advances in plant breeding have provided the tools necessary to study and apply knowledge derived from metabolomic, proteomic, and transcriptomic studies in the context of productive breeding populations. Here, we review the current understanding of the potential roles of environmental stress, ROS, and aflatoxin in the interaction between *A. flavus* and its host plants, and the current status in molecular breeding and marker discovery for resistance to *A. flavus* colonization and aflatoxin contamination in maize and peanut. We will also propose future directions and a working model for continuing research efforts linking environmental stress tolerance and aflatoxin contamination resistance in maize and peanut.

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1. Introduction

The colonization of maize and peanut by *Aspergillus flavus* and *Aspergillus parasiticus* (Link ex Fr. and Speare, respectively; teleomorphs: *Petromyces flavus* and *Petromyces parasiticus*) [1,2] results in contamination of their derived agricultural products with aflatoxins [3]. Aflatoxins are among the most potent mycotoxins, carcinogenic and teratogenic compounds, produced during infection and growth of fungi *A. flavus* and *A. parasiticus* on crops such as maize, peanut, cottonseed and tree nuts. Maize and peanuts are the most susceptible crops to aflatoxin contamination and serve as the main source of aflatoxin contamination for humans [4]. Aflatoxins not only have been associated with numerous diseases and disorders in humans and livestock, but also have a negative economic impact due to loss of crop value [5–7]. Resistance to *A. flavus* colonization and subsequent aflatoxin production is a complex phenomenon involving numerous genetic, physiological, and morphological factors and acts as a quantitative trait [8–10].

Examination of the functional composition of resistance mechanisms in maize and, to a lesser extent, in peanut using transcriptomic, proteomic, and metabolomic approaches has led to the elucidation of the roles of several specific genes, proteins, and signal molecules including pathogenesis-related proteins (such as PR-10, PR-10.1, 14-kDa trypsin inhibitor, chitinase, zeamatin, and B1,3-glucanase), stress-responsive proteins (such as catalase, superoxide dismutase, glyoxalase I, and glutathione-S-transferase), and reactive oxygen species (ROS) in regulating *A. flavus* resistance as well as their potential roles in cross-kingdom communication between host plants and *Aspergillus* spp. [11–20]. In addition, the link that exists between aflatoxin contamination and environmental stress, particularly drought stress, has also been a focal point of molecular research and applied breeding programs in recent years [5,21–25]. Hence, the use of drought tolerant germplasm with aflatoxin resistance has gained momentum for selection in various genetic studies [21,26].

Despite considerable advances in molecular research, a complete understanding of the details of the host-parasite interaction between *A. flavus* and its hosts including maize and peanut, namely the precise signaling mechanisms employed in this interaction, remains elusive and there is a need for continuing investigation. Therefore, in this review we focus on recent findings related to the biochemistry of defense regulation with regard to environmental stress, ROS, and inter-cellular communication between *A. flavus* and its host crops. In addition, recent advances in conventional and molecular breeding for aflatoxin resistance in maize and peanut, and the potential utilization of molecular markers for use in marker assisted selection (MAS) in breeding programs are highlighted.

2. Molecular biology of potential host-*A. flavus* interactions mediated by ROS

The molecular and biochemical bases of the interaction between the host crops and *A. flavus* has been the subject of numerous studies in recent years for identifying both the sources of resistance to *A. flavus* colonization, and the regulation of aflatoxin biosynthesis in *A. flavus* and other Aspergilli including *Aspergillus fumigatus* and *A. parasiticus*. Integration of the findings of these studies into a coherent model for explaining the subtleties of the interaction is lacking in the literature. In addition, the functional roles of the components/genes thought to be involved in the host-pathogen interactions were not well characterized. Hence, the potential components of the interaction and their implications for future research efforts are discussed.

2.1. *Pathogen recognition and upstream resistance gene expression regulation*

The plant-pathogen recognition is the first step in the interaction which causes rapid activation of appropriate defensive and infective mechanisms in the plant and the pathogen, respectively. Using maize as an example, the recognition of *A. flavus* by maize cells in contact with the pathogen and the subsequent transcriptional activation of the upstream defense signaling system constitute the first line of defense and response to infection. But the precise upstream recognition mechanisms employed by maize or peanut against *A. flavus* are not currently known. However, recent studies on WRKY transcription factors in maize [27] and model species such as *Arabidopsis thaliana* [28] may provide insight into this aspect of defense initiation.

WRKY transcription factors, which possess a rarely variable amino acid sequence of "WRKY" at the amino terminus of their DNA binding domain, function in the upstream regulation of various cellular processes in plants and other organisms, including pathogen defense response coordination [28]. It has been demonstrated that two WRKY transcription factor-encoding genes, *Zm*WRKY19 and *Zm*WRKY53, were significantly up-regulated by *A. flavus* inoculation in the resistant maize line TZAR101, and may play an important role in regulating upstream defense responses in developing maize kernels in response to *A. flavus* inoculation [27]. The ortholog of *Zm*WRKY19 in *Arabidopsis*, AtWRKY53, in contrast, functions in oxidative stress responses by promoting the expression of catalase and other antioxidant genes, and has been shown to interact with calmodulins [29,30]. *Zm*WRKY53 has been shown to enhance abiotic stress tolerance, including drought and salt stress [31]. Similarly, the WRKY genes were found to be associated with conferring tolerance to salinity in interspecific derivatives of peanut [32]. The ortholog of *Zm*WRKY53 in *Arabidopsis*, AtWRKY33, has also been demonstrated to function in necrotrophic pathogen defense responses and thermotolerance while its orthologs in wheat (*Ta*WRKY53) and rice (*Os*WRKY53) were shown to function in regulating chitinase and peroxidase gene expression [33].

Interestingly, the *Arabidopsis* orthologs of these WRKY transcription factors are directly regulated by mitogen activated protein kinase (MAPK) pathways, including MEKK1 and MPK3/6, in response to chitin perception by receptor kinases as a part of a pathogen associated molecular pattern (PAMP)-triggered immunity (PTI) mechanism [28,29,34–37]. This prospect of chitin perception as a trigger for PTI seems

plausible given that previous research demonstrated that resistant maize lines accumulate chitinase, which may provide a source for chitin monomers that can be perceived by receptor kinases [17]. Also, given the high level of expression of these orthologous WRKY genes in resistant maize, it is possible that such a signal transduction and receptor system may be present in maize and functional in the maize–*A. flavus* interaction [27]. In addition, appropriate studies need to be carried out in peanut to determine the role of WRKY genes in initiation of plant defense mechanisms. Furthermore, the expression of WRKY transcription factors in response to *A. flavus* inoculation might result in increased expression of antioxidant and pathogenesis-related genes in resistant maize lines providing enhanced oxidative stress tolerance and pathogen resistance (Fig. 1-A) [5,27].

