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CGIAR Research-for-Development Program on Mycotoxins

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Abstract

The major mycotoxins studied at the Consultative Group on International Agricultural Research (CGIAR) institutes are aflatoxins in maize, groundnut, sorghum and cassava, *Fusarium* toxins in maize, wheat and sorghum, and ochratoxin in cocoa and cashew. Genetic enhancement (both through plant breeding and biotechnology), biological control, habitat management, risk assessment, institutional capacity building and public awareness are among the tools in the "CGIAR research-for-development kit" to fight mycotoxins worldwide. A holistic approach should be pursued to deal with mycotoxins that includes the following elements: i) an integrated crop management package that combines mycotoxin-resistant germplasm, biological control, habitat control and soil-amendments; ii) low-cost mycotoxin detection technology for rapid appraisal that also should facilitate trade; iii) a participatory process for mycotoxin assessment in commercially important crops; and iv) a high-level panel composed of scientists, NGOs, farmers, traders, consumers, health officers and policy makers to monitor mycotoxin intervention strategies and to organize awareness campaigns.

Introduction

Food quality and safety are important traits used by people to select their diet. Internationally these standards are agreed through the *Codex Alimentarius* with national laws also having an important role (FAO, 2003). Contamination of staple foods is widespread in some locations, particularly in the developing world, and can occur at all levels of crop production: pre-harvest, harvest, and storage.

The safety and nutritional quality of food often is compromised by mycotoxins, which are metabolites produced by a few fungi that colonize both staple agricultural products and export crops from countries in the developed and developing world. The best known mycotoxin is aflatoxin, which is produced by a few species of the fungus *Aspergillus*, and commonly occurs in maize, groundnut, sorghum, and some root and tuber crops. *Fusarium* spp. also produce mycotoxins such as fumonisins, zearalenone, and deoxynivalenol. Myco-

toxins are regulated because they are hazardous to health. In addition to their carcinogenic properties, many mycotoxins are anti-nutritional factors that result in poor growth and immune suppression in young animals and children.

In the developed world, regulatory standards control exposure of humans and animals to dietary mycotoxins. These food safety regulations reduce the risks of morbidity and mortality associated with the consumption of contaminated food. In the developing world, particularly in sub-Saharan Africa, monitoring and enforcement of standards are rare. Mycotoxins also may form non-tariff trade barriers. The European Union has recently reduced regulatory limits for aflatoxin to 4 ng/g compared to the *Codex Alimentarius* Commission recommended standard of 20 ng/g in groundnuts. This dichotomy in legislation could cost some developing nations several hundred million dollars in export losses (Wu, 2004). The costs of food safety regulation includes the cost of production, compliance, and administration, and the deadweight loss associated with these costs. In countries with widespread aflatoxin occurrence, the best quality foods are exported and the poorer quality foods are consumed locally and harm the local population. Thus, mycotoxins degrade food quality, can be barriers to international trade, pose serious risks to health, and are directly and indirectly responsible for human deaths in Africa and Asia. Exposure to aflatoxin increases the incidence of acute toxicosis, liver cancer, and morbidity in children suffering from kwashiorkor.

The centers of the Consultative Group on International Agricultural Research (CGIAR) recognize mycotoxins as one of the most important constraints to the goal of improving human health and well-being through agriculture. The CGIAR centers pursue various strategies for the management of mycotoxins from the field to the fork. For example, aflatoxin management practices in farmers' fields and stores have been developed and are being implemented through national partners represented in several chapters elsewhere in this volume. Work continues on the dissemination of management practices, biological control through competitive exclusion strategies, and breeding for resistance. The level of fumonisin contamination in field and stored maize has been surveyed, and management processes that can affect these levels have been identified. Fungal-insect relationships both for *Aspergillus* spp. and *Fusarium* spp. in the field and in storage have been investigated. Several aspects of mycotoxin research-for-development need further attention. These include food basket survey, strategies to reduce the impact of mycotoxins on trade, bio-ecological aspects of mycotoxin production, biological control, resistance breeding, and the impact of mycotoxin management options and nutritional improvement on children's growth and health in high-risk zones.

