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Full Length Research Paper

Inheritance of resistance to kernel infection by *Aspergillusflavus* and aflatoxin accumulation in groundnut

OzimatiA.A[1], Rubaihayo P.R[1], Gibson P[1], Edema R[1], Kayondo I.S[1], Ntare B.R[2], Okello D.K[3]

[1]Department of Agricultural Production, School of Agricultural Sciences, Makerere University, P.O. Box 7062, Kampala, Uganda.
[2]International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), BP 320 Mali, Bamako.
[3]National Semi-Arid Resources Research Institute, Serere, Uganda. (NaSARRI)

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Aflatoxin contamination in groundnut is one of the major barriers in international groundnut trade. Aflatoxin may cause human cancer, hepatitis and liver cirrhosis. The objectives of the study were to determine: (i) the level of resistance to *Aspergillusflavus* and aflatoxin accumulation in selected groundnut genotypes. (ii) the gene action for resistance to kernel infection by *Aspergillusflavus* and aflatoxin accumulation and (iii) heritability for *Aspergillusflavus* and aflatoxin accumulation resistance. Eighteen groundnut genotypes were evaluated in the laboratory. Nine genotypes selected were crossed in North Carolina II mating design of four by five parents and theF_2 seeds obtained were evaluated in the laboratory for percent-severity kernel infection (PSKI3 and PSKI7), percentage kernel disease incidence (PKDI3 and PKDI7) and aflatoxin accumulation. Significant variation for PSKI3, PSKI7 and PKDI3 were observed. Baker's ratio for both PSKI3 (0.73) and PSKI7 (0.67) were high, whilelow Baker's ratio of 0.49 for aflatoxin accumulation was observed. Narrow–sense heritability of 66%-74% and 35%-46% for PSKI7 with *A.flavus* and aflatoxin accumulation were respectively observed. High narrow-sense heritability and additive variance for *A.flavus* indicate that the alleles for resistance to *A.flavus* can be easily transmitted to the offspring through recombination.

Key Words: *A.flavus,* aflatoxin, groundnut, *Arachishypogaea L*., heritability, North Carolina II.

INTRODUCTION

Groundnut (*Arachishypogaea*L.,) also commonly known as peanut is cultivated worldwide in tropical, sub-tropical and warm temperate areas (Okelloet al.,2010; Okelloet al.,2013). More than half of the world groundnut area of production is in Asia, followed by Africa and America. `China, India and the U.S.A are the largest producers of the groundnut in the world (Xue, 2004). Groundnut oil is the most important product of the crop in many parts of the world, especially in Asia where most of the seeds are crushed for oil (Okelloet al.,2010). The kernels may be eaten raw, roasted, boiled or salted and also the cake and the haulm are used for animal feed or may be used as a direct source of human food (Xue, 2004; Okello et al.,2013). In the U.S.A, about 60% of groundnuts are utilized in a variety of food products with the remainder used in approximately equal proportions for export and for production of edible oil (Xue, 2004). In Uganda groundnut is the second most important legume after beans *(Phaseolus vulgaris L*.) grown mainly in Eastern and Northern Uganda, but being consumed widely throughout the country (Okelloet al.,2010;Okelloet al.,2013). It is a high nutritional source of vitamin E, niacin, falacin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium (Okelloet al.,2010). With the ever increasing costs of animal protein, groundnut is becoming even more important source of protein in the lives of many Ugandans, for example, a kilogram of groundnut is high in food energy and provides approximately the same energy value as 2 kilograms of beef, 4 litres of milk or 36 medium-size eggs (Kayaa and Warren, 2006). These qualities make groundnut

*Corresponding author. E-mail: alfredozimati@yahoo.com

an imporant nutritional supplement to mainly cereal diets of maize, millet and sorghum of many Ugandans (Okelloet al.,2013).