2.2. Calcium signaling and reactive oxygen species (ROS) in defense regulation

In addition to MAPK signaling to promote the expression of defense-related genes, calcium signaling and reactive oxygen species (ROS) play a role in regulating defense responses. Recently, Ma and Berkowitz [38] reviewed the Ca^{2+}-calmodulin signaling and its role in regulating defense activation and hypersensitive cell death. Briefly, as a part of PTI responses, receptor kinase-bound nucleotidyl cyclases activate cyclic nucleotide gated ion channels (CNGCs) through cAMP or cGMP signal intermediates. This results in the influx of Ca^{2+} ions into the plant cell cytosol and the activation of calmodulins and calcium dependent protein kinases (CDPKs). These CDPKs then, in turn, activate the transmembrane protein complex NADPH

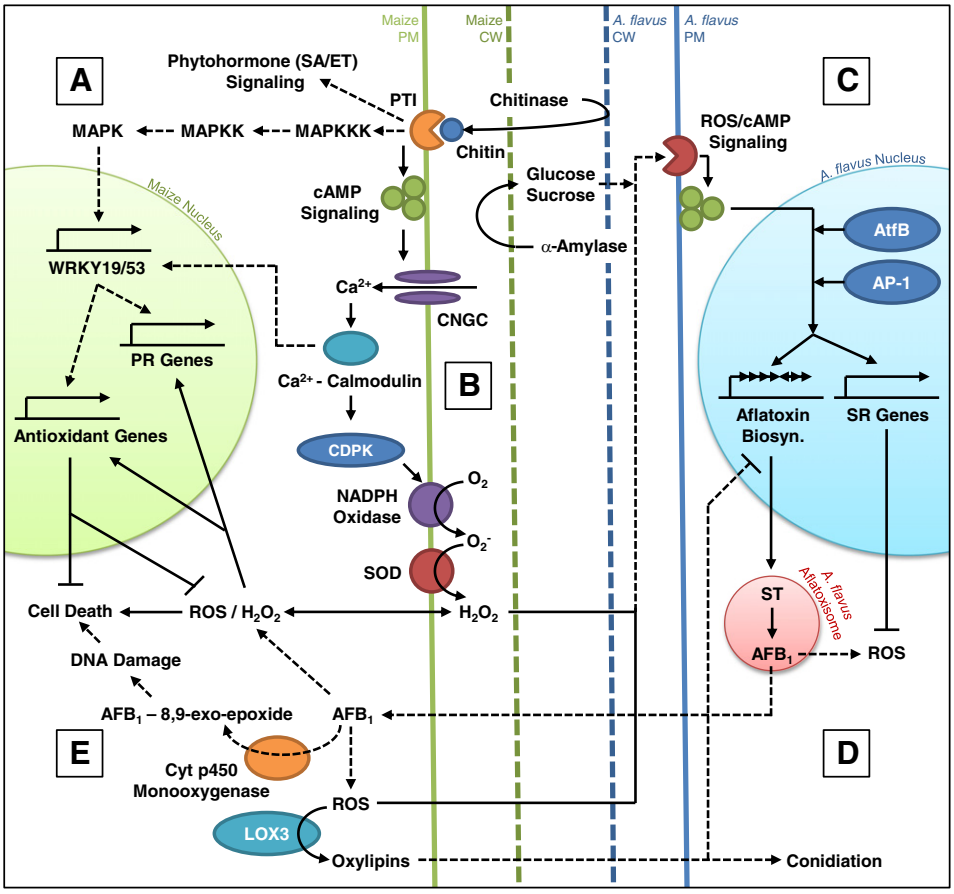

Fig. 1 – Hypothetical biochemical pathways and reactions present in the maize–*A. flavus* interaction. (A) The perception of chitin by receptor kinases activates a MAPK cascade leading to the expression of maize WRKY transcription factors *Zm*WRKY19 and 53 which promote the expression of antioxidant and pathogenesis-related gene expression; (B) PAMP triggered immunity (PTI) reactions lead to the activation of calcium signaling pathways resulting in the production of extracellular superoxide anions which are detoxified to hydrogen peroxide by superoxide dismutase (SOD); (C) Extracellular hydrogen peroxide functions in cross-kingdom communication between maize and *A. flavus* resulting in the stimulation of cAMP signaling and subsequent expression of genes encoding for stress response proteins and aflatoxin biosynthetic components; (D) The final stages of aflatoxin biosynthesis are confined to specialized structures known as aflatoxisomes while aflatoxin-derived and environmental ROS are detoxified by various stress response proteins. Host derived oxylipins may also stimulate conidiation and inhibit aflatoxin biosynthesis; (E) Aflatoxin is secreted from *A. flavus* and absorbed into the maize cell resulting in oxidative damage to DNA and other cellular components leading to cell death. Solid lines represent characterized pathways from the literature. Dashed lines represent hypothetical junctures between the components of the interaction.

oxidase which converts molecular O_2 to a superoxide anion (O_2^-). The superoxide anion is then detoxified by superoxide dismutase (SOD) to form H_2O_2 whose neutral charge allows it to pass through the plasma membrane and function in cytosolic defense signaling. In maize, Jiang and Zhang [39] demonstrated a similar mechanism functional in oxidative stress responses. Another study by Hu et al. [40] further demonstrated an interaction between Ca^{2+}/calmodulin signaling components and abscisic acid (ABA)-based ROS defense responses.

The presence of calcium/calmodulin signaling in maize is interesting because of the role of calmodulin in regulation of AtWRKY53, the ortholog of ZmWRKY19, in order to stimulate antioxidant gene expression [29,30]. Also, free mobility of H_2O_2 across cell membranes and its role as a source of oxidative stress, as H_2O_2 as a mobile signaling molecule, involves in cross-kingdom communication between maize and *A. flavus* or other invading pathogens (Fig. 1-B). The role of cytosolic levels of Ca^{2+} ions in stimulating these responses may also be relevant to the interaction between maize resistance to *A. flavus* and drought stress since cytosolic levels of Ca^{2+} would be proportionally higher due to water loss under drought stress conditions that activate the associated signaling mechanisms. However, detailed studies are needed to validate the role of Ca^{2+} signaling in regulating resistance to *A. flavus* in maize and other crop species.