Regional research efforts on mycotoxins in the developing world for information exchange, transfer, and eventual implementation of tested mycotoxin management strategies by various regions need coordination for synergies to develop. A research-for-development program network may be required to deal with mycotoxins, food safety, and trade (Fig. 1). An important step already made by the CGIAR is the development of inexpensive ELISA kits that use monoclonal and polyclonal antibodies to detect aflatoxins, fumonisins and ochratoxins in various crops, food (milk, confectionary, proceed meals) and feed samples (Reddy *et al.*, 2002). These assays enable rapid screening of samples for mycotoxins and speed the screening of breeding lines leading to quicker development of resistant cultivars. The cost-effective diagnostic kits provided a new impetus to the research to mitigate aflatoxin contamination, with high-throughput diagnostic labs established by the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in Malawi, Mozambique and India. Up to 300 samples per day can be analyzed for mycotoxins by ELISA in these labs.

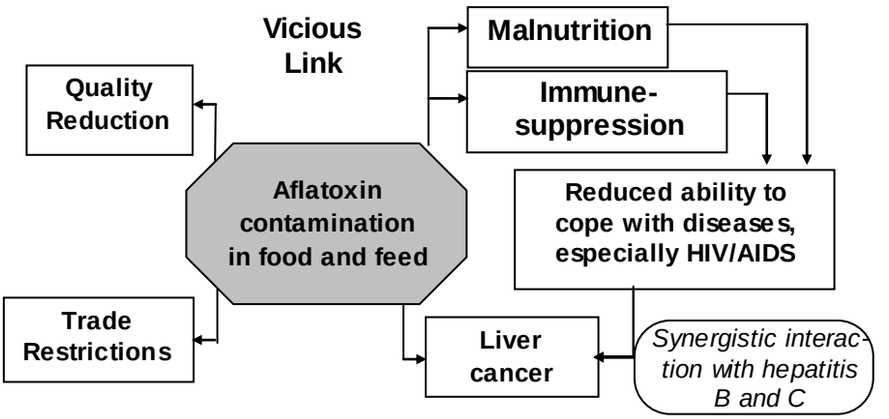

Figure 1. Effects of aflatoxin on trade and human health

Controlling mycotoxins in maize

One of the most important socioeconomic changes in the savannas of Africa has been the increasing production of maize in areas that previously were planted to millets and sorghum. The greater use of higher-yielding crop cultivars, increases in the availability and use of pesticides, and deregulation of the market for cereals also have changed these areas dramatically. Maize has essentially become a cash crop. Much of the increase in maize production has occurred in areas at significant risk of attack by pests of stored maize; *e.g.*, fungi that produce mycotoxins.

Aflatoxin contamination is widespread in Africa: in Benin and Togo, aflatoxin levels in maize averaged five times the safe limit of 20 ng/g in up to 50% of the household grain stores surveyed (Egal *et al.*, 2005; Gong *et al.*, 2002). As a result, people, especially children (Gong *et al.*, 2002), are being exposed to high levels of mycotoxins, often in mixtures, and the consequences have been largely ignored. For example, 99% of fully weaned children had ~2-fold higher aflatoxin-albumin adduct levels in their blood than do those receiving a mixture of breast milk and solid foods (Gong *et al.*, 2003, 2004). Surveys also indicate that *Fusarium* infection is prevalent in field and stored maize at many African locations. *Fusarium* spp. are found in all agroecological zones of Benin, but their prevalence is higher in the South than the North. The incidence of *Fusarium* infection is higher in the field than in storage (K. Hell, unpublished). *Fusarium* infection is usually reduced during storage. The most common species found were *F. verticillioides* and *F. proliferatum*. Fumonisins were found in the maize samples with levels often exceeding the limit of 4 µg/g recommended by the U.S. Food and Drug Administration, especially in villages in southern Benin: Yé (12 µg/g), Lainta (7 µg/g), Adjohoun (6.7 µg/g) and Kpomé (4.7 µg/g).

