

Review Article

Aflatoxin Resistance in Maize: What Have We Learned Lately?

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Aflatoxin contamination of maize grain is a huge economic and health problem, causing death and increased disease burden in much of the developing world and income loss in the developed world. Despite the gravity of the problem, deployable solutions are still being sought. In the past 15 years, much progress has been made in creating resistant maize inbred lines; mapping of genetic factors associated with resistance; and identifying possible resistance mechanisms. This review highlights this progress, most of which has occurred since the last time a review was published on this topic. Many of the needs highlighted in the last reviews have been addressed, and several solutions, taken together, can now greatly reduce the aflatoxin problem in maize grain. Continued research will soon lead to further solutions, which promise to further reduce and even eliminate the problem completely.

1. Introduction to the Problem

Although the detrimental health effects of aflatoxins and grain contamination have been known for over 50 years [1–5], a satisfactory solution has yet to be attained, from either a health or economic point of view. Since the publication of earlier reviews [6–11], encouraging advances have been achieved, but much remains to be done. The more recent reviews by Gorman and Kang [7]; Brown et al. [10]; and Moreno and Kang [11] highlighted the need for new sources of resistant germplasm, which have since been identified. QTL mapping was also called for, and several mapping studies and one meta-analysis have now presented the genetic architecture of aflatoxin resistance in maize in many of the most resistant lines. The suggestion of the use of nontoxigenic *A. flavus* in the review of Moreno and Kang [11] has been implemented with considerable success in some countries. Other suggestions as to specific genetic mechanisms of resistance, tools to study the problem including laboratory assays and simpler inoculation procedures, nixtamalization and chemical remediation treatments, have not yet contributed to appreciable progress outside of a limited scope. However, all information, taken together, and added to new proteomic, genomic, and genetic studies, is beginning to fill in the larger picture of this

very complicated disease problem of maize and other oil-seed crops.

2. Aflatoxins

Aflatoxins are hepatotoxic, carcinogenic secondary metabolites produced by some species of the fungal genus *Aspergillus*. Aflatoxin is one of the most toxic compounds found in nature, and it can be lethal to humans and other animals in amounts measured in parts per billion. In much of the world, the aflatoxin content in maize is highly regulated, making this more of an economic rather than a health problem, as infected grain is destroyed before it can enter the food stream. In some countries of the world, however, regulatory infrastructure does not enable inspection and enforcement. Thus, much of the infected maize is eaten, often within the household of the farmer who produced it. Long term exposure to sublethal doses of aflatoxin has been linked to liver cancer, stunted growth during childhood, and depressed immune systems [12, 13]. It has been suggested that epidemics of AIDS, malaria, tuberculosis, and other diseases in many developing world countries were exacerbated by long term exposure of the population to aflatoxin-infected maize [14].

3. Resistant Germplasm

Recent reviews identified a need for more germplasm with either genetic resistance to infection and growth of *A. flavus* in the grain or the ability to suppress fungal production of aflatoxin following infection [10, 11]. Early resistant germplasm characterization studies used the percentage of infected kernels in an ear or the grain aflatoxin levels to identify resistant lines [15–18]. These early sources of resistance include Mp313E, SC54, Mp420, and Tex6 [18, 19]. With the exception of Mp313E, expression of the resistance in these sources of germplasm tended to be highly dependent on the environment in which they were grown, and thus a line identified as resistant in Illinois might have been scored as susceptible in Texas. However, early studies using these lines determined that much of the resistance was highly quantitative tended to be inherited in an additive fashion and led to high general combining ability (GCA) in hybrids [16, 20–23]. Occasional epistatic, dominant, and reciprocal effects were also seen in diallel experiments, possibly limiting the utility of resistance in hybrids [16, 20, 22–24].