Groundnut production is faced with a number of constraints including, drought, pests such as leaf miners and aphids, diseases such as rosette, rust and leaf spot (Okelloet al.,2010). In addition to these constraints, groundnut is prone to infestation by two closely related species of fungi, *Aspergillusflavus* and *Aspergillusparasiticus*. *A. flavus* is commonly found in Africa, while *A. parasiticus* is dominant in America (Xue, 2004). These fungal species produce aflatoxin that pose a serious health hazard to humans and animals that consume the aflatoxin-contaminated groundnut. Aflatoxin is known to suppress the immune system of body leading to various diseases such as liver cirrhosis and cancer in humans(Xue, 2004). Katiyar et al.(2000) reported that there were synergistic effects between aflatoxin and hepatitis B virus infection causing cancer. Use of genotypes resistant to *A. flavus* and with diminished accumulation of aflatoxin is the best option for the resource constrained farmers to manage the problems of aflatoxin contamination (Kwemoi, 2011). However there is limited available knowledge on the inheritance of resistance to *A.flavus* and accumulation of aflatoxin in groundnuts. Mixon, (1976) in U.S.A. estimated broad-sense heritability of 75.6% for resistance to seed colonization by *A.flavus* in F_2, in a cross between resistant variety PI 337407 and susceptible variety PI 331326. Utomo et al.(1990) reported broad-sense heritability values of 55% and 63% for *in vitro* seed colonization by *A.flavus,* in F_2 derived from crosses between AR-4/NC7 and GFA-2/NC7, respectively and 23% and 21% for aflatoxin accumulation. The above studies reported varying heritability values for *A.flavus* colonization and aflatoxin accumulation with genotypes adapted to those environments. A study by Xue, (2004) estimated negligible additive genetic variance for both *A.flavus* and aflatoxin accumulation. Lianget al.,(2006) however reported that additive gene action was more important for the resistance to *A.flavus*. This study was intended to elucidate the genetics of inheritance resistance for *A.flavus* colonization and aflatoxin accumulation in the locally adapted genotypes in Uganda to establish an effective breeding strategy

MATERIALS AND METHODS

Culturing and Identifying *Aspergillusflavus*

A. flavus was isolated and cultured from groundnut samples, obtained from the local markets of Owino, Kalerwe and Bwaise located in Kampala. Kernels of the groundnuts obtained from the markets were surface disinfected for 1 minute with sodium hypochlorite (10% commercial bleach, Jik, Rickitt Benckiser, East Africa Ltd) followed by washing three times with 200 ml of sterile distilled water and placed directly on the surface of malt extract agar (Kwemoi, 2011). The non-osmophillic *Aspergillusflavus* was isolated from malt extract agar, prepared by mixing 33.6 g in 1 litre of distilled water, as recommended by the manufacturers (ScharlauChemis S.A Batch no 02-483). Ten kernels obtained from each of the three markets were placed directly onto the agar plates (petri-dish of 90 mm in diameter) and moistened by placing filter paper at the bottom of the petri-dishes. The plates were incubated upright at 30^oC for 42-72 hours to allow sufficient growth of *A.flavus*. Culture that was not easily identified was transferred onto potato dextrose agar and was isolated morphologically using the method suggested by (Pitt and Hocking, 1997).

Preparation of the Inoculums and Inoculation of the seeds

The pure inoculum of *A.flavus* that was isolated from naturally infected groundnuts obtained from the markets in Kampala was diluted with onelitre of distilled water. The spore suspension was then mixed thoroughly for uniform spore distribution by adding four drops of tween 20 and a sample of 2 ml of uniformly mixed spores was transferred onto a hemocytometer slide. The concentration of the spores was estimated by counting the number of spores that were visible in each of five large randomly selected squares on the hemocytometer slide and then the spore concentration was adjusted with distilled water to a concentration of about $1x10^6$ spores per ml of water (Oluret, 2012). Two drops of Tween 20 were then added per 100ml of water (Windham and William, 1998). The conidial suspension was then used to inoculate the kernels of each genotype by placing the seeds in 20 ml of the spore suspension for five minutes using a non-wounding technique suggested by (Ruming, 2004).

Scoring for *A. flavus* Colonization

Individual seeds were scored for severity of colonization of *A. flavus* on Oluret (2012) modified scale of 1-5, representing the percent-severity kernel infection (PSKI) on day 3 and 7 from the initiation of the experiment (Table 1).

Two types of measurements were generally used for screening *A. flavus* colonization in groundnut kernel;i) percent-severity kernel infection (PSKI), scored on each kernel on a scale of 1-5 and averaged over the number of kernels in the replication (Kwemoi, 2011) and ii) percentage kernel disease incidence (PKDI), determined by counting the number of kernels with visible greenish yellow spores, expressed as the percentage of total kernels in an experimental unit (Kwemoi, 2011).

Table 1. The modified rating scale used for severity of *A. flavus* on kernels.

Scale	Disease reaction	Description
1	Highly resistant	invisible mycelial growth
2	Resistant	1-20% surface coverage of mycelial growth on the kernels
3	Moderately resistant	21-50% surface coverage of mycelial growth on the kernels
4	Susceptible	51-70 % surface coverage of mycecial growth on the kernels
5	Highly susceptible	71-100% surface coverage of mycelial growth on the kernels