2.3. Potential role of ROS in aflatoxin biosynthesis and stress responsive gene regulation

As the defense signaling-derived ROS are generated extracellularly, they may stimulate the production of aflatoxin by *Aspergillus* spp. potentially as a part of an antioxidative defense mechanism [5]. A recent study by Roze et al. [41] demonstrated that aflatoxin biosynthesis and stress response are potentially linked in *A. parasiticus* by a transcription factor complex with the basic leucine zipper (bZIP) transcription factors AtfB and AP-1, in response to available carbohydrate or oxidative stress through a cAMP signaling mechanism. The protein complex directly promotes the expression of genes pertaining to secondary metabolism, particularly in aflatoxin biosynthesis. This study postulates that the protein complex or its components stimulate the expression of antioxidant defense genes and the promoters of the antioxidant genes are bound by bZIP transcription factors (Fig. 1-C). In addition, ROS cross-talk between the host plant cell and *Aspergillus* spp. may also result in the formation of oxylipins which regulate the reproductive development of Aspergilli as well as aflatoxin production [5,42–44] (Fig. 1-D, E).

2.4. Aflatoxin metabolism and potential effects on plant cell physiology

The connection between ROS-derived oxidative stress and aflatoxin production seems to indicate the antioxidant property of aflatoxin that may favor growth and function of *A. flavus* or other Aspergilli and would, therefore, be advantageous for fungal survivability. However, it is possible that the opposite is true, and this link may be useful to remediate oxidative stress caused by aflatoxin reacting with fungal cellular components. This seems plausible given two considerations.

First, the final stages of aflatoxin biosynthesis are confined to specialized, membrane-bound organelles termed aflatoxisomes [45–48]. This compartmentalization of aflatoxin biosynthesis followed by direct exocytosis lends itself to the possibility that mature aflatoxin compounds may be cytotoxic (Fig. 1-D). However, further experimentation will be required in order to examine the precise aflatoxin detoxification and damage remediation mechanisms employed by *Aspergillus* spp.

Second, aflatoxin may be metabolized by fungal or plant cells into toxic byproducts. Studies of the metabolism of aflatoxin B_1 (AFB_1) by human hepatocytes revealed that cytochrome p450 monooxygenases are capable of oxidizing AFB_1, resulting in the bioactivation of the toxin [49]. Specifically, cytochrome p450-3A4 converts AFB_1 into an epoxidized form, AFB_1-exo-8,9-epoxide, which readily reacts with DNA structures resulting in mutation and oxidative damage to various macromolecules [50]. Conversely, cytochrome p450-1A2 converts AFB_1 into AFB_1-endo-8,9-epoxide which is non-reactive and rapidly detoxified [50]. Since p450 monooxygenases are universally abundant in eukaryotic organisms, including maize [51], it is possible that aflatoxin is metabolized in a similar fashion in maize or peanut, resulting in oxidative damage to cellular components potentially leading to localized cell death (Fig. 1-E). However, for such a reaction to occur, the ability of aflatoxin to be absorbed by plant cells and its subsequent metabolism remain fundamental issues to be addressed in future research endeavors.

If indeed aflatoxin causes oxidative damage to cellular components of both pathogen and host, a question quickly arises. What is the advantage provided by the biosynthesis of aflatoxin? It was hypothesized in the literature that *A. flavus* functions as a facultative necrotroph during infection of maize kernel tissues [5,52]. Aflatoxin could enhance pathogenicity by causing localized death of host cells surrounding the invading fungal mycelia, while *A. flavus* is afforded protection by the co-expression of high levels of stress responsive genes [41]. In either case, detailed study of molecular mechanisms involved in aflatoxin biosynthesis in maize and peanut are needed to confirm these hypotheses.

3. Breeding for aflatoxin resistance in maize: biomarkers, quantitative trait loci (QTL) discovery, and applications in conventional programs

3.1. Biomarkers

The preceding discussion on the biochemistry of the interaction between maize and *A. flavus* presents both challenges and opportunities for continuing research, particularly while considering their potential applications. It has been established that there exists a correlation between the drought tolerance of maize lines and their relative resistance to aflatoxin contamination under hot and dry conditions [5,26]. These conditions are also known to result in the accumulation of ROS in plant tissues, and, given that recent reports demonstrate that ROS can regulate aflatoxin production in *Aspergillus* spp., this provides a potential link between aflatoxin production and host-derived oxidative stress [19,25,41,53]. Therefore, if host derived oxidative stress in

response to abiotic stress can possibly exacerbate aflatoxin production, the selection of components involved in antioxidant mechanism such as metabolites, proteins, and gene expression levels may allow them to be utilized as molecular markers, "biomarkers," for use in selection in breeding applications.

For instance, Pechanova et al. [18] reported that resistant maize lines accumulate high levels of superoxide dismutase, peroxidases, and chaperonins in rachis tissues. Such highly expressed proteins could be utilized for screening germplasm and populations for markers related to both aflatoxin resistance, as well as abiotic stress tolerance. Fountain et al. [54] reported that expression of the gene encoding the 14-kDa trypsin inhibitor known to inhibit fungal amylases, was highly expressed in kernel tissues of resistant maize lines compared to susceptible lines when infected by *A. flavus* under drought stress conditions. In addition, detoxifying enzymes such as glutathione-S-transferase (GST) and pathogenesis-related proteins such as PR-10 can also be used to screen for pathogen resistance or abiotic stress tolerance based on their respective biological activities [15,55,56]. Genomic and proteomic expression studies during the infection process have indicated that oxidative stress tolerance is vital to adaptive changes in fungal biology during infection [57]. Additional studies have also shown that maize lines with known resistance to drought and aflatoxin contamination are more recalcitrant to oxidative stress due to more stably expressed antioxidant components than susceptible lines [25]. Therefore, these oxidative stress tolerance mechanisms may serve as sources for selectable markers for use in breeding applications for aflatoxin contamination resistance and drought tolerance. By combining these and additional "biomarkers" in conjunction with traditional genetic markers such as insertion/deletions (indels), single nucleotide polymorphisms (SNPs), or simple sequence repeat (SSR)/microsatellite markers, the efficiency of selecting resistant germplasm could be enhanced. In addition, by selecting "biomarkers" that provide both abiotic stress tolerance and aflatoxin resistance, some of the confounding effects of genotype × environment interactions may be avoided. Future studies should examine the utilization and feasibility of potential multi-purpose "biomarkers" for use in large scale marker assisted selection (MAS) applications.

