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Pre-harvest host-resistance to *Aspergillus* infection and aflatoxin B_1 contaminations in groundnut (*Arachis hypogaea* L.) genotypes

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important oil crop in the tropical and sub-tropical countries. Pod and seed coat crack-inducing factors favour *Aspergillus* species infections and aflatoxin B_1 (AFB_1) contamination of groundnut. Aflatoxin B1 (AFB_1), a toxic secondary metabolite of *Aspergillus* species, remains a global concern due to its human and animal health, and economic impacts. Thus, the study was conducted at Babile in 2018 with the objective to identify groundnut genotypes resistant to pre-harvest fungal infections, aflatoxin contaminations and associated effects in crop physiology. Seventeen advanced groundnut breeding lines including one commercial cultivar (Werer-961), were evaluated using randomized complete block design and completely randomized design under field and with four replications for laboratory experiments, respectively. Aflatoxin B_1 analysis was carried out using Enzyme-Linked Immunosorbent Assay (ELISA) kits. Appropriate statistical procedures, including regression, were employed for data analyses. Highly significant ($p<0.01$) variation existed among the genotypes for *A. flavus* and *A. niger* infections, and the AFB_1 contamination ranged from 13.98 (G14) to 1990.86 ppb (G12). The more *A. flavus* infection, the more reduction in harvest yield and seedling vigour. Fortunately, 53 % of the test materials were found to be resistant to AFB_1 production, and frighteningly, none of the AFB_1 contaminated genotypes were within the acceptable limit of the lenient standard (10 ppb). All in all, the groundnut genotype (G4) was identified as a good source of pre-harvest resistance to *A. flavus* infection, AFB1 contamination and seedling vigour so that its inclusion in breeding programs is worthwhile utmost, specifically, in the test environment as pathogen-crop-environment interaction is natural. Since the experiment was employed at one location and for only one year, it is suggested to repeat the experiment across multiple locations and over seasons for reliable recommendation.

1. Introduction

Groundnut (*Arachis hypogaea* L., 2n = 4x = 40, AABB) is a *Leguminosae* and an annual legume crop adapted in the diverse environments of tropics and sub-tropics [1]. It is the second most important oilseed crop with average (1.8 t ha^{-1}) and potential (2.0–2.4 t ha^{-1}) seed yield and cultivated on 80,841.57 ha in the warm climates in Ethiopia [2–4]. The groundnut seed is a good source of protein, carbohydrate, fiber, calcium, zinc, magnesium, and fatty acids [5].

Aflatoxin contamination considerably affects the qualities of seed and seed products of groundnut, thereby impacting the farmers'

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income and consumers' health [6]. Hyphae, conidia and sclerotia of *Aspergillus* enter through the cracks of pods and seed coats and infect the cotyledon where they proliferate [7]. Basically, several factors, including low soil moisture stress at pod or seed maturity time and improper and poor post-harvest undertakings or handlings, can favour seed infection.

Aflatoxin B$_1$ (AFB$_1$) is the most toxic aflatoxin, which is a secondary metabolite of *Aspergillus* species that remains a global health and economic concern. Depending upon the concentration of aflatoxin contamination and extent consumption of the contaminated products cause immunosuppression, mutagenicity and teratogenicity [8–11]. Various pre-harvest management options, like organic manures and bio control agents (including non-toxigenic *Aspergillus* strains) in the field plots and the use of resistant varieties, immensely reduce infections due to *Aspergillus flavus* species and aflatoxin contamination. The use of biological control agents and resistant groundnut genotypes is the most effective; practically, the latter is magnificent in ease of application and circumvent recurrent costs [12].

