Recent developments in host plant resistance strategy 
of aflatoxin accumulation in crops

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ABSTRACT

Contamination of crops with aflatoxin, a naturally occurring toxin produced by *Aspergillus flavus* and *A. parasiticus*, frequently reduces the value and marketability of crops. The selection of resistant germplasm has great potential to reduce this problem, but highly quantitative nature of the trait makes this a difficult endeavor. However, to derive commercial benefit from these resistance markers like kernel resistance associated proteins needs to be identified to facilitate the transfer of resistance into commercially used lines of crops. In this review, the different approaches to eliminate aflatoxin contamination of crops are discussed with main emphasis on host plant resistance strategy.

**Keywords**: aflatoxin, germplasm, markers, proteins, host resistance strategy.

INTRODUCTION

Aflatoxins are the members of the diverse family of poisonous fungal metabolites know as mycotoxins. Aflatoxins have received increased attention from the food industry and general public mainly for two reasons. First, certain members of the aflatoxin family, specially, aflatoxin B$_1$ are extremely toxic and carcinogenic to animals and humans due to which they have been designated as biowarfare agents also [1]. Secondly, the incidence of these compounds in food and feed is ubiquitous and has occurred throughout the world, particularly in South East Asia, India, China, Russia and Africa [2-4]. The problem was first recognized after an outbreak of Turkey ‘X’ disease in United Kingdom in 1960 [5]. Research in the United Kingdom revealed that the disease was caused by aflatoxins. Since then extensive studies have been conducted on mycotoxins. Every year a significant percentage of the world’s grain and oilseed crops are contaminated with hazardous aflatoxins even after taking every precaution. Currently, more than 100 countries have regulations regarding levels of mycotoxins in food and feed industry [6]. US Food and Drug Administration (FDA) has limits of 20 ppb total aflatoxins on interstate commerce of food and feed [7].

CHEMISTRY AND METABOLISM

Aflatoxins are secondary metabolites which are produced by certain strains of *A. flavus* and *A. parasiticus* and chemically they are bifuranocoumarins. The major members are B$_1$, B$_2$, G$_1$ and G$_2$. The B series have a cyclopentenone ring structure replaced by a lactone in the G series. Aflatoxins fluoresce strongly in UV light, B$_1$ and B$_2$ produce blue fluorescence whereas, G$_1$ and G$_2$ produce a
green fluorescence [8]. This property makes it convenient for monitoring purification procedures and forms the basis for chemical analysis. The important physico-chemical properties of aflatoxins are given in table 1. Metabolism of aflatoxin plays an important role in determining the toxicity of aflatoxin. Aflatoxin B$_1$ is most potently toxic and carcinogenic of all the known aflatoxins, therefore, studies on the metabolism of aflatoxin B$_1$ (AFB$_1$) have been largely focused. Studies suggest that AFB$_1$ requires metabolic activation for its carcinogenic and mutagenic effects. *In vitro* studies have revealed that there are at least five types of metabolic transformation of AFB$_1$ viz., (i) reduction of aflatoxin (AFL), (ii) hydroxylation to aflatoxin M$_1$ (AFM$_1$) and Q$_1$ (AFQ$_1$), (iii) hydroxylation to aflatoxin B$_2$A (AFB$_2$A), (iv) O-demethylation to aflatoxin P$_1$(AFP$_1$), and (v) epoxidation to aflatoxin B$_1$-2,3 oxide (AFB$_{1-2,3}$-oxide). Aflatoxin B$_1$ may be transformed by mixed function oxidase enzymes (residing in liver microsomes and soluble cytosolic liver enzymes) into hydroxylated metabolites. These metabolites are amenable to conjugation with glucuronic acid and sulphate leading to detoxification.

Table 1. Physical and chemical properties of aflatoxins.

