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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.

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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

particular the SPAD chlorophyll meter reading (SCMR), leaf area and wilting symptoms, were recorded during the crop growth period. The maturity date recording, plants samples at harvest, determination of harvest index (HI), crop growth rate (C, kg ha^{-1} per day), pod growth rate (R, kg ha^{-1} per day) and partitioning (p, proportion of dry matter partitioned into pods) were described in previous papers (Hamidou et al., 2012, 2013). As these traits did not show significant relationship to drought tolerance, their results were not reported in this paper.

During the crop growing period, the maximum (Max) and minimum (Min) air temperatures and the relative humidity were recorded daily from a meteorological station located close to the experimental field. The meteorological station is installed in a regular field; the soil was always covered with weeds that were cut in the rainy season when they were too high. The soil temperature at 5 and 10 cm was measured at the hottest period of the day in the field where the meteorological station is installed, with no irrigation, and then where the soil temperature likely increased when the vegetation dried.

2.3. Aflatoxin measurement

Aflatoxin concentration in seeds was measured by an enzyme-linked immunosorbent assay (ELISA). In each trial, 100 g of seeds were sampled in each water stress plot. The 100 g of seeds were grounded into a fine powder in a mechanical blender. A sub-sample of 20 g of this fine powder was used for the extraction of aflatoxin by dissolving in 70% (v/v) methanol containing 0.5% (w/v) KCl and homogenized and filtered through Whatman No. 1 filter paper. The filtrate was diluted 1:15 with methanol and used in duplicate to estimate aflatoxin concentration by indirect competitive ELISA essentially as described by Reddy et al. (2001).

2.4. Statistical analysis

The results were performed with Gensat software, version 13. The data were subjected to analysis of variance (ANOVA) procedure for a linear mixed model. The Residual Maximum Likelihood (ReML) method of Genstat was used to obtain the unbiased estimate of the variance components and the best linear unbiased predictions (BLUPs) for the different parameters measured within each treatment, considering genotypes as random and replications as fixed effects. The significance of the genetic variability among accessions within treatment was assessed from the standard error of the estimate of genetic variance σ^2_g. Two way ANOVA analyses were also performed to assess the effects of environment (E) and genotype-by-environment (GxE) interaction, for the aflatoxin contamination trait. In this case, variation components involving G were considered as random effects whereas E and replication effects were considered as fixed. The significance of genetic variability across treatments or of the interaction effect was assessed in a manner similar to the above. The significance of the fixed effects was assessed using the Wald statistic that asymptotically follows a χ^2 distribution.

Since the analytical data had a wide range of values, logarithmic (base 10) transformations of aflatoxin concentration (ppb) were used in an analysis of variance to stabilize the variance. Log transformation did not reduce the experimental error as indicated by almost equal R^2 values for transformed and untransformed data (R^2 = 0.4827, R^2 = 0.4874 respectively) however, transformation did improve significance levels for genotype and environment (Table 1). Therefore, the log transformed results will be discussed. The means of aflatoxin contamination and pod yield ratio (pod yield ws/pod yield ww) were regressed for estimating their relationships (Fig. 2). This was done first by plotting the means of cohorts of the ten highest and lowest yielding genotypes under water stress across the MT, HT and combined MT–HT conditions and the respective mean of aflatoxin contamination. The choice of the cohorts of tolerant/sensitive genotypes was made in the earlier paper (Hamidou et al., 2013) where we discussed our choice to select these on the basis of a high yield under fully irrigated conditions and a contrasting yield under water stress. Then pod yield ratio and aflatoxin contamination values of individual genotypes being part of these cohorts were plotted.

3. Results

3.1. Weather

Environmental conditions were described in a previous paper (Hamidou et al., 2013). In particular, the averaged maximum air temperature (41 °C) was observed in high temperature seasons. At 5 and 10 cm of the soil, the temperatures reached 49 and 40 °C during high temperature seasons while during moderate seasons it reached 42 and 35 °C respectively (Fig. 1).

