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Although drought intensity increases aflatoxin contamination, drought tolerance does not lead to less aflatoxin contamination☆

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ABSTRACT

Drought stress is known to increase aflatoxin contamination in groundnut and establishing a possible relationship between drought tolerance and resistance to aflatoxin contamination could contribute to a more efficient selection of aflatoxin-resistant genotypes. In recent work, the reference collection of groundnut had been assessed across seasons varying for drought intensity, i.e. two moderate temperature (rainy season) and two high temperature (dry season) experiments under well-watered (WW) and water stress (WS) conditions (Hamidou et al., 2012, 2013). Here aflatoxin concentration (AC) in seeds is measured in these trials, first for possibly identifying germplasm with low aflatoxin concentrations and second for investigating possible relationships between aflatoxin concentration and drought tolerance. Drought stress intensity increased aflatoxin concentration in seeds and higher aflatoxin contamination was observed under combined drought and high temperature conditions than under drought alone. No germplasm with lower AC than resistant check (55-437) were found. Aflatoxin contamination showed very high GxE interactions, which suggest that selection for resistance to aflatoxin contamination must be specific to environment. Across trials, using means for each environment, there was a clear positive relationship between the aflatoxin concentration and the grain yield reduction due to drought, indicating that a higher drought severity led to higher aflatoxin concentration. However, within trial, the same relationships applied to individual genotypes, or to cohorts of tolerant/sensitive genotypes, were not significant. The major conclusion of this work is that while drought intensity did increase the level of aflatoxin contamination, as expected and previously reported, there seemed to be no direct relationship between tolerance to drought and aflatoxin concentration, suggesting that the mechanisms of drought tolerance and aflatoxin contamination are likely not common.

1. Introduction

Aflatoxin, a toxin produced by fungi *Aspergillus flavus* (*A. flavus*), is acutely toxic to some animals but also carcinogenic to humans (Thirumala-Devi et al., 2002). High level of aflatoxin content in groundnut-derived products for consumption is one of the main problems related to groundnut commercialization. Breeding groundnut for aflatoxin contamination resistance would have a broad impact on groundnut kernel quality, thereby enhancing the economic return and well-being of small-holder farmers, and health of consumers. However, contamination by aflatoxin is a multi-stage process and it is not clear what among these is the most critical to curb the final aflatoxin content (Liang et al., 2006; Cotty et al., 2007).

The fungi penetrate into the pods through small cracks that develop during pod maturation and drying (Robert et al., 1971; Sanders et al., 1984). Aflatoxin contamination indeed increases under drought stress (Girdthai et al., 2010a) because of decrease in the water activity, that creates cracks in pod wall that allow the penetration of the *A. flavus*. Damaged pods are likely to contain more aflatoxin than pods with undamaged shells (Sudhakar et al., 2007). Under prolonged drought conditions, groundnut genotypes which maintained high kernel moisture showed enhanced resistance and produced low aflatoxin (Cole et al., 1993). Other findings demonstrated that decrease of kernel water activity reduced phytoalexin

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production leading to increased aflatoxin contamination (Dorner et al., 1989). However, the relationship between seed infection percentage and aflatoxin production is not consistent (Sudhakar et al., 2007). These authors showed that aflatoxin production in kernels is mitigated when plant maintain high relative water content which allows phytoalexin production. Under drought conditions, phytoalexin production is inhibited and the low moisture favored *A. flavus* growth (Dorner et al., 1989). Thus, drought is a predisposing factor for aflatoxin production in groundnut (Waliyar et al., 2003b). However, aflatoxin production depends on many others factors besides *A. flavus* infection.

Recent studies in Niger demonstrated that drought stress for less than ten days was enough to cause significant aflatoxin contamination in the field (Waliyar et al., 2003a; Craufurd et al., 2006). The aflatoxin contamination is often related to the intensity of drought stress, the stage when drought stress occurs, and the soil and/or air temperature (Cole et al., 1989). Terminal drought effect on aflatoxin contamination is well documented (Sudhakar et al., 2007; Latha et al., 2007; Girdthai et al., 2010b). In the Sahel, groundnut production is often affected by an intermittent drought which is an episodic water deficit during plant growth. The question is whether screening for drought tolerant material can in part contribute to the search for genotypes that are resistant to aflatoxin contamination.