Similarly, nearly 35% of maize kernel samples collected from several villages during rainy and post-rainy seasons of 2004-2005 in Andhra Pradesh (India) contained 1 to 20 ng/g aflatoxin (F. Waliyar, unpublished). Six percent of the rainy season samples and 7.6% of the post-rainy season samples contained > 20 ng/g aflatoxins soon after harvest, whereas 20% of rainy season crop stored for up to 4 months had > 20 ng/g aflatoxins. This survey

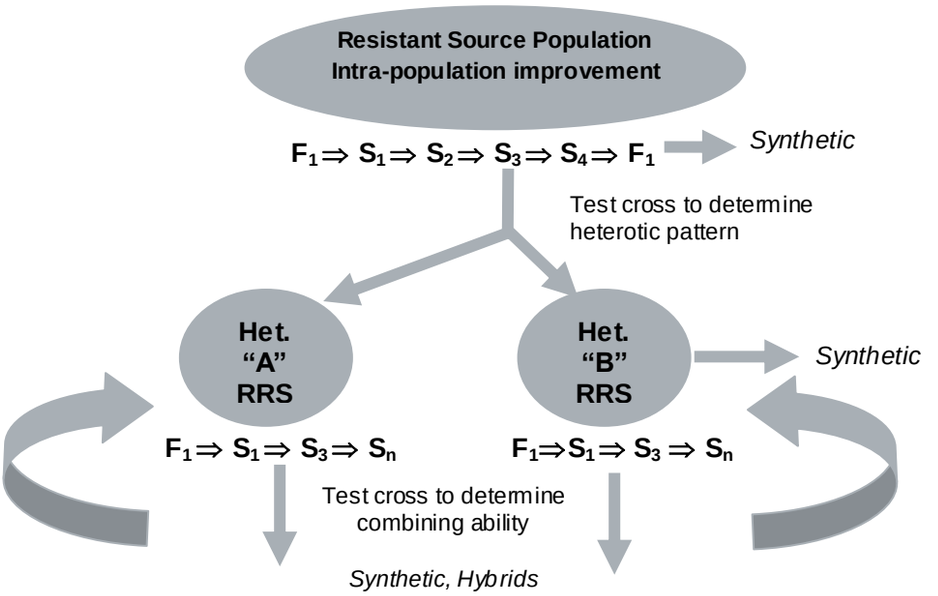

Figure 2. A comprehensive breeding strategy for developing biotic stress resistant maize germplasm. RRS = reciprocal recurrent selection.

suggests that even though toxin levels can be low at the time of harvest, they probably increase during grain storage.

Many insect species, drought and other environment factors, *e.g.*, nutrient stress, or pathogens are positively correlated with aflatoxin content in stored maize. These interactions between biotic and abiotic stresses not only reduce yield but also enable saprophytic fungi, such as *Aspergillus flavus*, to colonize the grain of stressed maize plants. In this regard, researchers at CIMMYT have been working to combine biotic and abiotic stress resistance, and to identify stress tolerant lines or hybrids that have a reduced incidence of *A. flavus*. Line recycling, *i.e.*, targeting crosses based on known traits in the parental lines, has used sources such as 'La Posta Sequía' for drought tolerance, lines extracted from the 'Multiple Insect Resistance Tropical' (MIRT) population for stem borer and armyworm resistance, stunt resistant populations (mainly 'P73' but also 'P76' and 'P79') and lines resistant to foliar diseases and ear rots (D. Bergvinson and D. Jeffers, unpublished). This broader approach to reducing the mycotoxin load has been successful and has led to efforts to breed source populations and synthetics against the maize weevil and larger grain borer, insects that serve as vectors for fungi and that breach the plant's external integrity allowing fungi to enter and colonize the plant. A comprehensive breeding strategy (Fig. 2) for developing biotic stress resistant maize germplasm has resulted in genetically enhanced lines with abiotic or biotic resistance to storage pests and aflatoxigenic fungi. Valuable sources for resistance to aflatoxin accumulation have been identified in several elite CIMMYT maize lines (Jeffers *et al.*, 2005). These and other maize germplasm sources developed by IITA (Fig. 3) are being incorporated into both tropical and southern United States maize breeding materials (Brown *et al.*, 2001).