3.2. Genotype, environment and genotype by environment interaction (GxE) effects

There were significant genotype (G), environment (E) and genotype-by-environment (GxE) interaction effects when data of the all four environments were analyzed (Table 1). The magnitude of the environment effect (*F* value) was by far the highest, followed by the GxE effects. Separating moderate temperature environments (MT) from high temperature environments (HT) eliminated genotypic effect and, decreased the environment and GxE interaction effects. The high magnitude of the environment effect indicates that the environment contributed to the large portion of variation in aflatoxin contamination. The genotype-by-environment (GxE) interaction effect had higher magnitude than the genotype (G) effect, and also contributed to the difference in aflatoxin contamination. The significance of GxE interaction indicates that genotypes were not contaminated equally across environments. By running the same analysis within moderate and high temperature conditions, taking the two years of field data in each condition, showed that the genotype had no significant effect (*F* value < 1.96) and that the magnitude of season effect was similar in MT and HT (Table 1). Earlier analysis had shown that the moderate temperature environment in 2008 (MT08) fell into a same mega-environment than the two high temperature environment (Hamidou et al., 2013), based on genotype and genotype by environment interaction (GGE) biplots. Therefore, the MT08 environment was combined to the two high temperature environments (HT09, HT10). There, the magnitude of the environment and GxE interaction effects increased compared to the magnitude of these two components in the two MT and the two HT environments (Table 1).

The heritability of aflatoxin contamination in the four environments was rather low (Table 2), in part explained by the relatively large variations in the aflatoxin content across replications within genotypes. The trial means of aflatoxin contamination were different in the four environments, with the highest means of aflatoxin contamination observed under high temperature conditions.

3.3. Drought and heat tolerance, and aflatoxin contamination across environment

Previous papers on the same subset of genotypes reported a large genotypic variation for drought tolerance (Hamidou et al., 2012, 2013). Since the genotype by environment interactions were highly significant, pod yield and aflatoxin contamination in each environment were analyzed separately. Regression of trial means of pod yield ratio (pod yield ws/pod yield ww) and aflatoxin

Table 1
Two way ReML analysis (*F* value) for aflatoxin contamination under water stress conditions during moderate temperature seasons 2008 and 2009 (MT08, MT09) and high temperature seasons 2009 and 2010 (HT09, HT10) where genotype (G), environment (E) and genotype-by-environment interaction (GxE) effects were tested.

	4 Environments (MT08-MT09-HT09-HT10)	2 Environments (MT08-MT09)	3 Environments (MT08-HT09-HT10)	2 Environments (HT09-HT10)
G	2.23*	0.88 ns	1.59 ns	0.34 ns
E	28.41***	5.68***	8.52***	5.66***
GxE	5.38***	3.7***	5***	3.1**

Significance at * 0.05, ** 0.01 and *** 0.001 level, ns = not significant.

