Original Research Article

Estimation of genetic variability in maize genotypes under infestation by the maize storage weevil , *Sitophilus zeamais* using multivariate analysis

Abstract

The maize weevil, *Sitophilus zeamais*, is an important field-to-store pest of maize; accounting for huge grain losses globally. Information on genetic variability and trait relationship is crucial to developing farmer-friendly approaches to mitigating the menace of the insect in sub-Saharan Africa. *Sitophilus zeamais*. Twenty-one maize genotypes were infested to investigate genetic variability for reaction to *S. zeamais* and the relationship of nutritional quality with insect attack. F_1 generation insects were introduced into test tubes containing 100 g of whole grains per genotype. Completely Randomized Design was used replicated twice. Nutritional components, seed weight (g), and data from feeding activity were subjected to principal component, cluster, and genotype × trait (GT) biplot analyses. The highest genetic distance (GD) was between NG/SA/07/122 and each of EVDT-Y2008 STR and the local check. Four clusters were obtained. The GT biplot revealed positive association of grain quality with tolerance traits, and genotypes EVDT-Y2008-STR, IFE-TZEE-WSTR, NA/SA/07/018, NG/SA/07/022, NG/SA/07/140, NG/SA/07/184, TZM 212, and TZB-SR B. Maize reaction to *S. zeamais* can be influenced through biofortification using the mentioned genotypes. Further research should focus on determining possible jettisoning of quality to improve resistance to *S. zeamais*.

Keywords: biofortification; biplot; maize; genetic distance; genetic variability; maize weevil

1. INTRODUCTION

Maize (*Zea mays* L.) which belongs to the family Poaceae or Gramineae, ranks second in area cultivated and first in production and productivity in order of world grain production (FAO, 2013). It is one of the most important cereal crops in the world after wheat and rice that ensures food security (Mustafa *et al*., 2013). It serves as a source of income to farmers because it is used as food, feed for livestock and also as raw material for industries. Maize is a significant part of the diet of millions of people all over the world, especially in Africa because of its nutritional value. The kernel contains 72 % starch, 10 % protein, 4.80 % oil, 8.50 % fiber, 3.0 % sugar, and 1.70 % ash (Chaudhry, 1983). In spite of the importance of maize as a means of feeding the ever-growing population of the world, its production and yield is greatly affected by biotic stresses such as pest and diseases among other environmental factors (Ali *et al*., 2016; Obok *et al*., 2021). *Sitophilus zeamais* is a major insect pest that affects maize production and reduces its yield adversely both on the field and in the store.

Sitophilus zeamais, commonly known as maize weevil, belongs to the beetle family Curculionidae. It is a primary insect of maize infesting the crop both before and after harvest. Infestations by the insect pest cause significant economic losses in grain yield, result in increased temperature as well as moisture conditions that later expose stored grains to secondary attack by pathogens causing diseases such as mycotoxin-producing fungi (Magan *et al*., 2010). Rees (2004) reported that post-harvest losses in maize caused by *Sitophilus zeamais* is about 20-30% on average. Over time, studies have been conducted on maize storage weevil and its impact on maize grain both before and after harvest; use of control measures especially through development of resistant varieties against the attack of the pest as well as the subsequent disease occurrence due to its effect (Pereira *et al*., 2009; Mwololo *et al*., 2012). There is little information available on the existing genetic variability and relationship among traits of importance that could aid in development and establishment of farmer-friendly approaches to mitigating the menace of the insect. Improvement efforts in maize in relation to infestation by maize storage weevil should focus on the amount of genetic variability and character association within the population.

In any breeding program, presence of genetic variability is one of the requirements essential in selection process. Maize improvement through successful selection programme can be achieved using valid information about the trait association and genetic variability with the aid of scientific tools such as correlation, principal component analysis and dendrogram. Knowledge of the nature and strength of association among traits will help in selection efficiency in bringing out genetic improvement in segregating population (Asish *et al*., 2008). Therefore, assessment of maize accessions to evaluate their genetic variability and trait association for infestation of *Sitophilus zeamais* is essential for future breeding program. This study was conducted to investigate genetic variability among the maize genotypes for reaction to *S*. *zeamais* as well as the relationship of nutritional quality traits of the maize seeds on the reaction of the genotypes to infestation.

