AFLATOXINS: FINDING SOLUTIONS FOR IMPROVED FOOD SAFETY

Managing Aflatoxin Contamination of Maize: Developing Host Resistance

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Aflatoxins are toxic and highly carcinogenic secondary products produced by the *Aspergillus flavus* (*A. flavus*) and *Aspergillus parasiticus* family of molds. When produced on a susceptible crop, aflatoxins contaminate maize grain products, threatening human and animal health. *A. flavus* is an opportunistic pathogen occurring with higher incidence on maize grown under stressed conditions, including late-season drought and high temperatures during kernel filling. Insect or mechanical damage to kernels can increase the infection rate of *A. flavus* and aflatoxin levels, which can also worsen with poor harvesting and storage conditions because grain that is insufficiently dried prior to storage provides an ideal environment for fungal growth. In addition to proper storage conditions, management strategies to reduce aflatoxin contamination also include biological control: the use of non–toxin producing *A. flavus* strains to prevent further infection by toxin-producing strains. Additionally, decontamination is sometimes possible, although the decontaminating agents themselves may be harmful and expensive. An important, safe, and preventative strategy for aflatoxin elimination is the development of host-plant resistance in order to inhibit fungal colonization or toxin production. Host resistance is an economical approach that is easy to disseminate, requires no additional production or management resources, leaves no harmful residues, and is compatible with other control measures, including proper storage and biological control. This brief highlights the advances that have been made to date in the identification of host resistance to *A. flavus* and aflatoxin accumulation while also laying out the breeding requirements for developing resistant maize cultivars.

Aflatoxin detection

Because aflatoxins are toxic at very low levels, detection methods must be sensitive and accurate. Although different methods can be used to detect and quantify aflatoxins, an inexpensive, robust, and high-throughput method is needed for large-scale breeding programs. The bright greenish-yellow fluorescent (BGYF) light technique can quickly detect maize lines supporting high fungal growth. This method uses a black light assay to observe fluorescence from kojic acid, a secondary metabolite produced by *A. flavus* in grain. Lines that show fluorescence are eliminated, and those with no fluorescence must be assayed using the more sensitive enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography or ultra-performance liquid chromatography (HPLC or UPLC), or affinity columns to determine aflatoxin levels. A cost-efficient ELISA technique is used at the International Maize and Wheat Improvement Center (CIMMYT) for routine detection and quantification of aflatoxins in breeding programs, providing results that correlate well with UPLC (Figure 1).

Tools to identify resistant germplasm

Maize kernel infection by *A. flavus* is highly variable under natural conditions. Selection of resistance genes relies on the ability to subject all plants both to equally high levels of active fungal infection to avoid escapes (plants that look resistant because they have not been infected) and to high-throughput phenotypic screening capacity. Aflatoxin trials must be carried out in replicated field plots over multiple years and locations because resistance is highly affected by the environment in which the infected plants are grown. Several techniques for mass inoculation of maize germplasm under field and laboratory conditions have been developed and are available (Brown et al. 1993). Standardized systems for data acquisition and exchange among breeding programs, such as those developed by CIMMYT, also help to accelerate both the identification of *A. flavus*– and aflatoxin-resistant germplasm and the development of tolerant maize cultivars.

Generation of resistant germplasm

Methods to achieve resistance to *A. flavus* and aflatoxin accumulation include (1) prevention of fungal infection of maize, which is especially important under stressed environmental conditions; (2) prevention of subsequent growth of the fungus once infection has occurred; (3) inhibition of aflatoxin production following infection; and (4) degradation of aflatoxins by the plant or fungus. Development of aflatoxin-resistant varieties is thus a complex process that may include direct selection for resistance to fungus and aflatoxin accumulation, indirect selection for resistance or tolerance to biotic and abiotic stresses, or selection for morphological traits such as ear, kernel, and husk characteristics that impede or delay fungal introduction or growth. Breeders at CIMMYT are evaluating known sources of aflatoxin resistance under drought conditions, as well as drought- plus heat-tolerant germplasm for possible resistance to *A. flavus* and aflatoxin accumulation. Sources of resistance to many of these factors have been identified and are now being combined to develop aflatoxin-resistant maize germplasm adapted to various agroecologies. Doubled haploid (DH) technology produces new pure breeding lines in a very short time as compared to the several generations needed to create pure lines via traditional self-pollination. DH technology is being used at CIMMYT to rapidly develop inbred lines combining *A. flavus* and aflatoxin resistance with other important agronomic traits. These DH lines are now being evaluated to identify new superior lines combining aflatoxin resistance, drought and heat tolerance, and good agronomic performance.