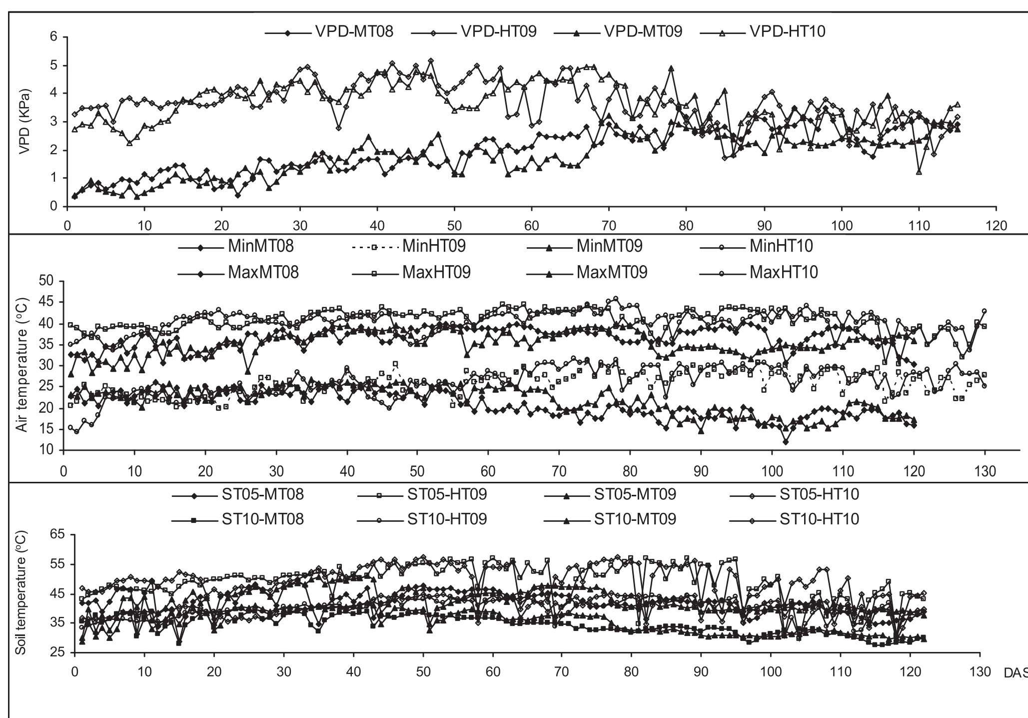

Fig. 1. Vapor pressure deficit (VPD) (A), air temperature (B), and soil temperature (C) at 5 and 10 cm depth (ST05 and ST10) during the experimental periods of the moderate temperature 2008 and 2009 seasons (MT08, MT09), and of the high temperature seasons (HT09, HT10).

contamination showed a strong correlation (r^2 = 0.96) showing that on a trial basis, an increase in the drought severity (low pod yield ratio) led to an increase in aflatoxin contamination (Fig. 2A). However, when the pod yield ratio was calculated for each individual genotype and plotted against the aflatoxin contamination of each individual genotype, no significant relationship was found (r^2 = 0.005), indicating that the tolerance of genotype, taken individually, was not a significant proxy to explain aflatoxin contamination (data not shown).

Table 2
Trial means, range of expected means (minimum, Min, and maximum, Max) of the aflatoxim content, F-probability (Prob.), coefficient of variation (CV%), standard error of differences (SED) within environment variance component (σ_g^2), standard error (SE), heritability ($h^2 = \sigma_g^2/(\sigma_g^2 + \text{SE})$) of aflatoxin contamination under different environments, i.e. moderate temperature (MT08, MT09) and high temperature (HT09, HT10) conditions. The Mean, Min and Max values are the log 10 of the aflatoxin contamination (ppb value in parenthesis).

Environment	MT08	HT09	MT09	HT10
Mean	1.23 (16.98)	1.38 (23.99)	1.13 (13.49)	1.3 (19.95)
Min	0.004 (1.01)	0.022 (1.05)	0.038 (1.09)	0.1 (1.26)
Max	2.87 (741.31)	2.91 (812.83)	2.69 (489.78)	2.93 (851.14)
Prob	4.93	3.95	2.85	1.36
Cv%	45.8	41.3	63.1	34.1
SED	0.31	0.3	0.29	0.27
σ_g^2	0.0959	0.0778	0.0632	0.009
SE	0.317	0.315	0.452	0.191
h^2	0.23	0.19	0.13	0.06

The same exercise was attempted on groups of contrasting genotypes. Our earlier paper reported groups of tolerant/sensitive germplasm, based on a high and low yielding genotypes in either the moderate temperature, high temperature, or across both environments (Hamidou et al., 2013). Here, we averaged the pod yield ratio and aflatoxin contaminations of the ten highest (tolerant) and lowest (sensitive) yielding genotypes under MT, HT or MT–HT conditions selected in previous paper (Hamidou et al., 2013). The values, along with those of 55-437 (resistant check) and Fleur 11 (sensitive check), are reported in Table 3. Regression of means of pod yield ratios and aflatoxin contamination of these high and low yielding genotypes under moderate (MT), high (HT) and across both moderate and high (MT–HT) temperature regimes showed a non-significant negative relationship (r^2 = 0.56, ns) between the ratio of pod yield and aflatoxin contamination (Fig. 2B). As indicated above, when we regressed the individual genotype data of pod yield ratio and aflatoxin contamination of these contrasting genotypes (10 tolerant and sensitive in each of the MT, HT, and MT–HT), no significant correlation was observed (r^2 = 0.005). In fact, it was quite clear that the trend of the relationship between the mean of cohorts of genotypes (r^2 = 0.56, ns) was the consequence of having high AC values for 3 genotypes in each of the three sensitive cohorts (Fig. 2B, open symbols) with AC values above a log (AC) of 2. Once these data points were removed, there was strictly no trend (data not shown). In other words, if there was a slight non-significant trend using the mean, which was driven by a minority of genotypes in the drought sensitive group having large AC values.

