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Research Article

Screening for new sources of resistance to *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) infestation in stored maize genotypes

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Abstract: New sources of maize resistance to the maize weevil, *Sitophilus zeamais* infestation are indispensable in successful breeding programs against post harvest grain damage. The study investigated the characters and mechanisms that confer resistance to weevil infestation using twenty elite maize genotypes. Detailed morphological and physical studies were conducted on whole-maize grain. The grain hull was separated from the whole-maize grain with a locally-fabricated machine and subjected to chemical analysis. Resistance was assessed at 33 days post infestation using weevil mortality, weevil survival, percent grain damage, weight of grain powder, percent weight loss and oviposition as indices rated on a scale developed by the present study. The resistant genotypes identified, particularly 2000SYNEE-WSTR and TZBRELD3C5 with very high degree of resistance should be used singly or best in an integrated pest management system for the control of *S. zeamais* infestation in stored maize. Though increases in the physical properties of grain hardness, weight, length and width increased varietal resistance, it was found that the bases of resistance were increased chemical attributes of phenolic acid, trypsin inhibitor and crude fiber while the bases of susceptibility were increased protein and starch. Characters that conferred resistance on the tested genotypes were found in the grain hull. The study identified antibiosis and antixenosis as the mechanisms of maize post harvest resistance to *S. zeamais* infestation.

Keywords: *Sitophilus zeamais*, maize, resistance, antibiosis, antixenosis

Introduction

Maize *Zea mays* L. is a member of the family Gramineae and an important food, cash and industrial crop (FAO, 2003). Though occupying less land area than either wheat or rice, maize gives a greater average yield per unit area (5.5 t/ha) thus fostering drive towards global food security (Sasson, 2012). Tongjura *et al.* (2010) stressed that maize provides families with much needed nutrients such as carbohydrates, proteins, fats, vitamin B and minerals. The importance of maize in West Africa is well-studied and yet, receiving increased attention (Nwosu, 2014). Nigeria cultivates 2 million of the 4 million hectares cultivated in West Africa and she produces about 1.5 million tons of grains annually (IITA, 1981).

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It had been reported that insects particularly the maize weevil, *Sitophilus zeamais* Motschulsky, cause severe losses in stored maize grain in Africa (Ofuya and Lale 2001). Declining food production, worsened by huge losses resulting from *S. zeamais* attack during maize storage expose farmers to different magnitudes of food shocks (Nwosu and Nwosu, 2012).

As a primary pest of stored maize, *S. zeamais* is capable of penetrating and infesting intact kernels of grain, in which immature stages develop (Lale and Ofuya, 2001) leaving the maize emptied of its nutritional and seed value culminating in outright rejection of the product at the local and international markets.

The widespread use of chemical insecticides for the management of stored products pests has been evoking global concern due to associated environmental hazards, development of resistance to chemicals by insect pest, presence of residues in food, adverse effects on non-target organisms and exorbitant prices of the insecticides (Nwosu and Nwosu, 2012). To that effect, the increased public awareness and concern for environmental safety has compelled reorientation of research focus to the development of alternative management strategies.

In the absence of sustainable remedy to insect pests' attack in stored maize grains, evidenced from increased reports on susceptibility of maize varieties to storage pests (Arnason *et al*., 1993, 2004; Adedire *et al*., 2011), urgent efforts are required to investigate the maize characters that have relationships with resistance to *S. zeamais* in particular, and develop sustainable management strategy for weevils infesting stored maize. Some workers have already hinted that resistance in stored maize to insect attack is related to some physical, chemical and biochemical characteristics of a maize variety (Sing and Mc Cain, 1963; Dobie 1977; Adedire *et al*., 2011). Grain color, shape, size, hardness, protein, moisture, sugar and phenol have been reported as the bases of resistance (Dobie, 1974; Osipitan and Odebiyi, 2007; Tongjura *et al*, 2010). Garcia-Lara (2004) found that increased phenolic acid, structural protein and diferulates of grain hull increased resistance to *S. zeamais*.

Incidentally, the bases and locus of maize grain resistance to stored products insects is still debatable (Dobie, 1974; Shafique and Chaudry, 2007; Astuti *et al*., 2013) and vary from insect to insect. Therefore, the objectives of this research were two folds. First to assess improved and newly-released maize genotypes for resistance to maize weevil infestation and secondly to determine specific maize grain characteristics that confer resistance and susceptibility to *Sitophilus zeamais* infestation.

Materials and Methods

Maize genotypes

Twenty improved maize genotypes tested for resistance to maize weevil in the study were TZBRCOMP.2C1F1, TZBRELD3C5, PVASYN3F2, PVASYN6F2, DTSYN-11-W, BR9943DMRSR, IWDC3SNY-W, WHITEDTSTRSYN, 2008DTMA-YSTR, 2000SYNEE-WSTR, ILE-1-0B, IFE MAIZE HYBRI-1,IFE MAIZEHYBRID-2, IFEMAIZE HYBRID-5, IFEMAIZEHYBRID-6, ART COMPOSITE-A-Y, ARTCOMPOSITE-B-Y, ART/98/SW1-Y, ART/98/SW4-0B and ART/98/SW5-OB. The first ten genotypes were obtained from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria and the remaining ones were got from Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, Nigeria.