Fortunately, efforts in the identification of major quantitative trait loci (QTLs) associated with resistance to aflatoxin production affirm the rapid genetic gain in the course of resistance breeding programs [13–16]. Actually, the biological resistance in groundnut can be attained either through the prevention of fungal invasion and/or aflatoxin production [17]. Generally, identification and development of genotypes resistant to pre-harvest fungal infection is the best management option to reduce adversities on crop physiology and human health risk since the complete elimination of pre-harvest produced aflatoxin is hardly possible [17,18]. The use of pre-harvest resistant genotype, followed by good post-harvest practices and other integrated management options, definitely reduce infection due to *Aspergillus* species and AFB$_1$ contamination. However, since the country has limited availability of pre-harvest groundnut resistant genotypes, the problem of infection by *Aspergillus* and AFB$_1$ contamination has been aggravated in groundnut seed and its products. Therefore, this study was carried out to examine the level of *Aspergillus* infection and AFB$_1$ contamination in advanced breeding lines of groundnut at Babile site (East Hararghe Zone), eastern Ethiopia.

2. Materials and methods

The study was conducted at Babile Research Sub-Station located at 9°08′N and 42°21′E in eastern Ethiopia. The altitude of the site is 1650 m above sea level (m.a.s.l.) and received 671 mm rainfall with a mean temperature ranging from 15.52 to 28.05 °C. The type of soil is sandy-loam soil. Seventeen drought-resistant advanced groundnut breeding lines, including the commercial cultivar (*Werer-961*), were examined (Table 1). The breeding lines were imported from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in Malawi and planted under rain-fed conditions in the 2018 main cropping season. The treatments for the field and laboratory experiments were arranged in a randomized complete block design (RCBD) with three replications and completely randomized design (CRD) with four replications, respectively. A block contained 9 m^2 plots. The planting spacing were 0.1, 0.6 and 3 m between plants and rows, and row length, respectively. Seeds were harvested from three central rows (5.4 m^2), sun-dried and studied in the Plant Protection Laboratory of School of Plant Sciences (Haramaya University).

Fungal identification: Seeds were randomly taken and plated onto a 90 mm diameter Petri-plate, containing freshly prepared potato dextrose agar (PDA) in three replications. Isolates were sub-cultured in Czapex Dox Agar to the species level and identified was performed according to the Laboratory Manual of [19].

Aflatoxin analysis (ELISA test): Exactly 100 g of seed sample was ground to a 1 mm particle size using a high-speed universal disintegrator (FW100) Grinder. Five grams of ground sample was added to 25 mL of 70 % methanol and stirred for aflatoxin extraction using a magnetic stirrer for 10 min. The extract was filtered through a Whatman No. 1 filter paper (Whatman International Ltd., Maid stone, UK), and 15 mL sterile distilled water (SDW) was added to 5 mL of the filtered solution. Tween 20 (0.25 mL) was added to the filtered solution and it was stirred for 2 min. Finally, an enzyme-linked immunosorbent assay (ELISA) kit was used for AFB$_1$ analysis and determined in parts per billion (ppb). All techniques, including the immune-affinity column procedure, were administered

Table 1
Descriptions of advanced groundnut breeding lines employed for the experiment during 2018 main cropping season.

No.	Pedigree	Genotype code	Source
1	RDRGVT (BAKA)	G1	Malawi
2	RDRGVT ICGV 14788	G2	Malawi
3	RDRGVT ICGV 00331	G3	Malawi
4	RDRGVT ICGV SM 01514	G4	Malawi
5	RDRGVT ICGV SM 03519	G5	Malawi
6	RDRGVT ICGV SM 3520	G6	Malawi
7	RDRGVT ICGV SM 3530	G7	Malawi
8	RDRGVT ICGV SM 05723	G8	Malawi
9	RDRGVT ICGV SM 06519	G9	Malawi
10	RDRGVT ICGV SM 8528	G10	Malawi
11	RDRGVT ICGV SM 8533	G11	Malawi
12	RDRGVT ICGV SM 8538	G12	Malawi
13	RDRGVT ICGV SM 8540	G13	Malawi
14	RDRGVT ICGV SM 8547	G14	Malawi
15	RDRGVT ICGV SM 8556	G15	Malawi
16	KAKOMA	G16	Malawi
17	Werer-961	G17	Ethiopia

according to R-Biopharm [9].

Seed oil content determination: It was carried out based on Near Infrared Reflectance Spectroscopy (NIRS) analytical technique [20].