Newer breeding lines and populations with high and repeatable resistance under varying environments have been released; these include Mp715, Mp717, GT-MAS:GK, CML176, CML269; CML322, and Tx114 [20, 25–28]. While stable resistance is a positive advancement in the efforts to breed resistant maize cultivars, all resistant breeding lines identified to date contain tropical germplasm in their backgrounds. Thus, they tend to be tall, late, and prone to lodging, in addition to lower yielding than commercial hybrid checks. Because of the highly quantitative nature of host plant genetic resistance to *A. flavus* infection and aflatoxin accumulation in maize, it has been very difficult to transfer the resistance from these older breeding lines into a more agronomically acceptable idiosyncrasy using only phenotypic selection. However, some of the newest breeding lines including Mp718, Mp719, Tx736, Tx739, and Tx740 [29, 30] that have recently been released show a much better plant type and high resistance.

There have been few recent large scale attempts to identify new resistant maize lines, but two have shown some promising results. First, the Genetic Enhancement of Maize (GEM) program has included aflatoxin accumulation resistance as a characterization criteria for some of the lines developed by GEM cooperators and has identified resistant germplasm in the process [31, 32]. These lines, displaying resistance in at least one field site, are in the process of further characterization. Second, an aflatoxin association mapping panel containing 300 maize lines has recently been publicly released, and 30 to 40 lines displaying good resistance in up to 7 environments are now available for use [33, 34]. Several of these inbreds have already been included in a new joint USAID/USDA project, together with two CGIAR centers, to incorporate as many of these lines into ongoing genetics studies and breeding activities as possible, with the goal of creating resistant OPV and hybrid cultivars as quickly as possible. The association mapping panel contained all known resistance sources identified up to the time of the assembly of the panel; lines suspected to be resistant due to origin or other stress resistance qualities; and many unknown lines to expand the overall levels of

diversity in the panel. Phenotyping of the panel revealed that the most resistant lines include the previously identified resistant germplasm and many new lines that may now be used for breeding and genetics studies. Pedigrees of a majority of the lines identified as resistant trace back, all or in part, to one Mexican maize landrace, Tuxpeño [33, 34]. This landrace has been used extensively in the creation of many of the maize breeding pools and populations of the International Maize and Wheat Improvement Center (CIMMYT) because it is a high yielding, agronomically superior dent population with good GCA.

4. Mapped Aflatoxin Resistance QTL

Past reviews of the aflatoxin problem in maize had also recommended the mapping of quantitative trait loci (QTL) for resistance to *A. flavus* infection or aflatoxin suppression. Eight QTL mapping experiments on *A. flavus* or aflatoxin resistance [35–42] and one additional on ear rot due to *A. flavus* [43] have been published to date. Most of these QTL studies have reported multiple QTL, most of which have been found in only one environment, and the majority of the QTL each account for less than 5% of the phenotypic variation observed in the population and the environment in which it was measured. Nevertheless, in every QTL mapping experiment, there has been at least one phenotypically large (explaining >10% of the observed variation, reaching up to >20% in some cases) and/or repeatable (occurring in more than one test environment or genetic background) QTL identified. Some of these QTL map to similar locations in different populations, and it has been posited that some of them are being caused by the same underlying gene in the different studies.

When data for more than three or four QTL studies are available, it is possible to run a meta-analysis on the data. This analysis combines mapping data and QTL effect data from all individual studies into a single projected map. This map will now contain all markers, all QTL, and those QTL that have overlapping confidence intervals will be re-calculated into a single QTL (generally with a smaller and more robust genetic interval). A meta-analysis including six of the QTL studies (for which sufficient data or information could be obtained by the authors at the time of analysis) has been published to test the hypothesis of co-location of QTL from different studies [42]. Six additional QTL studies of *Gibberella* (*Fusarium graminearum* (teleomorph: *Gibberella zeae*)) and *Fusarium verticillioides* (teleomorph: *Gibberella moniliformis*) ear rots were included to determine overlap of resistance QTL to different fungal ear rots. The meta-analysis found 62 meta-QTL on all chromosomes except 9 and 10 (Figure 1), and the majority of meta-QTL was created by the combination of QTL from multiple studies or ear rot traits. Meta-QTL confidence intervals were much smaller than those of QTL from individual experiments, which will greatly benefit marker-assisted breeding efforts. The meta-QTL on 4.08 was for aflatoxin accumulation only, but it contained QTL from four different studies and three independent resistance sources. The meta-analysis found a total of 12 independent QTL from all three ear rot fungi. Genetic intervals on the maize chromosomes