The grain samples were examined, cleaned and freed from any unwanted material prior to morphological, physical and chemical characterization and standardization for bioassays. The cleaned grains were kept in a deep freezer at -20 ± 2 ºC for 1 week to disinfest them and then stored at 4 ºC to prevent infestation (Sulehrie *et al*., 2003). Grains were kept for two weeks in muslin-covered plastic containers under experimental conditions for acclimatization and stabilization of moisture content at 12-13% (Abebe *et al*., 2009) before commencement of bioassays.

Morphological characteristics of genotypes

Ten grains from each of the twenty genotypes were randomly hand-picked and carefully examined for morphological characteristics.

Descriptions of genotypes were based on visual observation of color, appearance, shape, face-type and texture (Dobie, 1974; Adedire *et al.*, 2011). The texture was felt with hand to supplement visual observation.

Physical characteristics of genotypes

Ten grains from each of the twenty genotypes were examined. Micrometer screw gauge was used to determine the length, width and thickness of each of the maize genotypes while grain weight was measured using a digital weighing balance (Adventurer OHAUS, serial number: 8726170781**).** Grain hardness was determined with a compression machine (model: 200063 Milano, Italy). A grain was placed on the beam of the machine at a time and the lever was rolled down gradually until the grain produced a cracking sound. The bearing ratio/strength value was read, recorded and multiplied by a factor of 23.8 N to convert the strength value to Newton (N). The amount of force (N) needed to break the grain was taken as a measure of grain hardness (Sulehrie *et al.*, 2003).

Chemical characterization of grain hull of genotypes

Fifty grams of grain hull of each genotype were removed using a locally fabricated machine. The machine-produced mixture (grain coat and powder) was simply sieved (mesh length: 5 mm; mesh width: 2 mm) to assemble the hulls into a stainless basin. Hulls were milled and sieved through 0.4 mm sieve. The milled samples were subjected to chemical analysis at the central laboratory of Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. Protein, crude fiber, ash, starch and ether extract were determined using the standard method of AOAC (1990). Phenol was determined using the Prussian blue spectrophotometric method (Price and Bulter, 1977). Trypsin inhibitor activity in the maize genotypes was determined using the method developed and described by Kakade *et al.* (1974). Relationship of chemical constituents with maize resistance to *S. zeamais* was examined using correlation analysis.

Insect culture

Sitophilus zeamais population was obtained from the Storage Research Laboratory of Biology Department, Federal University of Technology Akure, Ondo, Nigeria. Cultures of the weevil were established and routinely maintained to provide weevils of similar age for the study. Twenty-five kilograms (25 kg) grain of the susceptible maize genotype, Bende White, was procured from Umuahia Main Market, Abia State, Nigeria, winnowed and cleaned to eliminate grains with visible damage symptoms. The cleaned grains were stored in a refrigerator at -4 ºC for 1 month after disinfestation for 1 week in a deep freezer at -20 ± 2 ºC). Grains were then transferred to plastic containers and kept at experimental conditions for two weeks. Weevils were maintained in 7 cylindrical containers (25 cm height and 15 cm diameter). Each of the containers had 100 adult *S. zeamais* per 500 g of grains. The containers were covered with muslin cloth (lid with a hole of 15 cm diameter each) to allow aeration and to prevent escape of the weevil. Seven days after oviposition, all parents *S. zeamais* were removed from each container and placed on another set of grains kept at the same conditions. Removal of parent *S. zeamais* and transfer to a fresh grain medium was repeated until sufficient numbers of weevils of known age were available for the experiments.

Screening the maize genotypes for relative resistance

The standard or control maize genotype, 2000SYNEE-WSTR, was identified as highly resistant maize using the guidelines provided by Osipitan and Odebiyi (2007).

Twenty grams of each maize genotype was weighed into a highly-transparent container (11 cm diameter and 4.5 cm height), in four replicates, and two pairs (2♀ + 2♂) of teneral adult *S. zeamais* (1-5 days old) were introduced into each container. Each container was covered with white muslin cloth to allow for aeration and to prevent exit and entry of insects. The infested maize was left in the laboratory for 33 days after which the numbers of dead (those that did not respond to probes with a pin) and live adults were counted. The numbers of undamaged and damaged maize grains (showing perforation and tunneling) were also counted and recorded. The grain damage was expressed as a proportion of the total number of grains sampled to give percent grain damage while grain weight loss was determined by subtracting the final from the initial weight of the grains sampled and expressed as a percentage (Tefera *et al*., 2011). Meanwhile, weight of frass (beetle excreta/grain powder) produced was weighed on a digital weighing balance.

Obligative oviposition bioassay

The experimental set up was similar to the screening test described earlier except that 10 g of maize was used. Two pairs (2♀ + 2♂) of teneral adult *S. zeamais* were introduced into each maize genotype and left for 7 days in the laboratory for the weevils to feed and oviposit. On the 8th day of infestation, adults were removed and discarded. Egg plugs were identified using the method of Frankenfeld (1948). The mean number of eggs laid (judging from 10 randomly selected seeds) were evaluated and recorded.

