

# Resistance to pre-harvest aflatoxin contamination in ICRISAT's groundnut mini core collection

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Accepted: 4 February 2016 / Published online: 10 February 2016  
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**Abstract** Aflatoxin contamination in groundnut by *Aspergillus flavus* has assumed global significance and is considered a potential threat to human and animal health. The present study focused on the screening and identification of stable and reliable resistance sources to pre-harvest aflatoxin contamination in ICRISAT's groundnut mini core germplasm accessions. Field studies were conducted during 2008 and 2009 in a randomized complete block design (RCBD) with three replications. Superior accessions ( $n = 34$ ) were selected and screened during 2010 and 2011 in a Lattice design with three replications. Seven best accessions with  $<1 \mu\text{g kg}^{-1}$  aflatoxin B<sub>1</sub> levels were further selected and screened during 2012 and 2013. Based on the evaluation in 2008 and 2009, four accessions had aflatoxin contamination within  $4 \mu\text{g kg}^{-1}$ , 50 accessions within  $10 \mu\text{g kg}^{-1}$ , 66 accessions within  $15 \mu\text{g kg}^{-1}$ , and 75 accessions within  $20 \mu\text{g kg}^{-1}$ . Of the 34 selected accessions evaluated in 2010 and 2011, eight accessions had  $<1 \mu\text{g kg}^{-1}$ . The analysis of 34 accessions over a

period of four years from 2008 to 2011 indicated that the mean toxin levels ranged from 0.9 to  $10.3 \mu\text{g kg}^{-1}$ . In total, 31 accessions had less aflatoxin accumulation than the resistant check, 55–437. The seven best accessions, ICGs 13,603, 1415, 14,630, 3584, 5195, 6703 and 6888, over six years (2008–2013) consistently accumulated very low levels of aflatoxin ( $<4 \mu\text{g kg}^{-1}$ ). These seven accessions could be potential sources for understanding the resistant mechanisms and can be further used in developing resistant cultivars or introgressing resistance in popular released varieties.

**Keywords** Groundnut · Aflatoxins · *Aspergillus flavus* · Mini core collection · Resistance

## Introduction

Groundnut (Peanut; *Arachis hypogaea* L.), an important food legume grown worldwide, is considered a rich source of protein for both humans and animals. However, aflatoxin contamination induced by *Aspergillus flavus* and *A. parasiticus* is a major constraint to the global trade of groundnut. According to World Health Organization (WHO), staple foods in developing countries are often contaminated with aflatoxins which result in health-related hazards (e.g., aflatoxicosis) when consumed. Important health implications of aflatoxin poisoning include immuno-suppression, impaired growth, various cancers, and death depending on the type, period and amount of exposure (Williams et al. 2004; Wagacha and Muthomi 2008).

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These health problems are more severe in African communities due to exposure to aflatoxins throughout their lives (Williams et al. 2004; Wild and Gong 2010). There have been increasing reports of aflatoxin contamination in freshly harvested groundnuts in several countries of sub-Saharan Africa. Recently, 22–54 % of groundnut samples collected during 2009 and 2010 from different groundnut growing districts in Mali showed  $>20 \mu\text{g kg}^{-1}$  of aflatoxins (Waliyar et al. 2015a). This contamination renders the commodity unfit for human consumption and unacceptable for trade in high-value markets. In Malawi, one of the important groundnut growing countries in East and Southern Africa, 21 % and 8 % of groundnut samples were found to contain aflatoxin levels of  $>20 \mu\text{g kg}^{-1}$  during 2008 and 2009, respectively (Monyo et al. 2012). About 78 % of aflatoxin contaminated raw groundnut samples were reported in Botswana with aflatoxin concentrations ranging from 12 to 329  $\mu\text{g kg}^{-1}$  (Barro et al. 2002).

Aflatoxin control and prevention strategies mainly include stopping the infection process of *A. flavus* by host-plant resistance/tolerance, biological control, managing environmental factors, pre-harvest and post-harvest crop management practices (Waliyar et al. 2008, 2015b; Hell and Mutegi 2011). Breeding the plant for resistance to seed infection and/or aflatoxin contamination is one of the economically feasible options in managing both pre- and post-harvest aflatoxin contamination in groundnut. Resistance breeding to groundnut aflatoxin contamination can be achieved by imparting resistance to pre-harvest seed infection, in vitro seed colonization (IVSC) by *A. flavus* and aflatoxin production (Nigam et al. 2009). However, independently inherited nature of these three types of resistance coupled with high G x E interaction makes it difficult to improve the host plant resistance in groundnut (Utomo et al. 1990; Upadhyaya et al. 1997; Nigam et al. 2009). Despite the constraints, many researchers have focused on the identification of new sources of resistances for all these three types. For example, the germplasm accession lines, PI 337394 F, PI 337409 and J 11, were confirmed to be resistant to IVSC by *A. flavus* (Zambettakis et al. 1981; Kisyombe et al. 1985). Additional sources such as Ah 78,223, U 4-47-7, Var 27, Faizpur and Monir 240–30 were also identified as being resistant to IVSC by *A. flavus* (Mehan 1989). Due to the lack of resistance of IVSC resistant genotypes under field conditions, the research focus has been shifted to identifying resistant sources to pre-harvest

infection and subsequent aflatoxin contamination. Later attempts have identified potentially resistant groundnut genotypes for pre-harvest aflatoxin contamination (Waliyar et al. 1994; Anderson et al. 1995; Upadhyaya et al. 2004). Three groundnut genotypes such as ICGV 87084, ICGV 87094 and ICGV 87110 were identified to be resistant to *A. flavus* and aflatoxin contamination as evaluated in Niger, Senegal and Burkina Faso in West Africa (Waliyar et al. 1994). Further improved groundnut germplasm lines such as ICGV 91278, ICGV 91283 and ICGV 91284 were registered as resistant to *A. flavus* seed infection (Upadhyaya et al. 2001). In China, a cultivar “Zhonghua 6” has been reported to be resistant to aflatoxin formation and is being widely cultivated in central China (Liao et al. 2009). However, the value of a resistant source depends on the level and stability of its resistance in view of the high G x E interaction for this important trait (Upadhyaya et al. 2002a). On the other hand, variability in the toxigenicity of *A. flavus* populations poses threat to the performance of resistant genotypes under varied climatic conditions and multiple environments.