Aflatoxin screening in maize kernels needs to be simple and cost-effective. Fluorescent screening uses a black light assay to observe fluorescence from kojic acid, a secondary me-

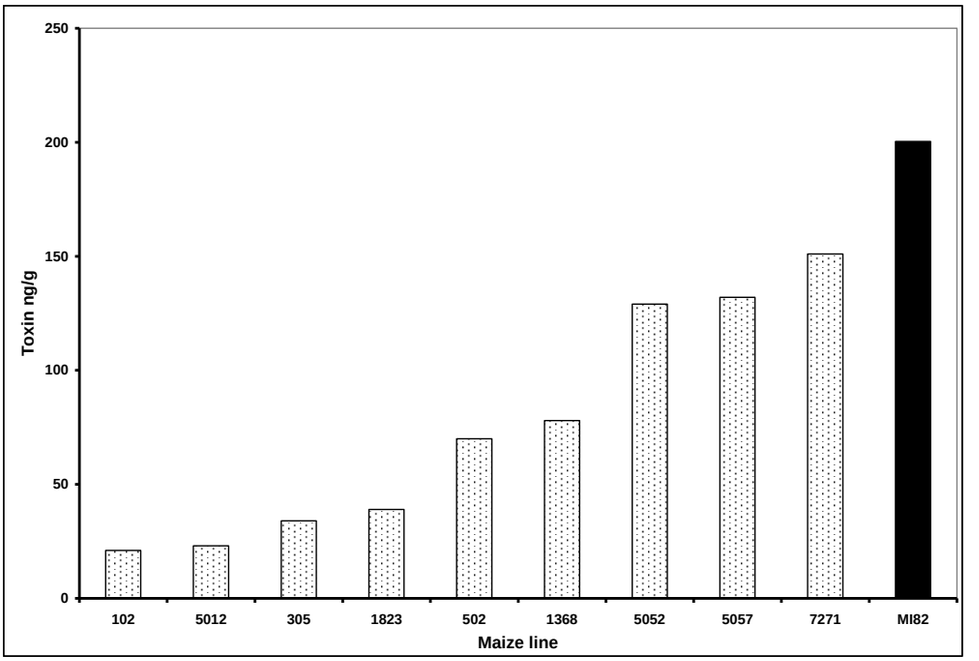

Figure 3. Aflatoxin accumulation in selected IITA maize inbred lines (▒) tested with a kernel screening assay. A susceptible hybrid (■) from the United States had 5300 ng/g [after Brown *et al.* (2001).

tabolite observed in colonized grain. Under black light, uninfected kernels are opaque and those infected are bright. This mass preliminary screening of breeding materials, allows selection of parental sources for further population improvement of maize.

Competitive exclusion (one strain competing to exclude another) is a relatively new, but very promising, biological control strategy for aflatoxin management in Africa. This control option leads to the best-adapted fungal strain being dominant in a given environment, and is a promising strategy for replacing toxigenic strains of *Aspergillus* with atoxigenic forms of same fungus. Several strains of *A. flavus* have been isolated and are being tested in Nigeria and Benin, with the goal of using atoxigenic strains to reduce aflatoxin contamination. Systematic knowledge of pre- and post-harvest practices can lead to complementary management of aflatoxins through cultural and storage practices (Turner *et al.*, 2005). For example, lodged maize plants, drought predisposition, high grain moisture and grain damage at harvest all increase the risk of aflatoxin production in storage. Thus, farmers need to follow good management practices at harvest time, and in drying, including using an appropriate storage structure and controlling insects. Management options include preventing rain-exposure of harvested cobs, storing maize in non-plastic bags, and the sorting out of kernels with insect damage and/or discoloration. Likewise, drying on black plastic sheets or cemented dry areas can reduce moisture content to safe levels after ~5 days, while drying maize cobs on the ground requires a minimum of 10 days.