Analysis and Quantification of Aflatoxin

After scoring for severity of *A. flavus* colonization, samples were oven dried at a temperature of 60^{o}C for 1 day to moisture content of less than 3%, the seed coats were removed and kernels were ground in a blender to make paste. Then aflatoxin were extracted from 25g of the ground samples that passed through 1mm mesh and quantified the total aflatoxin concentration in parts per billion (ppb) for each genotype, using the VICAM Aflatest immune-affinity flouro-metric method (VICAM, Watertown, MA, USA) as follows: ground samples of 25 g from each genotype were mixed with 125 ml of 70% methanol, 30% distilled water and 5 g of sodium chloride. The mixture was blended in an electric blender for 2 minutesand then passed three times through 90 mm diameter Whatmann paper to obtain a filtrate. Fifteen ml of the filtrate was then pipetted into a test tube and diluted with 30 ml of distilled water and mixed thoroughly. 15ml of the diluted filtrate was then passed through the immune-affinity column at a rate of 2 drops per second. The columns were washed with 10 ml of distilled water three times and 1 ml of methanol was added to recover the aflatoxin (VICAM, 2001). The filtrate reading was done using a calibrated VICAM Series-4 fluorescent set at 360 nm excitation and 450 nm emissions (VICAM, 1999), taken twice per genotype and considered a replication. This method produces an aflatoxin recovery of 85 percent and provides a detection limit of one part per billion (Azziz-Baumgartner et al., 2005). A logarithmic transformation of the value of aflatoxin content was used to equalize the variance and normalize the data.

Experimental Design for Screening of 18 Genotypes for *A.flavus* Resistance

The experiment for screening each seeds of genotype was carried out at Makerere School of Food Technology, Nutrition and Bio-Engineering microbiology laboratory, using a randomized completely block design, with three replications and 30 seeds per replication, with three Petri-dishes as an experimental unit. The inoculated seeds were placed on an incubator for seven daysat a temperature of 27^{o}C and relative humidity of 80-90%,maintained by moistened filter paper placed at the bottom of the Petri-dishes.

Analysis of the Data

GenStat 14th edition was used to perform the analysis of variance (ANOVA), correlation. Randomized completely block experimental design was used in the analysis of varianceto test the differences among the parental genotypes for the scoring of greenish-yellow *A.flavus* on the kernels and the levels of aflatoxin accumulation found in them. The analysis used the Linear Model for randomized completely block design.

$Y_{ij} = \mu + r_i + g_j + e_{ij}$

Where: Yij = Observed effect for ith replication and jth genotypes

μ = grand mean of the experiment

r_i= effect due to the ith replication

g_j= effect due to jth genotype

$\mathbf{e_{ij=}}$ effects due to the residual or random error of the experiment

Generation of F_2 Progeny

Nine parents were selected on the basis of their response to *A. flavus* colonization and aflatoxin accumulation. These were five susceptible farmer preferred varieties (Serenut 1R, Serenut 2T, Serenut 11T, Acholi white andRed beauty) and four resistant lines(Serenut 9T, Serenut4T, ICGV 94374 and Erududuru red) forcrossing in a North Carolina II (NC II) mating design. Crossing blocks were established at NaSARRI in the screen house to generate enough F_1 seeds per cross. Two staggered plantings were used in order to achieve flower synchronization (Fehr, 1993). The parental crossing blocks in the screen house were established in soil. The soil was packed into basin and each planted with two seeds. Crossing was done by hand pollination using the manual emasculation and hooking method (Fehr, 1993) with the resistant parents as the source of pollen (males) and susceptible genotypes as the female parents.F_1 seeds obtained were selfed to F_2 for laboratory evaluation of their response to *A. flavus* colonization and aflatoxin accumulation to determine their combiningabilities and gene action.

Table 2. Genotype means for percent-severity kernel infection (PSKI) and percentage kernel disease incidence (PKDI) scored on 18 groundnut genotypes, as scored on the 3rd and 7th day after inoculation.

Genotypes	Means PSKI3***	Mean PKDI3***	Mean PSKI7***	Description	Mean PKDI7*
Serenut 4T	12^{a}	13^{a}	26^{a}	Resistant	48^{a}
Serenut 9T	13^{a}	19^{a}	33^{a}	Resistant	70^{b}
Erudurudu red	16^{a}	23^{a}	48^{b}	Resistant	85^{bc}
Serenut 13T	17^{a}	55^{bcd}	70^{c}	Susceptible	96^{c}
Serenut11T	26^{ab}	61^{cde}	80^{d}	Susceptible	98^{c}
Serenut 1R	28^{ab}	74^{efg}	88de	Susceptible	96^{c}
Serenut 3R	31^{abc}	53^{bc}	91^{ef}	Susceptible	98^{c}
Serenut 7T	39^{bc}	68^{def}	93^{ef}	Susceptible	100^{c}
Serenut 12R	41^{bc}	87^{ghi}	96^{ef}	Susceptible	100^{c}
Serenut 14R	43^{bc}	72^{ef}	94^{ef}	Susceptible	100^{c}
Serenut 8R	45^{bc}	72^{ef}	98^{f}	Susceptible	100^{c}
Serenut 2T	45^{bc}	46^{b}	99^{f}	Susceptible	99^{c}
ICGV 094374	51^{cd}	94^{hi}	70^{c}	Susceptible	100^{c}
Serenut 10R	51^{cd}	82^{fgh}	98^{f}	Susceptible	100^{c}
Serenut 5T	52^{cd}	90^{hi}	91^{ef}	Susceptible	100^{c}
Red Beauty	70^{d}	93^{hi}	100^{f}	Susceptible	100^{c}
ICGV 03324	97^{e}	100^{i}	100^{f}	Susceptible	100^{c}
Acholi White	97^{e}	100^{i}	100^{f}	Susceptible	100^{c}
LSD	11.1	14.4	9.6		22.7
SEM	3.8	5.0	3.3		7.9
% C.V	1.9	2.9	1.5		3.0