Plant genetic resources are widely used in breeding programs for imparting resistance to various pests and diseases (Knauff and Gorbet 1989; Upadhyaya et al. 2010). Over the years, around 3000 groundnut germplasm accessions including wild *Arachis* germplasm accessions were evaluated for resistance to aflatoxin contamination (Thakur et al. 2000; Nigam et al. 2009; Holbrook et al. 2009). However, considering the number of groundnut germplasm accessions available in gene banks (around 15,000), many precious germplasm accessions might not get evaluated for this important trait. It is very difficult to screen such a huge collection under field conditions. Hence, researchers have developed “core” (Holbrook et al. 1993) and “mini core” collections (Upadhyaya et al. 2002b) of groundnut that represent the genetic variability of the entire collection and serves as handy germplasm sets for evaluating important biotic and abiotic stresses. Selection of resistant sources through systematic screening of mini core collection accessions is in practice for infusing genetic diversity (Upadhyaya et al. 2010). Although there are some reports on the screening of mini core collections for *A. flavus*/aflatoxin resistance (Yugandhar 2005; Kusuma et al. 2007; ICRISAT 2009), there is still lack of a detailed report on the availability of resistant sources among the ICRISAT mini core collection.

Considering the limitations and little progress obtained using conventional breeding, enhancing host-plant resistance to aflatoxin contamination using biotechnological approaches has also been explored in groundnut (Guo et al. 2009). However, the lack of good candidates with high levels of resistance and stability in multiple environments remains an important bottleneck to the further understanding of the resistance mechanisms in groundnut by employing genomics and other cutting-edge molecular biology tools. Hence, identifying highly resistant sources to pre-harvest infection and subsequent aflatoxin contamination is important and continues to be a thrust area. Therefore, the aim of this study was to evaluate the groundnut mini core germplasm collection in an on-station experiment for several years and identify the accessions that consistently showed a good level of resistance to pre-harvest aflatoxin contamination.

## Materials and methods

### Genotypes

In the present study, the groundnut mini core collection consisting of 184 germplasm accessions was used (Upadhyaya et al. 2002b). These accessions were selected from 1704 entries of the core collection of groundnut representing 14,310 accessions available in the ICRISAT gene bank (Upadhyaya et al. 2003). Basically, the mini core collection was a reduced subset of the global collection of groundnut germplasm (1 % of the entire collection). These germplasm accessions represent two subspecies (*fastigiata* and *hypogaea*) and six botanical varieties such as 37 from *fastigiata*, 58 from *vulgaris*, 85 from *hypogaea*, two from *peruviana*, and one each of *aequitioriana* and *hirsuta*. Two cultivars, 55–437 (resistant to pre-harvest aflatoxin contamination; Waliyar and Bockelee-Morvan 1989) and JL 24 (susceptible to pre-harvest aflatoxin contamination) were used as standard controls.

### Experimental location

Field screening of mini core collection accessions was performed during the rainy seasons of 2008–2013 at ICRISAT-Niamey, Sadore, Niger. The normal sowing time during the study period was 3rd week of June, and the experimental plots were harvested during the 3rd week of October. Niger was selected for screening since

it was a hot-spot location with significant pre-harvest aflatoxin contamination (Waliyar et al. 2003). The soils at Sadore were classified as sandy soils (93 % sand) (Waliyar et al. 1994; West et al. 1984). The average annual rainfall in Sadore, Niger is 562 mm (Sivakumar 1986).

### Preliminary field screening

During 2008, a total of 176 mini core germplasm accessions were screened. The mini core accessions ICGs 2857, 5051, 5827, 7190, 10,036, 10,554, 14,710 and 15,419 were excluded from the screening trials due to insufficient availability of seeds. Four other accessions ICGs 13,941, 13,942, 2738 and 156 were also included. In 2009, a total of 166 accessions comprising 162 mini core accessions and four other accessions, ICGs 13,941, 13,942, 2738 and 156 tested during 2008 were also screened. The mini core germplasm accessions, ICGs 2857, 5051, 5827, 7190, 10,036, 10,554, 14,710, 15,419, 6646, 6766, 6667, 2381, 11,088, 6022, 12,625, 4746, 12,000, 9905, 1668, 6993, 6913 and 11,426 were not included during this screening experiment. Two resistant (55–347 and J 11) and two susceptible checks (Fleur 11 and JL 24) for pre-harvest aflatoxin contamination were used during 2008 and 2009. The experiments were laid out in a Randomized Complete Block Design (RCBD) with three replications. The plot size during 2008 was 4.5 m<sup>2</sup> (1.5 m × 3.0 m) of three rows, each measuring 3 m with a gap of 50 cm between rows and 10 cm between seeds. During 2009, the plot size was 8 m<sup>2</sup> with four rows of 4 m length, with a similar arrangement of accessions.

A total of 34 accessions were screened during 2010 and 2011. Of these, 25 promising accessions were from 2008 and 2009 field experiments with a mean aflatoxin content of <7 µg kg<sup>-1</sup>. The accession ICG 6402 with mean aflatoxin content of 4.8 µg kg<sup>-1</sup> during 2008 and 2009 studies was not included due to insufficient seed. Further, nine promising accessions (ICGs 11,515, 12,988, 4543, 4670, 532, 81, 9157, 3775 and 13,603) from our earlier field studies (data not shown) were included. During both these years, 55–437 (resistant) and JL 24 (susceptible) were used as standard controls. The trials were laid out in a 6 × 6 Lattice design with three replications. The plot dimensions during 2010 were 8 m<sup>2</sup> (2 m × 4 m) of four rows, each measuring 4 m with a gap of 50 cm between the rows and 10 cm between plants. During 2011, the trial was laid out in a

plot size of 4.5 m<sup>2</sup> of three rows, each measuring 3 m with a gap of 50 cm between the rows and 10 cm between the plants. The performance of these accessions over four years (2008–2011) was analyzed to determine the stability of resistance. The accessions ICGs 13,603 and 12,988 were also included in the performance analysis.