3.2. QTL discovery

As previously stated, resistance to *A. flavus* colonization and aflatoxin contamination was demonstrated to be quantitative and heavily influenced by environmental interactions [5,9,58]. Therefore, recent breeding studies focused on the discovery and characterization of quantitative trait loci (QTL) for aflatoxin resistance were forced to consider the environment in obtaining phenotypic data, and have faced numerous challenges in identifying consistent QTL for aflatoxin resistance. For example, Willcox et al. [59] utilized an F_2 mapping population derived from Mp313E × Va35 (resistant × susceptible) to identify 20 QTLs with combined phenotypic variance explained (PVE) of 22–43%. However, when the mapping populations were grown in multiple environments, only 11 QTLs were found to be consistent with a combined PVE of 2.4–9.5%. An earlier study by Brooks et al. [60] also examined a population derived from Mp313E for the presence of QTL for aflatoxin resistance. A collection of 210 $F_{2:3}$ families derived from Mp313E × B73 (resistant × susceptible) was utilized for the study and a total of 85 polymorphic SSR markers was selected for genotyping and map construction. By analyzing phenotypic data from three locations, they were able to identify two consistent QTLs, one with a PVE of 7–18%, and a second with a PVE of 8–18%, indicating a nominal degree of variation between the environments. In another study, a single consistent QTL (PVE 8.42%) was identified using a recombinant inbred line (RIL) population of $228F_{8:9}$ RILs derived from RA × M53 (resistant × susceptible) utilizing 916 SNP markers for linkage map construction, and phenotyping data was obtained from two locations with contrasting environments for phenotypic analysis [61].

These studies demonstrate that resistance to *A. flavus* is not conferred by a single gene and is highly quantitative in nature. In addition, given the relatively low level of PVE provided by each QTL, it is likely that many QTL with low PVE (<10%) contribute to aflatoxin resistance and may be indicative of the polygenic nature of resistance and the involvement of multiple physiological and morphological traits in the overall resistance phenotype [5,62]. Interestingly, similar difficulties are also faced when examining drought tolerance QTL in maize. For example, Almeida et al. [63] recently performed a QTL analysis of drought tolerance in three populations: a RIL population derived from CML444 × MALAWI, a $F_{2:3}$ family set derived from CML440 × CML504, and a second $F_{2:3}$ family set derived from CML444 × CML441. They identified QTLs for grain yield under drought stress with PVE ranging from 2.6–17.8%, and for the anthesis–silking interval under drought stress with a PVE ranging from 1.7–17.8%. Genome wide association studies (GWAS), which rely on ancient recombination events among diverse inbreeds for mapping QTL, are limited in their detection of QTL with low PVE, and may not identify aflatoxin resistance and drought tolerance QTL unless high numbers of individuals and markers are used to increase the resolving power of the experiment [62,64].

Once identified, QTL function and composition must be determined in order to elucidate the mechanism being employed to produce a particular phenotype, namely aflatoxin resistance. In conjunction with QTL discovery in traditional bi-parental populations, the integration of functional genomics technologies has been shown to enhance QTL validation by confirming the expression and identity of genes present in QTL regions. A recent study by Kelley et al. [9] utilized microarray analysis to validate the expression of QTL-associated genes involved in *A. flavus* responses in the maize lines Mp313E and Va35. As this study illustrated, coupling functional genomics analysis with QTL discovery also allows the determination of the mechanism employed by maize in response to *A. flavus* infection by determining not only which genes within the QTL regions are regulated, but also whether they are up or down-regulated and the amount of regulation. Coupling expression and QTL studies with functional genetics, experiments can be conducted to determine the function of specific genes, such as pathogenesis-related defense genes through methods such as RNA interference-(RNAi) based gene silencing [14]. These studies will provide for the identification of the causal basis of a gene contribution to a QTL and provide possible explanations for the influence of environment on the detection and stability of QTL.

3.3. *Applications of biomarkers and QTL in conventional breeding programs*

Conventional breeding for resistance has formed the basis of generating aflatoxin contamination-resistant maize lines with recent efforts in this field resulting in the release of several lines with promising levels of aflatoxin contamination resistance [23,65–67]. As recently reviewed by Williams et al. [68], several aflatoxin resistant lines including GT601, GT602, GT603, Mp715, Mp717, Mp718, and Mp719 were derived from conventional breeding programs in the southeastern U.S. In addition, the incorporation of exotic lines with aflatoxin accumulation resistance and additional desirable traits into breeding programs to widen the genetic base of traditional temperate lines through cooperative efforts such as the Germplasm Enhancement of Maize (GEM) project will allow for further enhancement of previously identified resistant lines [68]. In addition to variety development, recent research has also focused on the evaluating the general and specific combining abilities of aflatoxin resistant lines to enhance hybrid development. For example, Williams et al. [69] performed a diallel cross using ten inbred lines with varying levels of resistance to aflatoxin contamination including: CI66, GA209, NC408, Mo18W, Mp313E, Mp494, Mp715, Mp717, SC212m, and T173. They found that the resistant lines Mp313E, Mp494, Mp715, Mp717, Mo18W, and NC408 possessed significant general combining ability (GCA) effects for resistance to aflatoxin and proposed that utilizing GCA data to plan crosses in resistance breeding will expedite progress in developing aflatoxin resistant hybrids.

This diallel study illustrates the potential utility of additional selection methodologies in enhancing aflatoxin resistance in maize. Currently, screening for aflatoxin resistance is carried out either in the field with direct inoculation which can produce variable results depending on environmental conditions and the method used, or in the laboratory with kernel screening assays (KSAs) for high throughput screening [70]. Given the potential for variability in these systems, the incorporation of molecular markers into breeding programs for use in MAS could provide for more consistent results. However, while many QTLs and associated polymorphic markers have been discovered in maize for resistance to aflatoxin contamination and *A. flavus* colonization, their utility in conventional breeding programs has been limited. This is likely due to the variable nature of the expression of these QTLs across multiple environments and conditions. Therefore, biomarkers selected based on their role in both aflatoxin resistance and abiotic stress responses may provide a method to account for environmental influences on aflatoxin resistance. When used in conjunction with traditional DNA-based marker systems and conventional resistance breeding, biomarkers may prove to valuable tools for breeder to enhance aflatoxin resistance and associated traits such as drought tolerance into improved germplasm.

4. Correlating environmental stress and aflatoxin resistance in peanut

Peanut (*Arachis hypogaea* L.) is an allotetraploid ($2n = 4x = 40$) crop grown in over 100 countries. One of the major concerns about the import/export of peanut is aflatoxin contamination (AC) which may result in the rejection of seed lots if levels of aflatoxin are above maximum prescribed limits [71]. AC in peanut is caused by two fungal pathogens, *A. flavus* and *A. parasiticus* with *A. flavus* being the most prevalent in infected pods. A typical pattern of fungal infection includes entry of the fungi through small cracks developed during pod maturation/drying in the ground [72].

