Genetic Resistance to Drought in Maize and Its Relationship in Aflatoxins Production

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1. Introduction

Maize (Zea mays L.) is one of the most important crops in the world. It is the third most important food grain crop in the developing world and is estimated that the demand for maize in developing countries will grow by 50%, from 558 million tons in 1995 to 837 million tons in 2020. Much of this increased demand will be needed by domestic supply for developing countries, which will require intensifying production on existing agricultural land (Ribaut and Ragot, 2007).

Drought is one of the prime abiotic stress in crops in the world. Crop yield losses due to drought stress are considerable. Particularly in maize, as an example, drought is the major stress affecting productivity in Africa leading up to 70% or total crop loss (Muoma et al., 2010; Ashraf, 2010). Although a variety of approaches have been used to alleviate the problem of drought, plant breeding, either conventional breeding or genetic engineering, seems to be an efficient and economic means of tailoring crops to enable them to grow successfully in drought-prone environments (Ashraf, 2010).

In turn, aflatoxins are found to contaminate a wide variety of important agricultural products such as corn, peanuts, tree nuts and cottonseed especially under extreme heat and drought conditions (Payne, 1998; Chen et al., 2003). Aflatoxin contamination significantly reduces the value of grain as an animal feed and export commodity (Chen et al., 2002).

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Therefore; aflatoxins have emerged as a major concern in agriculture and health sectors because of their harmful effects on human and animal health as well as for ubiquitous presence of aflatoxigenic fungi in many agricultural commodities under field and storage conditions; It is a great problem throughout the world. Aflatoxins are toxic secondary metabolites produced by Aspergillus flavus and *A. parasiticus*. They are potent carcinogens and poses health hazards to humans and domestic animals (Tubajika and Damann, 2001).

The production of aflatoxin is induced by *Aspergillus* species under high temperatures and low relative humidity (Widstrom et al., 2003). Infection of maize by *Aspergillus flavus* with the subsequent aflatoxin accumulation, it is why represents a serious risk in maize growing under drought conditions (Diener, et al.,1987; Payne etal., 1998). Drought can reduce the ability of maize to resist the invation of *A. flavus,* because it negatively affects the expression of genes encoding associated.resistance.

Substantial effort has been made to identify corn genotypes that resist infection by *A. flavus.* Although the most desirable and effective control of A. flavus and aflatoxin contamination is through the development of genetically resistant maize genotypes, successful management of aflatoxin in the field will require host resistance combined with management strategies such as appropriate nitrogen fertilization, population densities, insect control, and irrigation (Tubajika and Damann, 2001). Field studies demonstrate that reduction of drought stress by irrigation reduces aflatoxin contamination in corn and peanut. Drought tolerant corn varieties were also found to produce significantly less aflatoxins in the field under drought conditions compared to aflatoxin-resistant controls; this suggests a possible association between drought tolerance and aflatoxin resistance in corn (Brown et al., 2004).

Gene expression studies of plants in response to biotic or abiotic stress also found that disease resistance-related genes could be regulated by abiotic stress and vice versa. Further examination of host plant and pathogen interactions revealed that plant responses to abiotic stress and pathogen infections were mediated through several common regulatory genes or factors. The presence of "cross-talk" between responses to abiotic stress and biotic stress provides new approaches for enhancing host resistance to biotic stress through the up regulation of key signal transduction factors. Recent efforts to identify molecular and genetic markers for corn kernel resistance as well as studies in host plant-pathogen interactions have suggested a correlation between stress tolerance and plant disease resistance (Brown et al., 2004).

2. Maiz drought tolerance

Abiotic stress presents a major challenge in the quest for sustainable food production as this may reduce the potential yields by 70% in crop plants. Coping with plant environmental stress is the foundation of sustainable agriculture. Stress is a phenomenon that limits crop productivity or destroys biomass. Stress can be also biotic, caused by insects and diseases, or abiotic, which may include drought, flooding, salinity, metal toxicity, mineral deficiency, adverse pH, adverse temperature, and air pollution (Borlaug and Dowswell, 2005).

Within abiotic stress, drought is regarded as the most damaging. Moreover, water limitation is one of the most important constraints for agriculture and recently, global warming may be worsening this situation in most agricultural regions (Gosal et al., 2009; Xoconostle-Cázares et al., 2010).

The complex nature of drought tolerance limits its management through conventional breeding methods (Gosal et al., 2009). For example, stress hydric in flower formation stage reduces the number of kernels per plant, whereas in the grain filling stage the size of the kernel is reduced (Zarco et al., 2005; Grant et al., 1989).