PSKI3 & PSKI7 = Percent-severity kernel infection on day 3 and day 7 respectively. PKDI3 & PKDI7 = percentage kernel disease incidence on days 3 & 7, respectively. Significance levels: * P≤0.05, *** P≤0.001. T=tan seed coat (testa) and R=red seed coat (testa)

North Carolina II Analysis of Combining Ability and Gene Action

The analysis of combining ability and gene action were performed for the experimental design, randomized completely block design with threereplications, including the parents and crosses to estimate error variance, a method suggested by (Dabholkar, 1992). The error variance was then converted to an entry mean basis and used to calculate the variance components, to test for significance of the general combining ability (GCA) and specific combining ability (SCA) of both male and female parents and to evaluate the effects of GCA and SCA in the F_2s' response to *A.flavus* colonization and aflatoxin accumulation.

Analysis of combining ability in the NC 11 crosses wasdone as described by (Singh andChaudhary,2007).

$$\mathbf{Y = U + f_j + m_k + (fxm)_{jk} + e_{ijk}}$$

Where:

Y_{ijk}= effects observed due to r^{th} replications, j^{th} female and k^{th}male; U = Overall mean of the experiment; r_i= Observed effects due to i^{th}replication; f_j= GCA effects due the j^{th} female parent; m_k= GCA effects due the k^{th} male parent; $(fm)_{jk}$= Interaction between k^{th} and j^{th}; and e_{ijk}= Random error of the experiments and the table of ANOVA developed were presented in the table 2

Estimation of Heritability for *A.flavus* Colonization and Aflatoxin Accumulation in F_2 Populations.

In the analysis of the North Carolina II design, the parents used in the crosses were considered fixed in effects and estimating heritability was not appropriate and instead the analogous broad-sense and narrow-sense coefficients of genetic determination were estimated by the formulas suggested by (Ruming, 2004) as follows:

Broad-sense heritability;

$$(H) = \frac{\delta^2 GCA_f + \delta^2 GCA_m + SCA_f}{\delta^2 GCA_f + \delta^2 GCA_m + SCA_{fm+} \delta^2 e}$$

Narrow-sense coefficient genetic determination (heritability);

$$NS\text{-}CGD\ (=\sim h^2) = \frac{\delta^2 GCA_f + \delta^2 GCA_m}{\delta^2 GCA_f + \delta^2 GCA_m + SCA_{fm+} \delta^2 e}$$

A mid-parent-offspring regression approach in which narrow-sense heritability (h^2) was estimated directly from the slope "b'' of the mid-parent offspring regression (Lush, 1940), was used as a direct method of estimating narrow sense heritability from the regression:

Yij= a + b*MPij + Ei.

Yij = Performance of offspring of the i^{th} parent

Table 3. Genotype means of aflatoxin accumulation, measured in ppb and their log transformed values.

Genotype	Aflatoxins in ppb***	Description	Aflatoxins log10***
Serenut 9T	17[a]	Resistant	1.21[a]
Erudurudu Red	18[a]	Resistant	1.24[a]
Serenut 4T	23[a]	Susceptible	1.35[ab]
Serenut 2T	39[a]	Susceptible	1.58[bc]
ICGV094374	62[ab]	Susceptible	1.79[cd]
Serenut 5T	71[ab]	Susceptible	1.85[cd]
Serenut 7T	102[bc]	Susceptible	2.01[de]
Serenut 10R	103[bc]	Susceptible	2.01[de]
ICGV 03324	151[cd]	Susceptible	2.34[ef]
Serenut 12R	173[de]	Susceptible	2.24[efg]
Serenut 13T	220[ef]	Susceptible	2.34[fgh]
Serenut 3R	220[ef]	Susceptible	2.34[fgh]
Serenut 1R	273[fg]	Susceptible	2.44[fgh]
Acholi White	278[fg]	Susceptible	2.44[fgh]
Serenut 8R	287[g]	Susceptible	2.46[gh]
Serenut 14R	317[gh]	Susceptible	2.50[gh]
Red Beauty	350[hi]	Susceptible	2.54[h]
Serenut 11T	384[i]	Susceptible	2.59[h]
SEM	9.39		0.04
LSD	28.02		0.13
%CV	1.1		0.4