It has been demonstrated that pre-harvest aflatoxin contamination (PAC) is increased when abiotic stress such as drought stress is imposed on the crop. This may be due to reduced water activity during pod development which could lead to the creation of cracks in the pod wall. Hence, damaged pods tend to be more susceptible to PAC than undamaged pods [73,74]. Other studies point out that reduced kernel water content may decrease phytoalexin production thereby decreasing the plant's natural defense against infection leading to increased AC [75,76]. In addition to drought stress, heat stress has also been found to play an important role in PAC [75,77]. Apart from genetic sources of resistance in peanut, PAC management practices such as correct irrigation, fungicide applications, avoidance of mechanical damage, biological control, crop rotation, harvest timing, and good post-harvest storage conditions play critical roles in limiting AC in peanut [24,58].

Drought stress seems to function as a predisposing factor for PAC in peanut [78,79]. Therefore, a common idea arises that drought tolerant cultivars would assist in alleviating PAC, indicating that direct or indirect selection for PAC during drought tolerance would be appropriate. In order to understand the molecular mechanisms of aflatoxin biosynthesis, some genomic and proteomic studies have been carried out [80–83]. A positive correlation was found between 20 drought tolerant lines and PAC resistance [84]. Furthermore, the measures of several drought tolerance component traits such as SPAD (measured by a SPAD-502 meter: Minolta, Tokyo, Japan) chlorophyll meter reading (SCMR), and specific leaf area (SLA) also showed positive correlations with PAC resistance [73]. Conversely, the results of a recent study indicated that although drought tolerance increases PAC resistance in some lines, it does not universally apply to all genetic backgrounds. Hence, drought tolerance and resistance to PAC may involve different mechanisms in peanut [85].

The lack of high levels of resistance to aflatoxin contamination in cultivated germplasm and a reliable phenotyping protocol, poses challenges in using conventional breeding methods to identify resistance to PAC in peanuts. Nevertheless, a large scale screening effort consisting of 831 accessions in the US peanut core collection led to the identification of 19 accessions with low PAC and relatively high yield [86]. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) several resistant germplasms were identified for three types of resistance (i.e. PAC, resistance to in vitro seed colonization (IVSC), and aflatoxin production by *A. flavus*) after extensive screening of more than 2000 peanut accessions in a heavily infested field plot ("sick plot") under conditions of imposed drought [87].

Use of molecular markers for PAC resistance is very limited. For instance, a set of 6 amplified fragment length polymorphism (AFLP) markers with low PVE in *Arachis cardenasii*-derived

lines were identified [88] and in another study six QTLs for resistance to A. *flavus* infection with PVE up to 22.7% were identified [89]. Since resistance to PAC is a global problem, an international effort was recently undertaken under the ambit of the Peanut & Mycotoxin Innovation Lab (PMIL) initiated with collaboration between ICRISAT, the University of Georgia (UGA), and Institut Sénégalais de Recherches Agricoles (ISRA) in Senegal. This effort utilizes RIL populations, association mapping panels, multiparent advanced generation intercross (MAGIC) populations, interspecific introgression lines, and genomic selection approaches in order to enhance our understanding of the genetic components of PAC.

In summary, resistance to PAC in peanut is a complex trait with high G × E interaction, low heritability, and a lack of reliable phenotyping protocols. These limitations pose challenges in identifying and developing resistant germplasm. Unfortunately, there is no single, highly effective source of resistance that can be used to tackle this issue from a genetic perspective. Therefore, crop management in conjunction with enhanced genetic resistance should be the way forward for obtaining PAC resistance in peanut.

5. Conclusions and future perspectives

Resistance to A. *flavus* infection and aflatoxin contamination in maize and peanut is a complex trait that is heavily influenced by environmental factors. Current efforts in determining the biochemical basis of resistance and use of that knowledge in breeding programs has led to an increased understanding of elements of this plant–pathogen interaction. However, many questions remain to be answered as to the role of aflatoxin in the biology and ecology of *Aspergillus* spp. and its role in pathogenesis, including the role of aflatoxin as a source of cellular oxidative stress. In addition, the potential role of host-derived ROS in stimulating aflatoxin production is also in need of further study. Future work should also address the potential use of identified proteins, metabolites, and candidate genes as selectable biomarkers for use in MAS. By utilizing such markers, breeding programs can be optimized to select not only for aflatoxin resistance but also for associated abiotic stress tolerance.

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REFERENCES

[1] B.W. Horn, G.G. Moore, I. Carbone, Sexual reproduction in *Aspergillus flavus*, Mycologia 101 (2009) 423–429.
[2] B.W. Horn, J.H. Ramirez-Prado, I. Carbone, The sexual state of *Aspergillus parasiticus*, Mycologia 101 (2009) 275–280.
[3] U.L. Diener, R.J. Cole, T.H. Sanders, G.A. Payne, S. Lee, M.A. Klich, Epidemiology of aflatoxin formation by *Aspergillus flavus*, Annu. Rev. Phytopathol. 25 (1987) 249–270.
[4] F. Wu, P. Khlangwiset, Health economic impacts and cost-effectiveness of aflatoxin reduction strategies in Africa: case studies in biocontrol and postharvest interventions, Food Addit. Contam. A 27 (2010) 496–509.
[5] J.C. Fountain, B.T. Scully, X. Ni, R.C. Kemerait, R.D. Lee, Z.Y. Chen, B.Z. Guo, Environmental influences on maize–*Aspergillus flavus* interactions and aflatoxin production, Front. Microbiol. 5 (2014) 1–7.
[6] G.S. Shephard, Impact of mycotoxins on human health in developing countries, Food Addit. Contam. A 25 (2008) 146–151.
[7] C.P. Wild, Y.Y. Gong, Mycotoxins and human disease: a largely ignored global health issue, Carcinogenesis 31 (2010) 71–82.
[8] S. Amaike, N.P. Keller, *Aspergillus flavus*, Annu. Rev. Phytopathol. 49 (2011) 107–133.
[9] R.Y. Kelley, W.P. Williams, J.E. Mylroie, D.L. Boykin, J.W. Harper, G.L. Windham, A. Ankala, X. Shan, Identification of maize genes associated with host plant resistance or susceptibility to *Aspergillus flavus* infection and aflatoxin accumulation, PLoS ONE 7 (2012) 5.
[10] X. Liang, M. Luo, B.Z. Guo, Resistance mechanisms to *Aspergillus flavus* infection and aflatoxin contamination in peanut (*Arachis hypogaea*), Plant Pathol. J. 5 (2006) 115–124.
[11] P. Chadha, R.H. Das, A pathogenesis related protein, AhPR10 from peanut: an insight of its mode of antifungal activity, Planta 225 (2006) 213–222.
[12] Z.Y. Chen, R.L. Brown, K.E. Damann, T.E. Cleveland, Identification of a maize kernel stress-related protein and its effect on aflatoxin accumulation, Phytopathology 94 (2004) 938–945.
[13] Z.Y. Chen, R.L. Brown, A.R. Lax, B.Z. Guo, T.E. Cleveland, J.S. Russin, Resistance to *Aspergillus flavus* in corn kernels is associated with a 14-kDa protein, Phytopathology 88 (1998) 276–281.
[14] Z.Y. Chen, R.L. Brown, A. Menkir, T.E. Cleveland, Identification of resistance-associated proteins in closely-related maize lines varying in aflatoxin accumulation, Mol. Breed. 30 (2012) 53–68.
[15] Z.Y. Chen, R.L. Brown, K. Rajasekaran, K.E. Damann, T.E. Cleveland, Identification of a maize kernel pathogenesis-related protein and evidence for its involvement in resistance to *Aspergillus flavus* infection and aflatoxin production, Phytopathology 96 (2006) 87–95.
[16] Z.Y. Chen, R.L. Brown, J.S. Russin, A.R. Lax, T.E. Cleveland, A corn trypsin inhibitor with antifungal activity inhibits *Aspergillus flavus* α-amylase, Phytopathology 89 (1999) 902–907.
[17] K.G. Moore, M.S. Price, R.S. Boston, A.K. Weissinger, G.A. Payne, A chitinase from Tex6 maize kernels inhibits growth of *Aspergillus flavus*, Phytopathology 94 (2004) 82–87.
[18] O. Pechanova, T. Pechan, W.P. Williams, D.S. Luthe, Proteomic analysis of the maize rachis: potential roles of constitutive and induced proteins in resistance to *Aspergillus flavus* infection and aflatoxin accumulation, Proteomics 11 (2011) 114–127.
[19] L.V. Roze, S.Y. Hong, J.E. Linz, Aflatoxin biosynthesis: current frontiers, Annu. Rev. Food Sci. Tech. 4 (2013) 293–311.
[20] T. Wang, X.-P. Chen, H.-F. Li, H.-Y. Liu, Y.-B. Hong, Q.-L. Yang, X.-Y. Chi, Z. Yang, S.-L. Yu, L. Li, X.-Q. Liang, Transcriptome identification of the resistance-associated genes (RAGs) to