Table 3
Pod yield ratio (ratio-yield, i.e. pod yield under water stress/pod yield under fully irrigated conditions) and aflatoxin contamination (AC) of high and low yielding genotypes under moderate (MT), high (HT) and, moderate and high (MT–HT) temperatures conditions. Genotype 55-437 is an aflatoxin resistant check (RC), whereas Fleur 11 is an aflatoxin sensitive check (SC). AC ≤ 0.69 (log_{10} 5 ppb) = resistance, AC ≤ 1 (log_{10} 10 ppb) = tolerance, AC > 1 (log_{10} >10 ppb) = sensitive.

High yield-WS			Low yield-WS		
Genotypes	Ratio-yield	AC	Genotypes	Ratio-yield	AC
MT					
ICG 5891	0.88	1.05 (11.22)	ICG 188	0.33	1.27 (18.62)
ICG 6057	0.78	1.39 (24.55)	ICG 2738	0.49	1.23 (16.98)
ICG 9777	0.92	1.34 (21.88)	ICG 4670	0.39	1.33 (21.38)
ICG 9809	0.63	1.36 (22.91)	ICG 8083	0.35	1.08 (12.02)
ICG 11109	0.73	1.18 (15.14)	ICG 15390	0.51	1.26 (18.20)
ICG 11542	0.62	1.09 (12.30)	ICG 11862	0.21	1.41 (25.70)
ICG 12625	0.86	1.18 (15.14)	ICG 7897	0.39	2.06 (114.82)
ICG 15386	0.59	1.54 (34.67)	ICG 4746	0.46	0.46 (2.88)
J 11	0.91	1.30 (19.95)	ICG 6766	0.75	2.71 (512.86)
ICGV 97183	0.61	1.10 (12.59)	ICG 6667	0.55	2.34 (218.78)
Mean	**0.75**	**1.25 (17.78)**	Mean	**0.44**	**1.51 (32.36)**
RC					
55-437	**0.50**	**1.02 (10.47)**			
SC					
Fleur 11	**0.58**	**1.11 (12.88)**			
HT					
ICG 862	0.74	1.06 (11.48)	ICG 9905	0.97	1.12 (13.18)
ICG 8285	0.65	1.25 (17.78)	ICG 11862	0.37	1.20 (15.85)
ICG 1703	0.41	1.12 (13.18)	ICG 12189	0.95	1.20 (15.85)
ICG 4729	0.58	0.97 (9.33)	ICG 12682	0.90	1.14 (13.80)
ICGV-SM99504	0.55	1.32 (20.89)	ICG 1823	0.64	1.14 (13.80)
ICG 10053	0.71	1.36 (22.91)	ICG 7897	0.43	2.06 (114.82)
ICG 12991	0.54	1.21 (16.22)	ICG 6766	0.43	2.71 (512.86)
ICG 12879	0.94	1.28 (19.05)	ICG 6643	0.28	0.75 (5.62)
ICG-13943	0.53	1.49 (30.90)	ICG 4906	0.33	1.17 (14.79)
ICG 15042	0.36	1.27 (18.62)	ICG 6667	0.32	2.34 (218.78)
Mean	**0.60**	**1.23 (16.98)**	Mean	**0.56**	**1.48 (30.20)**
RC					
55-437	**0.48**	**1.18 (15.14)**			
SC					
Fleur 11	**0.41**	**1.42 (26.30)**			
MT–HT					
ICG 862	0.53	1.17 (14.79)	ICG 8083	0.24	1.22 (16.60)
ICG 6022	0.36	1.32 (20.89)	ICG 188	0.36	1.26 (18.20)
ICG 6646	0.51	1.37 (23.44)	ICG 15419	0.34	1.40 (25.12)
ICG 6813	0.58	1.26 (18.20)	ICG 6766	0.49	2.71 (512.86)
ICG 8285	0.40	1.36 (22.91)	ICG 11862	0.19	1.31 (20.42)
ICG 10053	0.55	1.31 (20.42)	ICG 7897	0.41	2.06 (114.82)
55-437	0.52	0.82 (6.61)	ICG 11426	0.25	1.24 (17.38)
ICG 10950	0.47	1.54 (34.67)	ICG 6643	0.33	0.75 (5.62)
ICG 12509	0.57	1.28 (19.05)	ICG 6667	0.53	2.34 (218.78)
ICG 12879	0.63	1.36 (22.91)	ICG 4906	0.41	1.17 (14.79)
Mean	**0.51**	**1.27 (20.42)**	Mean	**0.35**	**1.54 (35.48)**
RC					
55-437	**0.52**	**1.22 (16.60)**			
SC					
Fleur 11	**0.48**	**1.3 (19.95)**			

Values in parenthesis are the ppb value of AC.