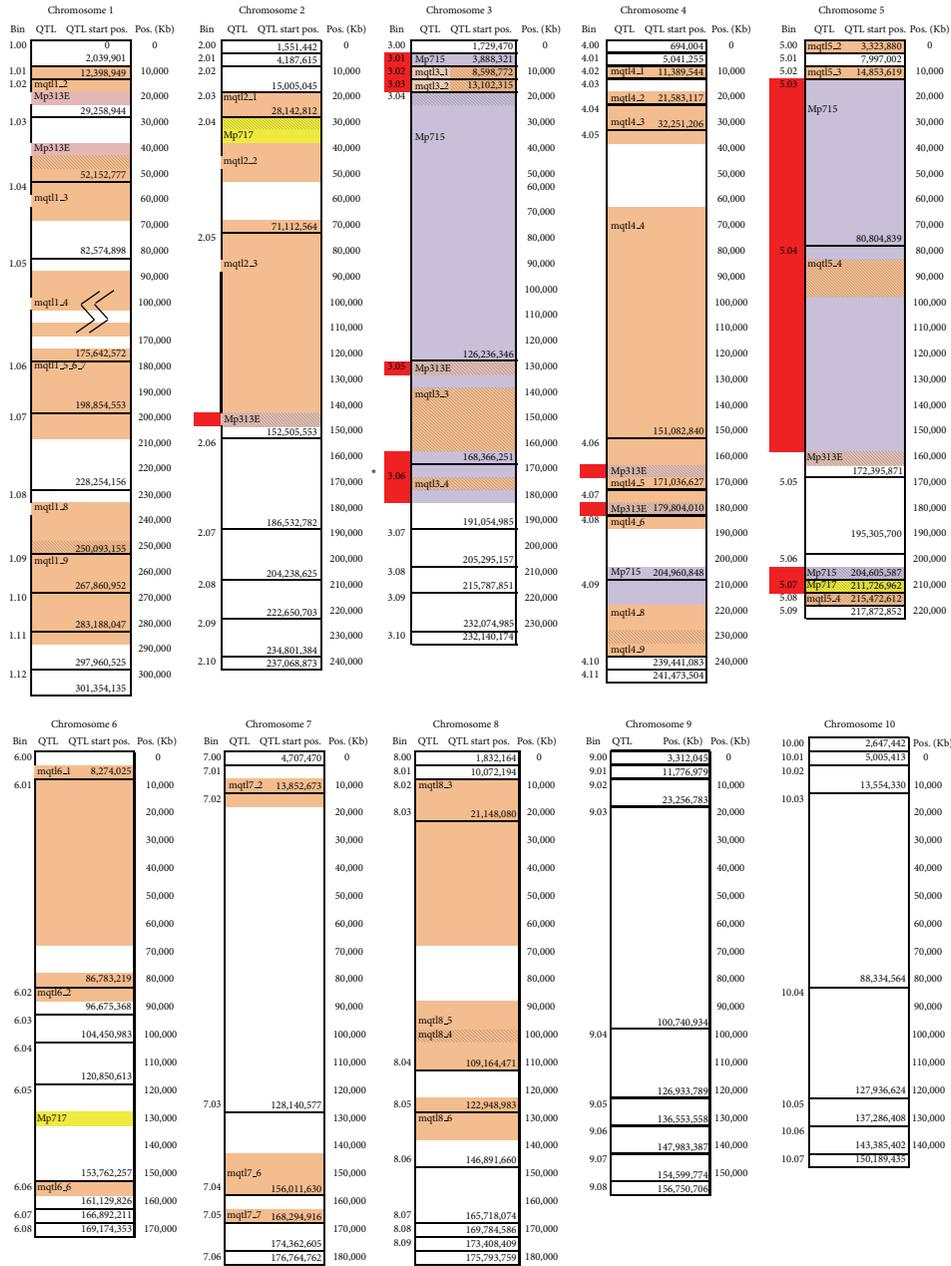


FIGURE 1: QTL for aflatoxin accumulation resistance from Mp313E (pink shaded), Mp717 (yellow shaded), and Mp715 (purple shaded). MetaQTL of 6 maps (orange shaded). QTL overlap indicated with hatched fill. QTL regions being introgressed into near isogenic lines highlighted in red at the left side of each chromosome.