T=tan seed coat (testa) and R=red seed coat (testa), Significance level: *** P≤0.001.

a = Mean of all parents evaluated
b = Linear regression coefficient
MPij = Mid-parent value for ijth cross
Ei = Experimental error associated with measurement of Yij

Genstat computer program 14th edition (Genstat release, 2011) was used to analyze the mid-parent-offspring regression. The regression coefficient "b" was used to directly estimate h^2 for *A.flavus* colonization and aflatoxin accumulation from the modal based on the following assumptions by (Fehr, 1993).

a. The character of interest had diploid Mendelian inheritance
b. The population was randomly-mated
c. The population was either in a linkage equilibrium or had no linkage
d. Parents used are non-inbreds
e. There was no environmental correlation between the performance of the parents and the offspring

Failure to meet the assumption could bias the heritability estimate offspring.

The means of the parents and the offspring were plotted against each other and the regression coefficient "b" was calculated as, according to (Fehr, 1993):

$h^2 = \delta^2_A / \delta^2_p$ and "b"= h^2

Where, h^2 = narrow sense heritability, δ^2_A = additive variance, $\delta^2_{p=}$ total phenotypic and "b" = regression coefficient

RESULTS AND DISCUSSION

The means for the genotypes are presented in Table 2 and indicated that Serenut 4T, Serenut 9T and Erudurudu red were resistant, as shown by a low PSKI7 values with *A. flavus* colonization, while the rest of genotypes were considered susceptible. None of the genotypes screened in this study was immune to *A.flavus* colonization when *in vitro* percent-severity kernel infection (PSKI) was undertaken, indicating that the isolate used in the study was virulent. Based on the scale provided by Thakur et al.(2000), the genotype scores for *in vitro* percent-severity kernel infection (PSKI7) with *A.flavus* on 7thdays after inoculation was used to group the genotypes by their response into three groups. These were: (a) resistant (Serenut 4T, Serenut 9T and Erudurudu red); (b) Susceptible (Serenut 13T) and (c) highly susceptible (14 genotypes).

The results presented in table 3, revealed that the genotypes were significantly different (P≤0.001) in their levels of aflatoxin accumulation. None of the genotypes proved to be free from aflatoxin accumulations, including two lines from ICRISAT Mali (ICGV 094374 and ICGV 03324) bred for resistance to aflatoxin production. In this

Table 4. Correlation of percentage kernel diseases infection (PKDI), percent-everity kernel infection (PSKI), and aflatoxin accumulation (in ppb) (based on 18 genotype means).

	PKDI7 (%)	PSKI7 (%)	Aflatoxin ppb
PKDI7 (%)			
PSKI7 (%)	0.78***		
Aflatoxin (ppb)	0.48*	0.44^{ns}	

* = Significant at P≤0.05, *** = significant at P≤0.001, ns = non-significant

Table 5. Mean square for combining ability for Percent-Severity Kernel Infection measured on day 3 &7

Sources	Df	m.s	v.c	m.s	v.c	Df		
		PSKI3		PSKI7				v.c
Rep	2	26.6^{ns}	-	99.9^{ns}	-	1	0.0036^{ns}	-
Crosses	19	153.8***	134.4	223.3***	181.6	19	0.0319***	0.0303
GCAf	3	441.3***	84.4	470.3***	85.7	3	0.0738***	0.0146
GCAm	4	199.7***	45.1	343.5***	75.5	4	0.0322***	0.0076
SCA	12	66.5***	47.2	121.6**	79.9	12	0.0213***	0.0197
Error	56	19.0	19.0	41.7	41.7	56	0.0016	0.0016
Baker's ratio			0.73		0.67			0.49
BS-CGD			0.90		0.85			0.96
NS-CGD			0.66		0.57			0.46

PSKI3 & PSKI7 = Percent-severity kernel infection with *A.flavus* on days 3 and 7 respectively, v.c = variance components, GCAf and GCAm= General combining abilities of female and male parents respectively, BSC-GD& NSC-GD = Broad-sense and narrow sense coefficients of genetic determination respective

study, only Serenut 9 and Erudu red qualified as resistant lines under United States Food and Drug Administration (USFDA), which allows the highest level of aflatoxin for interstate trade in food and feeds as 20 ppb (Agag, 2004) and while the rest of the genotypes were considered as susceptible, since they accumulated aflatoxin more than 20 ppb.