<table>
<thead>
<tr>
<th>Aflatoxin</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Melting point</th>
<th>UV absorption (306-363 nm)</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>B$_1$</td>
<td>C$<em>{17}$H$</em>{12}$O$_6$</td>
<td>312</td>
<td>268-269</td>
<td>21,800</td>
<td>425</td>
</tr>
<tr>
<td>B$_2$</td>
<td>C$<em>{17}$H$</em>{14}$O$_6$</td>
<td>314</td>
<td>286-289</td>
<td>23,400</td>
<td>425</td>
</tr>
<tr>
<td>G$_1$</td>
<td>C$<em>{17}$H$</em>{12}$O$_7$</td>
<td>328</td>
<td>244-246</td>
<td>16,100</td>
<td>450</td>
</tr>
<tr>
<td>G$_2$</td>
<td>C$<em>{17}$H$</em>{14}$O$_7$</td>
<td>330</td>
<td>237-240</td>
<td>21,000</td>
<td>450</td>
</tr>
</tbody>
</table>

Garner et al. [8] showed that metabolic activation of AFB$_1$ was necessary for its mutagenic activity. The metabolism of aflatoxin B$_1$ in animal tissues includes epoxidation of the 8,9 double bond, hydroxylation on both furan and lactone rings and oxidative demethylation resulting in a variety of polar metabolites. These water soluble conjugates can be classified as detoxification products because they are easily eliminated [9]. With respect to carcinogenicity, aflatoxin B$_1$-8,9-epoxide is the key active metabolite. Hydroxylated metabolite of AFB$_1$ (aflatoxin M$_1$, aflatoxin P$_1$ and aflatoxin Q$_1$) are assumed to represent detoxification products. Detoxification of aflatoxin B$_1$-8,9-epoxide may occur through conjugation with GSH. Hydrolysis of the epoxide forms a dihydrodiol that probably is still capable of causing toxicity via binding to protein but presumably in a less potent carcinogenic species than the epoxide. Thus, the amount of AFB$_1$ that is going to exert carcinogenic or toxic effects will depend on the amount converted to various metabolites as well as on the biological activity of these metabolites.

**AFLATOXIN CONTAMINATION**

Aflatoxin contamination in standing crops as well as stored grains of paddy, maize, sorghum, dry fruits and species have been reported with concentration ranging from 2 to 200 ppb. Dhavan and Choudhary [10] reported from a survey conducted during the period 1983-1993 comprising samples of cereals, oilseed cakes, compound feeds and other ingredients that highest incidence of aflatoxin contamination was observed in groundnut cake (96.35%), deoiled groundnut cake (96.20%) and the highest level of aflatoxin B$_1$, 8260 ppb was observed in maize. Bhat et al. [11,12] reported under a multicentre study conducted at different centres on the samples of groundnut collected from rural and urban areas of 11 states representing different geographical regions of the country that 21% of groundnut samples exceeded the permissible Indian regulatory limit of 30 µg/kg ranging from 5 µg/kg to 833 µg/kg, while study on maize samples collected from 11 states of India revealed that 666 fg/kg was maximum level of aflatoxin B$_1$ found in the state of Haryana. Dutta and Das [13]
found that out of different livestock and poultry feed samples collected from northern India, groundnut cake was highly contaminated with AFB1. Koirala et al. [4] reported the occurrence of aflatoxins in common food items like peanut butter/vegetable oil and cornflakes in Nepal.

**CONTROL OF AFLATOXIN CONTAMINATION**

Since the carcinogenic properties of aflatoxin were elucidated, the development of techniques for chemical quantitation in field samples resulted in the discovery that aflatoxin contamination occurs in both pre-and post-harvest stage. This allowed the first management of aflatoxin contamination through elimination of toxins from animal and human food streams. Aflatoxin control technique now being developed is based upon techniques in traditional crop management, enhancement of host plant resistance by breeding or genetic engineering interruption of the aflatoxin biosynthetic apparatus and biocontrol using atoxigenic strains [14]. Preventive measures include tillage systems and crop rotation which affect soil inoculum availability and root/soil interface and thereby prevent inoculum build up. Lisker and Liliehoj [15] observed that preventive measures in field to prevent aflatoxin accumulation include good cultural practices, harvesting at the optimum stage of maturity and rapid drying after harvesting and chemical control. Although the early harvesting is of limited usefulness in regions with little late season rainfall or where maturation occurs during hot periods of the year. Conventional methods like spraying of fungicide and insecticide (because *A. flavus* is a wound pathogen thereby correlating insect damage with aflatoxin accumulation) to limit aflatoxin levels and control insect damage have to prove cost effective and safe [16].