#### Final screening of selected germplasm accessions during 2012 and 2013

Seven accessions were selected and screened during 2012 and 2013. Of these, ICGs 1415, 14,630, 3584, 5195 and 6703 had a mean pre-harvest aflatoxin contamination level < 1 µg kg<sup>-1</sup> during 2010 and 2011. The other two accessions ICGs 13,603 and ICG 6888 with >1 µg kg<sup>-1</sup> were also included based on our earlier studies. The standard checks, 55–437 (resistant) and JL 24 (susceptible) were also included in a 3 × 3 Lattice design with a plot size of 6 m<sup>2</sup> with three replications.

In all the experimental plots year-wise, care was taken to ensure uniform depth of planting. Standard agronomic and cultural practices were followed during all the years of experimentation. Plot-wise harvesting of accessions was carried out at optimum maturity.

#### Data collection

Weather data for the years 2004 to 2013 were recorded daily inclusive of the crop growth periods by a nearby meteorological station from the experimental Fields. *maximum* and minimum air temperatures and precipitation were recorded for the study period. The mean minimum and maximum temperatures in Celsius over the experimental years ranged from: 9.5–30.1 to 25.4–44 (2008); 13.2–31.1 to 26–44.5 (2009); 12.7–31.5 to 25.5–45.9 (2010); 13.6–31.2 to 26.6–44.2 (2011); 11.1–31.0 to 26.2–44.5 (2012); and 12.1–30.7 to 24.5–44.0 (2013), respectively. The annual precipitation levels (mm) during the experimental years were 480.6 (2008), 515.6 (2009), 687.5 (2010), 497.8 (2011), 649.1 (2012), and 594.4 (2013).

Pod yields were recorded by harvesting each accession (replication-wise). For harvesting, the plants were uprooted by hand and brought to the threshing floor, stripped off, and then dried prior to yield assessment. For ascertaining natural seed infection by *A. flavus*, the pods were shelled, and 100 seeds/plot were selected.

The seeds from each replication were surface sterilized by immersion for 3 min in a 0.2 % aqueous solution of sodium hypochlorite. After three rinses in sterile distilled water, the seeds were transferred aseptically onto moistened filter paper in sterile Petri dishes. The Petri dishes were later incubated at room temperature (25 ± 1 °C) for a maximum of 7 days and fungi growing from the seeds were identified and the per cent *A. flavus* infection was enumerated (Waliyar and Bockelee-Morvan 1989). The *A. flavus* isolates were confirmed based on the microscopic and macroscopic characteristics using standard procedures (Raper and Fennel 1965). For aflatoxin estimation (AFB1), 500 g of pods were shelled and powdered, and 20 g was used for analysis through indirect competitive ELISA (Reddy et al. 2001). The limit of detection (LOD) of the assay was 0.01 µg/kg.

#### Data analysis

The individual and pooled data for the field studies during 2008–2013 were analyzed using Residual Maximum Likelihood (REML) with the GENSTAT statistical package (version 10.1; Rothamsted Experiment Station, Harpenden, Herts AL52JQ, UK). The significance of genetic variability among accessions within treatment was assessed from the standard error of the estimate of genetic variance  $\sigma^2$  g. REML analysis was 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. Similarly, the significance of genetic variability across treatments or the interaction effect was also assessed.

## Results

#### Climatic conditions in sadore, Niger

The analysis of weather records for a period of 10 years (2004 to 2013) in Sadore, Niger revealed that both the maximum and minimum temperatures corresponded with the peak in April and May, respectively. However, both the temperatures were the lowest in August and January respectively. The rainfall data for the same period indicated that August had consistently

recorded the highest rainfall, followed by the months of July and September. The data also revealed that the rainy season normally started in May and ended during October.

#### Statistical variances among accessions

##### *Aflatoxins*

Significant genotypic and G x E variances were observed in the pool analysis during the six years of field studies. The variance components due to genotypes ( $\sigma^2_g$ ) were quite high compared with G x E interaction ( $\sigma^2_{ge}$ ) in 2008 and 2009, indicating that the differences in aflatoxin contents were mainly contributed by the accessions in these years. Whereas in 2010–2013 and pooled season (2008–2011), the G x E variance component was quite high compared to the genotypic variance, indicating that genetic variability in aflatoxin resistance is masked by higher G x E interaction. The Wald statistic indicated a non-significant effect of environment based on the pooled data of six environments (Table 1).

##### *Seed infection by A. flavus*

Significant genotypic variances during 2008, 2009, and 2011 and for pooled seasons (2008 to 2011) separately and G x E interaction in the pooled analysis were observed. In 2008 and 2009, the variance component due to genotype was high over the G x E interaction. In 2011 and through the pooled data (2008 through 2011), a high G x E interaction was observed compared to genotypic variance. The Wald statistic indicated a

significant effect of environment on seed infection (Table 1).

##### *Pod and haulm yields*

Significant genotypic differences were observed during 2008–2011 for both pod and haulm yields. Significant G x E interaction was observed in haulm yields; however, the interaction was not significant for pod yields. The Wald statistic indicated a significant difference in pod and haulm yields (Table 1).