Traditional maize processing also may help reduce mycotoxin levels; *e.g.*, aflatoxin levels were reduced by 99% in Benin during the preparation of fermented mawè, and by 79%

and 43% when preparing fermented ogi and owo (or "paté de mais"). Roasting and treatment with alkali also reduces the level of aflatoxin present, whereas boiling and soaking of maize grain in lime-water can eliminate or greatly reduce the levels of aflatoxin in the final product. Last but not least, selective removal or isolation of contaminated portions of the food commodity remains the most widely used physical method for aflatoxin decontamination. Awareness campaigns are needed to sensitize the population to the risks posed by aflatoxins and to popularize management options to minimize its effects. For example, due to civil society-public action, more than 10 million people in Benin, Togo and Ghana became aware of the dangers posed by aflatoxin-contaminated feeds and foods.

Aflatoxin management in groundnut

Aflatoxins B_1 and G_1 are the most commonly produced forms in groundnut. These toxins are involved in several human diseases, particularly liver cancer and growth defects in children. Aflatoxin interactions with Hepatitis B and C viruses result in relatively high levels of primary hepatocellular carcinoma. Aflatoxins also are toxic to livestock, including ruminants, poultry, birds and fish, when contaminated meal is used in their feed. Due to its human and livestock health implications, aflatoxin contamination has become a major issue in the international trade of groundnuts and can directly impact the lives of poor farmers by reducing their income.

Infection of groundnut by *Aspergillus* spp. can occur both pre- and post-harvest. Pre-harvest infection by *A. flavus* and consequent aflatoxin contamination is important in crops grown under rain-fed conditions in the semi-arid tropics. End-of-season drought and damage to groundnut pods by soil pests increases the pre-harvest aflatoxin levels. Mechanical damage during harvest and post-harvest practices, *e.g.*, heaping, increase toxin levels in warm, humid areas. Poor harvesting and storage practices may lead to rapid development of the fungi and consequently to higher production of the toxin. Aflatoxin contamination occurs frequently in groundnut seeds, with very high toxin levels found in immature and small seeds. Small pods remaining in haulms, damaged and immature seeds often are used as cattle feed. Milk from cattle fed such contaminated fodder contains high levels of aflatoxin M_1.

Field and greenhouse screening methods have been used to increase the efficacy of evaluation of aflatoxin resistance in groundnuts. Sick plots with highly aggressive, toxigenic strains of *A. flavus*, and laboratory inoculation methods for selecting individual resistant seeds now enable the screening of large amounts of germplasm. As a result, sources of resistance to seed infection and aflatoxin production have now been identified and used to breed high-yielding lines with resistance to seed infection and aflatoxin contamination that have been registered and shared with national agricultural research systems (NARS) for further use in their programs, *e.g.*, ICGV 88145, 89104, 91278, 91283 and 91284 in Asia and ICGV 87084, 87094 and 87110 in West Africa.

The estimated heritability for seed colonization ranged from 0.55 to 0.79, for seed infection from 0.27 to 0.87, and for aflatoxin production from 0.2 to 0.47. Thus, the levels of resistance in available sources and in the groundnut breeding lines are not very high and do not suffice to effectively protect the crop from aflatoxin contamination under all conditions. Further, the diversity of these lines is very narrow. Hence, ICRISAT researchers have developed protocols for the transformation of groundnut to produce transgenic plants with anti-fungal genes, *e.g.*, chitinases that may increase the resistance to *A. flavus* (K.K. Sharma, personal communication).

Other options for aflatoxin control in groundnut include the use of isolates of *Trichoderma* and *Pseudomonas*, which provide biological control of *Aspergillus* in both field and greenhouse trials, and cultural practices that reduce aflatoxin contamination, *e.g.*, the application of far-

myard manure, lime, gypsum or cereal crop residues to the soil. Treatments including lime and farmyard manure can reduce aflatoxin contamination up to 90% in a highly susceptible cultivar such as "Fleur 11". Harvesting pods at the proper maturity, exclusion of damaged and immature pods, improved harvesting practices, the use of mechanical threshers, and proper seed storage bins are other cultural practices that help reduce aflatoxins in groundnuts.

