World Mycotoxin Journal, 2018; 11 (2): 187-199

Wageningen Academic Publishers

Relationship between drought and preharvest aflatoxin contamination in groundnut (*Arachis hypogaea* L.)

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Received: 18 September 2017 / Accepted: 16 November 2017

REVIEW ARTICLE

Abstract

Groundnut is a commercial oilseed crop that is prone to infection by *Aspergillus flavus* or *Aspergillus parasiticus*. Drought impairs the defence mechanism of the plant and favours the production of aflatoxin by the fungus. Aflatoxin is a carcinogen and its presence in food and feed causes significant economic loss. The answer to the question, 'how drought tolerance and aflatoxin resistance are related?' is not clear. In this review paper, the relationship of drought and preharvest aflatoxin contamination (AC), the relationship of drought tolerance traits and AC, and the approaches to enhance resistance to AC are discussed using up-to-date literature. Factors leading to AC are drought, high geocarposphere temperature, kernel/pod damage, and reduced phytoalexin synthesis by the plant. If the fungus colonises a kernel with reduced water activity, the plant cannot synthesise phytoalexin and then, the fungus synthesises aflatoxin. Breeding for resistance to AC is complicated because aflatoxin concentration is costly to measure, highly variable, and influenced by the environment. Since drought tolerant cultivars have resistance to AC, traits of drought tolerance have been used as indirect selection tools for reduced AC. The genetics of aflatoxin resistance mechanisms have not been made clear as the environment influences the host-pathogen relationship. Host-pathogen interactions under the influence of environment should be studied at molecular level to identify plant resistant factors using the tools of genomics, proteomics, and metabolomics in order to develop cultivars with durable resistance. Many candidate genes involved in host-pathogen interactions have been identified due to improvements in fungal expressed sequence tags, microarrays, and genome sequencing techniques. Moreover, research projects are underway on identifying genes coding for antifungal compounds, resistance associated proteins and quantitative trait loci associated with aflatoxin resistance. This review is expected to help those who wish to work on reducing AC in groundnuts.

Keywords: aflatoxin, drought, drought tolerance, peanut

1. Introduction

In rainfed areas of semi-arid tropics that suffer unpredictable droughts, groundnut (*Arachis hypogaea* L.) is cultivated (Wright and Nageswara Rao, 1994) and so, there is a real danger of contamination with mycotoxin known as 'aflatoxin' (Nageswara Rao *et al.*, 2002). Preharvest aflatoxin contamination (AC) in food and feed is a common problem all over the world. Aflatoxin is known to cause liver cancer (Hsu *et al.*, 1991). Wu *et al.* (2013) showed that consumption of large amounts of groundnuts contaminated with aflatoxins even at low levels is detrimental to health. The influence of AC on the agricultural economy is particularly destructive during droughts. Aflatoxins in US groundnuts caused >$25.8 million in losses per year during 1993 to 1996 (Schmale and Munkvold, 2017).

Aflatoxin is a secondary metabolite, made mostly by the fungi, *Aspergillus flavus* Link ex. Fries and *Aspergillus parasiticus* Speare. These fungi infect vulnerable crops including groundnut during cultivation, harvest, storage, and processing. They can enter groundnut pods through

ISSN 1875-0710 print, ISSN 1875-0796 online, DOI 10.3920/WMJ2017.2248

minute cracks that arise when the pods mature and dry (Sanders *et al.*, 1984). When *A. flavus* infects groundnut pods with insect or mechanical damage, AC may occur. On the other hand, postharvest AC can also occur if there are poor storage conditions. The Council for Agriculture Science and Technology (CAST, 2003) has described epidemics of severe aflatoxicosis due to infected food consumption by humans in India, Kenya, Malaysia, and Thailand. In 2004, aflatoxin-contaminated maize took the lives of 125 persons in Kenya (CDC, 2004; Probst *et al.*, 2007). Recently Bumbangi *et al.* (2016) reported high levels of AC in raw groundnut kernels sampled from open markets and supermarkets in Zambia, which posed a serious threat to health. Hence, these authors suggested that priority should be given to intervention strategies to lessen AC levels in groundnuts.

According to the US Food and Drug Administration (FDA), aflatoxin is often present in food (Williams *et al.*, 2004). The US FDA has set the maximum allowable limit of total aflatoxins (B_1, B_2, G_1 and G_2) in food/feed to 20 µg/kg, while the European Union has set a maximum level for aflatoxin B_1 of 2 µg/kg and for total aflatoxins of 4 µg/kg (EC, 2010). When the amount of aflatoxin is higher than the maximum allowable limits in food/feed products, they are destroyed in the developed countries, causing a loss of billions of dollars over the world every year. Acute epidemics of AC often take place in certain areas of southern US and lead to huge financial losses (Robens and Cardwell, 2005). As identification and detoxification policies are not feasible in developing countries, food safety is a most important issue in these countries. Regular use of food polluted with aflatoxin is prevalent in these countries due to food scarcity.

Torres *et al.* (2014) summed up the latest developments in preventing aflatoxins in several groundnut growing countries. Fountain *et al.* (2015) gave a detailed account of resistance to *Aspergillus flavus* in maize and groundnut with regard to molecular biology, breeding, environmental stress and future perspectives. Pooja *et al.* (2015) critically reviewed the biotechnological developments for fighting *A. flavus* and AC in crops. Sharanaiah *et al.* (2017) made a survey on the impact of biologically active aflatoxins and their control strategies. Recently, Udomkun *et al.* (2017) reviewed certain innovative technologies to manage aflatoxins in foods and feeds. Although drought is considered to be the main factor leading to preharvest AC in groundnut, an exclusive review on the relevance of drought on AC in groundnut has not been done. Therefore, this review will discuss the nature of aflatoxin, its relationship with drought and drought tolerance traits in groundnut, and the approaches to enhance resistance to AC.

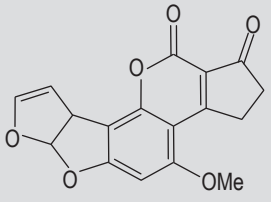
2. Types and structure of aflatoxins

Aflatoxins are members of a group of chemical substances with difuranocoumarins. There are four most important aflatoxins, named aflatoxin G_1, G_2, B_1 and B_2 (AFG_1, AFG_2, AFB_1 and AFB_2, respectively). This classification is derived from their capacity to show green (G) or blue (B) fluorescence under ultraviolet light and their relative mobility in thin-layer chromatography (Guo *et al.*, 2008b). Aflatoxins contain a lactone moiety (Figure 1) and highly oxygenated stable structure of 5 fused rings. AFB_1 is a strong cancer-causing agent (Squire, 1981). When cows are fed with feeds contaminated with AFB_1, they give milk containing aflatoxin M_1 which is a hydroxylated derivative metabolite of AFB_1 (Van Egmond, 1989). *A. parasiticus* produces AFB_1 and AFB_2, besides AFG_1 and AFG_2. On the other hand, *A. flavus* produces only AFB_1 (Horn *et al.*, 2009b). According to Klich and Pitt (1988), conidial wall texture was the most effective criterion for distinguishing *A. flavus* and *A. parasiticus*. They also found that very few *A. flavus* isolates produced type G aflatoxins.

The aflatoxin biosynthetic pathway has been determined and the molecular structures of the intermediates of the pathway described (Payne and Brown, 1998; Yu, 2004; Yu *et al.*, 2005). There are no less than 23 reactions catalysed by enzymes in the synthesis of aflatoxin and at least 15 structurally-defined intermediates in the aflatoxin biochemical pathway.

3. Relationship between drought and preharvest aflatoxin contamination

A number of studies in the past have shown that drought favours AC in groundnut. In this section, only the important findings in the past are discussed with reference to its impact on aflatoxin synthesis. Wotton and Strange (1987) noticed a negative relationship between irrigation and kernel colonisation by *A. flavus*, which is in agreement with the findings of Dorner *et al.* (1989) who noticed that low soil moisture favoured the growth of *A. flavus*. High aflatoxin levels are usually related to hot climate and drought in the field (Payne, 1998). Waliyar *et al.* (2003a) stated that drought is a predisposing factor for AC in groundnut. Severe drought



Aflatoxin B_1 (AFB_1)

Figure 1. Chemical structure of aflatoxin B_1.

encouraged the growth of *A. flavus*, leading to high AC (Arunyanark *et al.,* 2010; Craufurd *et al.*, 2006). Although the fungus is found in several climatic areas, it is often observed between latitudes 16° and 35° in warm climate zones and is not common above 45° latitudes (Klich, 2007). A negative correlation (r = -0.46) was observed between AC for seven elite accessions and rainfall during the crop season (Waliyar *et al.,* 2016). Wu *et al.* (2016) noticed a significant association between aflatoxin and meteorological conditions 30 days prior to harvest of groundnut. They observed maximum AC when the rainfall was occasional (leading to drought) and the approximate mean, minimum and maximum temperatures were 23, 20 and 29 °C, respectively. Recently, Kachapulula *et al.* (2017) found that quantities of crops unsafe for human consumption varied significantly (P<0.001) among agroecologies in Zambia with more AC (38%) in the warmest agroecology and the least (8%) in cool, wet agroecology.