Facultative oviposition bioassay

In the free choice test, one seed of each genotype was randomly selected from 10 g sample, coded and placed in a transparent plastic container (15 cm diameter). The seeds were equidistantly placed and the position of each was determined using table of random numbers. Two pairs of newly emerged (1-5 days) teneral adults were released in the centre of the dish and then covered with a netted-ventilated lid. The experiment was replicated four times. The number of eggs laid in each replicate was recorded after 7 days. Egg plugs were identified as described by Frankenfeld (1948).

Scale for rating varietal resistance to *Sitophilus zeamais* infestation

Number of dead adults, number of live adults, percent grain damage, weight of grain powder, percent weight loss and number of eggs laid were converted to scores on a scale from 1 to 5 (Table 1). The weighted average score was the final basis for the rating of the maize genotypes into one of the four widely recognized classes of resistance: highly resistant, resistant, moderately resistant and susceptible (Osipitan and Odebiyi, 2007).

Bases and mechanisms of resistance

The contributions of grain morphological factors of color, appearance, shape, face-type and texture to relative resistance were assessed in a discussion. It was suggested that grain physical and chemical characteristics determine resistance to stored product insect pests. To test this hypothesis, relationship of grain physical and chemical characteristics with maize resistance to *S. zeamais* infestation was examined using correlation analysis. Mechanisms of resistance were determined using the guidelines provided by Kumar (1984), Osipitan and Odebiyi (2007) and Munyiri *et al*. (2013).

Statistical analysis

The data on physical and chemical characteristics of the maize genotypes, growth performance and oviposition of *S. zeamais* on the genotypes were analyzed using one way analysis of variance (ANOVA) and Least Significance Difference (LSD) was used to determine significant differences between the genotypes (SPSS version 17.0) at P = 0.05. Grain physical and chemical characteristics of the different genotypes were correlated with varietal resistance weighted average score and significant results were pooled and interpreted.

Results

Morphological description of the elite maize genotypes

The genotypes differed in terms of color and shape but not in appearance, face-type and texture. Four color types were differentiated: red in PVASYN-6F2, PVASYN-3F2 and IFEMAIZEHYBRID-5, yellow in 2008DTMAYSTR and ART/98/SW1-OB, yellow red/ reddish yellow in IFEMAIZEHYBRID-6, ARTCOMPOSITE-A-Y and ARTCOMPOSITE-B-Y, white in the others. All sampled genotypes were opaque in appearance, had dent face and were smooth in texture, the shapes varied from oblong, to hexagonal, rectangular and oval types. The control or standard maize, 2000SYNEE-WSTR was among the genotypes with oblong shape.

Physical characteristics of the maize genotypes

Significant differences between all physical characteristics measured were observed (Table 2). IFEMAIZEHYBRID-1 had the longest grains (10.89 mm) while TZBRCOM.2CIFI had the shortest (8.51 mm). Whereas grain length of the former genotype was not significantly different from the grain length of the standard genotype (10.06 mm), that of the latter was significantly shorter.

DTSYN-11-W had the biggest width (9.08 mm) while PVASYN-3F2 had the smallest width (7.15 mm). The width of the standard genotype (7.96 mm) did not significantly differ from the highest and lowest widths obtained.

Genotype 2008DTMA-YSTR was the thickest (4.20 mm) while ARTCOMPOSITE-A-Y was the thinnest maize (3.16 mm). The thickness of the check maize genotype (3.30 mm) differed significantly from that of the thickest maize but not that of the thinnest genotype.

Grain weight of the standard genotype (0.32 g/10 grains) did not significantly differ from that (0.34 g) of the heaviest maize (BR9943DMRSR), but it was significantly higher than that ART/98/SWI-OB (0.24 g), the genotype with the lightest seeds.

The hardest maize was the check genotype (2000SYNEE-WSTR) and its strength (378.42 N) differed significantly from the 161.15 N obtained for the softest genotype (ART/98/SWI-OB).

Chemical composition of grain hull of the genotypes

Chemical characteristics of grain hulls are presented in Table 3. Significant differences were observed in the amount of chemical constituents of grain hull of the test genotypes. The content of the control genotype (2000SYNEE-WSTR) differed significantly from those of other genotypes. The maize genotypes with the lowest and highest content of the chemical parameters as well as genotypes of match (with the control genotype) are evident in Table 3.

Relative resistance of elite maize genotypes to weevil infestation

Scored growth performance of *S. zeamais* on the twenty maize genotypes is presented in Table 4. Approximately, all weevils (n = 4 per replicate) introduced into genotype 2000SYNEE-WSTR died, whereas there was no mortality in IFEMAIZEHYBRID-6 and COMPOSITE-A-Y. Number of dead weevils in the check maize genotype (2000SYNEE-WSTR) differed significantly from those of IFEMAIZE HYBRID-6 and ART COMPOSITE-A-Y. Percent grain damage ranged from 1.943 (in TZBRELD3C5) to 28.571 (in PVAASYN-3F2). The grain damage of the check genotype (3.15%) was not significantly different from the lowest value but showed a significant difference from the highest value.