**Physical treatments**

These include separation, boiling, autoclaving, UV irradiation, adsorption etc. Aflatoxins are quite stable to heat and are inactivated only at 250°C, but it also alters the nutritive value of commodities. Singh [17] suggested the participation of microsomal peroxidase of *A. flavus* in the *in vivo* degradation of endogenous aflatoxins under the influence of temperature. Later it was reported that high temperature suppresses aflatoxin formation by down regulating *afIR* (regulatory gene) along with other factors [18]. Aflatoxins are sensitive to UV light and gamma rays but the practical use of such treatment for destroying aflatoxins is questionable. Studies showed that at the doses required to effectively destroy aflatoxin, the irradiated commodity will also be destroyed [19]. Refai et al. [20] stated the control of aflatoxin B1 in meat by γ-irradiation. It was also observed that the irradiation of commodities did not result in complete detoxification [21]. Adsorbants including bentonite and activated charcoal can physically remove aflatoxins from liquid foods [22]. Groopman et al. [3] stated the use of phyllosilicate clays (hydrated sodium calcium alumino silicate) to chemi-sorb aflatoxin in aqueous suspensions including milk. But this still needs more research before being taken to the field.

**Chemical treatments**

This method of detoxification includes treatment with acids, bases, oxidizing agents, aldehydes, several gases and bisulfites. Aflatoxins are degraded by aqueous solutions of strong acids and bases. Among bases ammoniation process appears to be the promising, effective and safe in reducing aflatoxin level in corn, peanut meal cakes and whole cotton seed and cotton seed products by more than 99%. Ammoniation is done by using ammonium hydroxide or gaseous ammonia. But ammoniation process lowers the protein efficiency ratio of the product and enhances the production of off flavours and odours in the product [23]. Still ammoniation is used to reduce aflatoxin levels by more than 99% in corn [24]. Reduction in aflatoxin levels of peanut kernels/flours using ozone
gas (O₃) was also observed [25]. Groopman et al. [3] also observed reduction in the levels of aflatoxin with the use of ammonia either in solution or in gaseous form. Oxidizing agents like hydrogen peroxide, riboflavin, bisulfate etc. can also be used to degrade the aflatoxin in food and feed stuffs. Riboflavin and hydrogen peroxide can act together to inactivate aflatoxin M₁ in milk by generating singlet oxygen. Although, the amount of H₂O₂ and riboflavin used by Applebaum and Marth [26] are excessive and not suitable for practical application. Hagler et al. [27] observed the degradation of aflatoxins in corn by using sodium bisulfate which can react with aflatoxins at various temperatures and concentrations for various times to form water soluble products. Nitrogen fumigation in maize gave effective control of *A. flavus* growth and aflatoxin contamination. Urea (0.1-0.5%), citric acid (0.2-0.5%) and sodium propionate (0.1-0.5%) were also used to prevent fungal growth along with aflatoxins [28].