2. MATERIALS AND METHODS

2.1 Location of the experiment

The experiment was conducted at the Entomology Laboratory of the Department of Crop Production and Horticulture, Lagos State Polytechnic, Ikorodu, Lagos state, Nigeria. Lagos State Polytechnic is located at latitude 5^{o}10'N and longitude 3^{o}16'E of the Greenwich meridian, at an elevation of 50 m above sea level. The area has an average temperature between 25^{o}C and 29^{o}C and an annual rainfall ranging from 1670 mm to 2200 mm with a relative humidity between 65% and 68%.

2.2 Names and sources of maize genetic materials

Twenty-one maize genotypes were used in the study. Twenty (20) of the genotypes were obtained from Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria while seeds of an adapted cultivar, Igbogbo Local, were obtained from a local farmer and used a check. The 20 genotypes obtained from IAR & T were 06CHTTW19, EVDT-Y2008 STR, FE-TZEE-WSTR, NA/SA/01/018, NG/SA/07/022, NG/SA/07/029, NG/SA/07/044, NG/SA/07/122, NG/SA/07/140, NG/SA/07/184, NG/SA/07/72, Oba Super 11 F2, TB87/97/15, TSL Composite 4, TZB-SR A, TZB-SR B, TZM 1311, TZM 144, TZM 212, and TZM-1296.

2.3 Maize weevil

Adult weevils were collected from the Entomology Laboratory of Department of Crop Production and Horticulture, Lagos State Polytechnic, Ikorodu. Collected weevils were introduced to food (maize grains) and allowed to feed and multiply. The procedure was repeated with the progeny weevils. Insects in the second set of progeny were assumed to be of the same age and these were used to infest the maize grains.

2.4 Experimental procedure

Equal weight of whole grains of the 21 maize genotypes were drawn and introduced into different test tubes. The cultured weevils were introduced into the test tubes and were closed immediately with a net that was tightly fixed with a rubber band. The set up was placed under ambient condition. Grains were observed regularly for grain dust which was collected and weighed using a sensitive digital scale that measured in grams.

2.5 Data Collection

2.5.1 *Physical property of grain*

Data were collected on seed weight (g) by measuring the average weight of a seed of each genotype using a digital sensitive scale.

2.5.2 *Observations from insect feeding activities*

Number of holes (by visually counting the holes bored on maize seeds), weight loss (the difference between initial and final weights of seeds of each accession), Insect F_1 generation (number of progenies from weevils introduced into each test tube at the beginning of the experiment), and Weevil mortality (number of weevils found dead in each test tube) were recorded.

2.5.3 *Proximate and nutritional attributes of maize grains*

Proximate and nutritional analyses of seeds were done at Tetra ‘A’ Diagnostic and Analytical Laboratory, Abeokuta, Nigeria.

2.6 Data analysis

Data collected were subjected to principal component and cluster, and genotype × trait biplot analyses. The principal component and cluster analyses were performed in SAS (SAS Int., 2012) while genotype × trait biplot analysis was performed in R.

3. RESULTS AND DISCUSSION

3.1 Genetic distance estimates between all pair-wise maize genotypes

Table 1 is a summary of the estimates of genetic distance (GD) between paired maize genotypes for all measured traits. The highest genetic distance; estimated at 10.14 was between NG/SA/07/122 and the duo of EVDT-Y2008 STR and the local check while the lowest genetic distance of 2.07 existed between NG/SA/07/022 and IFE-TZEE-WSTR. A high GD estimate of 10.07 was also found between Igbogbo Local and EVDT-Y2008 STR. The observed pair-wise genetic distances is an indication of existing genetic variability which can be explored for improvement. With the pattern of variability among the genotypes, it is possible to develop elite hybrids that are improved for multiple traits, as well as improve the locally-adapted genotype through introgression. Choukan et al. (2006) similarly estimated genetic distances among maize inbred lines using SSR markers and agro-morphological traits.