Almost all the highest yielding genotypes in MT, HT and MT–HT had higher pod yield ratio than 55-437 but none of them had significant lower aflatoxin contamination than 55-437 (Table 3). This was in part the consequence of the experimental error associated with AC measurement, which is very common in aflatoxin studies. It was also in agreement with the lack of a relationship between drought tolerance and AC values (Fig. 2B). Across moderate temperature environments, ICG 5891, ICG 11542 and ICG 97183 had similar aflatoxin contamination to 55-437 while low yielding ICG 4746 showed very low aflatoxin contamination compared to 55-437. ICG 862, ICG 4729 and ICG 6643 showed similar aflatoxin contamination to 55-437 across high temperature environments. Fleur 11 showed low ratio and high aflatoxin contamination. There was no significant difference between mean of aflatoxin contamination of highest yielding genotypes under moderate and high temperature. The means of aflatoxin contamination of lowest yielding genotypes under moderate and high temperature were also similar (Table 3).

In each environment, we also ranked the aflatoxin contamination of genotypes, regardless of the pod yield ratios, for identifying those with significant lower aflatoxin contamination (resistant) than 55-437 and those with significant higher aflatoxin contamination (sensitive) than Fleur 11 (Table 4). The least significant difference (LSD) of the log values of aflatoxin contamination for

Table 4
Aflatoxin contamination (AC, in log 10 of ppb values in parenthesis) of aflatoxin resistant and sensitive genotypes in moderate (MT08, MT09) and high temperature (HT09, HT10) environments, and pod yield (Py) under water stress. MT08 and MT09 = moderate temperature in 2008 and 2009, HT09 and HT10 = High temperature in 2009 and 2010, LSD = least significant difference. 55-437 = resistant check genotype, Fleur11 = sensitive check genotype.

Genotype	AC	Py	Genotype	AC	Py	Genotype	AC	Py	Genotype	AC	Py
	MT08			HT09			MT09			HT10	
Resistant											
ICG 5663	0.20 (1.58)	103	ICG 12988	0.32 (2.09)	69	ICG 15380	0.70 (5.01)	148	ICG 7867	0.29 (1.95)	41
ICG 311	0.25 (1.78)	84	ICG 12235	0.36 (2.59)	53	ICG 4543	0.72 (5.25)	145	ICG-156	0.33 (2.14)	145
ICG15390	0.25 (1.78)	105	ICG 4684	0.39 (2.45)	77	ICG 14523	0.76 (5.75)	177	ICG 2857	0.33 (2.14)	54
ICG 5891	0.27 (1.86)	80	ICG 4729	0.41 (2.57)	107	ICG 11144	0.76 (5.75)	144	ICG 15233	0.34 (2.19)	72
ICG 163	0.37 (2.34)	114	ICG 1668	0.46 (2.88)	139	ICGV 96466	0.77 (5.89)	149	ICG 8760	0.36 (2.29)	147
ICG 12189	0.39 (2.45)	90	ICG 4598	0.48 (3.02)	56	ICG 6813	0.80 (6.31)	203	ICG 3992	0.39 (2.45)	120
ICG 4764	0.39 (2.45)	165	ICG 15396	0.53 (3.39)	72	ICG 12189	0.81 (6.46)	145	ICG 12988	0.41 (4.57)	56
55-437	**0.96 (9.12)**	**135**	**55-437**	**0.73 (5.37)**	**98**	**55-43**	**0.96 (9.12)**	**188**	**55-437**	**0.63 (4.27)**	**120**
Sensitive											
ICG 15386	2.39 (245.47)	100	ICGV 02022	2.53 (338.84)	94	ICG 163	2.03 (107.15)	184	ICGV-SM99504	2.43 (269.15)	83
ICG 405	2.48 (302)	117	ICG 10053	2.53 (338.84)	168	ICG 2106	2.03 (107.15)	155	ICG-13943	2.47 (295.12)	178
ICG 13491	2.56 (368.08)	81	ICG 532	2.48 (302)	87	ICG 7963	2.22 (165.96)	142	ICG 4670	2.48 (302)	101
FLEUR11	**1.23** (16.98)	**135**	**FLEUR11**	**1.92 (83.18)**	**81**	**FLEUR11**	**1.28 (19.05)**	**154**	**FLEUR11**	**0.76 (5.75)**	**127**
LSD	**0.90 (7.94)**	**0.51**		**0.90 (7.94)**	**0.58**		**0.71 (5.13)**	**0.48**		**0.97 (9.33)**	**0.55**