are defined as bins, which are numbered by chromosome and relative position along the chromosome. The 12 QTL from the meta-analysis fell into bins 4.07 to 4.09, a region spanning 65.7 Mb or 3% of the maize genome. Wisser et al. [44] also found that bins 4.07 and 4.08 contained more than twice the expected number of QTL for multiple diseases after correcting for gene density. These results indicate that the 4.07/8 region of the maize genome contains a cluster of genes influencing the response of maize to multiple pathogens. Additional meta-QTL for aflatoxin only were found on chromosomes 1–8 (Figure 1). On this figure, QTL from resistant lines Mp715 and Mp313E that are being used to develop

near isogenic lines (NILs) for independent validation of phenotypic effect are marked in red highlight.

5. Other Aflatoxin Resistance Candidate Genes

Several recent studies have investigated differential gene expression or proteins that may contribute to aflatoxin resistance. These studies often produce a list of possible candidate genes or proteins that may be prioritized based on strength of differential expression or possible biological importance of individual sequences to fungal resistance or, less certainly, possible disruption of aflatoxin production by *A. flavus*.

These studies identified differentially expressed proteins from resistant versus susceptible maize genotypes both with [45–48] and without [49, 50] infection of *A. flavus* during the experiment.

Genome-wide association studies (GWAS) have also been brought to bear on the aflatoxin problem in maize. Because most maize germplasms do not display resistance to *A. flavus* infection or aflatoxin accumulation, the association mapping panel developed by Warburton et al. [33] contains resistant germplasm that has been genotyped via Genotype By Sequencing (GBS, [51]). Two studies based on this germplasm have now been completed. The first, using some of the lines from the association mapping panel, found no genetic sequences associated with aflatoxin accumulation [52]. The second, using more SNPs for GWAS and the entire panel, found several gene sequences associated with maize grain aflatoxin levels, with a high level of probability [33, 34]. Twenty-one genetic regions were associated with aflatoxin reduction in grain at $P < 10^{-6}$ in more than one environment. These regions will be independently validated in NILs, RILs, or transgenic lines. Validation will give breeders the confidence necessary to begin large scale plant improvement via marker assisted selection with these genes.

Although these first large scale surveys of the genetic, genomic, and proteomic landscapes are an excellent first look at the possible genes contributing to *A. flavus* infection or aflatoxin accumulation resistance, they typically generate a long list of differentially expressed or associated genes and proteins, many of which will prove to have nothing to do with resistance. This is because the resistant and susceptible maize lines being compared are unrelated in all cases to date and will differ for many genes, not only resistance genes. High variation in gene and protein expression studies also obfuscates true differential expression. Thus, we see the need for follow-up experiments, preferably in independent genetic backgrounds, to ensure that these sequences cause resistance.

Validation of the sequences identified by the proteomics and genomics approaches has begun, mostly on a gene-by-gene basis. A candidate gene testing pipeline that includes QTL mapping and association mapping populations [53] has validated a chloroplast precursor identified by Mylroie et al. [54], a trypsin inhibitor, and three members of the chitinase gene family by Warburton et al. [55]. Transgenic or mutant lines overexpressing or silencing the gene of interest were used to study the trypsin inhibitor gene [56], the PR10 gene [57], and the genes encoding lipooxygenase ZmLox10 [58] and lipooxygenase ZmLox3 [59]. Quantitative PCR has confirmed the differential expression of genes and proteins, although their functions have not been characterized [47, 60]. Finally, reduced levels of aflatoxin can be correlated with a specific enzyme activity or protein component from infected maize kernels, for example, an aldose reductase [49], pericarp wax components [61], and 1- α Cys peroxiredoxin (PER 1), confirmed to be more expressed in one resistant maize inbred line than a susceptible inbred by Chen et al. [62] but not associated with aflatoxin resistance in an association mapping panel by Warburton et al. [33]. Similar validation studies continue with gene or protein sequences identified by means other than the genetics, genomics, and proteomics methods described

above. These include reports in the literature of antifungal properties in other organisms, such as the β -1,3-glucanase gene (confirmed to have an inhibitory effect on *A. flavus* growth in maize callus by [63]), other chitinases (see section below; [33, 55, 64]), and the maize ribosomal inactivating protein (RIP; [65]).