Table 1: Genetic distance estimates of pair-wise maize genotypes based on measured traits

GENOTYPE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
06CHTTW19 (1)	0.00																				
EVDT-Y2008 STR (2)	7.12	0.00																			
IFE-TZEE-WSTR (3)	4.28	6.83	0.00																		
IGBOGBO LOCAL (4)	7.32	10.07	7.28	0.00																	
NA/SA/07/018 (5)	2.25	6.38	3.70	6.67	0.00																
NG/SA/07/022 (6)	4.77	6.18	2.07	8.57	4.18	0.00															
NG/SA/07/029 (7)	2.88	7.86	3.53	7.91	2.74	3.94	0.00														
NG/SA/07/044 (8)	3.82	7.25	2.11	6.64	3.37	3.47	3.79	0.00													
NG/SA/07/122 (9)	5.87	10.14	5.76	10.14	6.22	5.81	5.40	6.69	0.00												
NG/SA/07/140 (10)	4.78	5.25	4.66	8.51	4.78	4.11	5.56	4.48	7.59	0.00											
NG/SA/07/184 (11)	2.86	6.78	4.11	7.69	3.48	4.30	3.61	4.06	6.51	4.41	0.00										
NG/SA/07/72 (12)	4.21	8.40	4.29	6.12	4.45	4.96	4.92	4.18	6.18	5.45	5.44	0.00									
OBA SUPER 11 F2 (13)	2.95	7.07	2.85	7.26	3.24	3.33	3.24	3.27	5.29	5.04	3.85	3.42	0.00								
TB87/97/15 (14)	5.38	9.66	6.42	5.10	5.22	7.19	5.76	5.42	9.12	6.74	6.29	4.41	6.08	0.00							
TSL COMPOSITE 4 (15)	4.15	6.45	2.34	7.86	3.62	2.10	3.31	3.26	6.10	4.09	4.39	3.88	2.80	6.04	0.00						
TZB-SR A (16)	4.64	8.91	4.38	6.09	4.26	4.93	4.49	4.31	5.93	5.72	5.50	2.53	4.32	3.83	4.21	0.00					
TZB-SR B (17)	5.95	6.60	4.85	7.65	5.62	4.53	6.02	4.56	8.87	3.45	4.85	6.23	5.83	6.31	4.91	6.03	0.00				
TZM 1311 (18)	5.39	9.23	3.46	9.50	5.26	3.73	3.95	4.64	3.56	6.82	5.76	5.48	4.10	8.14	4.16	5.24	7.41	0.00			
TZM 144 (19)	2.66	6.81	4.37	7.11	2.59	4.67	3.26	4.46	5.76	5.06	2.88	5.41	3.68	6.25	4.62	5.13	5.61	5.62	0.00		
TZM 212 (20)	4.70	6.02	2.91	7.94	3.91	2.26	4.37	3.31	6.81	3.69	4.24	4.94	4.21	6.16	3.19	4.39	3.67	5.00	4.72	0.00	
TZM-1296 (21)	3.74	8.15	4.29	8.35	3.84	4.56	3.21	5.04	4.64	6.78	3.82	5.72	3.21	7.54	4.72	5.64	7.26	4.17	3.06	5.44	0.00

3.2 Cluster analysis

Result of the Ward’s minimum variance cluster analysis depicted the existence of sufficient genetic variability among the maize genotypes by identifying clear heterotic groups (Figure 1). Using a genetic dissimilarity of 0.40, the dendrogram (Fig. I) classified the maize genotypes into four clusters. Cluster 1 was made up by Igbogbo local, NG/SA/07/72, TZB-SR A, and TB87197/15 while cluster 2 was composed of EVDT-Y2008 STR, IFE-TZEE-WSTR, NG/SA/07/022, TSL COMPOSITE 4, TZM 212, NG/SA/07/044, NG/SA/07/140 and TZB-SR B. Cluster 3 had 06CHTTW19, NG/SA/07/018, TZM 144, NG/SA/07/029, Oba Super II F2, TZM 1296, NG/SA/07/184, NG/SA/07/122, and TZM 1311. The clear clustering of the maize genotypes is an indication of sufficient phenotypic variability for the measured traits. The genotypes in a cluster are closely associated and this suggests that the variability within group could be used in the selection for improvement of associated traits. Invariably, heterosis could be exploited through genetic differences between groups where members of a cluster could serve as parents in crosses involving members from other clusters. Thus, hybrids could be developed to improve multiple target traits associated with distinct clusters. Cluster analysis has been used to elucidate genetic variability in maize (Oyetunde et al., 2021; Shrestha, 2016).