Seed quality attributes: (i.e. germination percentage, hundred seed weight, seedling dry weight, and seed vigour index one) were measured using International Seed Testing Association (ISTA) procedures [21].

2.1. Statistical data analysis

The percentage data was square-root transformed before running an analysis of variance (ANOVA). Statistical analysis system (SAS) computer software was used to perform ANOVA and Duncan multiple range test (DMRT) at 5 % probability level for the separation of means of genotype attributes. The linear regression analysis was also employed to determine the effects of *A. flavus* on seed yield and quality parameters [22,23].

3. Results and discussion

Aspergillus flavus and *A. niger* were isolated from freshly-harvested seeds of advanced groundnut breeding lines, verifying root vicinity is a source of inoculum for *Aspergillus* species in groundnut. The test genotypes revealed highly significant ($p < 0.01$) differences for hundred seed weight (HSW), germination percentage (GP), seedling dry weight (SDW), seed vigour index one (SVI–I) and infections due to *A. flavus and A.niger.* The variation among advanced groundnut lines might be due to its inherent potential and that suggests the existence of considerable variability among the genotypes to be exploited in groundnut breeding programs (Table 2). Similar to this study [24], found significant variation among groundnut genotypes for seed germination rate, seed vigour index, and seedling dry weight, whereas genotypes did not show any significant ($p < 0.05$) difference on seed oil content (SOC).

The genotype (G4) was resistant to A. flavus infection; thus, it can serve as a source of pre-harvest resistance to A. flavus infection. So, its inclusion in breeding programs is worthwhile utmost, specifically in the test environment as pathogen-crop-environment interaction is a natural phenomenon [25]. On the other hand, the highest levels of A. flavus infection was recorded in the genotypes G16, G11 and G2, respectively (Table 3). Similarly, G4 had also the least incidence of *A. niger* compared with G5, G15, G7 and G2, in which the highest occurrence was noted. Aspergillus niger was abundantly isolated compared to A. flavus, and none of the test genotypes was free of infection. All-in-all, variation in response to the *Aspergillus* infection was clearly noticed in genotypes tested in the same edaphic and other environmental conditions; such genetic variations for desirable traits have paramount importance in crops of the narrow genetic pool, like cultivated groundnut.

The genotype G4 was the best in GP and SDW that coincided with the level of resistance to soil-borne fungus, specifically A. flavus and *A. niger* infections. That indicates that fungal infections altered physiological traits, specifically GP and SDW. Normally, fungal pathogens land and affect cotyledons, thereby seedling attributes, including GP and SDW and finally the economic yield. Thus, it can be concluded that host resistance to pre-harvest seed infection is indispensable for better seed quality attributes and effective aflatoxin management [26,27].

The genotypes G5, G6 and G10 had the highest HSW, while G17 had the maximum SVI-I (Table 3). Overall, regression analysis revealed that HSW ($R = 0.992$, $F (1, 15) = 1831.454$, $p = 4.28E-17$), GP ($R = 0.361$, $F (1, 15) = 8.479$, $p = 0.011$), SDW ($R = 0.292$, $F (1, 15) = 6.174$, $p = 0.025$) and SVI-I ($R = 0.528$, $F (1, 15) = 16.792$, $p = 0.000$) were statistically significant, implying a pre-harvest *A. flavus* infection significantly affected HSW, GP, SDW and SVI-I, inferring that the more pre-harvest *A. flavus* infection, the more would be a reduction in instant harvest yield and seedling vigour in the subsequent cropping season (Fig. 1).

Groundnut genotypes showed significant variation in AFB_1 contamination (Fig. 2). Approximately 53 % (9 genotypes) of test materials had non-detectable aflatoxin AFB_1, suggesting the presence of host resistance to AFB_1 production and paramount importance for the downstream improvement program with improved post-harvest practices, being aflatoxigenic fungi are important at storage level.