Due to the large numbers of possible gene candidates being generated by these studies, an attempt to organize all the information into a searchable database has led to the creation of the Corn Fungal Resistance Associated Sequences database (CFRAS-DB; [66]), which is available to the public at <http://www.agbase.msstate.edu/cgi-bin/information/Maize.pl>. This database compiles all genetic and protein sequences and QTL regions that have been reported as associated with *A. flavus* or aflatoxin accumulation resistance in maize. These sequences and regions are all defined by their genomic location in relation to the B73 genomic sequence, so they are comparable and can be searched simultaneously. Identifying sequences with multiple lines of evidence for a role in resistance is therefore possible.

6. Other Traits Known to Influence Aflatoxin Resistance Indirectly

Insects are a common vector for spreading *A. flavus*. Thus, insect resistant maize germplasm was initially seen as a possible route to lower aflatoxin accumulation in maize as well. The highly quantitative nature of aflatoxin resistance itself and the high G x E associated with it made selection gain on the correlated insect resistance trait much easier. Studies presented work on corn ear worm (*Helicoverpa zea* Boddie) resistance by Widstrom et al. [67]; corn borer (*Diatraea grandiosella*) by Williams et al. [68] and Windham et al. [69]; ear borer (*Mussidia nigricornis*) by Setamou et al. [70]; weevil (*Sitophilus zeamais* Motschulsky) by McMillian et al. [71]; and several other less frequent arthropod carriers of *A. flavus* spores [72]. These studies indicated that the aflatoxin problem in maize could be partially remedied by conventional breeding or transgenic solutions to the insect vectors that introduce *A. flavus* spores directly into the developing maize ears. Unfortunately, it has since been shown that *A. flavus* can infect the maize ear via the silks and other entry points [73–75] other than insect damage. Thus, elimination of aflatoxin has not been achieved simply by combating insect vectors of transmission.

Other traits are linked to aflatoxin reduction in pre- or postharvest maize ears. A tight husk and non-upright ear prevent entry of spores and keep the ear dryer, making it a less conducive environment for fungal growth [76]. Harder kernels impede fungal penetration [77], and drought tolerant lines show significantly lower aflatoxin levels [78]. Maturity plays a role in reduction of aflatoxin, but only in some environments. It is not possible to predict in all cases if early or late maturing varieties will have less aflatoxin accumulation, because accumulation depends on the location and the weather [11, 75]. Although insect and drought resistance always play a positive role in reducing aflatoxin levels when insects or drought is present, other traits may be less useful, because the ideal maturity to escape *A. flavus* or aflatoxin

production cannot be predicted in advance, a problem further exacerbated by global climate change. Tight husks are not preferred by many US farmers, and optimal grain hardness is dictated by market pressures and end use, and not only by resistance. Thus, more targeted options for *A. flavus* resistance are necessary.