Previous works reported that drought tolerance mechanisms, either by escape, tolerance or avoidance, may impact the ability of genotypes to minimize aflatoxin production by maintaining kernel water activities allowing phytoalexin production. Investigation of pre-harvest aflatoxin contamination in 20 drought tolerant and susceptible peanut genotypes showed that drought tolerant lines had lower levels of aflatoxin contamination (Holbrook et al., 2000). A positive correlation was found between aflatoxin contamination and specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), which was used there as a surrogate for transpiration efficiency, itself taken as a proxy for drought tolerance (Girdthai et al., 2010a). This suggests that drought tolerant genotypes may possess some degree of tolerance to aflatoxin contamination and it has been argued that drought tolerance traits in peanut may have the potential to be used as indirect selection criteria for resistance to pre-harvest aflatoxin contamination (Arunyanark et al., 2009). If this was the case, the identification of drought tolerant genotypes would in part contribute to the identification of aflatoxin resistant genotypes. However, whether there is a direct relationship between drought tolerance, expressed as a yield reduction with regard to a fully irrigated control, and aflatoxin concentration in the seed, is still relatively unclear because preliminary evidences are based on a limited number of germplasm or on indirect traits. Therefore, this needs to be addressed with a large and representative set of germplasm, in which yield reduction under drought is measured along with aflatoxin contamination. Here we use such a data set from a recent study with 268 entries (Hamidou et al., 2013), in with contrasting germplasm for drought tolerance were identified, to investigate the robustness of possible relationships to AC.

Furthermore, it was demonstrated that heat stress plays an important role in the susceptibility to aflatoxin contamination (Abbas et al., 2002). Indeed, Dorner et al. (1989) reported that temperature increased kernel moisture loss, favored growth and aflatoxin production by *A. flavus* in peanut susceptible to contamination. As pod temperatures approached the optimum for *A. flavus* growth (35 °C), the proportion of kernels colonized and aflatoxin concentrations increased (Sanders et al., 1984). Moreover, Golombek and Johasen (1997) found that soil temperature 38/32 °C (day/night) imposed from the time of peg penetration induced low mature pod number due to low pod initiation rate at early reproductive stages. When the pod zone temperature ranges from 28 to 31 °C, the probability of aflatoxin contamination increased notably when those temperatures occurred in conjunction with water deficit (Hill et al., 1983). These authors observed that under low-moisture conditions, the critical threshold temperature for aflatoxin contamination in the geocarposphere is between 25 and 28 °C. Similarly, soil temperatures in the pod zone that are cooler than 29–31 °C also result in less aflatoxin concentration, even if a drought is imposed (Blankenship et al., 1984; Cole et al., 1989). However, these relations have not been verified under field conditions in West Africa where aflatoxin is a major problem. In a previous paper (Hamidou et al., 2013), we found that intermittent drought under field conditions had milder effects on yield under moderate temperature conditions than when a similar drought stress was imposed under higher temperature conditions. Therefore, we have here an ideal material to test whether at a trial level or at an individual genotype level, the yield reduction due to drought is related to aflatoxin contamination.

The objectives of this study were (i) to investigate variation in aflatoxin contamination in the groundnut reference collection of ICRISAT to possibly identify new sources of tolerance/resistance to aflatoxin contamination that can be used in breeding programs, (ii) assess the possible relationships between genotype tolerance to drought and to aflatoxin contamination and (iii) to investigate the combined effect of drought and high temperature on aflatoxin contamination.

2. Materials and methods

2.1. Experimental conditions and drought stress imposition

Two experiments were conducted during the rainy season in 2008 and 2009 (between August and December) occurring under moderate temperature conditions, and during the summer season 2009 and 2010 (between February and June), occurring under high temperature conditions. These experiments were planted in the field at the ICRISAT Sahelian Center (ISC) in Sadore, Niger, 45 km south of Niamey, 13°N, 2°E. The soils at ISC are arenosols (World Reference Base) with low pH, a very low water holding capacity, low inherent soil fertility and organic matter content. The agronomic results of these experimentations have been reported recently (Hamidou et al., 2012, 2013).

In all experiments, fertilizer NPK (15–15–15) and farm manure (200 kg ha^{-1}) were incorporated; the field was plowed and irrigated twice before sowing. Two hundred sixty eight (268) genotypes, including 259 entries of the groundnut reference collection, were evaluated. Seeds were sown by hand. The experimental design was an incomplete randomized block design with water treatment as main factor and genotypes as sub-factor randomized within each factor and replicated five times. Each plot (2 m^2) contained 2 rows (2 m each), with a 50 cm distance between row, and 10 cm spacing between plants per row. Calcium–ammonium–nitrate (200 kg ha^{-1}) and gypsum (200 kg ha^{-1}) were applied during pod formation.

2.2. Management of irrigation and measurements

Irrigation management for the four trials was described previously (Hamidou et al., 2013). The total water received from rainfall and irrigation in the moderate temperature seasons was 443 and 303 mm in 2008 (MT08) and 484 and 303.3 mm in 2009 (MT09), respectively for the well-watered (ww) and water stressed (ws) treatments. During high temperature experiments, the total water received from rainfall and irrigation was 642 mm and 362.4 mm in 2009 (HT09) and 672 mm and 392.1 mm in 2010 (HT10) for ww and ws treatments respectively. The morphophysiological traits, in