No grain powdering as a result of weevil infestation was observed in the check genotype. This differed significantly with highest weight of dust (0.18 g) recorded in the genotype, PVASYN-3F2. Meanwhile, no weight loss (0.0%) was observed in the check genotype and this again differed significantly from largest percent weight loss (5.10) experienced by PVASYN-3F2.

Effect of maize grain genotype on oviposition of *Sitophilus zeamais*

Scored results of obligative and falcultative oviposition bioassay of *S. zeamais* on the genotypes are presented in Table 4. In obligative oviposition, no egg was laid on the check maize genotype. Meanwhile, the weevil mostly preferred genotype PVASYN-3F2, laying an average of 11 eggs on it and this difference is significant.

In facultative oviposition bioassay of *S. zeamais*, significant differences were only observed between two maize genotypes (the standard maize-2000SYNEE-WSTR and PVASYN-3F2). No egg was laid on the check genotype while PVASYN-3F2 had a mean number of 1.50 eggs on it.

Table 1 Rating scale for determining the relative resistance of twenty elite maize genotypes to *Sitophilus zeamais* infestation.

Indices	Score	Indices	Score
Number dead		Grain weight loss (%)	
0.0-0.89	5	0.0-1.199	1
0.9-1.79	4	1.2-2.399	2
1.8-2.69	3	2.4-3.599	3
2.7-3.59	2	3.6-4.799	4
3.6-4.49	1	4.8-5.999	5
Number alive		Oviposition (Obligative)	
0.0-2.40	1	0.0-2.50	1
2.5-4.90	2	2.6-5.10	2
5.0-7.40	3	5.2-7.70	3
7.5-9.90	4	7.8-10.30	4
10.0 -12.40	5	10.4-12.9	5
Grain damage (%)		Oviposition (Facultative)	
0.0-6.80	1	0.00-0.30	1
6.9-13.70	2	0.31-0.61	2
13.8-20.60	3	0.62-0.92	3
20.7-27.50	4	0.93-1.23	4
27.6-34.40	5	1.24-1.54	5
Weight of powder (g)		Average	Resistance status
0.00-0.039	1	1.00-1.99	Highly resistant
0.04-0.079	2	2.00-2.99	Resistant
0.08-0.119	3	3.00-3.99	Moderately resistant
0.12-0.159	4	4.00-4.99	Susceptible
0.16-0.199	5		

Table 2 Physical characteristics of the twenty elite maize genotypes.

Maize genotypes	Length (mm)	Width (mm)	Thickness (mm)	Weight (g/10 grains)	Hardness (N)
DTSYN-11-W	10.42 ± 0.47	9.08 ± 0.35	3.67 ± 0.16	0.31 ± 0.01	303.45 ± 44.92
TZBRCOMP.2C1F1	8.51 ± 0.24	8.87 ± 0.22	3.33 ± 0.38	0.29 ± 0.04	256.35 ± 59.22
IWDC3SYN-W	9.25 ± 0.41	7.96 ± 0.71	3.63 ± 0.18	0.25 ± 0.02	291.55 ± 70.32
TZBRELD3C5	8.77 ± 0.21	8.68 ± 0.41	3.24 ± 0.28	0.32 ± 0.01	238.00 ± 38.87
PVASYN-6F2	9.26 ± 0.37	7.73 ± 0.43	3.66 ± 0.24	0.29 ± 0.01	268.00 ± 34.81
2000SYNEE-WSTR[1]	10.06 ± 0.19	7.96 ± 0.26	3.30 ± 0.32	0.32 ± 0.01	378.42 ± 68.01
2008DTMA-YSTR	9.39 ± 0.28	7.68 ± 0.15	4.20 ± 0.28	0.25 ± 0.02	349.86 ± 110.10
PVASYN-3F2	9.00 ± 0.33	7.15 ± 0.55	3.39 ± 0.22	0.28 ± 0.03	279.65 ± 70.32
WHITEDTSTRSYN	9.22 ± 0.50	7.56 ± 0.26	3.35 ± 0.35	0.27 ± 0.02	303.45 ± 63.99
BR9943DMRSR	9.30 ± 0.52	7.95 ± 0.57	3.20 ± 0.06	0.34 ± 0.02	273.70 ± 43.99
ILE-1-OB	10.09 ± 0.49	7.94 ± 0.40	3.24 ± 0.16	0.33 ± 0.02	297.50 ± 58.70
IFEMAIZEHYBRID-1	10.89 ± 0.33	8.17 ± 0.19	3.95 ± 0.20	0.29 ± 0.02	255.85 ± 35.53
IFEMAIZEHYBRID-2	9.06 ± 0.39	8.23 ± 0.25	3.74 ± 0.32	0.28 ± 0.02	190.40 ± 72.06
IFEMAIZEHYBRID-5	8.60 ± 0.25	8.05 ± 0.22	3.43 ± 0.21	0.25 ± 0.03	226.10 ± 83.86
IFEMAIZEHYBRID-6	9.69 ± 0.30	8.66 ± 0.27	3.86 ± 0.18	0.33 ± 0.00	177.90 ± 34.70
ARTCOMPOSITE-A-Y	10.24 ± 0.43	8.23 ± 0.17	3.16 ± 0.18	0.33 ± 0.01	368.90 ± 106.66
ARTCOMPOSITE-B-Y	9.41 ± 0.62	8.66 ± 0.56	4.16 ± 0.33	0.33 ± 0.02	340.34 ± 127.74
ART/98/SW1-OB	8.76 ± 0.25	7.31 ± 0.68	3.57 ± 0.63	0.24 ± 0.00	161.15 ± 26.58
ART/98/SW4-OB	9.84 ± 0.29	7.85 ± 0.46	3.82 ± 0.29	0.29 ± 0.01	196.35 ± 54.42
ART/98/SW5-OB	9.87 ± 0.33	7.99 ± 0.24	3.89 ± 0.24	0.31 ± 0.01	243.95 ± 53.55
LSD (0.05)	1.05	1.14	0.80	0.04	193.40