##### *Preliminary screening of mini core germplasm accessions*

Out of the 180 accessions screened in 2008, two accessions (1.1 % of the accessions screened) had  $<4 \mu\text{g kg}^{-1}$  AFB<sub>1</sub>. Overall, 39 accessions (21.7 %) had aflatoxin levels up to  $10 \mu\text{g kg}^{-1}$ . In addition, 30 accessions (16.7 %) were in the range of 10.1 to  $15.0 \mu\text{g kg}^{-1}$ , 13 accessions (7.2 %) in the range of 15.1 to  $20.0 \mu\text{g kg}^{-1}$ , and seven accessions (3.9 %) were in the range of 20.1 to  $30.0 \mu\text{g kg}^{-1}$ . Thus, during 2008, 89 accessions (49.4 %) had toxin levels of  $<30 \mu\text{g kg}^{-1}$  (Fig 1). The lowest aflatoxin content was recorded in ICG 6766 ( $2.6 \mu\text{g kg}^{-1}$ ), followed by ICG 6402 ( $3.4 \mu\text{g kg}^{-1}$ ). Two accessions, ICGs 14,475 and 14,482 had very high aflatoxin levels (2913.2 to  $4411.5 \mu\text{g kg}^{-1}$ , respectively). The highest aflatoxin content was recorded in ICG 11426 ( $5262.6 \mu\text{g kg}^{-1}$ ). The toxin levels were up to  $12.9 \mu\text{g kg}^{-1}$  for resistant checks, whereas they were up to  $3313.5 \mu\text{g kg}^{-1}$  for the susceptible checks.

In 2009, 20 accessions (12 %) of the 166 accessions screened had aflatoxin levels up to  $4 \mu\text{g kg}^{-1}$ . Thirty-

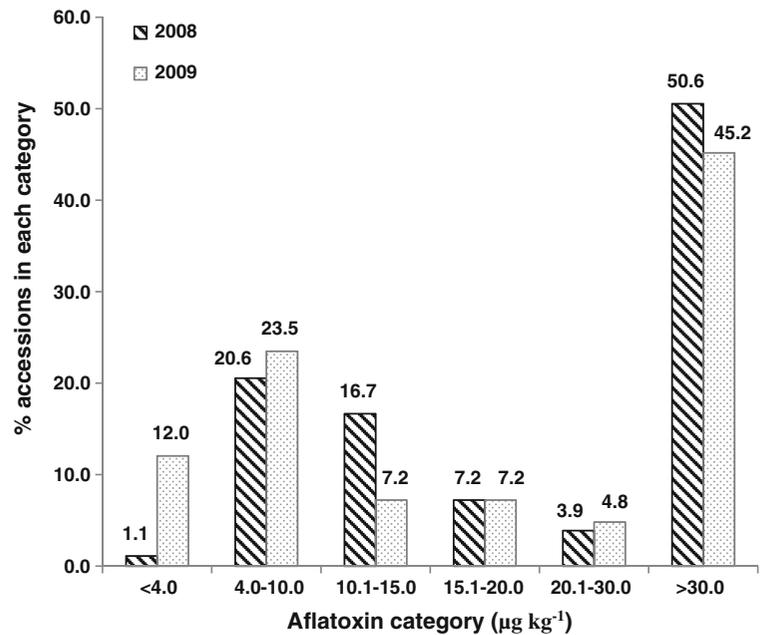
**Table 1** Residual maximum likelihood analysis (REML) for pre-harvest aflatoxin contamination, seed infection by *Aspergillus flavus*, pod and haulm yields in the mini core accessions of groundnut as evaluated during 2008 to 2013 at Sadore, Niger

Trait	$\sigma^2_g$							$\sigma^2_{g.e}$	Wald statistic (season)	F prob.
	2008	2009	2010	2011	2012	2013	2008–11 (4 seasons) pooled			
Aflatoxin (ppb)	501,606**	64,914**	4168.7**	1038.2**	14.2*	162.9*	16,780.1**	15,018.5**	3.53	0.322
<i>A. flavus</i> (%)	400.0**	62.9**	13.7	33.0*	3.65		35.68**	58.5**	75.29	<0.001
Pod Yield (t/ha)	0.25**	0.15**	0.02*	0.32**	0.12	0.21	4.48	0.09	620.23	<0.001
Haulm Yield (t/ha)	1.96**	0.91**	0.09*	1.79**	0.88	0.24	0.07	0.80**	527.6	<0.001

\*significant at 5 % level

\*\*significant at 1 % level

**Fig. 1** Reaction of groundnut mini core accessions to pre-harvest aflatoxin B<sub>1</sub> contamination during rainy seasons of 2008 and 2009 at Sadore, Niger



nine accessions (23.5 %) had toxin levels of 4.1 to 10.0 µg kg<sup>-1</sup>, 12 accessions (7.2 %) had 10.1 to 15 µg kg<sup>-1</sup>, 12 accessions (7.2 %) had 15.1 to 20.0 µg kg<sup>-1</sup>, and eight accessions (4.8 %) had 20.1 to 30.0 µg kg<sup>-1</sup>. Overall, 91 accessions had aflatoxin levels below 30 µg kg<sup>-1</sup> (Fig 1). Lowest toxin levels were recorded in ICG 1415 (1.4 µg kg<sup>-1</sup>), ICG 5195 (1.6 µg kg<sup>-1</sup>), and ICG 14630 (1.7 µg kg<sup>-1</sup>). In 2009, the highest toxin levels were recorded in ICG 4538 (990 µg kg<sup>-1</sup>), followed by ICG 9315 (988.3 µg kg<sup>-1</sup>). The toxin levels in the resistant checks were up to 7.6 µg kg<sup>-1</sup>, whereas they were up to 1048.3 µg kg<sup>-1</sup> in the susceptible checks.

The average performance of 166 accessions in resisting pre-harvest aflatoxin contamination for 2008 and 2009 are shown in Table 2. Four accessions had aflatoxins up to 4 µg kg<sup>-1</sup>. A total of 46 accessions had aflatoxins level in the range of 4.1 to 10.0 µg kg<sup>-1</sup>, 16 accessions had 10.1 to 15.0 µg kg<sup>-1</sup>, and nine accessions had aflatoxins in the range of 15.1 to 20.0 µg kg<sup>-1</sup>. Three accessions had aflatoxin levels ranging from 1000 to 1500 µg kg<sup>-1</sup>. The highest mean aflatoxin levels above 2000 to 2500 µg kg<sup>-1</sup> were recorded in ICG 14482 (Table 2). The mean aflatoxin levels for 2008 and 2009 indicated that 25 accessions had aflatoxin B<sub>1</sub> up to 7 µg kg<sup>-1</sup>. Overall, the mean toxin contents ranged from 2.9 to 2278.9 µg kg<sup>-1</sup>.