MT08, MT09, HT09 and HT10 were 0.9, 0.9, 0.71 and 0.97 respectively. Based on these LSD, no genotype had lower aflatoxin contamination than 55-437 in the 4 environments (Table 4). As for the sensitive genotypes, several germplasm had an aflatoxin contamination significantly higher than the sensitive genotype Fleur 11, the aflatoxin sensitive check. The resistant and sensitive genotypes were different from one environment to another. This corroborates the significant GxE interaction observed indicating that genotypes were not contaminated equally across environments.

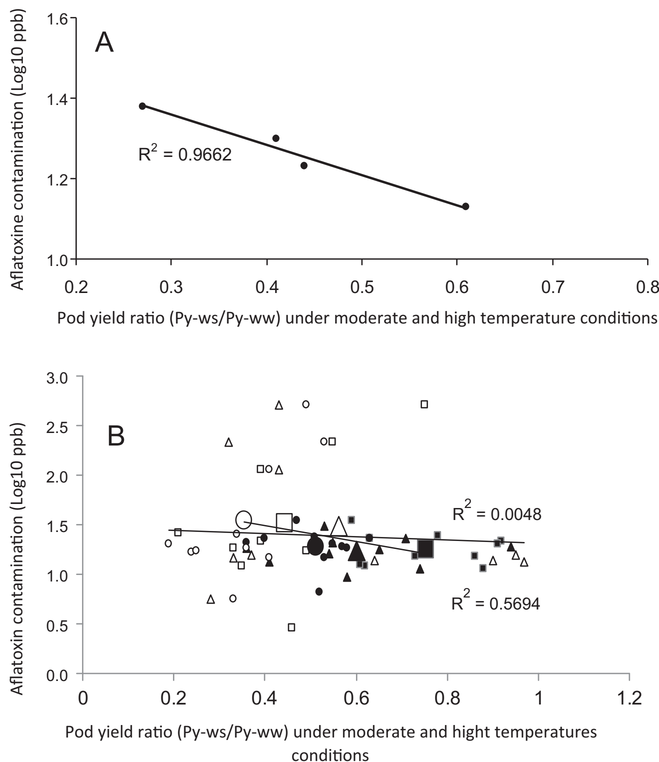

Fig. 2. Relationship between pod yield ratio (pod yield under fully irrigated conditions/pod yield under water stress) and aflatoxin contamination. Data are means of trials (A) and (B) the means of ten lowest – big symbols (□, Δ, o) and the ten highest – big symbols (■,▲, ●) yielding genotypes and the individual values of ten lowest – small symbols (□, △, ◯) and highest – small symbols (■, ▲, ●) yielding genotypes in moderate, high and moderate-high temperature conditions respectively.

4. Discussions

Large differences in aflatoxin contamination were mostly explained by environment and GxE interaction effects. As such, genotypes resistant to aflatoxin contamination were different in the four environments indicating that aflatoxin contamination was inconsistent across environment. Some drought tolerant genotypes showed low aflatoxin contamination (<5 ppb) although none had significantly lower aflatoxin contamination than resistant check 55-437. Aflatoxin contamination increased with drought stress severity, like those in the high temperature conditions. However, we have no data to assess whether the increase aflatoxin contamination could have been explained by temperature or by the higher drought effect due to temperature. The pod yield ratio of cohorts of drought tolerant and drought sensitive genotypes in each trial was not significantly correlated to aflatoxin contamination. The slight non-significant negative trend of that relationship was in fact driven by a minority of drought sensitive germplasm having large AC values. When taken individually, the drought tolerance index of individual genotypes showed no significant relationship to the aflatoxin contamination. Both analyses (by cohorts, or by individual genotypes) therefore suggest that the underlying mechanisms of drought tolerance and resistance to aflatoxin contamination are likely to be different.