[1] Control maize variety. Each value in the table is the mean ± standard error of ten replicates.

Table 3 Percent chemical composition of grain hull of the twenty elite maize genotypes.

Maize genotypes	Protein	Crude fiber	Ash	Starch	Ether Extract	Phenolic Acid	Trypsin inhibitor
DTSYN-11-W	2.80 ± 0.06	76.10 ± 0.06	0.80 ± 0.00	7.40 ± 0.00	0.93 ± 0.03	2.20 ± 0.00	0.57 ± 0.00
TZBRCOMP.2C1F1	2.00 ± 0.00	79.53 ± 0.09	0.70 ± 0.06	7.10 ± 0.00	0.83 ± 0.03	2.93 ± 0.03	0.82 ± 0.00
IWDC3SYN-W	3.40 ± 0.00	70.10 ± 0.10	0.80 ± 0.06	7.80 ± 0.06	0.77 ± 0.03	2.50 ± 0.06	0.50 ± 0.00
TZBRELD3C5	1.03 ± 0.26	80.10 ± 0.06	0.90 ± 0.05	6.90 ± 0.06	1.00 ± 0.00	4.03 ± 0.03	0.84 ± 0.00
PVASYN-6F2	2.60 ± 0.06	70.10 ± 0.06	1.00 ± 0.06	7.87 ± 0.03	1.10 ± 0.00	2.07 ± 0.03	0.40 ± 0.00
2000SYNEE-WSTR[1]	1.50 ± 0.06	80.10 ± 0.06	0.90 ± 0.00	6.73 ± 0.09	1.30 ± 0.00	4.00 ± 0.06	0.84 ± 0.00
2008DTMA-YSTR	2.40 ± 0.00	72.90 ± 2.65	1.50 ± 0.05	7.03 ± 0.03	0.70 ± 0.06	3.90 ± 0.00	0.81 ± 0.00
PVASYN-3F2	4.00 ± 0.00	69.80 ± 0.15	1.13 ± 0.03	8.30 ± 0.06	0.80 ± 0.00	1.50 ± 0.16	0.30 ± 0.05
WHITEDTSTRSYN	2.40 ± 0.06	77.70 ± 0.09	0.80 ± 0.00	7.20 ± 0.00	0.90 ± 0.00	2.90 ± 0.00	0.70 ± 0.00
BR9943DMRSR	2.60 ± 0.00	77.60 ± 0.00	0.70 ± 0.06	7.07 ± 0.09	1.03 ± 0.03	3.00 ± 0.05	0.70 ± 0.06
ILE-1-OB	3.70 ± 0.00	70.17 ± 0.03	0.50 ± 0.00	7.50 ± 0.00	1.13 ± 0.18	3.23 ± 0.03	0.47 ± 0.00
IFEMAIZEHYBRID-1	3.73 ± 0.03	70.73 ± 0.03	0.50 ± 0.06	7.20 ± 0.06	0.80 ± 0.00	1.83 ± 0.03	0.65 ± 0.06
IFEMAIZEHYBRID-2	3.97 ± 0.03	69.60 ± 0.00	0.60 ± 0.06	8.07 ± 0.09	1.00 ± 0.00	2.03 ± 0.03	0.52 ± 0.02
IFEMAIZEHYBRID-5	2.90 ± 0.06	70.83 ± 0.03	0.37 ± 0.03	7.30 ± 0.06	1.17 ± 0.00	3.02 ± 0.03	0.68 ± 0.00
IFEMAIZEHYBRID-6	3.60 ± 0.00	69.87 ± 0.03	0.47 ± 0.03	7.67 ± 0.03	0.87 ± 0.03	2.37 ± 0.03	0.60 ± 0.00
ARTCOMPOSITE-A-Y	3.60 ± 0.00	69.79 ± 0.01	0.50 ± 0.06	7.60 ± 0.00	0.80 ± 0.00	2.00 ± 0.00	0.71 ± 0.00
ARTCOMPOSITE-B-Y	3.00 ± 0.00	70.23 ± 0.03	0.20 ± 0.00	7.40 ± 0.00	1.07 ± 0.03	2.23 ± 0.03	0.58 ± 0.00
ART/98/SW1-OB	3.13 ± 0.03	70.69 ± 0.01	0.70 ± 0.00	7.30 ± 0.00	1.30 ± 0.06	2.30 ± 0.00	0.61 ± 0.06
ART/98/SW4-OB	3.80 ± 0.00	69.77 ± 0.03	0.50 ± 0.06	7.90 ± 0.00	1.30 ± 0.06	1.87 ± 0.03	0.49 ± 0.06
ART/98/SW5-OB	3.70 ± 0.00	70.10 ± 0.05	0.43 ± 0.03	7.50 ± 0.06	1.00 ± 0.00	2.10 ± 0.06	0.45 ± 0.00
LSD (0.05)	2.38	1.70	0.13	0.14	0.14	0.13	0.13