Conventional breeding and genetic engineering, are becoming an art through which crop varieties of high quality and yield are being developed. Breeding for any desired trait undoubtedly requires a significant amount of genetic variation at intra-specific, inter-specific or inter-generic levels. The contributions of plant breeding to food production at global level have been enormous during the 20th century. There has been most important plant breeding break-through for almost all commercially important crops including major ones such as maize, wheat, rice, cotton, among others. However, relatively little breeding work has been carried out on improving crops for drought tolerance. (Ashraf, 2010).

Through conventional breeding, genetic variability for drought tolerance among crops/crop cultivars or among sexually compatible plant species can be identified, and the genetic variation so identified can be introduced through different mating designs into cultivars/lines with good agronomic characteristics (Ashraf, 2010). Conventional breeders have made considerable strides in developing drought tolerant lines/cultivars of some important food crops. One of the breeding approach started in Mexico in the 1970s at the International Maize and Wheat Improvement Center (CIMMYT). This started with the intention of developing drought tolerant maize (Bänziger et al., 2004). In 1997, CIMMYT spanned its breeding program to southern Africa aimed at improving maize for the drought-hit areas. A number of maize hybrids developed by the CIMMYT scientists were found superior to all those developed by private enterprises. The maize hybrids were superior in terms of growth and grain yield under drought-prone environments (Bänziger et al., 2004).

In 2006, plant breeders at the Crops Research Institute (CRI) based at Kumasi, Ghana, have developed a highly drought tolerant maize cultivar 'Obatanpa GH' in collaboration with the International Institute of Tropical Agriculture (IITA), Ibadan, the CIMMYT, Mexico, and the Sasakawa Global 2000. Similarly, 16 early maturing maize inbred lines (from TZEI 1 to TZEI 16) resistant to a scrounging weed Striga hermonthica (Del.) Benth were produced by the IITA. All these lines were found to be highly resistant to water limited conditions (Ashraf, 2010).

Marker-assisted breeding (MAB) and transgenic approach are diverse biotechnologies. In MAB desirable genes can be tagged so they can be easily selected within the breeding population, whereas through the transgenic approach the desirable genes can be transferred from one species to another. A large number of genomic regions of a crop germplasm can be examined for their breeding value through MAB, which facilitates the breeder to pool genes of diverse origins (Vinh and Paterson, 2005).

3. Molecular markers for drought tolerance identified in maize

Given that the drought resistance or tolerance in maize is clearly a qualitative character (Zarco et al., 2005). Through MAB it is now possible to examine the usefulness of thousands of genomic regions of a crop germplasm under water limited regimes. By examining the breeding value of each of the genomic regions, the breeder can coalesce genes of multifarious origins in novel ways, which was not possible previously with conventional breeding tools and protocols (Ashraf, 2010).

Given that the drought resistance or tolerance in maize is clearly a quantitative character (Zarco et al., 2005). But, like tolerance to other abiotic stress, drought stress is controlled by many minor genes (polygenes) that have additive effects in their expression (Thi Lang and Chi Buu, 2008. The loci on chromosomes housing such types of genes are now referred to as quantitative trait loci (QTL) (Ashraf, 2010). In a QTL analysis, phenotypic evaluation is carried out in a large number of plants from a segregating population for a variety of genetic markers. Then, the whole population, or only a part of it, is genotyped. Finally, appropriate statistical analysis is performed to pinpoint the loci controlling a trait (Asins, 2002). Due to the intricacy of the abiotic stress tolerance and the problems encountered in phenotypic based selection, the QTL mapping has been considered as imperative to the use of DNA markers for improving stress tolerance (Ashraf, 2010).

Natural genetic variation of a crop can be exploited either via direct selection under stressful conditions (simulated or natural) or via mapping of QTL and subsequent marker-assisted selection (Ashraf et al., 2008). QTL mapping allows assessing the locations, numbers, magnitude of phenotypic effects, and pattern of gene action (Vinh and Paterson, 2005). The role of polygenes in controlling a trait has been widely assessed by traditional means, but the use of DNA markers and QTL mapping has made it convenient to dissect the complex traits (Ashraf, 2010).