**BIOTECHNOLOGICAL DETOXIFICATION**

Significant knowledge is now available about the molecular basis of aflatoxin biosynthesis that could suggest novel approaches in solving the aflatoxin problem through biotechnology. By utilizing the knowledge about the structure of inhibitor analogues, a new class of ecologically safe pesticides can be produced that are specifically inhibitory to aflatoxin synthesis and are non-toxic to plants or animals. Several natural plant products that are inhibitors of aflatoxin biosynthesis could be used for the development of ecologically safe biopesticides. Trail [29] has identified a compound in black pepper that inhibits transcription of aflatoxin biosynthetic genes but did not inhibit growth of the fungus. Inhibitory plant products could also serve as markers for enhancement of aflatoxin resistance traits in plants. It has been proposed that differential gene expressing during aflatoxin biosynthesis will help in reducing the aflatoxin contamination. A 70 kb DNA containing 25 gene cluster of the aflatoxin pathway has been characterized responsible for the enzymatic steps in toxin synthesis. Regulatory elements such as *afIR* and *aflS* (*aflJ*), nutritional and environmental factors also effect aflatoxin formation. It was also found that eight chromosomes of about 33-36Mbp estimated genome size harbor about 12,000 functional genes [30]. Acquisition of information on the molecular regulation of aflatoxin synthesis will be utilized in biotechnological strategies for genetic manipulation of the pertinent fungal genomes for the development of procedures for practical control of the aflatoxin contamination process.

**BIOLOGICAL DETOXIFICATION**

As the physical and chemical methods are not adequate to detoxify the aflatoxin, biological degradation or transformation of aflatoxin should be used. This system of detoxification of aflatoxin is accurate, specific and does not alter the nutritive value of the product. Many organism like bacteria, yeasts and molds are able to degrade aflatoxin. Different approaches of biological degradation can be classified as (i) atoxigenic strains, (ii) coinvading organisms, and (iii) organisms growing in different niche of the environment.

**Atoxigenic strains**

Atoxigenic strains are those which fail to produce enzyme(s) of the biosynthetic pathway and are able to grow along with toxigenic strains, thereby preventing aflatoxin contamination through interspecies competition. It was observed that the application of naturally occurring atoxigenic isolate of *A. flavus* to soils planted with cotton resulted in the competitive exclusion of aflatoxin, producing strains and a significant reduction in the levels of aflatoxin contamination of crop [31]. Cordwell and Coty [32] showed that atoxigenic strains are effective in reducing aflatoxin production.
in maize by both *A. flavus* and *A. parasiticus*. Identification of critical genes governing aflatoxin formation has led to the rational design of atoxigenic biocompetitive strains of toxigenic fungi through the use of gene disruption techniques. Now methods are being developed to reduce the aflatoxin producing potential of *A. flavus* communities by atoxigenic strains across the large regions.

**Coinvading organisms**

These are the organisms which are found associated with toxigenic strains in grains and seeds and are able to reduce the aflatoxin contamination by competing for the same nutrients and establishment on the same substrate. Wicklow et al. [33] demonstrated that *A. niger* and *T. viridi* on inoculation with *A. flavus* on corn prevent the production of aflatoxin. *Piper longum* also showed inhibitory effect on aflatoxin production [34]. It was reported that the natural contamination of *A. niger*, *Mucor racemous*, *Alternaria alternate* and *Rhizopus* and bacterium *Bacillus stearothermophilus* in the maize seeds reduce the aflatoxin level when they were inoculated with *A. flavus* on irradiated maize seeds [35]. Aziz and shahin [36] concluded that *T. viridi* and *A. niger* may be useful for biological control of aflatoxin contamination in maize kernels. Azab et al. [37] indicated that fragmentation of mycelium increased the ability of aflatoxin B1 degradation more by *Aspergillus flavus* than *A. parasiticus*.