4.1. Drought and aflatoxin contamination

Our results showed that the mean pod yield ratios for each trial were highly correlated ($r^2 = 0.96$) to the mean aflatoxin contamination in the four environments. This suggests that drought intensity increased aflatoxin accumulation in groundnut. Arunyanark et al. (2010) observed that severe drought promoted growth and persistence of *A. flavus* population leading to high aflatoxin contamination. Craufurd and colleagues (2006) came to the same conclusion. Previous works reported that drought tolerant genotypes may possess some degree of tolerance to aflatoxin contamination (Holbrook et al., 2000; Girdthai et al., 2010a). However, our findings revealed that among the 26 genotypes showing aflatoxin contamination as low as the resistant check 55-437 across environments, only ICG 5891, ICG 4729 and ICG 6813 figured among the drought tolerant (high yielding) genotypes in MT, HT and MT–HT conditions. Also contrary to previous reports (Arunyanark et al., 2009; Girdthai et al., 2010b; Sudhakar et al., 2007; Holbrook et al., 2000), this study suggests that there is no direct association

between drought tolerance of individual genotypes and their aflatoxin contamination. This was also shown by the absence of a significant relationship between AC values and the drought tolerance index of individual genotypes, or of cohorts of tolerant and sensitive genotypes across MT, HT, and MT–HT conditions, in any of the trials. This may suggest that the mechanisms of drought tolerance are likely unrelated to those of aflatoxin resistance. Therefore, while there is indeed an overall trend of an increase in aflatoxin contamination when drought conditions are more severe, and there are also a few drought sensitive genotypes having high AC values, the mechanisms of tolerance to drought and to aflatoxin contamination are likely to be quite different. Therefore, research on aflatoxin contamination resistance should be separated from research on drought tolerance, as it is very unlikely that a common mechanism leading to both drought tolerance and aflatoxin contamination resistance be identified. Of course these results will require further research because they challenge a common view that aflatoxin contamination and drought tolerance are related.

4.2. Environment and genotype by environment effect on aflatoxin contamination

In this study, combined analyses of variance for aflatoxin contamination showed significant environment (E) and genotype by environment interaction (GxE) effects. The high magnitude of the environment observed indicates that the environment contributed to the large portion of variation in aflatoxin contamination. The significance of GxE interaction, although its magnitude was smaller than environment magnitude, indicates that aflatoxin traits across environments were inconsistent among genotypes suggesting that selection of resistant genotypes to aflatoxin contamination must be specific to environment, making the selection of aflatoxin resistant genotype under drought conditions a difficult task. Similarly, Anderson et al. (1995) and Arunyanark et al. (2010) observed high GxE interaction for aflatoxin contamination. These authors reported that GxE interaction has long been recognized as the main reason for the lack of consistency of the performance of groundnut genotypes for aflatoxin contamination. Gorman et al. (1992) found also significant GxE interaction for aflatoxin contamination in maize and reported an inconsistency of infection by *Aspergillus* sp. Furthermore, heritability for aflatoxin contamination was different and relatively low across environments. Low genetic heritability for aflatoxin contamination in groundnut was previously reported (Anderson et al., 1996; Girdthai et al., 2010b; Arunyanark et al., 2010). Low heritability of seed aflatoxin contamination implies that it will be hard to improve this trait using a genetic approach. High GxE interaction and low heritability of aflatoxin contamination confound the selection of superior genotypes suggesting that alternative strategies need to be developed (Arunyanark et al., 2010). Other authors found close association between resistance to aflatoxin contamination and drought tolerant traits like drought tolerance index, specific leaf area, relative water content, SPAD chlorophyll meter reading and suggested these surrogates traits may be useful for indirect selection for improving aflatoxin contamination under drought conditions. In this study, no significant correlation was observed between the aflatoxin contamination and measured traits in the four environments (results not shown).

5. Conclusion

Our results showed significant but weak genotypic variation for aflatoxin contamination in one environment only, which support previous assertion that aflatoxin contamination is a complex trait and extremely variable in groundnut (Holbrook et al., 2000). Despite the existence of genetic variation for aflatoxin contamination among groundnut genotypes under drought conditions (Waliyar et al., 1994, 2003a,b; Girdthai et al., 2010a,b; Arunyanark et al., 2009), no new germplasm with AC values lower than resistant check 55-437 were found. The most salient result was the absence of a significant relationship between the drought tolerance index and aflatoxin contamination values, which suggest that mechanisms for drought tolerance and resistance to aflatoxin contamination are likely different. While this result deserves further study, it opens a new window of thinking, away from how drought and aflatoxin contamination are usually addressed.

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