[1] Control maize genotype. Each value in the table is the mean ± standard error of three replicates.

Scored performance of twenty elite maize genotypes screened for resistance to *Sitophilus zeamais*

Table 4 presents the performance of the maize genotypes investigated for resistance to *S. zeamais* based on the scoring scale developed. The check maize genotype and TZBRELD3C5 had mean weighted scores of 1.0 and 1.4286, respectively, thus putting them in the highly resistant category. PVASYN-3F2 with an average weighted score of 4.8571 was the sole susceptible genotype of the study. IFEMAIZEHYBRID-2 and ART/98/SW4-OB with mean scores of 3.4286 and 3.2857, respectively, were moderately resistant.

The other 15 maize genotypes whose weighted average scores ranged from 2.0 (TZBRCOMP.2C1F1 and 2008DTMA-YSTR) to 2.8571 (IFEMAIZEHYBRID-6 and ARTCOMPOSITE-A-Y) were rated resistant.

Bases and mechanisms for resistance or susceptibility to *S. zeamais* infestation

Bases for resistance or susceptibility to weevil infestation are recorded in Table 5. Correlation of the weighted seven resistance parameters with physical characteristics and chemical compositions of test genotypes showed both positive and negative relationships (Table 5).

Table 4 Scored performances of twenty elite maize genotypes screened for post harvest resistance to *Sitophilus zeamais.*

Maize genotypes	Dead weevil	Live weevil	% grain damage	Weight of powder	% weight loss	OO[1]	FO[2]	Weighted average	Resistance status[3, 4]
DTSYN-11-W	5	4	2	2	1	1	2	2.4286	R
TZBRCOMP.2C1F1	2	3	1	1	1	4	2	2.0000	R
IWDC3SYN-W	5	4	3	3	1	2	1	2.7143	R
TZBRELD3C5	2	3	1	1	1	1	1	1.4286	HR
PVASYN-6F2	4	4	3	3	1	2	2	2.7143	R
2000SYNEE-WSTR[5]	1	1	1	1	1	1	1	1.0000	HR
2008DTMA-YSTR	4	2	2	1	1	1	3	2.0000	R
PVASYN-3F2	5	4	5	5	5	5	5	4.8571	S
WHITEDTSTRSYN	4	3	1	3	1	2	1	2.1429	R
BR9943DMRSR	2	3	2	2	1	2	3	2.1429	R
ILE-1-OB	5	4	2	2	1	1	3	2.5714	R
IFEMAIZEHYBRID-1	5	4	2	2	1	1	1	2.2857	R
IFEMAIZEHYBRID-2	5	5	3	2	4	2	3	3.4286	MR
IFEMAIZEHYBRID-5	4	4	2	1	1	2	2	2.2857	R
IFEMAIZEHYBRID-6	5	4	2	1	1	2	5	2.8571	R
ARTCOMPOSITE-A-Y	5	4	2	1	1	2	5	2.8571	R
ARTCOMPOSITE-B-Y	5	4	2	1	1	1	3	2.4286	R
ART/98/SW1-OB	5	4	2	1	1	2	1	2.2857	R
ART/98/SW4-OB	5	5	3	3	1	2	4	3.2857	MR
ART/98/SW5-OB	5	4	2	1	1	1	4	2.5714	R

[1] OO: Obligative oviposition and [2] FO: Facultative oviposition.
[3] HR: Highly resistance R: Resistance, MR: Moderately resistance, S: Susceptible.
[4] Resistance scale of weighted average score has already been presented in Table 1.
[5] Control maize genotype.

Antibiosis was identified as a mechanism for resistance to weevil infestation and this is evident in Table 4. Antixenosis effect (by crude fiber, phenolic acid and trypsin inhibitor) was also observed (Table 5). Grain characteristics of the susceptible genotype, PVASYN-3F2 made it vulnerable to oviposition by adult *S. zeamais* (Table 4) and that mechanism of susceptibility is preference.