In 2010, aflatoxin accumulation in 34 accessions ranged from 0.06 to 12.81 µg kg<sup>-1</sup>. In 2011, the aflatoxin content in the same 34 accessions was in the range of 0.14 to 14.71 µg kg<sup>-1</sup>. The mean aflatoxin level of eight accessions was up to 1 µg kg<sup>-1</sup> for 2010 and 2011 and the mean toxin level of seven accessions ranged from 1.1 to 2.0 µg kg<sup>-1</sup> (Table 3). Aflatoxin levels in 55–437 (resistant check) and JL 24 (susceptible check) were 2.24 and 289.41 µg kg<sup>-1</sup>, respectively. The details of accessions with their respective range of mean aflatoxin levels in 2010 and 2011 are provided in Table 3.

The performances of 34 accessions resistant to pre-harvest aflatoxin contamination over four years (2008–2011) are depicted in Table 4. The mean toxin levels ranged from 0.98 (ICG 13603) to 10.34 µg kg<sup>-1</sup> (ICG 12988) (Table 4). The other accessions with aflatoxin levels below 2 µg kg<sup>-1</sup> include ICGs 14,630, 1415, 5195, 3584, 8490, and 6703. The toxin content of the resistant check (55–437) was 5.7 µg kg<sup>-1</sup>. Out of the 34 accessions, 31 had aflatoxin levels below that of the resistant check (55–437). The susceptible check (JL 24) recorded the highest toxin content of 861.9 µg kg<sup>-1</sup>. Pod yields ranged from 0.86 (ICG 513) to 1.91 t ha<sup>-1</sup> (ICG 3584). The pod yields in resistant check (55–437) and susceptible checks (JL 24) were 1.68 t ha<sup>-1</sup> and 1.37 t ha<sup>-1</sup>, respectively. Haulm yields ranged from 2.71 to 4.38 t ha<sup>-1</sup> among the accessions (Table 4).

**Table 2** Reaction of groundnut mini core accessions to pre-harvest aflatoxin contamination during 2008 and 2009 at Sadore, Niger

Aflatoxin levels ( $\mu\text{g kg}^{-1}$ )	No. of accessions	Details
0.0–4.0	4	ICGs 5195, 14,630, 1415, 6703
4.1–10.0	46	ICGs 3584, 6407, 6263, 8490, 5663, 6402, 862, 7153, 513, 6201, 4750, 7181, 12,697, 6888, 721, 1711, 1142, 8285, 332, 6654, 6375, 9666, 15,309, 4543, 5609, 11,515, 4670, 12,988, 532, 13,858, 13,941, 11,322, 11,457, 14,106, 81, 875, 11,249, 9157, 9249, 3775, 14,118, 163, 2106, 3053, 13,099, 10,890
10.1–15.0	16	ICGs 14,523, 3027, 15,287, 13,603, 111, 11,687, 156, 12,370, 1519, 76, 334, 1137, 3421, 2019, 2879, 5779
15.1–20.0	9	ICGs 36, 11,144, 4729, 8083, 1973, 9809, 13,942, 297, 12,672
20.1–30.0	14	ICGs 2773, 1274, 11,109, 115, 13,723, 15,190, 12,921, 15,042, 2772, 7969, 9507, 9842, 14,127, 4998
30.1–100	22	ICGs 118, 3343, 11,862, 5745, 9777, 442, 188, 13,856, 11,651, 8567, 10,092, 3746, 9961, 13,491, 10,474, 8760, 11,855, 3681, 14,705, 3240, 13,787, 4412
100.1–500	34	ICGs 1399, 434, 6057, 2738, 5327, 5475, 928, 14,008, 5494, 4955, 7243, 14,466, 10,384, 5016, 12,189, 4156, 4684, 5891, 4911, 5236, 6813, 4527, 4598, 4343, 2777, 4389, 5662, 11,219, 8106, 13,982, 7906, 7000, 14,985, 7963
500.1–1000	16	ICGs 12,682, 9037, 3102, 10,479, 10,566, 12,276, 8517, 5286, 3992, 6892, 10,185, 2511, 397, 3673, 2925, 9315
1000.1–1500	3	ICGs 4538, 5221, 9418
1500.1–2000	1	ICG 14475
2000.1–2500	1	ICG 14482

Aflatoxin levels are estimated at harvest using ELISA

Aflatoxin values are means of 2008 and 2009 data

#### *Final screening of selected mini core accessions in 2012 and 2013*

In 2012, all the seven elite accessions (ICGs 1415, 14,630, 3584, 5195, 6703, 6888 and 13,603) had aflatoxins below  $1 \mu\text{g kg}^{-1}$ . Toxin content was the least in ICG 13603 and ICG 14630 ( $0 \mu\text{g kg}^{-1}$ ), whereas it was the highest in ICG 6888 ( $0.86 \mu\text{g kg}^{-1}$ ). The resistant check (55–437) recorded  $1.58 \mu\text{g kg}^{-1}$ , whereas the susceptible check (JL 24) recorded about  $11.68 \mu\text{g kg}^{-1}$ . The mean *A. flavus* infection of seeds for these accessions ranged from 1.30 % (ICG 1415) to 2.7 % (ICG 6888) (Table 5). The pod yields ranged from 1.40 (ICG 14630) to 2.22 t ha<sup>-1</sup> (ICG 1415). The resistant check has

a pod yield of 1.94 t ha<sup>-1</sup>, whereas the susceptible check recorded 2.16 t ha<sup>-1</sup>.

In 2013, three accessions (ICGs 6888, 1415 and 13,603) had aflatoxins up to  $1 \mu\text{g kg}^{-1}$ , and four accessions (ICGs 3584, 5195, 6703 and 14,630) had aflatoxins below  $3 \mu\text{g kg}^{-1}$  (Table 6). The resistant check (55–437) had recorded aflatoxin levels of  $5.5 \mu\text{g kg}^{-1}$ , whereas susceptible check (JL 24) recorded  $40 \mu\text{g kg}^{-1}$ . The pod yields for these seven accessions ranged from 3.7 to 4.6 t ha<sup>-1</sup>.