Recent molecular biology tools have undoubtedly led to the development of DNA markers that have been effectively used to identify QTL a number of traits in different crops. Ashraf et al. (2008) have listed a variety of DNA markers such as RFLPs, RAPDs, CAPS, PCRindels, AFLPs, microsatellites (SSRs), SNPs, and DNA sequences being currently in use to examine the inheritance of stress tolerance. QTL mapping for the drought tolerance trait has been done in different crops, the most notable being maize, wheat, barley, cotton, sorghum, and rice (Sari-Gorla et al., 1999; Ashraf, 2010).

Associations between markers and traits were first reported in maize by Stuber and Moll (1972) using isozymes. The advent of abundant DNA-based molecular markers allowed the construction of genetic maps. In maize, a linkage analysis between the manifestation of some key characteristics like male and female flowering time, anthesis-silking interval, plant height, and molecular markers [RFLP, microsatellites (SSR) and AFLP] was carried out under different water regimes using a maize population consisting of 142 RILs derived from selfing the F1 population from a cross B73×H99. Linkage analysis showed that, the QTL identified for male flowering time and plant height were the same under well-watered and water-stressed conditions (Sari-Gorla et al., 1999).

A marker-assisted backcross (MABC) selection program for improving grain yield under water limited conditions in tropical maize was conducted at CIMMYT, Mexico, which involved the crossing of drought resistant line Ac7643 with a drought susceptible line CML247. Marker-based selection was carried out stepwise on all four generations (from BC1F1 to BC2F3). After the four consecutive MABC cycles, the 70 BC2F3 individuals exhibiting the closest allelic composition at target and non-target loci were bred with two CIMMYT testers (CML254 and CML274). Thirty genotypes were selected on the basis of their performance in terms of grain yield and some key agronomic traits. However, the best five MABC-derived hybrids produced yield about 50% more than that of control hybrids, but in contrast, under mild water stress, there was no difference between MABC-derived hybrids and the control plants. This confirms that the expression of genetic variation for drought tolerance mainly depends on the severity of drought stress (Ribaut and Ragot, 2007).

4. Correlations between drought and heat stress tolerance and resistance to Aspergillus infection in maize

Some studies have shown that heat stress plays an important role in the susceptibility of corn to aflatoxin contamination (Payne, 1998; Abbas et al., 2002). Under these conditions stress the plant can result in high aflatoxin levels. Plant stress facilitates greater colonization of corn kernel and infection by *A. flavus* in the field. In general it appears that there is a relationship between temperature-moisture and fungal infection and consequently aflatoxin contamination. It has been reported that when conditions of high temperature and drought occur together during the growing season, increases growth of the fungus and toxin production in grains (Payne, 1998). In this direction, there are results that indicate that irrigating corn fields to reduce drought stress also reduced fungal infection and aflatoxin production (Jones et al. 1981) or lower soil temperature reduces aflatoxin contamination in peanut (Hill et al., 1983).There are another results that reinforce this notion in peanuts (Cole et al., 1985; Dorner et al. 1989). Studies on the influence of irrigation and subsoiling on infection and aflatoxin production in corn, have suggested that water stress appears to be a major factor affecting aflatoxin contamination (Payne et al. 1986). Even it has been found in peanuts an inverse relationship between the amount of water supplied and fungal colonization with aflatoxin production (Wotton and Strange 1987).On the other hand, Tubajika and Damann, (2000) found that corn drought tolerant lines all had significantly lower levels of ear rot and aflatoxin contamination compared to the aflatoxin resistant. In summary, available information suggests a possible association between drought tolerance and aflatoxin resistance in corn. But it has been difficult to detect genetic markers or chromosomal regions associated with kernel resistance quantitative trait loci (QTL) because enormous influence of environmental factors on phenotypic expression of resistance (Davis et al ., 1999, Paul et al., 2003).

Chen et al., (2002) performed a proteomic approach to identify proteins whose level of expression was associated with kernel resistance against *A. flavus* infection and aflatoxin production. They compared resistant with susceptible genotypes using large format two-dimensional gel electrophoresis. Over a dozen proteins spots were identified and sequenced. These proteins were categorized as follows: storage proteins (globulin 1 and globulin 2), late embryogenesis abundant (LEA) proteins related to drought or desiccation (LEA3 and LEA14), water- or osmo-stress related proteins (WSI18 and aldose reductase), heat-stress related proteins (HSP16.9), and antifungal proteins which include a trypsin inhibitor (Chen et al., 2002). The majority of those proteins were stress-related proteins and highly hydrophilic storage proteins which suggest that kernel resistance may require high levels of these kinds of proteins (Brown et al., 2004).