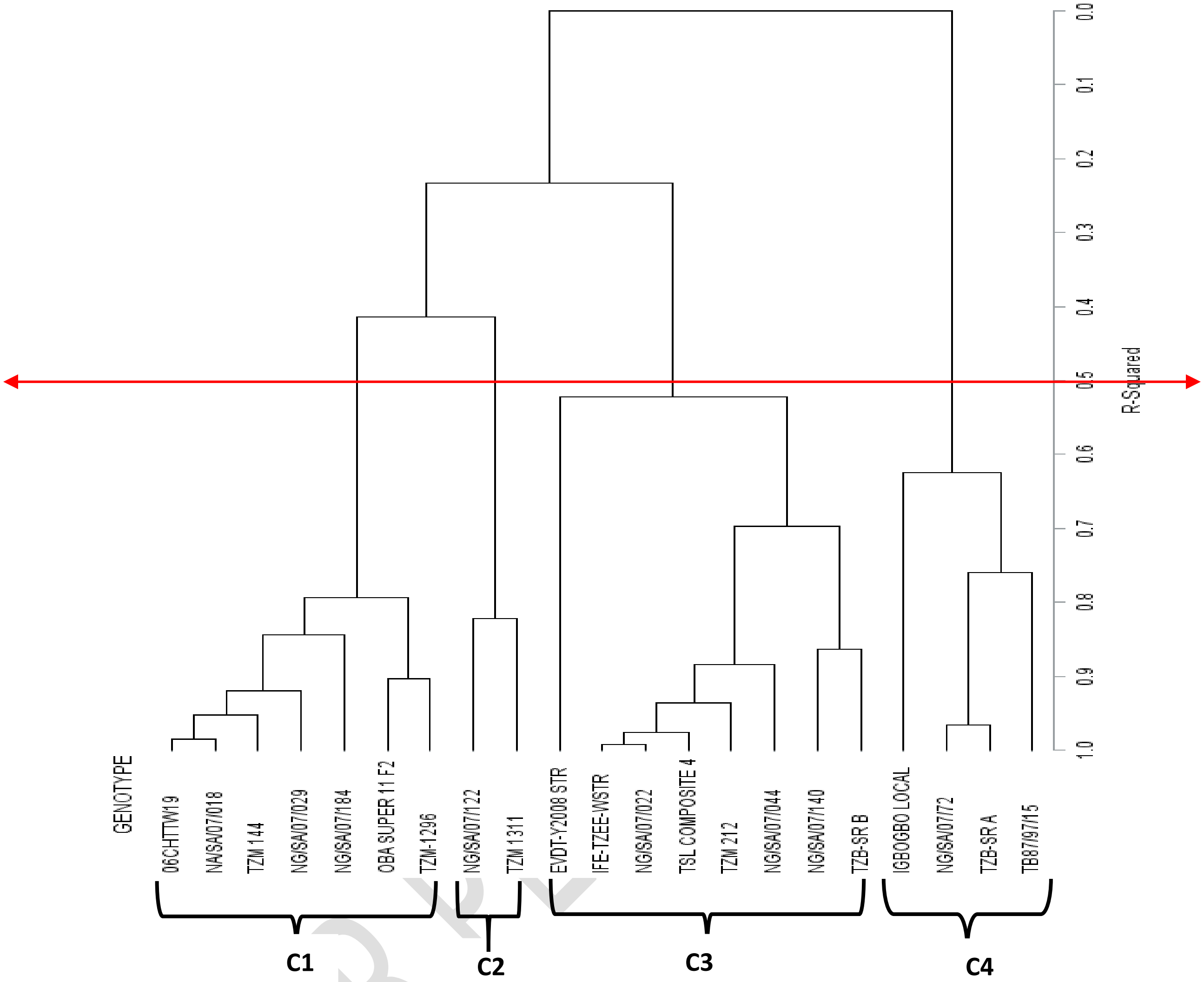


Fig. 1. Dendrogram of relatedness among 21 maize genotypes, (X-axis), based on genetic dissimilarity (Y-axis) from Ward's minimum variance cluster analysis. The red double-arrowed line groups the genotypes into clusters at approximately 0.50 level of dissimilarity; C1, C2, C3, C4 are clusters 1, 2, 3, 4 respectively.