7. Seeing the Big Picture in *A. flavus* and Aflatoxin Resistance

Known basal fungal resistance mechanisms that evolved in plants include chitinolytic enzymes that digest fungal cell wall components. Chitinases and related hydrolases have been shown to limit the infection and colonization of maize by *A. flavus* [55, 63, 64, 79, 80]. Tissue-specific expression of potent chitinases may be one approach for building resistant maize. The vast numbers of candidate gene and protein sequences identified to date have highlighted other possible specific resistance mechanisms against infection and growth of *A. flavus*, or the production of aflatoxin by the fungus. For example, Woloshuk et al. [81] found an *A. flavus* α -amylase to play a role in the production of aflatoxin, and Chen et al. [82] found a maize trypsin inhibitor that reduced activity of the *A. flavus* α -amylase. Expression of α -amylase inhibitors from maize, or stronger inhibitors from other organisms, may limit the production of aflatoxin by limiting the availability of simple sugars. However, it may not stop the infection of *A. flavus* and subsequent ear rot and yield loss. Increased expression of jasmonic acid, the upstream phytohormone which increases the production of α -amylase, thus increases other protease inhibitors and plant polyphenol oxidase (PPO) enzymes, all of which may interfere with fungal growth. Jasmonic acid is a versatile signaling mechanism for many other pathways as well, and investigation into how it specifically affects *A. flavus* continues.

Another class of enzymes brought to light by previous studies and by association mapping in this laboratory includes the lipoxygenase enzymes encoded for by the LOX family of genes that produce at least 500 distinct oxygenated fatty acid species, collectively known as oxylipins, which may influence fungal growth, sporulation, and toxin production. These genes regulate reproduction, development, and secondary metabolism in both flowering plants and fungi; they are known to directly facilitate both plant resistance and susceptibility to pests and pathogens depending on specific molecular species of oxylipins they produce and specific plant-pathogen interaction. Growing evidence suggests that fungal performance on compatible hosts is a complicated and evolving communication system utilizing oxylipin signals where each species tries to get the upper hand over the other [83, 84]. Oxylipins such as jasmonates exert many of their functions through their ability to regulate signal cross-talk with other hormones [84].

Phenylpropanoids (phenolics and flavonoids such as chlorogenic acid), alkaloids, terpenoids, polyamines, and volatiles including plant signaling molecules are all known to regulate fungal aflatoxin production by either modifying the growth environment in the maize kernel, inhibiting signaling circuits upstream of the biosynthetic pathway, or inhibiting

gene or enzymatic expression in the pathway [85, 86]. These volatile signaling molecules include phytoalexins, which are known to decrease production of aflatoxin in cotton [87], and are produced in response to *A. flavus* infection in peanut [88]. Nonvolatile terpenoid phytoalexins are also being studied in maize as possible elicitors of defense against mycotoxin-producing fungal pathogens [89]. Lipids are known to be involved in cross-species signaling, especially between hosts and pathogens. The role of these diverse compounds in the inhibition of aflatoxin, like the α -amylase inhibitors, may lead to a native or transgenic solution to aflatoxin contamination, but may or may not control ear rot.

Constitutive resistance in the kernels of maize germplasm that does not allow unregulated growth of the fungus may include barriers to penetration and growth of the hyphae. Wax thickness and composition in the pericarp layer of the kernel have been reported to inhibit initial infection [61, 90]. Recent microscopy studies of *A. flavus* hyphal growth inside inoculated maize kernels suggest barriers inside the kernel may also prevent spread through the hilar layer through which the *A. flavus* mycelia typically enters the kernel [91] or colonization within the kernel, in an apparent attempt to protect the embryo itself [92]. The basal endosperm transfer cells (BETCs) within the endosperm tissue are the site of increased invertase activity in developing seeds [93], and invertase has been shown to increase resistance to biotic and abiotic stress [94]. Other genes expressed in this layer may slow fungal growth as well. The composition of the kernel itself, beyond simple kernel hardness or internal physical barriers, may play a significant role in the slowing of infection or growth of *A. flavus*. Mideros et al. [75] found a significant correlation of resistance to aflatoxin accumulation with kernel composition traits including fiber, ash, and carbohydrate. Brown et al. [95] found a correlation between resistance and high lipid content in the seed embryo and Chen et al. [49] found modified storage proteins (globulin 1 and 2) are associated with resistance. It may be that the maize seed can be modified in order to make it less "palatable" or hospitable to the colonizing *A. flavus*.