5. Stress-related proteins

Recent studies have found higher levels of stress-related proteins and highly hydrophilic storage proteins in kernels of resistant genotypes compared with susceptible genotypes. This may enable resistant kernels to effectively induce an active defense response upon fungal attack, even under stress caused by heat or drought (Chen et al., 2004).

Chen et al. (2004) conducted an study conducted in which a GLX-I protein was identified on the basis of peptide sequence analysis. This protein was expressed at higher levels in resistant maize kernel embryos in contrast with the susceptible ones. Sequence homology

comparisons indicate that maize GLX-I belongs to the long-type glyoxalase family (280 to 295 amino acids), which contains two highly homologous domains. GLX-I is present in many organisms, such as fungi, plants, and animals. It catalyzes the conversion of MG, a potent cytotoxic compound, to nontoxic D-lactate in the presence of glutathione and GLX-II (Johansen et al., 2000). MG is known to arrest growth and react with DNA and protein and increase sister chromatid exchanges (Payne, 1998). Recent studies found that, in addition to dehydrin and group 3 late embryogenesis abundant proteins, *glx-I* was induced during drought stress in *Sporobolus stapfianus*, and in response to salt and water stress in *B. juncea* and tomato, suggesting an important role for GLX-I in conferring tolerance to plants under those stress conditions (Payne, 1998; Chen et al., 2004). Levels of GLX-I activity were examined in dry, noninfected, and infected kernels of resistant and susceptible lines and it was found that resistant lines generally have higher constitutive levels of GLX-I activity than susceptible ones. In addition, the level of MG did not increase in resistant genotypes. This lack of increase could be due to the relatively higher levels of GLX-I activity observed in resistant infected kernels. An elevation in MG content in susceptible genotypes, combined with low GLX-I activities, could weaken the kernel's ability to defend against fungal infection (Chen et al., 2004).

6. Antifungal proteins

Plants are exposed to a large number of pathogenic fungi. Although they do not have an immune system, plants have evolved a variety of potent defense mechanisms, including the synthesis of low-molecular-weight compounds, proteins, and peptides that have antifungal activity (Selitrennikoff, 2001). These proteins appear to be involved in either constitutive or induced resistance to fungal attack. Several classes of antifungal proteins involve inhibition of the synthesis of the fungal cell wall or disrupt cell wall structure and/or function; others perturb fungal membrane structure, resulting in fungal cell lysis (Selitrennikoff, 2001).

Plants when exposed to pathogens such as fungi and viruses produce low-molecular-weight antimicrobial compounds called phytoalexins, antimicrobial peptides, and small proteins and up-regulate a number of antimicrobial proteins. These plant proteins, called pathogenesis-related (PR) proteins, have been classically divided into five groups, PR-1, -2, -3, -4, and -5, based on serological and amino acid sequence analyses (Van Loon, 1985). PR-1 proteins are accumulated to high levels after pathogen infection and are antifungal both in planta and in vitro (Niderman et al., 1995). PR-1 proteins have been found in rice, wheat, maize, tobacco, *Arabidopsis thaliana* barley, and many other plants (Selitrennikoff, 2001).

PR-2 proteins have (1,3) β-endoglucanase activity in vitro and have been grouped into three classes on the basis of amino acid sequence analysis. The antifungal activity of plant (1,3)β-glucanases is thought to occur by PR-2 proteins hydrolyzing the structural (1,3)β glucan present in the fungal cell wall, particularly at the hyphal apex of filamentous molds where glucan is most exposed, resulting in a cell wall that is weak. This weakened cell wall results in cell lysis and cell death (Belfa et al., 1996).

A number of enzymatic assays have shown PR-3 (chitinases) proteins to have in vitro chitinase activity. Chitinases have been isolated from fungi, plants, and bacteria and have potent antifungal activity against a wide variety of human and plant pathogens. PR-3 proteins are endochitinases that cleave cell wall chitin polymers in situ, resulting in a weakened cell wall and rendering fungal cells osmotically sensitive (Selitrennikoff, 2001).

PR-4 proteins are chitin-binding proteins PR-4 proteins have been isolated from potato, tobacco, barley, tomato, and many other plants. The antifungal activity of these kinds of proteins is likely the result of protein binding to nascent fungal cell wall β-chitin. By mechanisms not understood these results in disrupted cell polarity, with concomitant inhibition of growth (Bormann et al., 1999).