Correlations between *in vitro* PSKI7 with *A.flavus* and aflatoxin production (Table 4) was not significant in this study.The non-significant correlation observed in this study between *in vitro* PSKI7 values and aflatoxin accumulation, suggested that *in vitro* resistance to *A.flavus* colonization and aflatoxin accumulation were controlled by different genes in the set of the materials used in the study. In a similar study by Utomo et al.(1990) and Upadhyayaet al.(1997), very low correlation was observed between *in vitro* seed colonization by *A.flavus* in resistant genotypes and aflatoxin accumulation. They suggested that the genetic mechanisms for resistance to these two traits were independent. Combining the different genes controlling the two traits on the same genetic background would provide resistance to both *A.flavus* colonization and aflatoxin accumulation in a cultivar.

The results of the analysis of variance for combining ability of PSKI for day 3 and 7 (Table 5), indicated that additive gene action was more important than non-additive for resistance to *A.flavus* colonization, as shown by a high Baker's ratio of 0.73 for PSKI3 and 0.67 for PSKI7. This suggests that selection for *A.flavus* resistance should be effective in early generations in F_2 or F_3 thus reducing the use of resources required in progressing the generations into advanced stages. While the results of analysis of variance in the F_2 generation for aflatoxins (ppb) log transformed values presented in table 5, indicated that the, general combining ability for female parents and for the male parents as well as specific combining ability were all highly significant (P≤0.001). The GCA male and GCA female mean squares approximated to the SCA mean square, as shown by the Baker's ratio of 0.49, suggesting dominance or epistatic behavior were responsible for resistance to aflatoxin accumulation. Under this situation, selection is delayed or genetic improvement is made difficult unless an effective strategy for capturing non-additive gene effect is in a place (Teklewold and Becker, 2005).

The results of general combining ability effects of male and female parents, for percentage kernel severity infection at day 7 (PSKI7) for *A.flavus* colonization and aflatoxin accumulation are presented in table 6. The male parents, Erudurudu red, Serenut 4T, Serenut 9T revealed negative combining ability effects for resistance to *A.flavus* colonization based on PSKI7. The results were not significant (P≤0.05) while ICGV 094374 had highly sign-

Table 6. General combining ability effects of male parents, for percentage kernel severity infection (PKSI7), with *A.flavus* and aflatoxin accumulation.

Parents		
Resistant (Male lines)	***A.flavus* infection**	**Aflatoxins (ppb)**
Erudurudu Red	-3.6^{ns}	28.6***
Serenut 4T	-5.4^{ns}	-29.6***
Serenut 9T	-5.5^{ns}	-47.6***
ICGV 094374	14.5***	48.6***
Susceptible female lines		
Acholi White	12.8***	43.6***
Red beauty	2.1^{ns}	3.8^{ns}
Serenut 1R	3.2^{ns}	-29.7***
Serenut 11T	-8.9**	19.1***
Serenut 3R	-9.2**	-36.7***

*** Significant at P≤0.001**,** ns=significant

ificant positive (P≤0.001) general combining ability, for resistance to *A.flavus* colonization based on PSKI7. On the other hand, the general combining ability effects for resistance to aflatoxin production were highly significant (P≤0.001) in all four male resistant parents, with Serenut 4T and Serenut 9T having negative GCA effects while ICGV 094374 and Erudurudu red had positive GCA effects for resistance to aflatoxin accumulation. GCA is the mean performance of a line in all its mating combinations, expressed as a deviation from the overall population mean. The negative GCA effects for the resistant male parents (Serenut 9T and Serenut 4T) for *in vitro* PSKI7 with *A.flavus*, suggested that Serenut 9T and Serenut 4T provided a high contribution to *A.flavus* resistance in the F_2 progeny. The resistant male parents (Erudurudu red and ICGV 094374) showed positive GCA effects indicating that they contributed toward susceptibility in the F_2 progeny. The results of GCA effects for female parents at PSKI7 for resistance to *A.flavus* showed that Acholi White, Red beauty and Serenut 1R, had positive GCA effects for resistance to PSKI7 with *A.flavus*, although the GCA effects were statistical non-significant for Red beauty and Serenut 1R. The positive GCA effect for Acholi White infection was on the other hand highly significant (P≤0.001) at PSKI7 for *A.flavus*. Significant negative (P≤0.01) effects for infection were observed for Serenut 11T and Serenut 3R with PSKI7 for *A.flavus*.