Discussion

Effect of grain morphology and physical characteristics on maize susceptibility

Variations recorded in the color of sampled maize genotypes are in agreement with observations of previous studies (Adegbola, 1992; Adedire *et al*., 2011). These authors indicated that the color of maize grains differ among genotypes, ranging from white, yellow, red, purple to black. None of the tested genotypes was purple or black in color; rather color combination observed on few of the genotypes seemed to favor resistance.

Table 5 Significant results of the correlation between physicochemical characteristics of the maize genotypes and *S. zeamais* resistance weighted average[1].

Grain characters	r	Bases for resistance or susceptibility
Protein	0.582*	Basis for susceptibility
Starch	0.851*	Basis for susceptibility
Crude fiber	-0.700*	Basis for resistance
Phenolic acid	-0.764*	Basis for resistance
Trypsin inhibitor	-0.814*	Basis for resistance

[1] Resistance weighted average score has an inverse relationship with resistance, but a direct relationship with susceptibility (Kumar, 1984).
r: Correlation coefficients.
*Significant at P = 0.05 (1-tailed).

Since 83.33% of white colored genotypes including all the highly resistant genotypes were at least resistant to the weevil, it is hereby suggested that presence of white color on a maize grain is also a positive indicator of good resistance to *S. zeamais*. Possibly, brightness of color white repelled the insects from infesting the grains. Dobie (1974) had earlier identified color as one of the factors contributing to resistance to weevil infestation. The observation that all sampled maize genotypes appeared opaque corroborates the findings of Adedire *et al*. (2011). That all genotypes were smooth in texture was a matter of chance, since a genotype can also have rough surface. The contributions of shape and face-type to grain resistance were not clear and therefore merit further investigation.

The occurrence of significant differences in the physical factors of the test genotypes agree with the findings of other workers (Osipitan and Odebiyi, 2007; Makanjuola *et al*., 2009; Tonjura *et al*., 2010). In this study, comparatively larger grain length, width, weight and hardness conferred good resistance to weevil infestation. However, it is well established that larger grain sizes are indicators of resistance against infestation and damage by *Sitophilus zeamais* (Guadrups *et al*., 2001; Makanjuola *et al*., 2009). The present result also corroborates the findings of Omoloye and Amodu (2006) that smaller-sized sorghum were comparatively more susceptible to infestation by *S. zeamais* than larger-grained type. On the contrary, Tongjura *et al*. (2010) documented that smaller seeds which must be hard and compact, with less moisture were more resistant to the maize weevil attack. Though with few exceptions (Olusanya, 1981; Osiptan and Odebiyi, 2007), various authorities have reported that grain hardness in particular, is one of the most important physical properties conferring certain degree of resistance to weevil attack on a maize variety (Tongjura *et al*., 2010; Adedire *et al*., 2011). Incidentally, the check maize genotype with best resistance in the study had the greatest hardness, agreeing with the reports of these authorities. It is now obvious why 85% of all tested maize genotypes were resistant to the weevil, since their hardness exceeded tremendously the "standard" (7 x 23.8 N = 166.60 N) reported by Osipitan and Odebiyi (2007). Dobie (1974) simply identified grain hardness as a basis of resistance after correlation analysis.

Effect of grain hull chemical composition on maize susceptibility

The significant variations in the chemical constituents of the genotypes are fairly well-documented (Singh and Mc Cain, 1963; Dobie, 1974; Arnason *et al*., 2004; Osipitan and Odebiyi, 2007; Adedire *et al*., 2011). The findings of other researchers suggest that the list of grain chemical characters that confer resistance to a genotype against weevil infestation is still debatable.

In this study, all the protein values recorded were lower than the range reported by Fageer and ElTinay (2004) and this is probably attributed to varietal differences.

Resistance of twenty elite maize genotypes

Significant variations were found among the tested maize genotypes with respect to weevil mortality, live weevil, percent of grain damage, weight of powder, percent of weight loss and oviposition preference. Observed differences in the resistance parameters and final susceptibility of the maize genotype is simply a measure of the inherent ability (antibiosis) of a particular genotype to resist *Sitophilus zeamais* infestation.

Judging from the performance of the tested genotypes, 2000SYNEE-WSTR (the control maize) and TZBRELD3C5 were the most resistant (highly resistant). This is because they had highest weevil mortality and lowest percent grain damage, coupled with no weight loss, no powdering and no oviposition. Previous study showed that TZBRELD3C series was highly resistant to *S. zeamais* (Adedire *et al*., 2011).

On the contrary, the genotype PVASYN-3F2 was the only susceptible maize in this study because it recorded significantly highest percent grain damage, weight loss, powdering and considerable low weevil mortality. In addition, it showed the highest oviposition preference. Therefore, detailed comparative genetic study should be conducted on genotypes 2000SYNEE-

WSTR (highly resistant) and PVASYN3F2 (susceptible) to possibly establish sources of resistance.

The fact that seventeen genotypes of the twenty elite genotypes (85%) screened for post harvest resistance were resistant agrees with the information that some sources of resistance have been incorporated into elite maize genotypes (Bergvinson, 2001).