#### *Multi-year analysis of Superior accessions against pre-harvest aflatoxin contamination*

Field performance of the seven best accessions (ICGs 1415, 14,630, 3584, 5195, 6703, 6888 and 13,603) for

**Table 3** Reaction of selected groundnut mini core accessions to pre-harvest aflatoxin contamination during 2010 and 2011 at Sadore, Niger

Aflatoxin levels ( $\mu\text{g kg}^{-1}$ )	No. of accessions	Details
0–1.0	8	ICGs 14,630, 3584, 8490, 1415, 332, 7153, 5195, 6703
1.1–2.0	7	ICGs 8285, 862, 9666, 7181, 4750, 513, 6263
2.1–3.0	9	ICGs 1142, 532, 5663, 6888, 721, 4543, 6407, 9157, 4670
3.1–4.0	5	ICGs 6201, 12,697, 6654, 1711, 11,515
4.1–5.0	2	ICGs 81, 3775
>5.0	3	ICGs 6375, 12,988, 13,603

Aflatoxin levels are estimated at harvest using ELISA

Aflatoxin values are means of 2010 and 2011 data

**Table 4** Evaluation of groundnut mini core accessions for their resistance to pre-harvest aflatoxin contamination during the rainy seasons of 2008, 2009, 2010 and 2011 at Sadore, Niger

Genotype	Aflatoxin (ppb)*	Pod yield (t/ha)	Haulm yield (t/ha)
ICG 13603	0.98	1.37	2.99
ICG 14630	1.43	1.14	3.65
ICG 1415	1.32	1.34	3.45
ICG 5195	1.36	1.17	3.22
ICG 3584	1.60	1.91	3.71
ICG 8490	1.93	1.04	2.92
ICG 6703	1.69	1.47	4.08
ICG 7153	2.40	1.33	4.02
ICG 862	2.51	1.19	4.17
ICG 6263	2.58	0.95	2.90
ICG 5663	2.78	1.31	3.73
ICG 6888	2.42	1.51	3.74
ICG 6407	2.89	1.10	3.81
ICG 513	3.00	0.86	2.84
ICG 7181	3.06	1.09	3.39
ICG 332	3.09	1.45	3.52
ICG 4750	3.17	1.47	4.02
ICG 8285	3.39	1.32	3.30
ICG 9666	3.60	1.27	4.38
ICG 6201	3.82	1.41	3.97
ICG 721	3.87	1.01	3.31
ICG 12697	3.96	1.19	3.37
ICG 1142	3.95	0.88	3.19
ICG 1711	4.44	1.13	2.91
ICG 6654	4.46	1.03	2.71
ICG 4543	4.61	1.39	3.75
ICG 532	4.63	1.04	3.24
ICG 4670	4.83	1.49	3.83
ICG 11515	5.11	1.11	3.22
ICG 9157	5.33	1.46	2.72
ICG 6375	5.58	1.33	3.17
ICG 81	5.78	1.70	4.03
ICG 3775	6.22	0.91	3.34
ICG 12988	10.34	1.78	4.11
55–437	5.69	1.68	3.62
JL 24	861.9	1.37	3.17
<b>LSD (5 %)</b>	37.31	0.33	0.79
<b>SEm (±)</b>	13.42	0.119	0.285
<b>CV (%)</b>	69.12	32.63	29.36

55–437 was the resistant check and JL 24 was the susceptible check

Values are means of four years with three replications/treatment/year

\*significant at 1 % level

six years (2008, 2009, 2010, 2011, 2012 and 2013) indicated that the mean aflatoxin levels ranged up to  $5.8 \mu\text{g kg}^{-1}$  (ICG 13603). Lowest aflatoxin levels were recorded with ICG 1415 ( $1.5 \mu\text{g kg}^{-1}$ ), followed by ICG 5195 ( $1.7 \mu\text{g kg}^{-1}$ ), and ICG 3584 ( $1.8 \mu\text{g kg}^{-1}$ ). For

other accessions, the aflatoxin levels were  $1.9 \mu\text{g kg}^{-1}$  (ICG 14360),  $2.0 \mu\text{g kg}^{-1}$  (ICG 6703), and  $3.1 \mu\text{g kg}^{-1}$  (ICG 6888). The resistant check recorded aflatoxin levels of  $5.3 \mu\text{g kg}^{-1}$ , whereas the susceptible check recorded  $581.8 \mu\text{g kg}^{-1}$  (Fig 2). The mean pod yields

**Table 5** Field evaluation of selected groundnut mini core accessions for pre-harvest aflatoxin contamination at Sadore, Niger in 2012 (rainy season)

Accession	Aflatoxin levels* ( $\mu\text{g kg}^{-1}$ )	% Kernel <i>A. flavus</i> infection	Pod yield ( $\text{t ha}^{-1}$ )
ICG 13603	0.00	1.54 (4.63)	1.98
ICG 1415	0.42	1.29 (3.80)	2.22
ICG 14630	0.00	2.0 (6.24)	1.39
ICG 3584	0.30	1.76 (5.82)	1.92
ICG 5195	0.54	1.76 (5.82)	1.91
ICG 6703	0.67	2.23 (7.25)	2.02
ICG 6888	0.86	2.70 (7.27)	1.98
55–437	1.58	1.76 (5.82)	1.94
JL 24	11.68	1.99 (6.24)	2.16
<b>LSD (5 %)</b>	1.51	1.84 (4.67)	0.76
<b>SEm (<math>\pm</math>)</b>	0.52	0.63 (1.59)	0.23
<b>CV (%)</b>	50.2	99.49 (76.81)	31.39

55–437 was the resistant check and JL 24 was the susceptible check

Values in parentheses are angular transformed values

% *A. flavus* infection is enumerated at harvest using standard protocols

\*significant at 5 % level

ranged from 1.6 to 2.2  $\text{t ha}^{-1}$  for these seven accessions. The highest pod yields were recorded with ICG 3584

**Table 6** Field evaluation of selected groundnut mini core accessions for pre-harvest aflatoxin contamination at Sadore, Niger in 2013 (rainy season)