Horn *et al.* (1995) reported that *Aspergillus* spp. are exclusively thermo-tolerant, xero-tolerant and can stay alive efficiently in the field during water deficit and temperature stress. Water deficit decreases soil water activity, which in turn, lessens the growth of amoebae, competing bacteria and fungi. Hence, proliferation of xerophiles, such as *A. flavus* and *A. parasiticus,* increases (Pitt *et al.*, 2013). *A. flavus* survives as conidia or sclerotia in the soil. It is present in the form of mycelia inside plant tissues. Horn *et al.* (2009a) reported that sclerotia produce conidia and probably ascospores with harsh environments. Structural and regulatory aflatoxin biosynthetic genes of the fungus have been significantly influenced by environmental stresses, such as high temperature and drought (Medina *et al.*, 2014). Reactive oxygen species (ROS) accumulates within the tissues of host plant due to drought and/or heat stress and these ROS may have a vital role in communicating with *A. flavus* (Fountain *et al.*, 2015) to synthesise aflatoxin. Hence, drought has a significant role in host-pathogen interactions.

Hill *et al.* (1983) found that a lower soil temperature reduced AC in groundnut. Cole *et al.* (1985) noticed that when there was a drought and high soil temperature, there was high AC in groundnut. Similar findings were reported by Arunyanark *et al.* (2009). Fungal attack and aflatoxin formation in groundnut pods are due to lengthy drought stress, increased soil temperature (>22 °C) and any physical damage to pods during the kernel filling stage (Cole *et al.*, 1989; Horn, 2005; Nageswara Rao *et al.*, 2002). Drought and pest infestation make groundnuts more susceptible to toxigenic *Aspergillus* infection and AC (Pitt *et al.*, 2013). The chief features favouring infection by *A. flavus* and *A. parasiticus* in groundnuts are found to be kernel damage by insects, drought and increased soil temperatures (Torres *et al.*, 2014).

Literature shows differences in pod zone temperature (geocarposphere) that favoured AC. Under the influence of drought, the reported geocarposphere temperatures that favoured AC were 28-31 °C (Hill *et al.*, 1983), 35 °C (Sanders *et al.*, 1984) and 29.6-31.3 °C (Cole *et al.*, 1985). Williams and McDonald (1983) reported that the mean, threshold, and geocarposphere temperature required for AC were between 25.7-27 °C. The optimum temperature for the growth of *A. flavus* is 25-42 °C (Klich *et al.*, 1992). Interestingly, AC is found to occur if the pods are subjected to water deficit even if crop roots received enough water (Sanders *et al.*, 1993). This reveals that the geocarposphere temperature increases when the pods are subjected to drought. An increase in the geocarposphere temperature favours the fungi to infect the pods. This is supported by Cole *et al.* (1989) who reported that geocarposphere temperatures cooler than 29-31 °C can lead to less aflatoxin, even under water deficit conditions. Craufurd *et al.* (2006) related fungal infection and subsequent AC in groundnut with the occurrence of drought during pod filling stage when soil temperatures are near optimal for *A. flavus*. It is relevant to note here that drought in the absence of high soil temperature does not result in AC (Craufurd *et al.*, 2006).

Sudhakar *et al.* (2007) reported that high aflatoxin levels are usually found in damaged pods compared to pods with intact shells. Extreme water deficit results in injuries to the pods and testas and enables the fungus to enter and infect the kernels (Okello *et al.*, 2010). The fungus can also infect through flowers (Styer *et al.*, 1983). Cole *et al.* (1985) suggested that when a kernel is infected with *A. flavus* or *A. parasiticus*, aflatoxin synthesis occurs only after the natural defence mechanisms stop due to drought and high temperature. When there is no drought, fungal infection induces the synthesis of plant phytoalexins, which decrease the growth of fungi and consequent AC (Basha *et al.*, 1994; Wotton and Strange, 1987). Sudhakar *et al.* (2007) reported inconsistent associations between AC and seed infection percentage and inferred that AC in kernels is lessened when there is high leaf relative water content (RWC) in the leaf which permits synthesis of phytoalexin. A phytoalexin compound present in groundnut kernels is resveratrol (Sanders *et al.*, 2000).

However, if there is drought, the canopy of groundnut diminishes (Cole *et al.*, 1985) and hence, the soil temperatures increase, which in turn will result in dry soil, decreased plant water status and finally a reduction in kernel water activity. Cole *et al.* (1989) said that a decrease in kernel water activity can lead to a decrease in phytoalexin synthesis which favours *A. flavus* growth and synthesis of aflatoxin. The AC is frequently connected with drought intensity, crop's growth stage of drought occurrence and the temperature of soil and/or air (Cole *et al.*, 1989). Payne (1998) reported that water deficit and elevated temperature, which frequently happens simultaneously in the course of a

growing season, have probably been involved in poor kernel development and, thus, favoured fungal growth leading to AC. Craufurd *et al.* (2006) reported that drought for <10 days in the field was adequate to bring about considerable AC, especially in the last 4-6 weeks of a growing season (Cole *et al.*, 1985; Hill *et al.*, 1983). Two factors, namely water deficit and greater *A. flavus* load in the soil act together and lead to kernel colonisation by *A. flavus* and consequent AC (Arunyanark *et al.*, 2009).

It is well known that kernel invasion or colonisation by the fungus does not always result in AC (Basha *et al.*, 1994) because drought determines AC, as can be seen in Figure 2, which depicts the sequence of events responsible for AC in groundnut. This figure summarises the information collected for this review.

It can be concluded that the factors responsible for AC in groundnut are drought, high soil temperature in the pod zone, kernel damage by insects, physical damage to pods, and reduced phytoalexin synthesis by the plant. A combination of reduced soil water activity and high soil temperature favours the load of toxigenic thermo-tolerant *Aspergillus* in the soil by decreasing the population of competing soil microflora. High *Aspergillus* load in the soil leads to kernel colonisation by the fungus. Reduced kernel water activity due to drought weakens the plant's defence mechanism and thereby, phytoalexin synthesis is inhibited and AC occurs. It is pertinent to note here that phytoalexin production by the plant and aflatoxin production by the fungus are inversely proportional.