The female parents GCA effects for aflatoxin accumulation were positive for Acholi White, Red beauty and Serenut 11T, but statistically non-significant (P≤0.05) for Red beauty and highly significant for Acholi White and Serenut 11T (P≤0.001). It was observed that Serenut 1R and Serenut 3R had negatively significant (P≤0.01, P≤0.001) GCA effects for aflatoxin accumulation respectively. Similarly the susceptible female parents (Acholi White, Serenut 1R and Red beauty) contributed to susceptibility in the F_2 generation in the crosses in which they were involved. Serenut 3R as a female parent showed highly negative GCA effects and therefore contributed to resistance in the crosses in which it was involved. Parents with significant GCA effects in the desired direction for a trait of interest are the best for hybridization (Dabholker, 1992). The male parents (Serenut 9T and Serenut 4T) could be used in the hybridization program because they had negative GCA effects, which are in the desired direction. The female parents Serenut 11T and Serenut 3R showed negative GCA effects for aflatoxin production being in the desired direction (Dabholker, 1992), indicated they could be effectively used in a breeding program as the best female parents. On the other hand male parents Erudurudu red and Serenut 9T showed negative GCA effects, also in the desired direction and could be used as good male parents. In most cases, the parents that are good combiners showed low aflatoxin levels themselves, suggesting that the parent may be chosen either on the basis of the GCA or the parental mean, or a combination of the two.

Specific combining ability values (SCA) for PSKI7 with *A.flavus* and aflatoxin (ppb) are presented in Table 7. The results revealed that the F_2's from a cross of Serenut 1R x Serenut 4T showed significantly negative SCA effects at PSKI7 for *A.flavus* (-12.91*). Similarly, F_2's from the crosses of Serenut 1R x Erudurudu red, Serenut 3R x Serenut 4T, Serenut 3R x Erudurudu Red, Red beauty x ICGV 094374, Red beauty x Serenut 9T, Serenut 11T x Serenut 4T, Serenut 11T x ICGV 094374 and AcholiWhite x Serenut 9T all displayed negative SCA effects, but were statistically non-significant for PSKI7 with *A.flavus*. Red beauty x Serenut 4T, however had the most positive and significant SCA effect for PSKI7 with *A.flavus* (18.01**), while crosses Serenut 1R x ICGV 094374, Serenut 1R x Serenut 9T, Serenut 3R x Serenut 9T,

Table 7. Specific combining ability (SCA) effects for percentage kernel severity infection (PSKI7) with *A.flavus,* and aflatoxin accumulation (ppb)

Crosses	PSKI7	Aflatoxins (ppb)
Serenut 1R/ Serenut 4T	-12.9*	10.8ns
Serenut1R/Eruduurud Red	-7.1ns	21.7*
Serenut 1R/ICGV 094374	9.2ns	-88.3***
Serenut 1T/Serenut 9T	10.8ns	55.8***
Serenut 3R/Serenut 4T	-7.2ns	-27.7**
Serenut 3R/ Erudurudu red	-6.8ns	-0.3ns
Serenut 3R/ ICGV 094374	5.2ns	6.7ns
Serenut 3R/ Serenut 9T	8.9ns	21.3*
Red beauty/ Serenut 4T	18.0**	44.3***
Red beauty/ Erudurudu Red	1.1ns	-63.3***
Red beauty/ ICGV 094374	-7.9ns	19.7ns
Red beauty/ Serenut 9T	-11.2ns	-0.7ns
Serenut 11T/ Serenut 4T	-5.9ns	-49.9***
Serenut 11T/ Erudurudu red	7.6ns	32.5***
Serenut 11T/ ICGV 094374	-3.8ns	65.5***
Serenut 11T/ Serenut 9T	2.1ns	-47.9***
Acholi White/ Serenut 4T	8.0ns	22.6*
Acholi White/ Erudurudu Red	5.1ns	9.5ns
Acholi White/ ICGV 094374	-2.6ns	-3.6ns
Acholi White/ Serenut 9T	-10.6ns	-28.5*
SEM	6.46	9.82
SED	9.14	13.89

* Significant at P≤0.05, ** significant at P≤0.01, and *** significant at P≤0.001. PSKI7 = Percent-severity kernel infection on day 7 and ns = non-significant

Red beauty x Serenut 9T, Acholi White x Serenut 4T and Acholi White x Erudurudu red, had positive SCA effects at PSKI7 with *A.flavus,* though statistically non-significant.

The crosses of Serenut 1R x ICGV 094374, Serenut 3R x Serenut 4T and Red beauty x Erudurudu red, Serenut11T x Serenut 4T and Serenut 11T x Serenut 9T had negative and statistically significant SCA effects for aflatoxin production. The F2s from the crosses of Serenut 3 x Erudurudu red, Red beauty x Serenut 9T and Acholi White x ICGV 094374 also had negative but non-significant SCA effects. Crosses of Serenut 1R x Erudurudu red, Serenut 1T x Serenut 9T, Red beauty x Serenut 4T, Serenut 11T x Erudurudu red, Serenut 11T x ICGV 094374 and Acholi White x Erudurudu red all showed positive and significant SCA effects (either at P≤0.05, 0.01 or 0.001) for aflatoxin production, while crosses Serenut 1R x Serenut 4T, Serenut 3R x ICGV 094374, Red beauty x ICGV 094374 and Acholi White x Erudurudu red, had positive SCA effects that were statistical insignificant. SCA effects were significant for both *A.flavus* and aflatoxin accumulation suggesting that the level of resistance in certain parental combinations were significantly higher or lower in the progeny than would have been predicted based on the parents' GCA values. The crosses of Serenut 1R x Serenut 4T and of Serenut 1R x Red beauty displayed the most negative SCA effects for resistance to *A.flavus* and to production of aflatoxin, respectively. The combination of these crosses would be desirable in hybridization, since they produced the desired high frequency of resistant progeny, as suggested by (Dabholkar, 1992).