Many candidate genes do not offer obvious clues as to a resistance mechanism, and the sheer number of associated sequences has complicated the search for resistance mechanisms. An attempt by the laboratory of the authors of this review is being made to use all genes from the GWAS results of Warburton et al. [33] in a pathway analysis in order to find commonalities between some of the candidate genes and identify metabolic or enzymatic pathways that increase resistance. Similar analyses may be run on gene expression studies of *A. flavus* resistance in maize as they become available. It is likely that there is more than one mechanism by which resistance to *A. flavus* and aflatoxin accumulation can be increased. Although complete resistance has never been reported in maize, some breeding lines with very low levels of aflatoxin in inoculated field trials have been found. These resistant lines are the ones used by most breeders trying to increase resistance in their selection programs and have been used as the parents of most QTL mapping projects. Although many of these lines may trace their ancestry back to the Tuxpeño maize landrace from Mexico mentioned earlier,

some of the resistant lines appear to be highly unrelated to the majority of the resistant germplasm. It is therefore logical to assume that they may have evolved different mechanisms for resistance.

Because the final trait— aflatoxin levels in maize grain—is a result of many prior steps (initial infection; fungal growth; toxin production) and each step is dependent on a raft of factors (environmental conditions; multiple host genotypes; multiple pathogen genotypes), the opportunities for the plant to avoid colonization by the fungus and accumulation of aflatoxin are numerous. Unfortunately, however, since the toxin has a minimal negative effect on the maize plant itself, and because *A. flavus* is not an aggressive pathogen, natural selection has not favored the development of strong resistance. Furthermore, because the importance of aflatoxin to the fungus is still not well understood [96], developing an effective strategy to block its production becomes even more difficult. Casting a wide net to find multiple sources of resistance, and pyramiding them, may be a better bet.

8. Will These New Results Lead to Practical Resistance?

The increased number of maize breeding lines showing resistance to *A. flavus* infection or spread, and aflatoxin accumulation, gives breeders ever increasing options for their own improvement programs. Although the background of most, if not all, resistant lines are tropical, many of the newest lines are products of crosses and backcrosses between tropical and temperate germplasm. Many of these lines were developed from breeding crosses and hybrids obtained through the GEM project [22, 23, 29]. The most resistant breeding lines are also actively being used by two centers in the Consultative Group on International Agriculture Research (CGIAR), the International Institute for Tropical Agriculture (IITA), and the International Maize and Wheat Improvement Center (CIMMYT) and have been requested by two small seed companies in sub-Saharan Africa (data not shown).

Several QTL and one meta-QTL study of aflatoxin or *A. flavus* induced ear rot have provided genomic locations of regions from the resistant lines that provide a stable increase in resistance. Each of these QTL generally explains between 5% and 20% of the phenotypic variation for resistance. The effect of some of these QTL may have been overestimated due to the Beavis effect [97]. However, MIM models of resistance including the top 5–8 QTL can account for over half of the phenotypic variation, and therefore it is hoped that these will be worth breeding for via marker assisted selection (MAS). Because at least one large-effect QTL (in bin 4.08) has been found in multiple genetic mapping populations and verified in a recombinant inbred line population [42], there is reason to believe that some of these QTL are not simply the result of a statistical amplification effect. To ensure this, the larger-effect QTL regions (and those that are stable across more than one environment) are being validated in near isogenic lines (NILs) in the Corn Host Plant Research Resistant Unit (CHPRRU, to which the authors belong). These QTL are being backcrossed to different maize lines, in order to verify the stability of effect in different genetic backgrounds. The

NILs created with these QTL are just now nearing fixation, the point where the background is nearly identical to the recurrent (originally susceptible) parent except for the QTL being transferred, which will be homozygous for the donor (resistant parent) allele). Early phenotypic trials with nearly-fixed lines are very encouraging [34]. In addition to NILs created with QTL, the SNPs found to be associated with aflatoxin reduction in the GWAS study by Warburton et al. [33] are being used in initial crosses to create NILs.