In contrast, AFB1 was detected on nearly 47 % of the studied groundnut genotypes (G1, G2, G6, G9, G12, G14, G16 and G17) with the lowest (G14, 13.98 ppb) and highest (G12, 1990.86 ppb) concentrations. Alarmingly, none of these records was below the maximum permissible limit (10 ppb) of African AFB1 standards [28,29]. Thus, it is commendable to exclude aflatoxin susceptible genotypes from the breeding program unless they have special desirable/elite traits and used as a testing materials. Generally, rejection of AFB_1 contaminated genotypes and retention of such promising and potential genotypes for host resistance in the earliest possible time in groundnut improvement program enables the best and judicious use of resources, thereby enhancing the breeding efficiency.

Table 2
Analysis of variance for seed attributes and *Aspergillus* infections in 17 advanced groundnut breeding lines.

Source	Mean squares						
	A. flavus	*A. niger*	SOC	HSW (g)	GP	SDW (mg)	SVI-I
Genotype	6.071***	3.381***	0.007[NS]	27.593***	1.044***	2735.3***	229846.5***
Error	0.0730 (34)	0.1542 (34)	0.004 (34)	9.611 (34)	0.177 (51)	878.255 (51)	44123.1 (51)

[†]Numbers in parenthesis are error degree of freedom *Significant at P < 0.05; **Highly significant at P < 0.01. **DF**, degree of freedom; **GP**, germination percentage; **HSW**, hundred seed weight; **SDW**, seedling dry weight; **SOC,** seed oil content and **SVI-I**, seed vigour index one.

Table 3

Mean incidences of *Aspergillus* species and associated effects in groundnut seed attributes under laboratory conditions.

G	*A. flavus*[a,b]	*A. niger*[a,b]	SOC[a]	HSW (g)[b]	GP[a,b]	SDW (mg)[b]	SVI-I[b]
G1	2.69 (7)[i]	8.23 (67.7)[c]	6.77 (46.0)	38.05[cd]	8.80 (77.5)[abcde]	273.0[ab]	1825.5[bcd]
G2	5.13 (26)[bc]	9.66 (93.3)[ab]	6.75 (45.0)	37.73[cd]	9.23 (85.5)[ab]	297.0[a]	1872.8[abc]
G3	4.64 (22)[de]	7.67 (58.9)[cde]	6.74 (46.6)	43.05[ab]	8.89 (79.0)[abcde]	271.0[ab]	1876.4[abc]
G4	0.00 (0)[j]	6.23 (38.9)[f]	6.74 (45.0)	42.63[abc]	9.29 (86.5)[a]	300.0[a]	1953.8[abc]
G5	3.15 (10)[hi]	9.77 (95.5)[a]	6.73 (44.4)	44.72[a]	8.83 (78.0)[abcde]	278.5[a]	1864.9[abc]
G6	3.55 (13)[gh]	6.97 (61.1)[e]	6.87 (45.0)	44.29[a]	9.02 (81.5)[abc]	275.5[ab]	1934.2[abc]
G7	3.39 (12)[gh]	9.48 (90.0)[ab]	6.75 (45.0)	41.31[abc]	8.91 (79.5)[abcde]	285.50[a]	1783.2[bcd]
G8	4.82 (23)[cd]	7.27 (53.3)[de]	6.74 (45.3)	41.96[abc]	8.57 (73.5)[bcdef]	255.0[abcd]	1625.6[dc]
G9	2.77 (8)[i]	7.15 (51.1)[de]	6.66 (46.2)	41.24[abc]	8.97 (80.5)[abcd]	218.0[cd]	1818.2[bcd]
G10	4.82 (18)[cd]	7.07 (50.0)[de]	6.74 (45.9)	44.72[a]	8.60 (74.0)[abcdef]	287.0[a]	1616.7[dc]
G11	5.48 (30)[b]	7.81 (61.1)[cd]	6.78 (45.5)	38.17[bcd]	8.04 (65.0)[f]	212.5[d]	1478.8[d]
G12	3.55 (13)[gh]	7.45 (55.5)[de]	6.73 (45.4)	39.77[abcd]	7.23 (52.5)[g]	260.5[abc]	1149.4[e]
G13	3.79 (14)[fg]	7.53 (56.7)[cde]	6.76 (44.8)	43.05[abc]	8.31 (69.0)[def]	292.5[a]	1614.9[dc]
G14	3.38 (12)[gh]	7.31 (53.5)[de]	6.75 (45.5)	44.16[ab]	8.24 (68.0)[ef]	255.5[abcd]	1602.6[dc]
G15	4.06 (17)[f]	9.19 (84.4)[ab]	6.71 (46.9)	37.73[cd]	9.16 (84.0)[ab]	264.5[abc]	2023.8[ab]
G16	6.49 (42)[a]	9.00 (81.1)[b]	6.76 (46.0)	41.62[abc]	8.51 (72.5)[cdef]	276.5[ab]	1612.3[dc]
G17	4.19 (23)[ef]	7.80 (48.8)[cd]	6.84 (46.5)	34.27[d]	8.88 (79.0)[abcde]	228.0[bcd]	2182.6[a]
Mean	3.88	7.97	6.75	41.08	8.68	266.5	1755.03
CV (%)	6.97	4.92	0.98	7.55	4.85	11.12	11.97