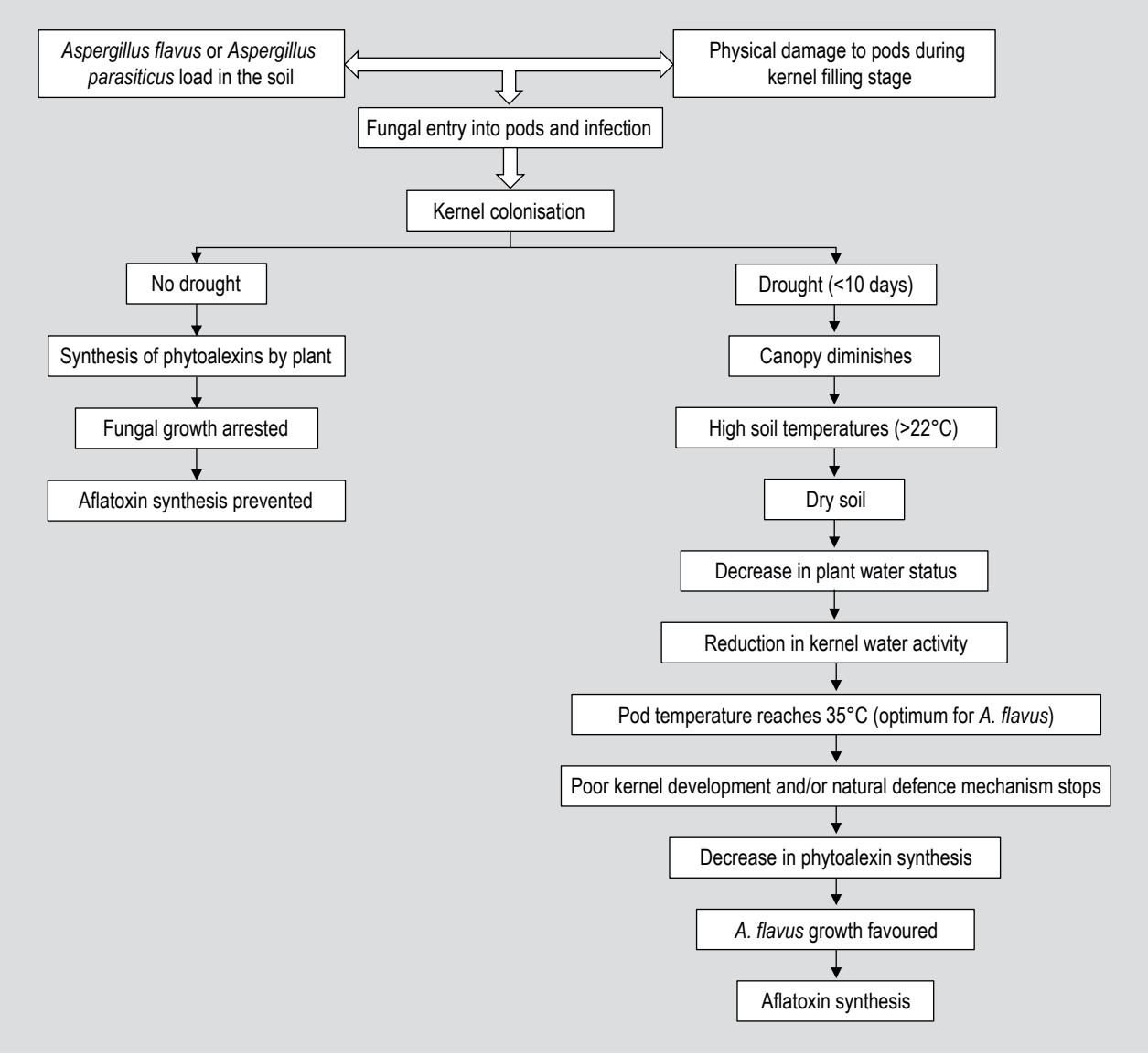


Figure 2. Sequence of physiological events responsible for aflatoxin contamination in groundnut.

4. Relationship between drought tolerance and resistance to preharvest aflatoxin contamination

In plant breeding programs, traits for drought tolerance are being used as indirect selection criteria to select for reduced AC. There are three reasons for this. First, measurement of aflatoxin concentration is expensive. Second, aflatoxin concentration is highly variable as it is mostly influenced by the environment. Third, in numerous studies the drought tolerance traits have been associated with lower AC.

Cole *et al.* (1993) stated that under prolonged water deficit the groundnut varieties higher in kernel water were more resistant to fungi and contained less aflatoxin. After assessing resistance to AC in groundnut varieties with varying levels of drought tolerance, Holbrook *et al.* (2000) concluded that drought tolerant varieties had significantly less AC. There is a potential for the drought tolerance traits to be employed as indirect selection criteria for resistance to AC. These results imply that drought tolerant varieties may acquire a certain level of resistance to AC. Arunyanark *et al.* (2009) were of the opinion that the mechanisms governing genotypic variation for resistance to AC may be connected with differences in drought tolerance.

Transpiration efficiency (TE) is a difficult physiological trait to measure. It is the ratio of biomass produced and the quantity of water transpired by a plant. Wright *et al.* (1994) noticed a statistically significant correlation between TE and an easy to measure trait, specific leaf area (SLA). When the SLA is low, the leaves are thicker. Thicker leaves have a high chlorophyll density per unit leaf area. Therefore, the varieties of groundnut with low SLA show a greater CO_2 assimilation efficiency. So low SLA is correlated with lower AC. The work by Girdthai *et al.* (2010) supports the findings by Wright *et al.* (1994); Girdthai *et al.* (2010) found a positive correlation between AC and SLA, which was used as a surrogate trait for drought tolerance in their work. According to Arunyanark *et al.* (2009), the cuticle layer may be thicker in thicker leaves and the thicker cuticle is considered to be the principal component contributing to avoid water loss through transpiration.

Rooting efficiency is defined as the plant's capability to alter the distribution of its roots to use water from the deeper layers of soil. It is a significant physiological characteristic that has a relationship with drought tolerance (Songsri *et al.*, 2008). A plant's capacity to obtain water from deeper layers of soil is indicated by higher root length density (RLD) at deeper depths of soil. Water extraction from soil might allow cell turgor maintenance in order to contribute to drought tolerance. Traits of drought tolerance, namely SLA and RLD, might contribute to resistance to AC (Arunyanark *et al.*, 2009). These authors suggested that three traits (SLA, RLD and kernel colonisation) in combination may be used as selection criteria for choosing genotypes that resist to AC.

Holbrook *et al.* (2009) experimented with traits that were less variable and inexpensive to measure. These authors concluded that visual stress ratings, leaf temperature and yield under water deficit could be used as tools for indirect selection for less AC. They selected these traits because of positive and significant relationships of AC with leaf temperature and visual stress ratings and also due to a negative but significant relationship of AC with yield. According to Girdthai *et al.* (2010), the best characteristics that can be used as tools for indirect selection for less AC include SLA, RWC, chlorophyll density and drought stress ratings among which RWC and SLA are cheaper and less variable. The correlation between RWC and SLA was investigated by Nautiyal *et al.* (2002) who suggested that conservation of plant water status in the course of a water deficit is favoured by low SLA and so, metabolic activities are maintained, which, in turn, results in the maintenance of favourable leaf temperature. Wotton and Strange (1985) hypothesised that the ability of groundnut

Table 1. Noteworthy traits of drought tolerance associated with less aflatoxin contamination (AC).

Traits contributing to less AC[1]	Difficulty level of measurement	References
Kernel water content (high)	easy	Dorner and Cole (1997)
Transpiration efficiency (high)	difficult	Arunyanark *et al.* (2009)
Specific leaf area (low)	easy	Arunyanark *et al.* (2009)
Root length density (high)	difficult	Arunyanark *et al.* (2009)
Visual stress ratings (no stress)	easy	Holbrook *et al.* (2009)
Leaf temperature (low)	easy	Holbrook *et al.* (2000)
Yield with water deficit (high)	easy	Hamidou *et al.* (2014)
Leaf relative water content (high)	easy	Girdthai *et al.* (2010)
Chlorophyll density (high)	difficult	Girdthai *et al.* (2010)

[1] Words in parenthesis indicate the degree of the particular trait.

kernels to synthesise phytoalexin depends on the capacity to conserve the water status of the plant during a water deficit.

On the other hand, Hamidou *et al.* (2014) reported an absence of a significant relationship between the drought tolerance index and AC values. Hence, they inferred that although drought intensity increases AC, drought tolerance in groundnut does not lead to less AC. This suggests that the mechanisms of drought tolerance and resistance to AC are different. Important selected drought tolerance traits associated with less AC are given in Table 1. The difficulty level of measuring these traits is also given.

It has been proven beyond doubt that drought leads to AC. Therefore, there is a great possibility for a link to exist between the mechanisms regulating aflatoxin resistance and drought tolerance in groundnut. It can be inferred from published literature that relationships have been established between these two traits only based on correlation coefficients (*r*). No direct relationships between these two traits have been demonstrated at cellular, physiological, biochemical, or molecular level. Until such a direct relationship is shown, it should be admitted that such relationships are merely speculative. Therefore, more research efforts are required to unravel the mystery behind the relationship between these two traits. Identifying common metabolite(s)/protein(s)/gene(s) expressed during fungal infection and drought may be useful to trace how these two traits are related. Meanwhile, traits of drought tolerance listed in Table 1 will continue to serve as indirect selection tools for reduced AC.