Aspergillus flavus infection in pre-harvested peanut (*Arachis hypogaea*), Funct. Plant Biol. 40 (2013) 292–303.
[21] B.Z. Guo, Z.Y. Chen, R.D. Lee, B.T. Scully, Drought stress and preharvest aflatoxin contamination in agricultural commodities: genetics, genomics and proteomics, J. Int. Plant Biol. 50 (2008) 1281–1291.
[22] T. Jiang, J. Fountain, G. Davis, R. Kemerait, B. Scully, R.D. Lee, B. Guo, Root morphology and gene expression analysis in response to drought stress in maize (*Zea mays*), Plant Mol. Biol. Report. 30 (2012) 360–369.
[23] B.T. Scully, M.D. Krakowsky, X. Ni, J.P. Wilson, R.D. Lee, B.Z. Guo, Preharvest aflatoxin contamination of corn and other grain crops grown on the U.S. Southeastern Coastal Plain, Toxin Rev. 28 (2009) 169–179.
[24] A.M. Torres, G.G. Barros, S.A. Palacios, S.N. Chulze, P. Battilani, Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination, Food Res. Int. 62 (2014) 11–19.
[25] L. Yang, J.C. Fountain, T. Jiang, B.T. Scully, R.D. Lee, R.C. Kemerait, S. Chen, B. Guo, Protein profiles reveal diverse drought-responsive signaling pathways in maize kernels, Int. J. Mol. Sci. 15 (2014) 18892–18918.
[26] B.Z. Guo, J. Yu, X. Ni, R.D. Lee, R.C. Kemerait, B.T. Scully, Crop stress and aflatoxin contamination: perspectives and prevention strategies, in: B. Venkateswarlu, A.K. Shankerk, C. Shanker, M. Makeswari (Eds.), Crop Stress and Its Management: Perspectives and Strategies, Springer, New York 2012, pp. 399–427.
[27] J.C. Fountain, Y. Raruang, M. Luo, R.L. Brown, B.Z. Guo, Z.Y. Chen, Potential roles of WRKY transcription factors in regulating host defense responses during *Aspergillus flavus* infection of immature maize kernels, Physiol. Mol. Plant Pathol. 89 (2015) 31–40.
[28] P.J. Rushton, I.E. Somssich, P. Ringler, Q.J. Shen, WRKY transcription factors, Trends Plant Sci. 15 (2010) 247–258.
[29] Y. Miao, T. Laun, P. Zimmermann, U. Zentgraf, Targets of the WRKY53 transcription factor and its role during leaf senescence in *Arabidopsis*, Plant Mol. Biol. 55 (2004) 853–867.
[30] S.C. Popescu, G.V. Popescu, S. Bachan, Z. Zhang, M. Seay, M. Gerstein, M. Snyder, S.P. Dinesh-Kumar, Differential binding of calmodulin-related proteins to their targets revealed through high-density *Arabidopsis* protein microarrays, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 4730–4735.
[31] H. Li, Y. Gao, H. Xu, Y. Dai, D. Deng, J. Chen, *Zm*WRKY33, a WRKY maize transcription factor conferring enhanced salt stress tolerances in *Arabidopsis*, Plant Growth Regul. 70 (2013) 207–216.
[32] S.K. Bera, B.C. Ajay, A.L. Singh, WRKY and Na^+/H^+ antiporter genes conferring tolerance to salinity in interspecific derivatives of peanut (*Arachis hypogaea* L.), Aust. J. Crop. Sci. 7 (2013) 1173–1180.
[33] S.S. Gill, N. Tuteja, Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants, Plant Physiol. Biochem. 48 (2010) 909–930.
[34] T. Eulgem, I.E. Somssich, Networks of WRKY transcription factors in defense signaling, Curr. Opin. Plant Biol. 10 (2007) 366–371.
[35] Y. Miao, T.M. Laun, A. Smykowski, U. Zentgraf, *Arabidopsis* MEKK1 can take a short cut: it can directly interact with senescence-related WRKY53 transcription factor on the protein level and can bind to its promoter, Plant Mol. Biol. 65 (2007) 63–76.
[36] J. Wan, S. Zhang, G. Stacey, Activation of a mitogen-activated protein kinase pathway in *Arabidopsis* by chitin, Mol. Plant Pathol. 5 (2004) 125–135.
[37] B. Zhang, K. Ramonell, S. Somerville, G. Stacey, Characterization of early, chitin-induced gene expression in *Arabidopsis*, Mol. Plant-Microbe Interact. 15 (2002) 963–970.