PR-5 proteins are known as TL proteins several TL proteins cause cell permeability changes in fungal cells with a cell wall but have no or little effect on protoplasts. For example, zeamatin (a TL protein from corn) caused very rapid cell lysis of *N. crassa*, even at 4°C; lysis occurred primarily at subapical regions (Roberts and Selitrennikoff 1990).

7. Biochemical pathways

Biotic or abiotic stress alone was able to induce the expression of genes involved in both biotic and abiotic stress responses. Fungal infection represents a unique kind of stress to host plants. In response to such stress, plants not only induce specific antifungal genes, but also up regulate general stress-related genes (Brown et al., 2004).

An increasing body of evidence suggests that a subset of plant responses to biotic and abiotic stress is shared, such as the generation of reactive oxygen species (ROS), the activation of mitogen-activated protein kinases (MAPKs), and hormone modulations (Brown et al., 2004). Plant–pathogen recognition causes the rapid activation of appropriate defenses. Some of the components in the signal transduction pathways have been identified and characterized.

One of the mayor consequences of drought stress is the loss of protoplasmic water leanding to the concentration of ions such as Cl- and NO3-. At high concentrations these ions effectively inhibit metabolic functions (Hartung et al., 1998). Also, the concentrations of protoplasmic metabolites and the loss of water from the cell lead to the formation of a glassy state. Under this condition, the chances of molecular interactions can cause protein denaturation and membrane fusion (Hoekestra et al., 2001). To maintain cell turgor and metabolic functions under drought stress, plants generate reactive oxygen species (ROS), including hydrogen peroxide (Inze and Montagu,1995). ROS production is recognized as a common event in plant response to biotic and abiotic stress. The mechanism of how ROS leads to downstream responses is still not clear, however, the requirement of specific MAPKs has been implicated (Kovtun et al., 2000). Accumulation of hydrogen peroxide, eventually, induce the expression of detoxification and stress protection proteins as Heat Shock Proteins (HSPs)(Kovtun et al., 2000). Some reports suggests that HSPs function as molecular chaperones which are involved in ATP-dependant protein unfolding or assembly/disassembly actions and prevent protein denaturation during stress (Pelham, 1986). Termotolerance have been associated to expression of HSPs in maize (Preczewski et al., 2000)

The mitogen activated protein kinase (MAPK) cascades are the major components downstream of receptors that transduce extracellular stimuli into intracellular responses (Zhang and Klessig, 2001). One of the mechanisms by which different stimuli converge onto one MAPK is believed to involve several unrelated kinases that function as MAPKKKs to initiate the MAPK cascade. Several plant kinases have been identified as MAPKKKs, including EDR1 and NPK1/ANPs (Zhang and Klessig, 2001)

A variety of plant hormones, including salicylic acid (SA), jasmonate (JA), ethylene, and abscisic acid, have been implicated in mediating responses to a wide range of biotic and

abiotic stress. The roles of these hormones are dependent upon the particular hostpathogen interaction (Diaz et al., 2002; Brown et al., 2004). The the effect of phytohormones is also regulated by other factors. For example, the MAPK kinase kinase, EDR1, negatively regulates SA-inducible defenses, whereas MAPK 4 appears to differentially regulate SA and JA signals. These findings also suggest that MAPK modulates cross-talk between different plant defense pathways (Hammond-Kosacky and Parkerz, 2003).

Everything discussed in this article, on the complex role of drought in relation to aflatoxin corn contamination, shows the current research situation and paths toward solving this important and serious public health problem

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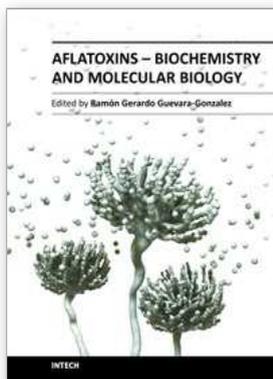
Aflatoxins - Biochemistry and Molecular Biology

Edited by Dr. Ramon G. Guevara-Gonzalez

Aflatoxins â€" Biochemistry and Molecular Biology is a book that has been thought to present the most significant advances in these disciplines focused on the knowledge of such toxins. All authors, who supported the excellent work showed in every chapter of this book, are placed at the frontier of knowledge on this subject, thus, this book will be obligated reference to issue upon its publication. Finally, this book has been published in an attempt to present a written forum for researchers and teachers interested in the subject, having a current picture in this field of research about these interesting and intriguing toxins.

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