5. Approaches to enhance resistance to preharvest aflatoxin contamination

AC is costly to determine and varies a lot in groundnuts (Holbrook *et al.*, 2000). A decrease in aflatoxin content is a significant goal in breeding programs. Groundnut breeding programs for resistance to AC would have a comprehensive effect on quality of seeds, thus improving the financial profit and welfare of agriculturalist and well-being of consumers (Hamidou *et al.*, 2014). There is no one time AC in groundnut as AC may occur at any time when ideal conditions are available. Holbrook *et al.* (2009) gave two conditions for obtaining groundnut varieties that resist AC: (1) there have to be genetic divergence in terms of AC resistance, so that gene(s) for resistance can be inserted into varieties; (2) there must be dependable and effective selection methods to find varieties with resistance genes.

Mitigation of AC using a plant breeding approach has been a long-standing objective of researchers (Arunyanark *et al.*, 2009). But, significant and inexplicable G×E interactions and huge assay expenses for aflatoxins have slowed this research (Anderson *et al.*, 1995; Blankenship *et al.*, 1985). Arunyanark *et al.* (2010) stated that it is essential to find alternate approaches to select genotypes that resist AC, because low heritability of AC and high G×E interactions confuse the selection process. Hamidou *et al.* (2014) observed that AC had high G×E interactions and hence, suggested that selection for resistance to AC must be specific to a given environment. These authors noticed that genotypes found to resist AC did not have a uniform degree of resistance in different environments, indicating that AC was not consistent across environments. As a result, there is a requirement to investigate alternate approaches including the use of indirect selection criteria for resistance to AC. Groundnut varieties that effectively resist AC may be possible, provided that the appropriate traits for drought tolerance that also favour resistance to AC are found. In other words, breeding for resistance to AC in groundnut requires the inclusion of drought tolerance traits as surrogate traits.

The genetics of resistance mechanisms for AC have not been so far made clear. According to Pooja *et al.* (2015), the allelic relationship among several sources for resistance characters that can aid to pyramid the non-allelic genes for each resistance mechanism is unknown. Also, it has been found (Hamidou *et al.*, 2014; Waliyar *et al.*, 2003b) that certain groundnut cultivars resist *A. flavus* infection under laboratory conditions but not under field conditions. Crop's resistance to aflatoxin has been grouped under three categories such as resistance to infection in the pod wall, resistance to invasion and colonisation in the seed coat, and resistance to aflatoxin synthesis in the cotyledon. Many groundnut cultivars with these three types of resistances have been identified in many studies and resistance traits were transferred to different genetic backgrounds for the development of several breeding lines. However, no relationships have been noticed among these three resistance categories in any studies. This is attributed to the influence of environmental factors on the crop-fungus relationship.

Many methods have been suggested for containing AC genetically. One of these methods is to develop and utilise groundnut genotypes that resist insects and that tolerate water-deficits and elevated temperatures (Guo *et al.*, 2008b). Holbrook *et al.* (2009) stated that the two most hopeful mechanisms of resistance to AC found in groundnut are drought resistance and root knot nematode resistance. When *Meloidogyne arenaria* infects groundnut while there is a water deficit during maturation of pods, the result is a rise in AC of kernels (Timper *et al.*, 2004). This is correct for the USA, however, nematodes are not necessarily a problem in many other countries. But of course, drought and other soil born insects and pathogens can also play a major role.

A method to improve resistance of groundnut to AC is to pyramid genes from different and diverse sources (Upadhyaya *et al.*, 2002), such as genes encoding three

types of aflatoxin resistances, as well as fungal and insect resistances. Sullivan and Holbrook (2007) had noticed encouraging outcomes in the use of ground-based canopy reflectance remote sensing as a selection principle for aflatoxin- and drought-resistant varieties of groundnut. Studies of Wu *et al.* (2016) suggested a basis for forecasting AC using weather conditions, which can help in carrying out precautions to lessen pre-harvest AC of groundnuts. Besides, research on the influence of various types of soil on AC in crops is necessary because properties of soil can possibly affect AC (Graham, 1982). According to Torres *et al.* (2014), light sandy soil encourages fast fungal growth especially with drought, and heavier soils have good water-holding capacity, so the possibility for the occurrence of a water deficit is minimised which partially contributes to low AC in heavier soils.

Biological control is a very hopeful approach to decrease AC in groundnuts. Aflatoxin was removed by a bacterium, *Flavobacterium aurantiacum* B-184 from solutions (Ciegler, 1979). *Enterococcus faecium* can detoxify AFB_1 which binds to the cell wall constituents of the bacteria (Haskard *et al.*, 2001), such as polysaccharides and peptidoglycans. Under laboratory conditions, some saprophytic yeast species, such as *Candida krusei* and *Pichia anomala* (Masoud and Kaltoft, 2006), and certain bacteria such as *Bacillus subtilis*, *Burkholderia* spp., *Lactobacillis* spp., *Pseudomonas* spp. and *Ralstonia* spp. (Palumbo *et al.*, 2006) inhibit *Aspergillus* growth.

Using non-aflatoxigenic strains of *A. flavus* and *A. parasiticus*, reductions in AC have been achieved in several crops, including groundnut. These non-aflatoxigenic strains are genetically stable and mostly asexual, and cannot recombine with native aflatoxigenic strains and so, they have been used as biocontrol strains (Ehrlich and Cotty, 2004). As these biocontrol strains occupy the same niches in soil (Yin *et al.*, 2008), they can compete with aflatoxigenic strains for the infection sites and essential nutrients, and finally, displace them. Dorner (2008) observed consistent and substantial decreases in AC up to 90% in groundnut using non-aflatoxigenic *Aspergillus* strains. In the recent study of Alaniz Zanon *et al.* (2016), biocontrol of aflatoxins has been reported using the application of naturally occurring non-aflatoxigenic *A. flavus* to soil as an antagonist. Non-aflatoxigenic *A. flavus* competed and interfered with the proliferation of indigenous aflatoxigenic *A. flavus* resulting in a competitive exclusion of aflatoxigenic *A. flavus* and, in turn, in less AC. Fungal community structure influences AC, which suggest the use of atoxigenic *A. flavus* as biocontrol agent to reduce AC (Kachapulula *et al.*, 2017; Mallikarjunaiaha *et al.*, 2017). Currently, such biocontrol products are sold under the trade name AflaSafe® in Africa, and Afla-guard® and AF36® in the USA. However, there are certain challenges in using this biocontrol method due to the vast diversity in *A. flavus*, which can form heterokaryotic reproductive forms. Contrary to the findings of Ehrlich and Cotty (2004), Razzaghi-Abyaneh *et al.* (2014) reported that non-aflatoxigenic *A. flavus* could participate in sexual recombination, which may further increase their functional and genetic diversity. There is a possibility that sexual recombination may increase the population of aflatoxigenic strains, thus, the biocontrol strategy may become ineffective. Hence, studies on genetic variations among *Aspergillus* spp. are necessary to develop an efficient biocontrol strategy to reduce AC.

Waliyar *et al.* (2013) reported that by following certain management procedures in the field, the occurrence of AC can be diminished to a certain extent. These include well-timed sowing, keeping an ideal plant population, providing appropriate nutrition, preventing water deficit, containing pathogens, weeds and insect pests and harvesting appropriately. However, according to Liang *et al.* (2006), these methods might not be suitable for small-scale agriculture particularly in the tropical regions of developing countries.

Host-pathogen interactions under the influence of environment (especially drought and heat stress) are to be studied at the genetic level to identify plant resistant factors. Developments in recombinant DNA technology coupled with genomics, proteomics, and metabolomics will make it possible to develop cultivars with durable resistance soon. Many candidate genes involved in host-pathogen interactions that lead to AC have already been identified due to improvements in fungal expressed sequence tags, microarrays, and genome sequencing techniques (Cleveland *et al.*, 2006). The aflatoxin biosynthesis pathway has been studied adequately with reference to the enzymes, genes, intermediates, and regulatory mechanisms (Bhatnagar *et al.*, 2003; Ehrlich, 2009). Guo *et al.* (2008a) developed expressed sequence tags to identify genes encoding resistance to aflatoxin produced by *A. parasiticus* and then, a groundnut microarray was developed (Guo *et al.*, 2011) for the identification of candidate genes providing resistance to infection by *A. flavus*. Brown *et al.* (2013) reported that RNA interference gene silencing could permit genetically engineered crops to express resistance against *A. flavus*. With this method, fungal DNA sequences could be used to recognise and inhibit fungal growth in the plant. Mallikarjunaiaha *et al.* (2017) characterised non-aflatoxigenic *A. flavus* strains genetically and found six different deletion patterns for thirteen examined genes from the aflatoxin biosynthesis pathway. Among the observed deletion patterns, the most frequently absent gene was found to be aflR.