Accession	Aflatoxin levels* ( $\mu\text{g kg}^{-1}$ )	Pod yield ( $\text{t ha}^{-1}$ )
ICG 13603	1.0	4.6
ICG 1415	1.0	4.1
ICG 14630	2.9	3.5
ICG 3584	1.7	4.2
ICG 5195	1.8	3.8
ICG 6703	2.2	3.7
ICG 6888	0.2	3.9
55–437	5.5	3.9
JL 24	40.0	4.5
<b>LSD (5 %)</b>	1.46	0.88
<b>SEm (<math>\pm</math>)</b>	0.50	0.30
<b>CV (%)</b>	13.88	17.12

55–437 was the resistant check and JL 24 was the susceptible check

\*significant at 5 % level

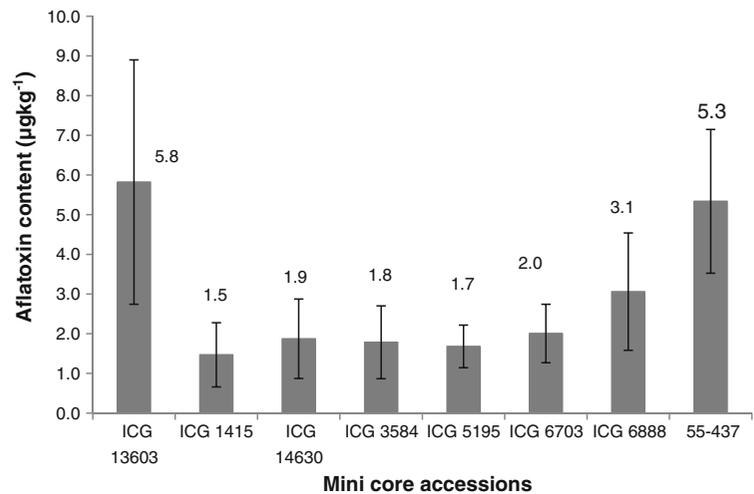
(2.2  $\text{t ha}^{-1}$ ), followed by ICG 6888 and ICG 13603 (2.0  $\text{t ha}^{-1}$ ). ICGs 1415 and 6703 have recorded pod yields up to 1.9  $\text{t ha}^{-1}$ , whereas ICG 5195 and ICG 14630 recorded 1.7 and 1.6  $\text{t ha}^{-1}$ , respectively. Pod yields in resistant and susceptible checks were about 2.1 and 2.0  $\text{t ha}^{-1}$  respectively. Haulm yields for the accessions ranged from 3.4 to 4.2  $\text{t ha}^{-1}$  (Fig 3).

## Discussion

Genetic resources make an important contribution to crop improvement programs. Hence screening of various germplasm sets, such as core and mini core collection accessions, is important for the identification of resistant sources. Previous researchers have demonstrated that valuable genes in germplasm collections can be effectively identified by using a core or mini core collection (Anderson et al. 1996a; Franke et al. 1999; Holbrook and Anderson 1995; Holbrook et al. 2000a; Isleib et al. 1995). Mini core germplasm accessions have been widely screened in several crops, including groundnut, for identifying resistant sources to different biotic (Kusuma et al. 2007) and abiotic stresses (Vadez et al. 2007). As per the previous documentation on the ICRISAT groundnut mini core collection, 28 accessions were resistant to abiotic stresses, 30 accessions were resistant to biotic stresses, 18 accessions were agronomically desirable but susceptible to different stresses, and 16 accessions were nutrient dense (Upadhyaya et al. 2014). For example, ICG 8760 and ICG 3787 were reported to be resistant to late leaf spot and rust diseases in India (Kusuma et al. 2007). Similarly, for *Sclerotinia* blight disease of groundnut, 67 new sources of resistance from ICRISAT mini core accessions were identified using a molecular marker (Chamberlin and Melouk 2011).

In our present study, varied reactions to pre-harvest aflatoxin contamination were observed in the ICRISAT groundnut mini core accessions during 2008 and 2009. For example, two accessions below 4  $\mu\text{g kg}^{-1}$  were observed in 2008, whereas about 20 accessions in the similar range were observed in 2009. This trend was also observed in other aflatoxin ranges of accessions during both the years. A more reliable data for assessing the overall performance of ICRISAT groundnut mini core collection against pre-harvest aflatoxin contamination is the mean data of both 2008 and 2009 as given in Table 2. Four accessions accumulated aflatoxins up to 4  $\mu\text{g kg}^{-1}$ , which is in accordance with the European

**Fig. 2** Performance of a few groundnut mini core accessions showing resistance to pre-harvest aflatoxin contamination as evaluated during the rainy seasons of 2008 to 2013 at Sadore, Niger. 55-437 was the resistant check. JL 24 was used as a susceptible check with an aflatoxin content of  $581.8 \mu\text{g kg}^{-1}$ . Aflatoxin levels were estimated at harvest and values are the means of six years



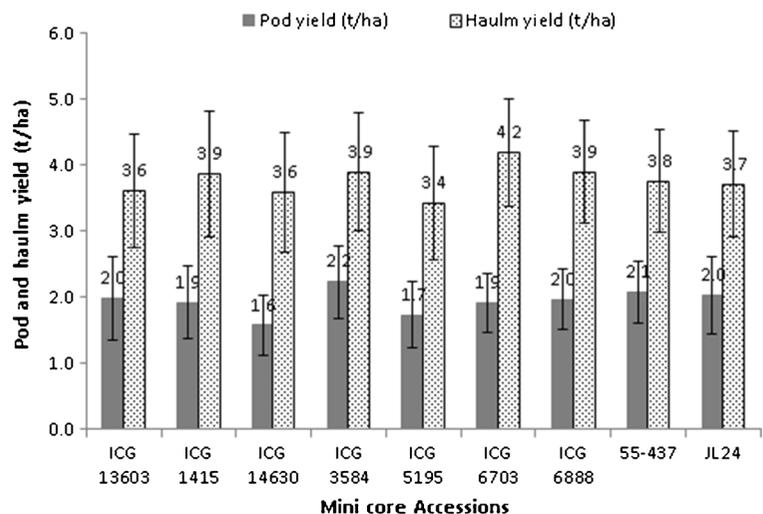
55-437 was the resistant check

JL 24 was used as a susceptible check with an aflatoxin content of  $581.8 \mu\text{g kg}^{-1}$ . Aflatoxin levels were estimated at harvest and values are the means of six years