**Organisms growing in different niche of the environment**

These are the organisms especially bacteria occupying a different niche in the ecosystem and are able to detoxify the toxin. *Flavobacterium auranticum* can be used to remove aflatoxin from non-defatted and partially defatted peanut milk. Later it was proved by radiolabeling that *F. auranticum* on degradation of radiolabeled aflatoxin B1 leads to the release of $^{14}$CO$_2$ by live cell and not by the dead cells [38]. It was also reported that the degradation of aflatoxin by *F. auranticum* is a mineralization process in which it detoxifies aflatoxins without utilizing exogenous energy sources, thereby, facilitating its use in fermentation reactions [39]. It was reported by Hosono and Husamatsen [40] that an inhabitant of intestines of healthy animals and human (*Enterococcus faecalis* FK-23) removed aflatoxins under *in vitro* conditions which showed its important protective function in the intestine. It was noted that *Lactobacillus* spp. have some proteins of molecular weight ranging from 6000-14,000 that greatly inhibited aflatoxin production [41]. It was reported by Vaithianathan [42] that *Bacillus firmus* isolated from soil degrades the aflatoxin B1. Further it was concluded after treatment with HCl biodegrading aflatoxin B1 ability of *Lactobacillus* increased because of the formation of stable intracellular AFB1-bacterial complex [37]. Oluwafemi et al. [43] used lactic acid bacteria, which is Generally Regarded As Safe Status (GRAS), as a bio-detoxification agent for aflatoxins.

**DEVELOPING RESISTANT CULTIVARS**

Although there are several management strategies that may reduce aflatoxin contamination in crops, the pre-eminent strategy for elimination of aflatoxin is to develop pre-harvest host resistance of aflatoxin accumulation. This strategy has gained even greater prominence due to recent discoveries of natural resistance in crop that can be exploited in plant breeding strategies. Secondly, pre-harvest host resistance of aflatoxin contamination of crop focuses on inhibition of fungal colonization of the host plant and/or toxin production by *Aspergillus* spp. on the host plant. Thirdly, it would eliminate that need to detoxify large quantities of aflatoxin contaminated seed and avoid the uncertainties inherent in gaining regulatory agency approval for detoxification procedures.
Varietal differences in aflatoxin production in crops

Screening for resistant cultivars to aflatoxin has been done in corn as well as in other crops. Mixon [44] studied the potential for developing agronomically suitable cultivars of peanut genotypes that exhibit resistance to seed colonization of the fungus producing aflatoxin. Later Holbrook et al. [45] observed the effect of reduced linoleic acid composition on pre-harvest aflatoxin contamination of seven peanut genotypes. Screening for resistance against aflatoxin B₁ production in sorghum was done by Miguel and Andres [46] to conclude that toxin production depends on both the strains of fungus and substrate. Seenapa et al. [47] on screening different cowpea cultivars suggested the partial resistance of some cowpea lines which on further selection and breeding could provide resistant cultivars against fungal invasion and toxin production. Nagrajan and Bhat [48] on screening seven maize hybrids postulated involvement of a low molecular weight protein in conferring resistance and whose concentration varied according to the resistance of susceptible behaviour of the corn. Workers classified different maize varieties into resistant, moderately resistant and susceptible categories on the basis of aflatoxin production and observed that the resistant line had fewer kernels infected and lower aflatoxin concentration in the grains at harvest. It was also found that no maize cultivar supported zero aflatoxin production [49,50]. Windham and Williams [51] compared temporal differences in infection of *A. flavus* and aflatoxin levels in resistant and susceptible maize hybrid. Later it was suggested that additive gene action is controlling the resistance to aflatoxin contamination in maize kernels due to some proteins acting synergistically [52,53].

Factors contributing to the resistance to aflatoxin production

Plants defend themselves against fungal attack by complex mechanisms. Delineation of causal effects among varieties or hybrids is difficult to assess because multiple parameters such as, insect damage and agronomic conditions are integral factors. Intrinsic factors and latent mechanisms that can affect fungal growth range from simple organic compounds to macromolecules such as complex polypeptides and polyphenols [54]. Attempts have been made to correlate differences in susceptibility to *A. flavus* aflatoxin contamination with differences in the chemical make up of various susceptible and resistant genotype. They may include pericarp structures such as thickness and surface wax and sub-pericarp components such as preformed or induced proteins (including lectin, enzyme inhibitors and antifungal proteins) that inhibit fungal growth or aflatoxin production [55,56]. Zeringue et al. [57] found an association between C₆-C₁₂ alkanal and alkenal contents in several maize genotypes and the aflatoxin contamination. They suggested the correlation of decay products of polyunsaturated fatty acids (linoleic acid) and plant disease resistance and indicated that the lipoxygenase pathway may contribute to this resistance. Later they observed that the presence of more concentration of furfural (2-furan carboxyaldehyde) in resistant maize genotypes appears to contribute to a defense mechanism for protecting the developing maize kernel from fungal attack [58].