An interesting research area to combat *A. flavus* infection is to identify genes coding for compounds that inhibit fungal growth, such as defensins, thionins, pathogenesis-related proteins, lectins, ribosome inactivating proteins (RIP), etc.

These genes can then be transferred to elite groundnut cultivars using a gene transfer method. Certain genes, namely glucose oxidase gene, ribosome-inactivation protein gene, stilbene synthase gene and tabtoxin acetyltransferase gene, have been introduced into plants to increase resistance against fungi (Wani *et al.*, 2010). Transgenic groundnut had enhanced resistance to *A. flavus* and AC when expressing tobacco glucanase gene (Sundaresha *et al.*, 2010), and rice chitinase gene (Prasad *et al.*, 2013).

Identification of resistance associated proteins is a proteomics based research area, with an aim to reduce AC. Resistant groundnut lines when infected with *A. flavus* exhibited a 3- to 4-fold rise in β-1,3-glucanase, which may play a role in plant defence mechanism (Liang *et al.*, 2005). Wang *et al.* (2010) compared the differential expression of seed protein profiles between a resistant cultivar and a susceptible cultivar, infected with *A. flavus* under drought conditions to identify proteins involved in the resistance to AC. Major result of this study is the identification of six protein spots including low molecular weight heat shock protein precursor, RIO kinase, L-ascorbate peroxidase, iso-Ara h3, 50 S ribosomal protein L22 and putative 30 S ribosomal S9, that were significantly up-regulated in the resistant cultivar.

Targeting Induced Local Lesions in Genomes (TILLING), a reverse-genetics approach, was used by Knoll *et al.* (2011), to screen mutagenised groundnut populations for induced changes in allergen genes, with an aim of creating a cultivar with reduced allergenicity. *Ara h 1* and *Ara h 2* are two major allergen genes. The significant mutations identified were a disrupted start codon in *Ara h 2.02* and a premature stop codon in *Ara h 1.02*. Homozygous individuals were recovered in succeeding generations for each of these mutations, and elimination of *Ara h 2.02* protein was confirmed.

The cultivated groundnut is an allotetraploid with a large genome size and hence, interpretation of genomic data is complex. Moreover, the cultivated groundnut has relatively narrow genetic diversity (Moretzsohn *et al.*, 2004). To conduct genetic and genomic studies in cultivated groundnut, simple sequence repeats (SSR) are the most desirable molecular markers. As many as 15,518 SSR markers were generated between 2002-2012 (Guo *et al.*, 2013), which are valuable inputs for the research works on molecular genetics and breeding in cultivated groundnut. Kanyika *et al.* (2015) identified 139 informative SSR markers associated with resistance to certain groundnut diseases (early leaf spot, rosette disease, rust & AC), that have been mapped to the *Arachis* genome earlier and can be employed in quantitative trait loci (QTL) mapping. Molecular markers are useful in constructing genetic linkage maps, which is a pre-requisite for QTL studies.

Use of molecular markers for resistance to AC in groundnut is very limited. Many QTLs associated with drought tolerance related traits in groundnut, such as transpiration, transpiration efficiency, specific leaf area, leaf area and SPAD chlorophyll meter reading, have been identified (Gautami *et al.*, 2012; Ravi *et al.*, 2011; Varshney *et al.*, 2009). However, QTLs associated with resistance to *A. flavus* and AC are scarce. Identifying QTL for resistance to AC is expected to be very important in allowing the use of marker assisted selection to transfer aflatoxin resistance into elite lines of groundnut. In *Arachis cardenasii* derived lines, Milla *et al.* (2005) identified a set of six amplified fragment length polymorphism (AFLP) markers with low phenotypic variance explained (PVE). Liang *et al.* (2009) identified six QTLs for resistance to *A. flavus* infection with PVE ranging from 6.2 to 22.7%. Fountain *et al.* (2015) asserts that crop's resistance to *A. flavus* colonisation and AC should be quantitative and that resistance is severely influenced by environmental interactions. Consequently, identifying consistent QTL for resistance to AC is a very difficult task because breeding efforts to discover and characterise QTL for resistance to AC were forced to consider the environment in obtaining phenotypic data (Fountain *et al.*, 2015).

Until now, it has not been possible to develop a groundnut variety that can resist AC consistently in different seasons/localities. Breeding for reduced AC remains cumbersome due to certain major problems which include lack of knowledge on the mechanism(s) responsible for regulation of aflatoxin biosynthesis by the fungus, expensive aflatoxin assay and high G×E interactions. Hence, indirect selection criteria for resistance to AC namely, drought tolerance, insect/nematode resistance are employed currently. However, it is of paramount importance to study the complex interactions among the plant, fungus and environment, which may help to throw light on plant resistance factors. Although the non-aflatoxigenic biocontrol strains have been used successfully for reducing AC, further studies are required on the diversity of *Aspergillus* spp. so that, there will not be any possibility for the development hyper-competitive toxigenic strains in the long-run. More research works need to be carried out using gene expression analysis tools, so as to understand the functions of the groundnut genes. The modern genomic tools have great potential to disentangle the complex aflatoxin resistance mechanisms in groundnut.

6. Future thrusts

An important task is to lessen and remove AC from food and feed crops. Even though a lot of investigations pointed out positive relationships between aflatoxin resistance and drought tolerance, the foundation of the association remains uncertain and requires more research. Further investigations are specifically required to evaluate the

connection between AC and drought tolerance during brief episodes of acute water deficit near the end of the growing season.

It is still unknown whether the two traits namely, drought tolerance and resistance to AC are controlled by same genes or not. The failure to establish genetic relationship between these traits hinders the development of groundnut varieties that resist AC consistently. Quite a lot of aflatoxin resistant genotypes were identified at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) based on three types of resistance viz., resistance to preharvest seed infection, resistance to *in vitro* seed colonisation (IVSC), and resistance to aflatoxin production by the fungus (Nigam *et al.*, 2009). Liao *et al.* (2009) reported that a cultivar 'Zhonghua 6' which is resistant to AC, has been extensively cropped in central China. Rahmianna *et al.* (2015) identified a genotype 'GH 51' that had AC under the safe level (≤10 µg/kg), which has been released by the Ministry of Agriculture, Indonesia. Recently, Waliyar *et al.* (2016) identified seven best accessions, ICGs 13603, 1415, 14630, 3584, 5195, 6703 and 6888 which were found to accumulate consistently very low levels of aflatoxin (<4 µg/kg) over a period of six years (2008-2013). However, these elite genotypes may not express their resistance under all the environmental conditions. This is mainly attributed to the variability in the toxigenicity of *Aspergillus* spp. in different localities that makes the resistant genotypes not to perform uniformly. Consequently, breeding for AC resistance needs to continue.

Torres *et al.* (2014) stated that total prevention of aflatoxin is not practically possible using available methods and the most promising approach for reducing AC seems to be crop resistance enhancement. This approach requires: (1) inhibiting fungal infection during a drought; (2) inhibiting consequent fungal growth; (3) preventing AC, and (4) crop or fungi mediated aflatoxin degradation. Hamidou *et al.* (2014) proposed that studies on drought tolerance should be separated from studies on AC resistance, as it is very doubtful that a common mechanism leading to both drought tolerance and AC resistance can be identified.

The most important trait of interest that is to be genetically engineered in groundnut is 'resistance to AC' which has high degree of plasticity and complexity. The complex nature of this trait is mainly due to reasons such as, (1) aflatoxin measurement is difficult, (2) AC shows very high G×E interactions, and (3) the heritability of AC is low. Hence, research works to reduce AC in groundnut are very difficult. A complex network of genes that are regulated in unknown manner under the influence of environment must be unravelled to understand the molecular mechanism underlying resistance to AC. Besides, the ecological role of aflatoxin to the fungus should be studied, so that it may be possible to design strategies to arrest aflatoxin production by the fungus at molecular level.

Acknowledgements

Financial support provided by Arba Minch University (GOV/AMU/TH2/CNS/Bio/06/2009) is gratefully acknowledged.

References

Alaniz Zanon, M.S., Barros, G.G. and Chulze, S.N., 2016. Non-aflatoxigenic *Aspergillus flavus* as potential biocontrol agents to reduce aflatoxin contamination in peanuts harvested in Northern Argentina. International Journal of Food Microbiology 231: 63-68.