[38] W. Ma, G.A. Berkowitz, Ca^{2+} conduction by plant cyclic nucleotide gated channels and associated signaling components in pathogen defense signal transduction cascades, New Phytol. 190 (2011) 566–572.
[39] M. Jiang, J. Zhang, Cross-talk between calcium and reactive oxygen species originated from NADPH oxidase in abscisic acid-induced antioxidant defence in leaves of maize seedlings, Plant Cell Environ. 26 (2003) 929–939.
[40] X. Hu, M. Jiang, J. Zhang, A. Zhang, F. Lin, M. Tan, Calcium–calmodulin is required for abscisic acid-induced antioxidant defense and functions both upstream and downstream of H_2O_2 production in leaves of maize (*Zea mays*) plants, New Phytol. 173 (2007) 27–38.
[41] L.V. Roze, A. Chanda, J. Wee, D. Awad, J.E. Linz, Stress-related transcription factor AtfB integrates secondary metabolism with oxidative stress response in aspergilla, J. Biol. Chem. 286 (2011) 35137–35148.
[42] K.J. Affeldt, M. Brodhagen, N.P. Keller, *Aspergillus* oxylipin signaling and quorum sensing pathways depend on G protein-coupled receptors, Toxins 4 (2012) 695–717.
[43] X. Gao, M.V. Kolomiets, Host-derived lipids and oxylipins are crucial signals in modulating mycotoxin production by fungi, Toxin Rev. 28 (2009) 79–88.
[44] C.M. Grice, M. Bertuzzi, E.M. Bignell, Receptor-mediated signaling in *Aspergillus fumigatus*, Front. Microbiol. 4 (2013) 26.
[45] A. Chanda, L.V. Roze, S. Kang, K.A. Artymovich, G.R. Hicks, N.V. Raikhel, A.M. Calvo, J.E. Linz, A key role for vesicles in fungal secondary metabolism, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 19533–19538.
[46] A. Chanda, L.V. Roze, J.E. Linz, Aflatoxin export in *Aspergillus parasiticus*: a possible role for exocytosis, Eukaryot. Cell 9 (2010) 1724–1727.
[47] J.E. Linz, A. Chanda, S.Y. Hong, D.A. Whitten, C. Wilkerson, L.V. Roze, Proteomic and biochemical evidence support a role for transport vesicles and endosomes in stress response and secondary metabolism in *Aspergillus parasiticus*, J. Proteome Res. 11 (2011) 767–775.
[48] L.V. Roze, A. Chanda, J.E. Linz, Compartmentalization and molecular traffic in secondary metabolism: a new understanding of established cellular processes, Fungal Genet. Biol. 48 (2011) 35–48.
[49] L.L. Bedard, T.E. Massey, Aflatoxin B_1-induced DNA damage and its repair, Cancer Lett. 241 (2006) 174–183.
[50] F.P. Guengerich, W.W. Johnson, T. Shimada, Y.F. Ueng, H. Yamazaki, S. Langouët, Activation and detoxication of aflatoxin B1, Mutat. Res. 402 (1998) 121–128.
[51] N. Jameson, N. Georgelis, E. Fouladbash, S. Martens, L.C. Hannah, S. Lal, Helitron mediated amplification of cytochrome P450 monooxygenase gene in maize, Plant Mol. Biol. 67 (2008) 295–304.
[52] S.X. Mideros, G.L. Windham, W.P. Williams, R.J. Nelson, *Aspergillus flavus* biomass in maize estimated by quantitative real-time polymerase chain reaction is strongly correlated with aflatoxin concentration, Plant Dis. 93 (2009) 1163–1170.
[53] M. Farooq, A. Wahid, N. Kobayashi, D. Fujita, S.M.A. Basra, Plant drought: effects, mechanisms, and management, Agron. Sustain. Dev. 29 (2009) 185–212.
[54] J.C. Fountain, Z.Y. Chen, B.T. Scully, R.C. Kemerait, R.D. Lee, B.Z. Guo, Pathogenesis-related gene expressions in different maize genotypes under drought stressed conditions, Afr. J. Plant Sci. 4 (2010) 433–440.
[55] M. Hajheidari, A. Eivazi, B.B. Buchanan, J.H. Wong, I. Majidi, G.H. Salekdeh, Proteomics uncovers a role for redox in drought tolerance in wheat, J. Proteome Res. 6 (2007) 1451–1460.
[56] A. Kakumanu, M.M. Ambavaram, C. Klumas, A. Krishnan, U. Batlang, E. Myers, R. Grene, A. Pereira, Effects of drought on gene expression in maize reproductive and leaf meristem tissue revealed by RNA-Seq, Plant Physiol. 160 (2012) 846–867.