Some workers generalized that hybrids with good husk coverage and insect resistance have been found to accumulate less toxin. Later, it was observed that pericarp being the outermost layer of corn kernels may provide considerable protection against invasion of the kernels by pathogens. They concluded that wax and cutin layers of maize kernel pericarps may play a role in resistance to aflatoxin accumulation in MAS gk and other genotypes [59]. Goh et al. [60] also demonstrated that *A. flavus* can produce extracellular cutinase (or non-specific esterases or both) which may be involved in infection of intact corn kernels in the field [60]. They proposed that resistance in some genotypes may be due to greater waxing on kernel surfaces, which can restrict the entry of fungus and to the internal factors which can restrict growth of fungus within kernels. Some other workers...
like Abdollahi and Buchanan [61] observed that glycolysis has an important role in aflatoxin biosynthesis and fermentable sugars are optimal sources for aflatoxin biosynthesis. Widstrom et al. [62] stated that maize genotypes with sugary kernels support more fungal growth and higher aflatoxin production than genotypes with starchy kernels while others suggested that fungus produces greater amount of aflatoxin on simple sugars such as glucose, sucrose and maltose [63]. Flaherty et al. [64] detected an aflatoxin inducing activity in kernel extracts colonized by *A. flavus* with GUS reporter assay where the inducing activity was determined to be glucose, maltose and maltotriose. Evidence of α-amylase present in the kernel extracts suggested that these sugars were produced by the action of α-amylase from *A. flavus* on kernel starch. Later, Woloshuk et al. [65] observed that best inducers of aflatoxin biosynthesis are carbon sources readily metabolized via glycolysis. They also suggested that α-amylase produced by *A. flavus* supplies a burst of fermentable sugars which in turn induce aflatoxin biosynthesis.

A 14 KDa trypsin inhibitor (T1) protein has also been identified by Chen et al. [66] which is associated with resistance to *A. flavus* in corn genotypes. This protein also caused spore rupture and abnormal hyphal development. The purified T1 inhibited the growth of *A. flavus* and some other fungal pathogens thereby suggesting its potential role in aflatoxin contamination of corn and other crop. They also suggested that inhibition of fungal growth by T1 may be partially due to its inhibition of fungal α-amylase production and to a lesser extent, its activity thereby limiting the amount of carbon source for fungal growth [67]. Some other workers working on α-amylase deficient mutant of *A. flavus* suggested that α-amylase plays a role in the aflatoxin production. Later they suggested that α-amylase facilitates aflatoxin production and growth of *A. flavus* from a wound in the endosperm to the embryo. They also isolated a 36 kDa protein from the legume *Labab purpureus* which inhibited the activity of *A. flavus* α-amylase by 50% and affected the germination of fungus as starch was not converted to sugars which were utilized by fungus for growth and aflatoxin production [68-70].

Nagrajan and Bhat [48] proposed that a protein of low molecular weight present in higher concentration in resistant variety Opaque-2 may have a powerful inhibitory action for aflatoxin production. Later it was observed that the inhibition of normal metabolic pathway in *A. flavus* growth was induced by polypeptides from maize. It was also observed that salt soluble protein fractions of molecular weight 16 kDa and a group of low molecular weight protein (less than 14 kDa) might interact with fungal spores and this in turn leads to the inhibition of fungal growth [71,72]. Antifungal proteins such as ribosome inactivating proteins (RIPs) which modify and inactivate foreign ribosomes may play a role in seed protection against the fungal attack. Loesch et al. [73] proposed a role for b-32(RIP) in maize for defense against pathogens by an increase in its concentration during susceptibility to fungal attack. Later on it was proposed that the coordinate Opaque-2 controlled synthesis of this maize RIP and the major seed storage proteins provides the germinating seedling with both nutritional benefits and protection against pathogen invasion of the surrounding endosperm [74]. Guo et al. stated that RIP synthesized during seed development in kernels and may protect against fungal infection of the kernels during storage and germination may act synergistically in defensive roles [75].