Anderson, W.F., Holbrook, C.C., Wilson, D.M. and Matheron, M.E., 1995. Evaluation of preharvest aflatoxin contamination in several potentially resistant peanut genotypes. Peanut Science 22: 29-32.

Arunyanark, A., Jogloy, S., Wongkaew, S., Akkasaeng, C., Vorasoot, N., Kesmala, T. and Patanothai, A. 2010. Heritability of aflatoxin resistance traits and correlation with drought tolerance. Field Crops Research 117: 258-264.

Arunyanark, A., Jogloy, S., Wongkaew, S., Akkasaeng, C., Vorasoot, N., Wright, G.C., Rachaputi, R.C.N. and Patanothai, A., 2009. Association between aflatoxin contamination and drought tolerance traits in peanut. Field Crops Research 114: 14-22.

Basha, S.M., Cole, R.J. and Pancholy, S.K., 1994. A phytoalexin and aflatoxin producing peanut seed culture system. Peanut Science 21: 130-134.

Bhatnagar, D., Ehrlich, K.C. and Cleveland, T.E., 2003. Molecular genetic analysis and regulation of aflatoxin biosynthesis. Applied Microbiology and Biotechnology 61: 83-93.

Blankenship, P.D., Cole, R.J. and Sanders, T.H., 1985. Comparative susceptibility of four experimental peanut lines and the cultivar Florunner to preharvest aflatoxin contamination. Peanut Science 12: 70-72.

Brown, R.L., Bhatnagar, D., Cleveland, T.E., Chen, Z.Y. and Menkir, A., 2013. Development of host maize resistance to aflatoxigenic fungi. In: Razzaghi-Abyaneh, M. (ed.) Aflatoxins: recent advances and future perspectives. InTech, Rijeka, Croatia, pp. 3-22.

Bumbangi, N.F., Muma, J.B., Choongo, K., Mukanga, M., Velu, M.R., Veldman, F., Hatloy, A. and Mapatano, M.A., 2016. Occurrence and factors associated with aflatoxin contamination of raw peanuts from Lusaka district's markets, Zambia. Food Control 68: 291-296.

Centers for Disease Control and Prevention (CDC), 2004. Outbreak of aflatoxin poisoning – eastern and central provinces, Kenya, January-July 2004. Morbidity and Mortality Weekly Report 53: 790-793.

Ciegler, A., 1979. Control measures for aflatoxin contamination of agricultural commodities. Annals of the New York Academy of Sciences 329: 285-292.

Cleveland, T.E., Yu, J., Chen, Z., Brown, R.L., Cary, J.W., Rajasekaran, K., Bhatnagar, D., Campbell, B.C., Nierman, W.C. and Payne, G.A., 2006. The use of crop proteomics and fungal genomics in elucidating fungus-crop interactions. In: Proceedings of the Myco-Globe Conference. September 26-30, 2006, Bari, Italy, pp. 32.

Cole, R.J., Sanders, T.H., Dorner, J.W. and Blankenship, P.D., 1989. Environmental conditions required to induce pre-harvest concentration in groundnut. In: Hall, S.D. (ed.) Proceedings of International Workshop on Aflatoxin Contamination in Groundnut, Summary of Six Years Research. October 6-9, 1987. ICRISAT Center, Patancheru, India, pp. 279-287.

Cole, R.J., Sanders, T.H., Hill, R.A. and Blankenship, P.D., 1985. Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. Mycopathologia 91: 41-46.

Cole, R.J., Sobolev, V.S. and Dorner, J.W., 1993. Potentially important sources of resistance to prevention of pre-harvest aflatoxin contamination in peanuts. Proceedings of the American Peanut Research and Education Society 25: 78.

Council for Agricultural Science and Technology (CAST), 2003. Mycotoxins: risks in plant, animal and human systems. Task force report No. 139. Ames, IA, USA.

Craufurd, P.Q., Prasad, P.V.V., Waliyar, F. and Taheri, A., 2006. Drought, pod yield, preharvest *Aspergillus* infection and aflatoxin contamination on peanut in Niger. Field Crops Research 98: 20-29.

Dorner, J.W., 2008. Management and prevention of mycotoxins in peanuts. Food Additives and Contaminants Part A 25: 203-208.

Dorner, J.W. and Cole, R.J., 1997. A method for determining kernel moisture content and aflatoxin concentrations in peanuts. Journal of the American Oil Chemists Society 74: 285-288.

Dorner, J.W., Cole, R.J., Sanders, T.H. and Blankenship, P.D., 1989. Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts. Mycopathologia 105: 117-128.

Ehrlich, K.C., 2009. Predicted roles of uncharacterized clustered genes in aflatoxin biosynthesis. Toxins 1: 37-58.

Ehrlich, K.C. and Cotty, P.J., 2004. An isolate of *Aspergillus flavus* used to reduce aflatoxin contamination in cottonseed has a defective polyketide synthase gene. Applied Microbiology and Biotechnology 65: 473-478.

European Commission (EC), 2010. Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official Journal of the European Union L 50: 8-12.

Fountain, J.C., Khera, P., Yang, L., Nayak, S.N., Scully, B.T., Lee, R.D., Chen, Z.Y., Kemerait, R.C., Varshney, R.K. and Guo, B., 2015. Resistance to *Aspergillus flavus* in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives. Crop Journal 3: 229-237.

Gautami, B., Pandey, M.K., Vadez, V., Nigam, S.N., Ratnakumar, P., Krishnamurthy, L., Radhakrishnan, T., Gowda, M.V.C., Narasu, M.L., Hoisington, D.A., Knapp, S.J. and Varshney, R.K., 2012. Quantitative trait locus analysis and construction of consensus genetic map for drought tolerance traits based on three recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). Molecular Breeding 30: 757-772.

Girdthai, T., Jogloy, S., Vorasoot, N., Akksaeng, C., Wongkaew, S., Holbrook, C.C. and Patanothai, A., 2010. Associations between physiological traits for drought tolerance and aflatoxin contamination in peanut genotypes under terminal drought. Plant Breeding 129: 693-699.

Graham, J., 1982. The occurrence of aflatoxin in peanuts in relation to soil type and pod splitting. Food Technology, Australia 34: 208-212.

Guo, B., Chen, X., Dang, P., Scully, B.T., Liang, X., Holbrook, C.C., Yu, J. and Culbreath, A.K., 2008a. Peanut gene expression profiling in developing seeds at different reproduction stages during *Aspergillus parasiticus* infection. BMC Developmental Biology 8: 12.

Guo, B., Chen, Z-Y., Lee, R.D. and Scully, B.T., 2008b. Drought stress and preharvest aflatoxin contamination in agricultural commodity: genetics, genomics and proteomics. Journal of Integrative Plant Biology 50: 1281-1291.

Guo, B., Fedorova, N.D., Chen, X., Wan, C.H., Wang, W., Nierman, W.C., Bhatnagar, D. and Yu, J., 2011. Gene expression profiling and identification of resistance genes to *Aspergillus flavus* infection in peanut through EST and microarray strategies. Toxins 3: 737-753.

Guo, B., Pandey, M.K., He, G., Zhang, X., Liao, B., Culbreath, A., Varshney, R.K., Nwosu, V., Wilson, R.F. and Stalker, H.T., 2013. Recent advances in molecular genetic linkage maps of cultivated peanut. Peanut Science 40: 95-106.

Hamidou, F., Rathore, A., Waliyar, F. and Vadez, V., 2014. Although drought intensity increases aflatoxin contamination, drought tolerance does not lead to less aflatoxin contamination. Field Crops Research 156: 103-110.

Haskard, C.A., El-Nezami, H.S., Kankaanpää, P.E., Salminen, S. and Ahokas. J.T., 2001. Surface binding of aflatoxin B_1 by lactic acid bacteria. Applied and Environmental Microbiology 67: 3086-3091.

Hill, R.A., Blankenship, P.D., Cole, R.J. and Sanders, T.H., 1983. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Applied and Environmental Microbiology 45: 628-633.

Holbrook, C.C., Guo, B.Z., Wilson, D.M. and Timper, P., 2009. The U.S. breeding program to develop peanut with drought tolerance and reduced aflatoxin contamination. Peanut Science 36: 50-53.