[57] M. Reverberi, M. Punelli, V. Scala, M. Scarpari, P. Uva, W.I. Mentzen, A.L. Dolezal, C. Woloshuk, F. Pinzari, A.A. Fabbri, C. Fanelli, G.A. Payne, Genotypic and phenotypic versatility of *Aspergillus flavus* during maize exploitation, PLoS ONE 8 (2013) (e68735).
[58] B.Z. Guo, N.W. Widstrom, R.D. Lee, D.M. Wilson, A.E. Coy, Prevention of preharvest aflatoxin contamination: integration of crop management and genetics in corn, in: H. Abbas (Ed.), Aflatoxin and Food Safety, CRC Press, Boca Raton, Florida 2005, pp. 437–457.
[59] M.C. Willcox, G.L. Davis, M.L. Warburton, G.L. Windham, H.K. Abbas, J. Betrán, J.B. Holland, W.P. Williams, Confirming quantitative trait loci for aflatoxin resistance from Mp313E in different genetic backgrounds, Mol. Breed. 32 (2013) 15–26.
[60] T.D. Brooks, W.P. Williams, G.L. Windham, M.C. Willcox, H.K. Abbas, Quantitative trait loci contributing resistance to aflatoxin accumulation in maize inbred Mp313E, Crop Sci. 45 (2005) 171–174.
[61] Z. Yin, Y. Wang, F. Wu, X. Gu, Y. Bian, Y. Wang, D. Deng, Quantitative trait locus mapping of resistance to *Aspergillus flavus* infection using a recombinant inbred line population in maize, Mol. Breed. 33 (2014) 39–49.
[62] M.L. Warburton, W.P. Williams, Aflatoxin resistance in maize: what have we learned lately? Adv. Bot. 2014 (2014) 10.
[63] G.D. Almeida, D. Makumbi, C. Magorokosho, S. Nair, A. Borém, J.M. Ribaut, M. Bänziger, B.M. Prasanna, J. Crossa, R. Babu, QTL mapping in three tropical maize populations reveals a set of constitutive and adaptive genomic regions for drought tolerance, Theor. Appl. Genet. 126 (2013) 583–600.
[64] P.M. Visscher, D. Posthuma, Statistical power to detect genetic loci affecting environmental sensitivity, Behav. Genet. 40 (2010) 728–733.
[65] B.Z. Guo, M.D. Krakowsky, X. Ni, B.T. Scully, R.D. Lee, A.E. Coy, N.W. Widstrom, Registration of maize inbred line GT603, J. Plant Regist. 5 (2011) 211–214.
[66] B.Z. Guo, N.W. Widstrom, R.D. Lee, A.E. Coy, R.E. Lynch, Registration of maize germplasm GT601 (AM-1) and GT602 (AM-2), J. Plant Regist. 1 (2007) 153–154.
[67] B.T. Scully, M.D. Krakowsky, X. Ni, P.J. Tapp, J.K. Knoll, R.D. Lee, B.Z. Guo, Registration of maize inbred line 'GT888', J. Plant Regist. 9 (2015) (in press).
[68] W.P. Williams, M.D. Krakowsky, B.T. Scully, R.L. Brown, A. Menkir, M.L. Warburton, G.L. Windham, Identifying and developing maize germplasm with resistance to accumulation of aflatoxins, World Mycotoxin J. 8 (2014) 193–209.
[69] W.P. Williams, G.L. Windham, P.M. Buckley, Diallele analysis of aflatoxin accumulation in maize, Crop Sci. 48 (2008) 134–138.
[70] R.L. Brown, A. Menkir, Z.Y. Chen, D. Bhatnagar, J. Yu, H. Yao, T.E. Cleveland, Breeding aflatoxin-resistant maize lines using recent advances in technologies — a review, Food Addit. Contam. A 30 (2013) 1382–1391.
[71] J.M. Wagacha, J.W. Muthomi, Mycotoxin problems in Africa: current status, implications to food safety and health and possible management strategies, Int. J. Food Microbiol. 124 (2008) 1–12.
[72] T.H. Sanders, P.D. Blankenship, R.J. Cole, R.A. Hill, Effect of soil temperature and drought on peanut pod and stem temperatures relative to *Aspergillus flavus* invasion and aflatoxin contamination, Mycopathologia 86 (1984) 51–54.
[73] T. Girdthai, S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C.C. Holbrook, A. Patanothai, Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought, Plant Breed. 129 (2010) 693–699.
[74] P. Sudhakar, P. Lathat, M. Babitha, P.V. Reddy, P.H. Naidu, Relationship of drought tolerance traits with aflatoxin contamination in groundnut, Indian J. Plant Physiol. 12 (2007) 261–265.
[75] J. Dorner, R. Cole, T. Sanders, P. Blankenship, Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in pre-harvest aflatoxin contamination of drought-stressed peanuts, Mycopathologia 105 (1989) 117–128.
[76] J.I. Pitt, M.H. Taniwaki, M.B. Cole, Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of Food Safety Objectives, Food Control 32 (2013) 205–215.
[77] S.D. Golombek, C. Johasen, Effect of soil temperature on vegetative and reproductive growth and development in three Spanish genotypes of groundnut (*Arachis hypogaea* L.), Peanut Sci. 24 (1997) 67–72.
[78] A. Arunyanark, S. Jogloy, S. Wongkaew, C. Akkasaeng, N. Vorasoot, G.C. Wright, R.C.N. Rachaputi, A. Patanothai, Association between aflatoxin contamination and drought tolerance traits in peanut, Field Crop Res. 114 (2009) 14–22.
[79] T. Girdthai, S. Jogloy, N. Vorasoot, C. Akkasaeng, S. Wongkaew, C.C. Holbrook, A. Patanothai, Heritability of, and genotypic correlations between, aflatoxin traits and physiological traits for drought tolerance under end of season drought in peanut (*Arachis hypogaea* L.), Field Crops Res. 118 (2010) 169–176.
[80] B.Z. Guo, J. Yu, C.C. Holbrook, T.E. Cleveland, W.C. Nierman, B.T. Scully, Strategies in prevention of preharvest aflatoxin contamination in peanuts: aflatoxin biosynthesis, genetics and genomics, Peanut Sci. 36 (2009) 11–20.
[81] B.Z. Guo, C.Y. Chen, Y. Chu, C.C. Holbrook, P. Ozias-Akins, H.T. Stalker, Advances in genetics and genomics for sustainable peanut production, in: N. Benkeblia (Ed.), Sustainable Agriculture and New Biotechnologies, CRC Press, Boca Raton, Florida 2012, pp. 341–367.
[82] T. Wang, E.H. Zhang, X.P. Chen, L. Li, X.Q. Liang, Identification of seed proteins associated with resistance to pre-harvested aflatoxin contamination in peanut (*Arachis hypogaea* L.), BMC Plant Biol. 10 (2010) 267–278.
[83] Z. Wang, S. Yan, C. Liu, F. Chen, T. Wang, Proteomic analysis reveals an aflatoxin-triggered immune response in cotyledons of *Arachis hypogaea* infected with *Aspergillus flavus*, J. Proteome 11 (2012) 2739–2753.
[84] C.C. Holbrook, C.K. Kvien, K.S. Rucker, D.W. Wilson, J.E. Hook, Preharvest aflatoxin contamination in drought tolerant and intolerant peanut genotypes, Peanut Sci. 27 (2000) 45–48.
[85] F. Hamidou, A. Rathore, F. Waliyar, V. Vadez, Although drought intensity increases aflatoxin contamination, drought tolerance does not lead to less aflatoxin contamination, Field Crops Res. 156 (2014) 103–110.
[86] C.C. Holbrook, B.Z. Guo, D.M. Wilson, P. Timper, The U.S. breeding program to develop peanut with drought tolerance and reduced aflatoxin contamination, Peanut Sci. 36 (2009) 50–53.
[87] S.N. Nigam, F. Waliyar, R. Aruna, S.V. Reddy, P.L. Kumar, P.Q. Craufurd, A.T. Diallo, B.R. Ntare, H.D. Upadhyaya, Breeding peanut for resistance to aflatoxin contamination at ICRISAT, Peanut Sci. 36 (2009) 42–49.
[88] S.R. Milla, T.G. Isleib, S.P. Tallury, Identification of AFLP markers linked to reduced aflatoxin accumulation in *A. cardenasii*-derived germplasm lines of peanut, Proc. Am. Peanut Res. Educ. Soc. 37 (2005) 90.
[89] X. Liang, G. Zhou, Y. Hong, X. Chen, H. Liu, S. Li, Overview of research progress on peanut (*Arachis hypogaea* L.) host resistance to aflatoxin contamination and genomics at the Guangdong Academy of Agricultural Sciences, Peanut Sci. 36 (2009) 29–34.