[a] Numbers in parentheses are the records in percentage.

[b] same letters within the same column indicate no significant difference between means. **CV,** coefficient of variation; **DF,** degree of freedom; **G,** genotype; **GP,** germination percentage; **HSW,** hundred seed weight; **SDW,** seedling dry weight; **SOC,** seed oil content; and **SVI-I,** seed vigour index one.
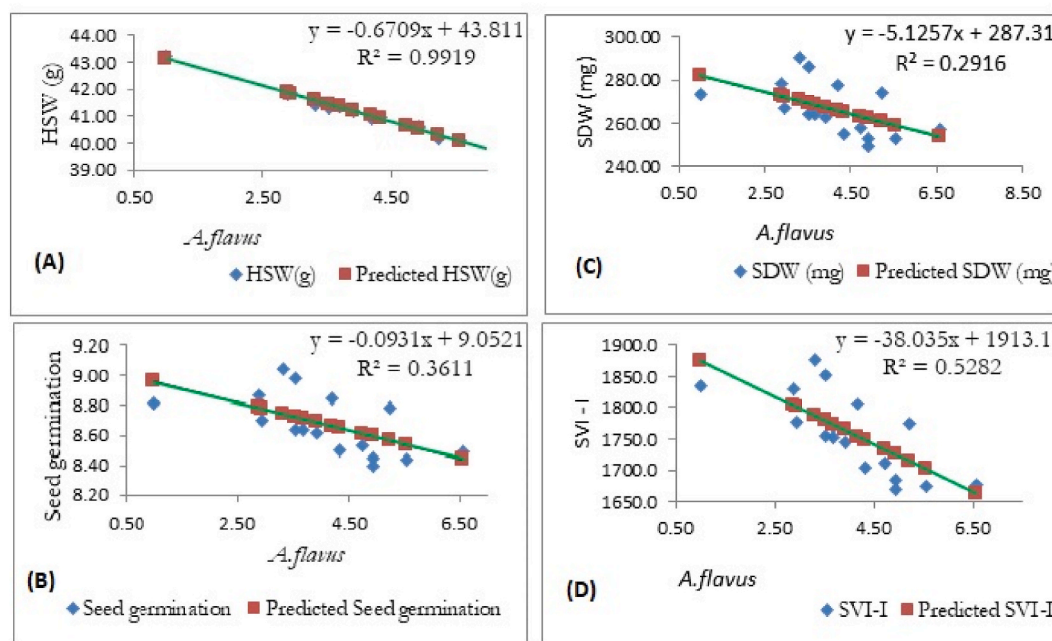
Fig. 1. Fitted line plots of *A.flavus* and hundred seed weight (A), seed germination (B), seed dry weight (C) and seed vigour index I (D).