It is hoped that these NILs will validate the phenotypic effect and allow a convenient resource to pyramid QTL or associated SNPs into a single genetic background. Once the NILs are fixed for one or more regions that increase resistance, crossing the NILs created with the same genetic background and with alternative QTLs or SNPs allows the fixation of multiple SNPs and QTL in one line via MAS with small populations and only a few generations for fixation. With a few such steps of crossing and fixing, a line can be created containing all known genomic regions that increase aflatoxin resistance. It is hoped that such a line will be as resistant (or more) as the most resistant lines found to date, but more stable in the expression of resistance across diverse environments. This is because each QTL may express differently in diverse environments, probably because distinct mechanisms of resistance are expressed in response to different environmental cues. One line containing all mechanisms, especially all that behave with an additive gene action, will certainly provide stable resistance in many environments.

The total number of markers possibly associated with resistance coming out of the mapping experiments reviewed here, as well as other studies including Brown et al. [98] and Chen et al. [99], is very large. Because the creation of NILs is costly and time consuming, many of the SNPs with smaller phenotypic effects, and those only identified in one environment, will not be used in an NIL validation experiment. While the possibilities of these SNP associations being nothing more than false positives, having nothing to do with resistance, are fairly high in each case, as a group, many of them will increase resistance of lines containing the resistant form or the sequence polymorphism. Although MAS with each would be a poor use of resources, using these SNPs in an index selection or to guide genomic selection is a real possibility [100]. Furthermore, a recurrent reciprocal selection scheme via MARS may allow for the efficient creation of new, stably resistant inbred lines. The additive nature of many of the associated SNPs and QTL encourages this approach.

Understanding the specific mechanisms and pathways involved in resistance will lead to the ability to manipulate the key genes in a pathway to increase the expression of the trait. This has been done with considerable success in maize with carotenoid content and pro-Vitamin A content using natural variants present in the maize primary gene pool [101, 102]. With the more complicated and quantitative aflatoxin resistance trait, such straightforward selection may not be possible, but key points in pathways will certainly become targets of selection or transgenic engineering.

A better understanding of the fungus and the conditions under which it produces aflatoxin will also lead to solutions. Developing maize that can block the environmental cues

promoting aflatoxin synthesis by the fungus may be one way to reduce toxin levels in the plant. In essence, rather than selecting plants that resist the fungus, one would select plants that tolerate the fungus but inhibit toxin expression, similar to the logic of slow-rusting wheat plants, which allow for the slow growth of the fungal pathogen *Puccinia* ssp. Rather than trying to suppress infection (thus guaranteeing continued evolution of yet more virulent forms of the fungus), slow-rusting wheat tolerates a fungal load that does not appreciably reduce yield [103]. Such a breeding scheme would be similar to the strategy whereby nontoxigenic strains of *A. flavus* are applied to compete with and suppress the growth of the toxigenic forms. A better understanding of the fungus and the role of aflatoxin metabolism may also lead to more direct methods for the elimination of the fungus or toxin. The creation of transgenic lines that use RNA interference (RNA-i) or synthetic peptides is being advanced now [104, 105]. These technologies could have near term application for host induced gene silencing, as targets in the aflatoxin biosynthesis pathway are identified.

9. Conclusions

Solving the aflatoxin problem in maize will require a multi-pronged and coordinated approach. In the developing world, better postharvest storage and food preparation (i.e., nixtamalization) will provide the largest benefits quickly. Biocontrol and the use of atoxigenic *A. flavus* may reduce the problem in the short term as well, and over time the level and durability of control will become apparent. In the long term, the creation of resistant germplasm will be the most economically efficient control measure for all markets. New breeding lines, validated QTL and SNPs associated with resistance, and optimized markers for MAS, MARS, and GS, will speed the effort; these tools are now, or will soon be, available. Transgenic approaches are feasible and may spur interest in working on the trait in the private sector. Progress in preventing aflatoxin in maize has been slow. Some of the hoped for technologies and advances, such as short cuts for evaluating resistance to aflatoxin accumulation in the laboratory and more efficient procedures for evaluating resistance in the field, are still in development. More work is needed to understand the reasons and conditions under which aflatoxin is produced. However, there have been significant advances since the last reviews highlighted the needs and the direction of research, and they have been surveyed in this article. With further education for producers and consumers, and the deployment of resistant germplasm created from available inbred parents, the problem of aflatoxin in maize could soon be solved.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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