Bases and mechanisms for resistance

The physical properties of grain such as hardness, length, width, thickness and weight did not significantly correlate with the resistance weighted unit and this implies that these characteristics were neither responsible for resistance nor susceptibility of the tested maize genotypes to weevil infestation. This result differed markedly with the findings of Dobie (1974) and Osipitan and Odebiyi (2007) that identified grain hardness (among other maize grain physical characteristics) as a basis for resistance to *S. zeamais* infestation in stored maize. However, Olusanya (1981) strongly eliminated grain hardness and other physical characteristics of grain as the bases for resistance and this agrees with the findings of the present study. The same author suggested that the roles of other factors should rather be investigated.

Significant and positive correlations found between protein and starch of the maize genotypes and the resistance weighted unit indicated that increases in amounts of the constituents in maize reduced resistance to weevil infestation. Therefore, it is concluded that the listed chemical constituents are the characteristics that confer susceptibility to a maize genotype. This probably accounts for the observation of Ichiro *et al*. (2009) that insects consume starch and proteins in grains to grow and to lay eggs. Similarly, Osipitan and Odebiyi (2007) identified the nutritive factor of starch as a basis for susceptibility to maize weevil infestation. It is easily discernible from this study that *S. zeamais* performs best on proteins and starch-rich grains.

On the contrary, significant and negative correlations found between the crude fiber, phenolic acid and trypsin inhibitor contents of the maize varieties and the resistance weighted unit implies that increases in their amounts increased resistance. This observation corroborates the findings of Garcia-Lara *et al*. (2004) that named phenolic acid of grain coat as a basis for resistance to the maize weevil. The study has identified crude fiber, phenolic acid and trypsin inhibitor as the bases for resistance to *S. zeamais* infestation in stored maize. Indeed, plants are known to produce proteinase inhibitors that protect their seeds against insect attack (Matsumoto *et al*., 1997; Jongsma and Bolter, 1997). These substances impede the nutritional metabolism of insects by inhibiting their digestive enzymes (Ichiro *et al*. 2009). Crude fiber, phenolic acid and trypsin inhibitor have been identified in this study as growth inhibiting substances against *S. zeamais* in stored maize. The three chemical characters conferred combination of antixenosis (non-preference) and antibiosis effects on the elite maize genotypes. The resistance in the elite maize genotypes generally affected adversely the survival and development of *S. zeamais* and this suggests antibiosis effect. The significant number of eggs oviposited in the susceptible maize, PVASYN-3F2 implies that its grain characteristics conferred preference on the genotype. The mechanisms of resistance observed in this study tally with the reports of Painter (1951, 1958), Pathak (1969), Kumar (1984), Osipitan and Odebiyi (2007) and Munyiri *et al*. (2013) on mechanisms of resistance to *S. zeamais* infestation in stored maize and rice.

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غربال برای منابع جدید مقاومت به شپشه ذرت *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) در ژنوتیپ‌های ذرت

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چکیده: جستجو برای دستیابی به منابع جدید مقاومت به شپشه ذرت *Sitophilus zeamais* برای برنامه‌های اصلاحی نسبت به خسارت آفت در مرحله پس از برداشت ضروری است. در این مطالعه خصوصیات و مکانیسم‌های دخیل در مقاومت ۲۰ ژنوتیپ ممتاز ذرت مورد بررسی قرار گرفت. خصوصیات شکل‌شناسی و فیزیکی دانه‌های ذرت به تفصیل مورد بررسی قرار گرفت. پوشش بیرونی دانه از کل دانه ذرت جدا شد و ترکیبات شیمیایی آن مورد شناسایی قرار گرفت. ارزیابی مقاومت ۳۳ روز پس از آلوده‌سازی انجام گرفت. در این مطالعه افراد مرده و زنده تعیین شد. هم‌چنین درصد خسارت دانه، وزن پودر تولید شده، درصد کاهش وزن دانه و میزان تخم‌ریزی به‌عنوان شاخص‌های مقاومت تعیین شدند. ژنوتیپ‌های مقاوم شامل 2000SYNEE-WSTR وTZBRELD3C5 بودند که می‌تواند برای مدیریت شپشه ذرت در انبار مورد استفاده قرار گیرند. گرچه خصوصیات فیزیکی مانند سختی دانه، وزن و طول و عرض دانه در افزایش مقاومت مؤثر است، اسید فنولیک، بازدارنده تریپسین و میزان فیبر هم مؤثر هستند اما مقدار بالای پروتئین و نشاسته حساسیت بذر به آفت را افزایش می‌دهد. خصوصیات دخیل در مقاومت در ژنوتیپ‌های مطالعه شده در پوشش بذر مشاهده شد. در این مطالعه مکانیسم‌های مقاومت آنتی بیوزی و آنتی زنوزی ژنوتیپ‌های ذرت به شپشه ذرت در مرحله پس از برداشت تعیین شدند.

واژگان کلیدی: *Sitophilus zeamais*، ذرت، مقاومت، آنتی‌بیوز و آنتی‌زنوز