Another type of antifungal protein found in maize kernels is zeamatin which increases the permeability of fungal cell membranes. High zeamatin concentrations permeabilize fungal membranes, causing cell death whereas lower concentrations do not cause cell death directly but may interact with the membrane to facilitate penetration of other compounds. Brown et al. [76] suggested that post-harvest resistance to aflatoxin contamination in resistant populations is related to the metabolic activities of the living maize embryo. Guo et al. [77] proposed that a zonal distribution of antifungal proteins seems to occur along similar zones where fungal infection takes
place. They also observed that RIP was found primarily in the aleurone layer of the endosperm and glandular layer of scutellum, whereas zeamatin was located mainly in the kernel embryo where they uniquely protect kernels from pathogen. Huang et al. [78] concluded that two kernel proteins might contribute to resistance to aflatoxin production in a resistant inbred TeX 6. One protein with a molecular mass of 28 kD inhibited *A. flavus* growth while a second protein with a molecular mass of less than 100 kD inhibited toxin formation with little effect on fungal growth. Brown et al. [79] determined the biochemical characterization of corn kernels resistant to the infection of *A. flavus* and *F. moniliforme* so that common protein related to the resistance to either fungus as well as uniquely expressed proteins would be identified. Peethambaran et al. [80] observed that antifungal activity present in resistant maize lines may be due to the higher chitinase activity in silk proteins. Chen et al. [81] suggested the identification of potential selectable markers associated with the resistance in corn by using proteome comparisons of corn kernels resistant or susceptible of *A. flavus* infection. Later they found using proteome comparisons that stress related proteins along with antifungal proteins are associated with kernel resistance to aflatoxin production in maize [82].

Plants respond to fungal attack by the synthesis of pathogenesis related proteins (PR proteins) which include hydrolytic enzyme such as chitinases, glucanases and other hydrolases which have an antibiotic role in plants that is they do not attack endogenous carbohydrates but rather protect plant from fungal pathogens by digesting the invading fungal cell walls. Nassser et al. [83] identified and characterized maize PR protein out of which 4 were chitinases. Chitinase induction was found by northern blot analysis of total RNA extracted from both maize kernels and callus after challenge with *A. flavus* [84]. Lozovaya et al. [85] indicated that β-1-3-glucanase activity may have a role in the inhibition of the growth of the *A. flavus* and the maize embryogenic callus and kernels do respond to the presence of this fungus. Later it was concluded that only particular isoforms of the 2 fungal degradative enzymes might be involved in combating *A. flavus* in maturing corn kernels. They demonstrated the pattern of enhanced or inducible proteins in maturing corn kernels in response to *A. flavus* infection and that only particular isoforms of two hydrolytic enzymes are involved in the maturing corn kernel infected with *A. flavus* at the milk stage [86-88]. Thus, comparisons of kernel protein profiles between susceptible and resistant genotypes may shorten the time it takes to identify resistance associated proteins. The identification of these proteins may provide markers for plant breeders and facilitate the introduction of resistance through genetic engineering into corn and other aflatoxin susceptible crops.

**CONCLUSION**

Host plant resistance as a strategy for eliminating aflatoxin accumulation in crops may be regarded as reality due to the discovery of genotypes having natural resistance to aflatoxin contamination and the development of new inbred lines through breeding. Further exploitation of this strategy requires the identification of markers like resistance associated proteins in order to transfer the resistance for aflatoxin accumulation in commercially used lines of crops. However, more work is required to determine the role of different categories of proteins as antifungal, stress related and storage contribute to the total resistance in crops. This would inturn assist in meeting the challenges of aflatoxin and other mycotoxin producing fungi along with enhancing the understanding of host plant interactions with fungi.

**REFERENCES**