Fusarium head blight host-plant resistance in wheat

Fusarium graminearum is the main pathogen causing scab, or head blight, of wheat. Other species involved, depending on the climate and crops grown in rotation with wheat, are *F. culmorum*, *Michrodochium nivale*, *F. avaneaceum*, *F. poae*, and *F. sporotrichioides*. These pathogens affect grain yield and quality due to their ability to produce mycotoxins. *F. graminearum* and *F. culmorum* produce the trichothecene mycotoxins, deoxynivalenol and 15-acetyl-deoxynivalenol, and zearalenone in North America, or nivalenol, zearalenone, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol and fusarenon-X in Asia. Deoxynivalenol is associated with plant disease aggressiveness and may have been responsible for large-scale human poisonings in the last century in China and India. This toxin also causes vomiting and feed refusal in domestic animals and immuno-suppression in mice. The closely related nivalenol toxin is toxic to bone-marrow in experimental animals. Zearalenone, a chemically unrelated compound has oestrogenic effects in domestic pigs and experimental animals.

Fusarium head blight negatively affects wheat grain quality due both to lower weight of the affected grain and to the reduction in quality that accompanies mycotoxin contamination, and may result in significant economic losses directly to the farmers. CIMMYT, through funding from special grants from the Government of Japan and other donors, provides a global platform for international collaboration on scab research by facilitating the sharing of knowledge and genetically enhanced wheat germplasm and other breeding materials and tools. This global platform capitalizes on the knowledge accumulated on both host plant resistance and genetic improvement of the wheat crop against scab. For example, DNA markers are being mapped and used to incorporate three different types of resistance to the pathogen. These are: (I) resistance to initial infection or penetration, (II) resistance to fungal spread within plant tissues, and (III), degradation of mycotoxins. Chromosome 2D carries quantitative trait loci (QTLs) for type I and type II resistance, which are in the same region as QTLs for heading date and spike length. Although there are a few markers in this chromosomal region, new DNA markers associated with toxin tolerance are being mapped by *in silico* expressed sequence tag mapping that takes advantage of the synteny of the short arm of wheat chromosome 2D with that of rice chromosomes 4 and 7 (T. Ban, unpublished).

Scab screening with a spike inoculation test remains complex, unstable and low throughput. Easy, stable assessment methods for wheat breeders are being developed at CIMMYT that use the primary leaf. When a drop of a conidial suspension is placed on the wounded portion (~1 mm in diameter) of a leaf, the pathogen can infect and produce an oval lesion. This assay can distinguish resistant and susceptible cultivars (J. Murakami, unpublished). This new screening method, when coupled with the advances in genetic enhancement, should lead to novel resistance sources that carry genetically characterized R-loci. It also should be possible to assess transgressive R-segregating genotypes that combine distinct resistance genes, with the aim of pyramiding disease resistance genes in locally adapted wheat germplasm.

Towards a CGIAR-facilitated food safety program

The main role of science in agriculture has been to propel the evolutionary process that allows increased production with less land and less effort (Douthwaite and Ortiz, 2001). Who benefits from these advances depends on who controls the technology, who innovates, how selection decisions are made, and how innovations are enacted. We advocate a "research-for-development end-user-driven" approach that replaces the disconnected concept of research *and* development, in which researchers deal with technology generation and developers test this technology with potential end-users (Ortiz and Hartmann, 2003). Research-for-development needs society-conscious, committed scientists who are willing to transform into developers, by bringing a technology focus to their work. The research products resulting from this work are demand- not supply-driven, by end-users and not by "ivory tower" scientists. Hence, this approach closes the gap between research and development, and ensures that from the start of the research process, *i.e.*, its planning, that development goals are driving the agenda. Two metaphors: "from thinking to acting" and from "research to decision" define this new research-for-development approach, in which advanced research institutes, development organizations, the private sector, development investors and national governments are all partners and share the responsibility for accelerated agricultural diversification and commercialization for the small-scale agricultural sector. Research-for-development, keeping in mind the end-users, operates within a continuum that uses a "means" (research) for an "end" (development), thereby leading to impact on both people's livelihoods and science. With this approach, a working culture evolves in which management rewards internally the top performers following this framework, and externally encourages staff to broaden alliances or partnerships for development in their community of practice. Networking is a *must*, because organizations, which do not always have the same goals, see the advantage of teaming-up to successfully meet the objectives in a target area.