Holbrook, C.C., Kvien, C.K., Ruckers, K.S., Wilson, D.M. and Hook, J.E., 2000. Preharvest aflatoxin contamination in drought tolerant and intolerant peanut genotypes. Peanut Science 27: 45-48.

Horn, B.W., 2005. Colonization of wounded peanut seeds by soil fungi: selectivity for species from *Aspergillus* section Flavi. Mycologia 97: 202-217.

Horn, B.W., Greene, R.L. and Dorner, J.W., 1995. Effect of corn and peanut cultivation on soil populations of *Aspergillus flavus* and *A. parasiticus* in Southwestern Georgia. Applied and Environmental Microbiology 61: 2472-2475.

Horn, B.W., Moore, G.G. and Carbone, I., 2009a. Sexual reproduction in *Aspergillus flavus*. Mycologia 101: 423-429.

Horn, B.W., Ramirez-Prado, J.H., and Carbone, I., 2009b. The sexual state of *Aspergillus parasiticus*. Mycologia 101: 275-280.

Hsu, I. C., Metcalf, R. A., Sun, T., Welsh, J. A., Wang, N. J. and Harris, C. C., 1991.Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature 350: 427-431.

Kachapulula, P.W., Akello, J., Bandyopadhyay, R. and Cotty, P.J., 2017. Aflatoxin contamination of groundnut and maize in Zambia: Observed and Potential Concentrations. Journal of Applied Microbiology 122: 1471-1482.

Kanyika, B.T.N., Lungu, D., Mweetwa, A.M., Kaimoyo, E., Njung'e, V.M., Monyo, E.S., Siambi, M., He, G., Prakash, C.S., Zhao, Y. and Villiers, S.M., 2015. Identification of groundnut (*Arachis hypogaea*) SSR markers suitable for multiple resistance traits QTL mapping in African germplasm. Electronic Journal of Biotechnology 18: 61-67.

Klich, M.A., 2007. *Aspergillus flavus*: The major producer of aflatoxin. Molecular Plant Pathology 8: 713-722.

Klich, M.A. and Pitt, J.I., 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Transactions of the British Mycological Society 91: 99-108.

Klich, M.A., Tiffany, L.H. and Knaphus, G., 1992. Ecology of the aspergilli of soils and litter. In: Bennett, J.W. and Klich, M.A. (eds.) *Aspergillus*: biology and industrial applications. Butterworth Heinemann, Boston, MA, USA, pp. 329-353.

Knoll, J.E., Ramos, M.L., Zeng, Y., Holbrook, C.C., Chow, M., Chen, S., Maleki, S., Bhattacharya, A. and Ozias-Akins, P., 2011. TILLING for allergen reduction and improvement of quality traits in peanut (*Arachis hypogaea* L.). BMC Plant Biology 11: 81.

Liang, X., Zhou, G., Hong, Y., Chen, X., Liu, H. and Li, S., 2009. Overview of research progress on peanut (*Arachis hypogaea* L.) host resistance to aflatoxin contamination and genomics at the Guangdong Academy of Agricultural Sciences. Peanut Science 36: 29-34.

Liang, X.Q., Holbrook, C.C., Lynch, R.E. and Guo, B.Z., 2005. β-1,3-Glucanase activity in peanut seed (*Arachis hypogaea* L.) is induced by inoculation with *Aspergillus flavus* and copurifies with a conglutin-like protein. Phytopathology 95: 506-511.

Liang, X.Q., Luo, M. and Guo, B.Z., 2006. Resistance mechanisms to *Aspergillus flavus* infection and aflatoxin contamination in peanut (*Arachis hypogaea*). Plant Pathology Journal 5: 115-124.

Liao, B., Zhuang, W., Tang, R., Zhang, X., Shan, S., Jiang, H. and Huang, J., 2009. Peanut aflatoxin and genomics research in China: progress and perspectives. Peanut Science 36: 21-28.

Mallikarjunaiaha, N.H., Jayapalaa, N., Puttaswamyb, H. and Ramachandrappa, N.S., 2017. Characterization of non-aflatoxigenic strains of *Aspergillus flavus* as potential biocontrol agent for the management of aflatoxin contamination in groundnut. Microbial Pathogenesis 102: 21-28.

Masoud, W. and Kaltoft, C.H., 2006. The effects of yeasts involved in the fermentation of *Coffea arabica* in East Africa on growth and ochratoxin A (OTA) production by *Aspergillus ochraceus*. International Journal of Food Microbiology 106: 229-234.

Medina, A., Rodriguez, A. and Magan, N., 2014. Effect of climate change on *Aspergillus flavus* and aflatoxin B_1 production. Frontiers in Microbiology 5: 1-7.

Milla, S.R., Isleib, T.G. and Tallury, S.P., 2005. Identification of AFLP markers linked to reduced aflatoxin accumulation in *A. cardenasii*-derived germplasm lines of peanut. Proceedings of American Peanut Research and Education Society. 37: 90.

Moretzsohn, M.C., Hopkins, M.S., Mitchell, S.E., Kresovich, S., Valls, J.F.M. and Ferreira, M.E., 2004. Genetic diversity of peanut (*Arachis hypogaea* L.) and its wild relatives based on the analysis of hypervariable regions of the genome. BMC Plant Biology 4: 11.

Nageswara Rao, R.C., Wright, G.C. and Krosch, S., 2002. Management practices to minimise pre-harvest aflatoxin contamination in Australia peanuts. Australian Journal of Experimental Agriculture 42: 595-605.

Nautiyal, P.C., Nageswara Rao, R.C. and Joshi, Y.C., 2002. Moisture-deficit-induced changes in leaf water content, leaf carbon exchange rate and biomass production in groundnut cultivars differing in specific leaf area. Field Crops Research 74: 67-79.

Nigam, S.N., Waliyar, F., Aruna, R., Reddy, S.V., Kumar, P.L., Craufurd, P.Q., Diallo, A.T., Ntare, B.R. and Upadhyaya, H.D., 2009. Breeding peanut for resistance to aflatoxin contamination at ICRISAT. Peanut Science 36: 42-49.

Okello, D.K., Kaaya, A.N., Bisikwa, J., Were, M. and Oloka, H.K., 2010. Management of aflatoxins in groundnuts: A manual for farmers, processors, traders and consumers in Uganda. Available at: http://tinyurl.com/y9jdyf95.

Palumbo, J.D., Baker, J.L. and Mahoney, N.E. 2006. Isolation of bacterial antagonists of *Aspergillus flavus* from almonds. Microbial Ecology 52: 45-52.

Payne, G.A. and Brown, M.P., 1998. Genetics and physiology of aflatoxin biosynthesis. Annual Review of Phytopathology 36: 329-362.

Payne, G.A., 1998. Process of contamination by aflatoxin-producing fungi and their impact on crops. In: Sinha, K.K. and Bhatnagar, D. (eds.) Mycotoxins in Agriculture and Food Safety. Marcel Dekker, New York, NY, USA, pp. 279-306.

Pitt, J.I., Taniwaki, M.H. and Cole, M.B., 2013. Mycotoxin production in major crops as influenced by growing, harvesting, storage and processing, with emphasis on the achievement of food safety objectives. Food Control 32: 205-215.

Pooja, B-M., Sowmini, S., Madhurima, B-P., Farid, W. and Kiran, K.S., 2015. Biotechnological advances for combating *Aspergillus flavus* and aflatoxin contamination in crops. Plant Science 234: 119-132.

Prasad, K., Bhatnagar-Mathur, P., Waliyar, F. and Sharma, K.K., 2013. Overexpression of a chitinase gene in transgenic peanut confers enhanced resistance to major soil borne and foliar fungal pathogens. Journal of Plant Biochemistry and Biotechnology 22: 222-233.

Probst, C., Njapau, H. and Cotty, P.J., 2007. Outbreak of an acute aflatoxicosis in Kenya in 2004: Identification of the causal agent. Applied and Environmental Microbiology 73: 8.

Rahmianna, A.A., Purnomo, J. and Yusnawan, E., 2015. Assessment of groundnut varietal tolerant to aflatoxin contamination in Indonesia. Procedia Food Science 3: 330-339.

Ravi, K., Vadez, V., Isobe, S., Mir, R.R., Guo, Y., Nigam, S.N., Gowda, M.V., Radhakrishnan, T., Bertioli, D.J., Knapp, S.J. and Varshney, R.K., 2011. Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). Theoretical and Applied Genetics 122: 1119-1132.