4. Conclusion

Currently, *Aspergillus* species and the associated aflatoxin is among the priority issues due to their toxic effects on human and animal health in groundnut seeds and seed products and rejections of contaminated products from domestic and international markets across the globe. Aflatoxin producing *Aspergillus* species likely start groundnut pod and seed infections in the field; consequently, affected seed yield and qualities of seed products, and aggravated under drought stress conditions. In the present study, variations were noticed in *A. flavus* and *A. niger* infections and aflatoxin B_1 (AFB$_1$) contamination, ranging from 13.98 to 1990.86 ppb. The test genotypes showed highly significant ($p < 0.01$) differences for hundred seed weight (HSW), seed germination percentage (GP), seedling dry weight (SDW) and seed vigour index-1 (SVI–I), while meaningfully did not exhibit significant ($p < 0.05$) difference for seed oil
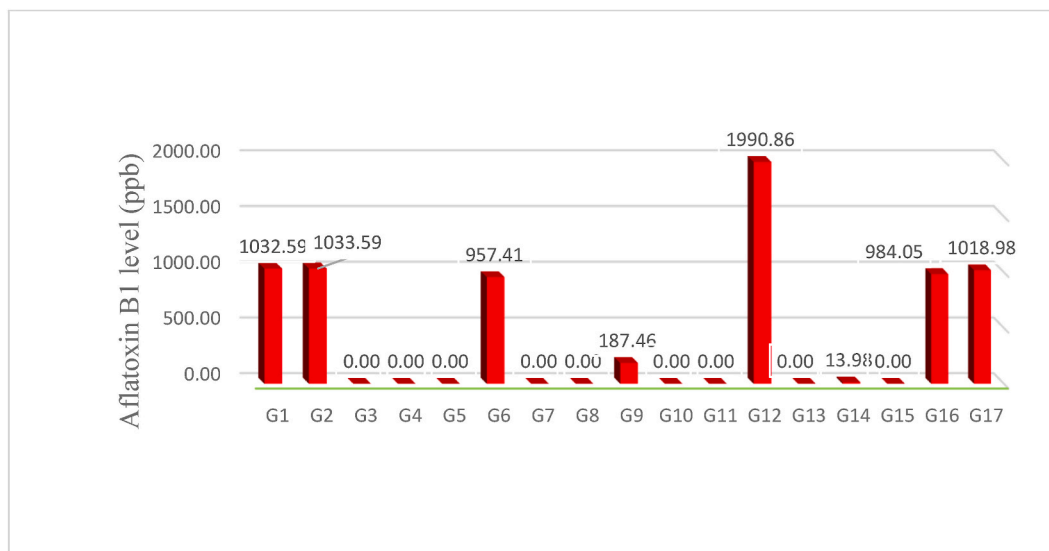
Fig. 2. Levels of aflatoxin B_1 concentration in 17 advanced groundnut lines.

content (SOC). The genetic variations for these traits, indicating the genotypes can be selected for enhancing seed yield in groundnut.

A pre-harvest A. *flavus* infection caused adversities on HSW, GP, SDW and SVI-I. Consequently, the more A. *flavus* infection, the more reduction in harvest yield and seedling vigour in the succeeding cropping inferred. Among the tested groundnut genotypes, G4 showed non-infections due to A. *flavus* and least by A. *niger*. All tested groundnut materials other than G3, G4, G5, G7, G8, G10, G11, G13, and G15 had detectable levels of AFB_1 beyond the maximum limits set for African standards (10 ppb), indicated susceptibility for A. *flavus* infections and aflatoxin accumulations. Altogether, the genotype G4 can be used as a source of pre-harvest resistance to A. *flavus* infection and AFB_1 contamination so that its inclusion in breeding programs is valuable, specifically in the test environment as pathogen-crop-environment interaction is a natural process. The use of host resistance to pre-harvest seed infection is a crucial management option for better seed quality attributes and sustainable groundnut production and productivity. Since the experiment was employed at one location and for only one season, it is suggested to repeat across locations and over seasons for reliable recommendation.

Data availability statement

Data will be available on request.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Zeyede Akale: Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation. **Abdi Mohammed:** Writing – review & editing, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Amare Kebede:** Writing – review & editing, Software, Formal analysis, Conceptualization. **Seltene Abady:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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