Due to the complex nature of agricultural problems in the developing world, solutions cannot be based on a "one size fits all approach." Research is required to develop decision-making processes that take natural resource fragility, community vulnerability, risk profiles, asset resilience, market options, service provision capacity and competitive advantage into account when designing solutions for specific client needs. Researchers must offer a broad array of products, because low input environments require yield-stabilizing technologies, and high-yield potential technologies should be developed for high-input environments. Such moving targets needs must be addressed by a heterogeneous, but dynamic moving strategy, which may change at any given point of time. Researchers following this trajectory must be able to use all of the available research tools for development.

Increasing productivity per unit area results in more food to consume or sell and may diversify the crops being planted. Similarly, higher and more stable yield potential and profitability permits poor farmers to invest in inputs for producing more food and income. High yields also may lead to reduced food prices for the urban and rural poor and to monetarization of rural areas, whose inhabitants may prefer "money in the pocket" (income generation) rather than "a meal on the table" (food security). High yielding crops also may provide employment opportunities for poor people throughout the trade chain (from harvest to processing). Thus, the outputs from research-for-development efforts should be linked to a well-resourced capacity-building program so that farmers will be equipped with plant or animal genetic resources to cope with changing environments and the entrepreneurial skills to assess and take advantage of agricultural market opportunities.

Researchers, farmers, and policymakers should remember that economic phenotype performances (P) are influenced by many factors and their interactions, *i.e.*,

P = Genotype × Environment × Crop Management × Policy (affecting both people and markets) × Institutional Arrangements × Social Demographics

Decentralized (through networking) end-user participatory research with local partners may provide a means for working in marginal, low input, stressful environments. Such decentralization rearranges priorities as local research partners target crop and resource management and as other responsibilities, *e.g.*, technology testing and the development of new materials through research or selection, shift from a central research station to become local targets. In this way, individual research programs, irrespective of their size, deliberately maintain diversity across locations. Such an approach should be driven by the needs of the rural poor to ensure that the work has a positive impact on their livelihood.

The major mycotoxins studied at the CGIAR institutes are aflatoxins in maize, groundnut, sorghum and cassava, *Fusarium* toxins in maize, wheat and sorghum, and ochratoxin in cocoa and cashew. Genetic enhancement (both through plant breeding and biotechnology), biological control, habitat management, risk assessment, institutional capacity development and public awareness are among the tools in the "CGIAR research-for-development kit" to fight mycotoxins worldwide. These "tools" resulted from strategic, applied and adaptive research by scientists at the international agricultural research centers in partnership with their counterparts in the national agricultural research systems and advanced research institutes. Decades of research-for-development by the CGIAR centers suggest that a holistic approach should be pursued to deal with mycotoxins and should include the following elements:

- An integrated crop management package that combines mycotoxin resistant germplasm, biological control, habitat control and soil-amendments.
- Low-cost mycotoxin detection technology for rapid appraisal that also facilitates trade.
- Participatory process for mycotoxin assessment in commercially important crops.
- A high-level panel composed of scientists, NGO staff, farmers, traders, consumers, health officers and policy makers to monitor mycotoxin intervention strategies and to organize awareness campaigns.

In this way, the CGIAR addresses the Millennium Development Goals; *i.e.*, mycotoxin-free food is key for better health, and is especially important for the health of post-weaning children, particularly in sub-Saharan Africa, and to cope with malnutrition and HIV/AIDS.

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