The results of mid-parent offspring regression coefficient analysis are presented in Table 8. The regression mean squares for PSKI3 and PSKI7 with *A.flavus* were significant (P≤0.001), while the mean square for log transformed aflatoxin was not significant. Regression coefficient "b", which is a direct estimate of h^2 (Vogelet al.,1980) was run to obtain the narrow sense heritability from the slope of the regression equation. These two methods estimated the narrow sense heritability values as 66-74% at PSKI3, 57-70% at PSKI7 and 35-46% for aflatoxin accumulation estimated from kernel exposed to *A.flavus* for 7 days.

High broad-sense heritability values of 55-78.5% have been reported in various studies Utomo et al.(1990); Upadyhaya et al.(1997) for *in vitro* seed colonization by *A.flavus*. Utomo et al.(1990) also reported in the same material a broad sense heritability range of 21-23% for resistance to aflatoxin production. In the present study high

Table 8. Mid-parent offspring regression Mean Squares and Narrow Sense Heritability estimates for PKSI3, PSKI7 with *A.flavus*, and Aflatoxin Accumulation.

Traits	MS	h^2 = "b" regression coefficient
PSKI3	1594^{***}	0.54
PSKI7	2398^{***}	0.70
Aflatoxins log_{10}	0.0455^{ns}	0.35

*** Significant at P≤0.001**,** ns=significant

values of broad sense coefficient of genetic determination (BS-CGD) with *A.flavus* were recorded for PSKI3 (90%), PSKI7 (85%), and 96% for aflatoxin production on a genotype mean basis for kernels exposed to *A.flavus* for seven days. These values approximated the H, which reflects all the genetic contribution to the phenotypic variance, including additive and non-additive effects (Falconer and Mackay, 1996). These high values BS-CGD implied that the environment did not played a key role in the expression of resistance to aflatoxin accumulation (Kwemoi, 2011).

High narrow sense heritability which estimated the ratio of additive genetic variance to phenotypic variance was recorded in this study using two methods of heritability estimatesby (Lianget al., 2006) estimated a high narrow sense heritability of 58% for resistance to *A.flavus*. Similarly, in this study high narrow sense heritability estimates of 66-74% for PSKI3 and 57-70% for PSKI7 with *A.flavus* were observed, suggesting that resistance to *A.flavus*in this set of crosses was controlled mainly by additive genetic variance. Most breeders of self-pollinated crops use narrow sense heritability to adopt an appropriate selection strategy and to predict the rate of phenotypic changes (Falconer and Mackay, 1996). On the other hand, in this study low estimates of narrow sense heritability (35-46%) were found for resistance to aflatoxin accumulation, indicating that high non-additive genetic variance, coupled with environmental variation, were key to controlling the resistance traits.

CONCLUSION

This study revealed that there was genetic variability among 16 locally adapted and 2 exotic lines of germplasm used. Sources of resistance to both *A.flavus* and aflatoxin accumulation were identified as Serenut 4T, Serenut 9T and Erudurudu red. Serenut 4T and Serenut 9T are commercial varieties in Uganda released in 2002 and 2011 respectively whereas Erudurudu red is a landrace popularly grown in Teso region in Eastern Uganda (Okelloet al.,2013). Their use as cultivars and breeding lines should be promoted as one of the methods in aflatoxin management.

Analysis of combining ability for *A.flavus* revealed that both additive and non-additive gene actions were important for resistance to *A.flavus* and aflatoxin accumulation. Additive was more important than non-additive gene action for resistance to *A.flavus,* while non-additive was more important than additive for the resistance to aflatoxin production.

Broad sense heritability estimates were high for *A.flavus* and aflatoxin accumulation, implying low environmental effects in the overall phenotypic expression. The narrow sense coefficient of genetic determination for fixed parents was high for *A.flavus*, suggesting that the performance offspring in multiple crosses could be predicted from the parent. However narrow sense heritability estimates were low for aflatoxin production for the same fixed parents in both NC II and regression analyses, implying that the offspring's performance is better only in specific crossing combinations and therefore cannot be predicted for a wide range of crosses.

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