Commission standards (of total aflatoxins) for human consumption (Wilson and Otsuki 2001). Similarly, 50 accessions were within the limits stated by the Kenya Bureau of Standards, ( $10 \mu\text{g kg}^{-1}$  of total aflatoxins) and Nigeria. A total of 66 accessions had aflatoxin level within the permissible limits ( $15 \mu\text{g kg}^{-1}$  of total aflatoxins) of countries such as South Africa, Australia, New Zealand, Canada, and Gulf Cooperation Council (GCC) countries like Saudi Arabia, USE, Kuwait, Bahrain, Oman, Yemen and Qatar. Similarly, 75

accessions had toxin level within the limits of United States Food and Drug Administration (FDA) safety limits ( $20 \mu\text{g kg}^{-1}$ ). Overall, 89 of the groundnut mini core accessions have met the limits set by India ( $30 \mu\text{g kg}^{-1}$ ). The rest of the accessions were with aflatoxin levels of  $>30 \mu\text{g kg}^{-1}$ . In addition, the analysis of the 34 selected accessions from the 2008–2011 field studies indicated that 23 accessions were within aflatoxin limits of  $4 \mu\text{g kg}^{-1}$  (European Commission Standards). Overall, all the accessions have met the

**Fig. 3** Pod and haulm yields of certain groundnut mini core accessions evaluated for pre-harvest aflatoxin contamination during the years 2008 to 2013 at Sadore, Niger. 55-437 was the resistant check and JL 24 was the susceptible check. Values are the means of six years



55-437 was the resistant check and JL 24 was the susceptible check

Values are the means of six years

allowable aflatoxin limits set by Kenya and Nigeria. Further, the final screening of seven best accessions (ICGs 13,603, 1415, 14,630, 3584, 5195, 6703 and 6888) in 2012 and 2013 indicated that pre-harvest aflatoxin contamination is  $<4 \mu\text{g kg}^{-1}$ , which is in accordance with the limits of European Commission (Tables 5 and 6). Further, the kernel infection by *A. flavus* for these accessions was up to 2.70 % in 2012 (Table 5). However, in African countries, the legislation on aflatoxin limits are restricted only to the export crops and thus, there is a significant risk of rural poor being exposed to aflatoxins constantly with serious health impacts (Wagacha and Muthomi 2008).

Aflatoxin production in groundnut is significantly influenced by environmental parameters such as temperature and soil moisture. Due to high G x E interaction effects, inconsistencies among genotypes offering resistance to aflatoxins have been reported under different climatic conditions (Anderson et al. 1995, 1996b; Holbrook et al. 1994). For example, drought intensity enhances aflatoxin accumulation in groundnut (Hamidou et al. 2014). Earlier studies have established that severe drought promoted growth and persistence of *A. flavus* population in soils, thereby resulting in high aflatoxin levels in crops (Arunyanark et al. 2010). Drought tolerant genotypes have been also reported to have tolerance to aflatoxin contamination (Holbrook et al. 2000b; Girdthai et al. 2010). For selection of resistance sources under field conditions, Niger was selected because it is one of the hottest countries in the WCA region. High temperatures and drought during the end of crop season (October/November) in Sadore, Niger, are conducive for pre-harvest aflatoxin contamination. Another reason for selecting the experimental site at Niger is that the chances of *A. flavus* infection at field level and aflatoxin contamination are high, even when the stress periods are short (Craufurd et al. 2006). This resulted in sufficient mold pressure on accessions during field experimentation. In our studies, the aflatoxin contamination for seven elite accessions was found to be negatively correlated with rainfall during the crop season ( $r = -0.46$ ). In addition, there were no rains during November, thus, creating drought which favored aflatoxin contamination. Medina et al. (2014) reported that environmental factors such as temperature and water stress have significantly influenced structural and regulatory aflatoxin biosynthetic genes. In view of the high G x E interactions pertaining to aflatoxin resistance in groundnut, multi-location testing of accessions under

varied climatic conditions was considered to be important.

Field research at ICRISAT, Sadore, Niger has significantly contributed to the understanding of aflatoxin resistance in groundnut. Several years of field studies with groundnut mini core germplasm accessions at this site allowed the identification of significantly higher levels of resistance. In our present study, we have identified seven mini core accessions, ICGs 13,603, 1415, 14,630, 3584, 5195, 6703 and 6888, as unique with consistently low levels of pre-harvest aflatoxin contamination. ICG 13603 (Origin: Indonesia), ICG 1415 (Origin: Senegal), ICG 14630 (Origin: Brazil), ICG 3584 (Origin: India), ICG 5195 (Origin: Sudan), and ICG 6888 (Origin: Brazil) belonged to *Arachis hypogaea* sub sp. *fastigiata*. ICG 6703 (Origin: Paraguay) belonged to *Arachis hypogaea* sub Sp. *hypogaea*. These seven accessions were considered to be good candidates for a better understanding of the resistant mechanisms as well as breeding for aflatoxin resistance. However, multi-location, multi-environmental testing of these seven accessions is desirable under different climatic conditions. This is because such testing will further enhance the knowledge on the uniformity and stability of resistance of these accessions to aflatoxin contamination. Moreover, these accessions can be incorporated for field research by National Agricultural Research Systems (NARS) partners of ICRISAT in their breeding programs. While our efforts continue to identify and develop even superior aflatoxin resistance lines in groundnut, there is a need to screen these accessions in Asia, North America, South America and other African regions prior to incorporating them into groundnut breeding programs.

**Acknowledgments** We are thankful to CGIAR Consortium for the partial financial support rendered through CGIAR Research Program on Grain Legumes. ICRISAT is a member of the CGIAR Consortium.

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