Razzaghi-Abyaneh, M., Chang, P., Shams-Ghahfarokhi, M. and Rai, M.M., 2014. Global health issues of aflatoxins in food and agriculture: challenges and opportunities. Frontiers in Microbiology 5: 420.

Robens, J.F. and Cardwell, K., 2005. The cost of mycotoxin management in the United States. In: Abbas, H.K. (ed.) Aflatoxin and food safety. CRC Press, Boca Raton, FL, USA, pp. 1-12.

Sanders, T.H., Blankenship, P.D., Cole, R.J. and Hill, R.A., 1984. Effect of soil temperature and drought on peanut pod and stem temperatures relative to *Aspergillus flavus* invasion and aflatoxin contamination. Mycopathologia 86: 51-54.

Sanders, T.H., Cole, R.J., Blankenship, P.D. and Dorner, J.W., 1993. Aflatoxin contamination of peanuts from plants drought stressed in pod or root zones. Peanut Science 20: 5-8.

Sanders, T.H., McMichael, R.W. and Hendrix, K.W., 2000. Occurrence of resveratrol in edible peanut. Journal of Agricultural and Food Chemistry 48: 1243-1246.

Schmale, D.G. and Munkvold, G.P., 2017. Mycotoxins in crops: a threat to human and domestic animal health. Available at: http://tinyurl.com/hjxnz86.

Sharanaiah, U., Honnayakanahalli, M.M., Bhadvelu, C., Prahlad, S., Jayanna, S.K., Sri, R., Prakasha, A., Marahel, S., Tumkur, R.B., Sollepura, B.R., Murali, N., Govinda gowda, V.R., Mohankumar, S. and Harishchandra, S.P., 2017. Aflatoxins and food pathogens: impact of biologically active aflatoxins and their control strategies. Journal of the Science of Food and Agriculture 97: 1698-1707.

Songsri, P., Jogloy, S., Vorasoot, N., Akkasaeng, C., Patanothai, A. and Holbrook, C.C., 2008. Root distribution of drought resistant peanut genotypes in response to drought. Journal of Agronomy and Crop Science 194: 92-103.

Squire, R.A., 1981. Rating animal carcinogens: a proposed regulatory approach. Science 214: 877-880.

Styer, C.H., Cloe, R.J. and Hill, R.A., 1983. Inoculation and infection of peanut flowers by *Aspergillus flavus*. Proceedings of the American Peanut Research and Education Society 15: 91.

Sudhakar, P., Lathat, P., Babitha, M., Reddy, P.V. and Naidu, P.H., 2007. Relationship of drought tolerance traits with aflatoxin contamination in groundnut. Indian Journal of Plant Physiology 12: 261-265.

Sullivan, D.G. and Holbrook, C.C., 2007. Using ground-based reflectance measurements as selection criteria for drought- and aflatoxin-resistant peanut genotypes. Crop Science 47: 1040-1050.

Sundaresha, S., Manoj Kumar, A., Rohini, S., Math, S.A., Keshamma, E., Chandrashekar, S.C. and Udayakumar, M., 2010. Enhanced protection against two major fungal pathogens of groundnut, *Cercospora arachidicola* and *Aspergillus flavus* in transgenic groundnut over-expressing a tobacco β-1,3 glucanase. European Journal of Plant Pathology 126: 497-508.

Timper, P., Wilson, D.M., Holbrook, C.C. and Maw, B.W., 2004. Relationship between *Meloidogyne arenaria* and aflatoxin contamination in peanut. Journal of Nematology 36: 167-170.

Torres, A.M., Barros, G.G., Palacios, S.A., Chulze, S.N. and Battilani, P., 2014. Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. Food Research International 62: 11-19.

Udomkun, P., Wiredu, A.N., Nagle, M., Müller, J., Vanlauwe, B. and Bandyopadhyay, R., 2017. Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application – a review. Food Control 76: 127-138.

Upadhyaya, H.D., Waliyar, F. and Nigam, S.N., 2002. Breeding for resistance to aflatoxin contamination in groundnut. Aflatoxin 1-6. Available at: http://www.oar.icrisat.org/4180

Van Egmond, H.P., 1989. Current situation on regulations for mycotoxins. Overview of tolerances and status of standard methods of sampling and analysis. Food Additives and Contaminants 6: 139-188.

Varshney, R.K., Bertioli, D.J., Moretzsohn, M.C., Vadez, V., Krishnamurthy, L., Aruna, R., Nigam, S.N., Moss, B.J., Seetha, K., Ravi, K., He, G., Knapp, S.J. and Hoisington, D.A., 2009. The first SSR-based genetic linkage map for cultivated groundnut (*Arachis hypogaea* L.). Theoretical and Applied Genetics 118: 729-739.

Waliyar, F., Osiru, M., Sudini, H. and Njoroge, S., 2013. Reducing aflatoxins in groundnuts through integrated management and biocontrol. In: Aflatoxins – finding solutions for improved food safety. International Food Policy Research Institute, Washington, DC, USA, pp. 1-2.

Waliyar, F., Reddy, S.V., Subramaniam, K., Reddy, T.Y., Rama Devi, K., Craufurd, P.Q. and Wheeler, T.R., 2003a. Importance of mycotoxins in food and feed in India. Aspects of Applied Biology 68: 147-154.

Waliyar, F., Traore, A., Fatondji, D. and Ntare, B.R., 2003b. Effect of irrigation interval, planting date, and cultivar on *Aspergillus flavus* and aflatoxin contamination of peanut in a sandy soil of Niger. Peanut Science 30: 79-84.

Waliyar, F., Vijay Krishna Kumar, K., Diallo, M., Traore, A., Mangala, U.N., Upadhyaya, H.D. and Sudini, H. 2016. Resistance to pre-harvest aflatoxin contamination in ICRISAT's groundnut mini core collection. European Journal of Plant Pathology 145: 901-913.

Wang, T., Zhang, E., Chen, X., Li, L. and Liang, X., 2010. Identification of seed proteins associated with resistance to pre-harvested aflatoxin contamination in peanut (*Arachis hypogaea* L). BMC Plant Biology 10: 267.

Wani, S.H., 2010. Inducing fungus-resistance into plants through biotechnology. Notulae Scientia Biologicae 2: 14-21.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M. and Aggarwal, D., 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. American Journal of Clinical Nutrition 80: 1106-1122.

Williams, R.J. and McDonald, D., 1983. Grain molds in the tropics: problems and importance. Annual Review of Phytopathology 21: 153-178.

Wotton, H.R. and Strange, R.N., 1985. Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to *Aspergillus flavus*. Journal of General Microbiology 131: 487-494.

Wotton, R.A. and Strange, R.N., 1987. Increased susceptibility and reduced phytoalexin accumulation in drought stressed peanut kernels challenged with *Aspergillus flavus*. Applied and Environmental Microbiology 53: 270-273.

Wright, G.C. and Nageswara Rao, R.C., 1994. Groundnut water relations. In: Smartt, J. (ed.) The groundnut crop: a scientific basis for improvement. Chapman and Hall, London, UK, pp. 281-325.

Wright, G.C., Nageswara Rao, R.C. and Farquhar, G.D., 1994. Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. Crop Science 34: 92-97.

Wu, F., Stacy, S.L. and Kensler, T.W., 2013. Global risk assessment of aflatoxins in maize and peanuts: are regulatory standards adequately protective? Toxicological Sciences 135: 251-259.

Wu, L.X., Ding, X.X., Li, P.W., Du, X.H., Zhou, H.Y., Bai, Y.Zh. and Zhang, L.X., 2016. Aflatoxin contamination of peanuts at harvest in China from 2010 to 2013 and its relationship with climatic conditions. Food Control 60: 117-123.

Yin, Y.N., Yan, L.Y., Jiang, J.H. and Ma, Z.H., 2008. Biological control of aflatoxin contamination of crops. Journal of Zhejiang University Science 9: 787-792.

Yu, J., 2004. Genetics and biochemistry of mycotoxin synthesis. In: Arora, D.K. (ed.) Fungal biotechnology in agricultural, food, and environmental applications. Marcel Dekker, New York, NY, USA, pp. 343-361.

Yu, J., Cleveland, T.E., Nierman, W.C. and Bennett, J.W., 2005. *Aspergillus flavus* genomics: Gateway to human and animal health, food safety, and crop resistance to diseases. Revista Iberoamericana de Micología 22: 194-202.