Quantitative trait loci (QTL) for both fungal and aflatoxin accumulation resistance have been mapped, and the transfer of these QTL into new elite germplasm is underway. This is a challenge for breeders, as *A. flavus* and aflatoxin resistance is controlled by a large number of genes with small effects whose performance varies by environment. Markers linked to QTL or genes associated with aflatoxin resistance may enable rapid selection gains for resistance. The recent elucidation of the fungal aflatoxin biosynthetic pathway and the regulatory genes for this pathway provide the

potential for developing kernel mechanisms that directly inhibit aflatoxin biosynthesis.

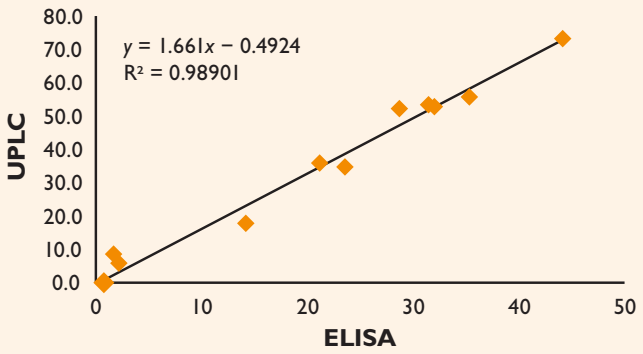
Promising new technologies to speed up aflatoxin resistance

Genes, QTLs, and genetic mechanisms contributing to *A. flavus* and aflatoxin resistance are being identified using new tools and techniques. Next-generation sequencing and association mapping is being used to identify DNA and RNA sequences involved in resistance. Final confirmation of genomic regions providing improved resistance using near lisogenic lines is nearing completion for several QTL and gene sequences at CIMMYT and the US Department of Agriculture's Agriculture Research Service (USDA-ARS). Finally, new techniques involving RNA interference (RNAi) gene silencing may allow transgenic maize plants to resist infection by *A. flavus*, using DNA sequences from the fungus itself to allow recognition and prevent growth of the fungus in the plant.

Technology-implementation challenges

Accumulation of aflatoxins in maize occurs following a complex series of interactions among maize, the environment, the pathogen, insects, and crop-management practices. Selection must be done simultaneously for multiple stresses in order to combine drought and heat tolerance, resistance to insects (especially ear-feeding insects), and resistance to the pathogen. These stress tolerances must be combined with improved agronomic performance in new maize varieties for adoption of aflatoxin-resistant cultivars to occur, as farmers will not grow low-yielding varieties regardless of aflatoxin resistance. The negative impacts of aflatoxin consumption are generally slow and difficult to recognize, while hunger due to insufficient food is immediate and pressing. The challenge is to systematically identify the best sources of resistance, introduce them into adapted maize germplasm, and make the germaplasm available in areas where aflatoxin contamination is a problem. Established procedures for field inoculation, measurement of aflatoxin levels, and generation of doubled haploids, together with new techniques for implementation of marker-assisted breeding, gene expression studies, proteomics, and RNAi, will lead to more opportunities for efficient development of aflatoxin-resistant elite maize cultivars. Work has progressed on the development of resistant maize varieties, and resistance is being pyramided and combined with other disease and abiotic (heat and drought) stress-resistance genes by several collaborating institutions. Because this is a long-term process, no new cultivars are yet ready for release. Good progress has been made, yet final testing of new breeding lines must be performed in replicated field trials before release. Support for aflatoxin resistance breeding must continue in the meantime.

FIGURE 1 **Relationship between aflatoxin quantification using ELISA and UPLC**



Source: Author's calculation, 2012.
Notes: ELISA = enzyme-linked immunosorbent assay. UPLC = ultra-performance liquid chromatography. The high correlation (r = 0.99) reveals the reliability of ELISA to detect and quantify aflatoxins in breeding programs.

FOR FURTHER READING

Brown, R. L., D. Bhatnagar, T. E. Cleveland, Z. Y. Chen, and A. Menkir. 2013. "Development of Maize Host Resistance to Aflatoxigenic Fungi." In *Aflatoxins - Recent Advances and Future Prospects,* edited by M. Razzaghi-Abyaneh, 1–22. InTech, www.intechopen.com/books/aflatoxins-recent-advances-and-future-prospects.

Krska, R. 2008. "Mycotoxin Analysis: An Update." *Food Additives and Contaminants* 25 (2): 152–163.

Widstrom, N. W., A. Butron, B. Z. Guo, D. M. Wilson, M. E. Snook, T. E. Cleveland, and R. E. Lynch. 2003. "Control of Pre-Harvest Aflatoxin Contamination in Maize by Pyramiding QTLs Involved in Resistance to Ear Feeding Insects and Invasion by *Aspergillus* Spp." *European Journal of Agronomy* 19 (4): 563–572.

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