3.3 Principal component analysis

The principal component analysis (PCA) was able to identify among the measures traits, traits that were important to the observed genetic variability among the evaluated maize genotypes. The first four principal components (PCs) (Table 2) together controlled approximately 77% of the observed variability. The PCs 1, 2, 3, and 4 had Eigen vectors of 5.24, 3.93, 2.04, and 1.17 respectively which explained about 33, 25, 13, and 7% of the total variation respectively. The PC 1, which explained about 33% of the total variation, was characterized by moisture, dry matter, crude fibre, crude protein, ash, and fat contents with loadings of -0.399, 0.399, 0.392, 0.369, 0.369, and 0.354 in that order. In PC 2, starch content, beta carotene, seed weight, sugar content, and amylase had significant loadings of 0.454, 0.429, -0.367, 0.337, and 0.337 respectively. Additionally, the PC 3 was characterized by number of holes on seeds, F_1 insect generation and carbohydrate content with loadings of 0.585, 0.549, and 0.359 respectively while PC 4 was characterized by seed weight loss, amylase content, and sugar content which had loadings of 0.607, -0.564, and 0.335 respectively.

The loadings of moisture, dry matter, crude fibre, crude protein, ash, and fat content indicated the important roles of the traits in the variation observed among the maize genotypes. This suggests that these traits are crucial in maintaining variability within the germplasm and should be considered for selection in maize improvement programme. This observation is comparable to those of Magudeeswari et al. (2019) and Semassa *et al*. (2017) that the PCA was effective to identify important traits to variation among maize germplasm lines.

The PCA biplot grouped the maize genotypes into clusters over the four quadrants (Figure 2) based on the contributions of the measured traits (Figure 3) as explained by the PCs. The genotypes were scattered on the component score plot with NG/SA/07/72 and Oba Super 11 F_2 placed away from other genotypes. The local check had close association with genotypes 06CHTTW19, Ife-TZEE-WSTR, NG/SA/07/022,

NG/SA/07/029, NG/SA/07/140, NG/SA/07/72, TZM1311, and TB87/97/15 which are associated with numbers of F_1 generation insects and holes on seeds. Genotypes NG/SA/07/122, TZB-SR A, and TZM 144 which were positioned in the top right quadrant had relationship with weight loss and crude protein content. The bottom left quadrant, composed of NG/SA/07/184 and TSL Composite 4 seed weight and carbohydrate content as the associated traits. The grouping of measured traits into quadrants on the PCA biplot revealed the pattern of association among the traits while couple with the scatter pattern of the genotypes on the component score plot, information can be derived to associate individual genotypes or groups of genotypes with measured traits. Thus, selection for improvement of specific traits can be effective.

Table 2: Trait loadings from principal components of maize genotypes subjected to infestation by *S. zeamais*

Measured traits	Principal component axis 1	Principal component axis 2	Principal component axis 3	Principal component axis 4
Seed weight	-0.118	-0.367	0.119	0.107
Moisture	-0.399	0.127	-0.159	0.104
Dry matter	0.399	-0.127	0.159	-0.104
Fat	0.354	-0.068	0.174	0.036
Ash	0.369	-0.079	-0.150	-0.023
Crude fiber	0.392	-0.101	0.088	-0.149
Crude protein	0.369	0.120	-0.159	-0.040
Carbohydrate	-0.053	-0.266	0.359	-0.095
Starch	0.013	0.454	-0.025	-0.148
Sugar	0.126	0.337	-0.206	0.335
Amylose	-0.055	0.337	0.085	-0.564
Beta Carotene	0.140	0.429	0.053	-0.092
Weevil mortality	0.161	-0.121	-0.155	-0.195
F_1 insect generation	0.013	0.206	0.549	0.256
Number of holes on seeds	-0.056	0.182	0.585	0.008

Seed weight loss	0.213	0.139	-0.010	0.607
Eigen value	5.243	3.934	2.039	1.169
Proportion of variance (%)	0.328	0.246	0.127	0.073
Cumulative variance (%)	0.328	0.574	0.701	0.774

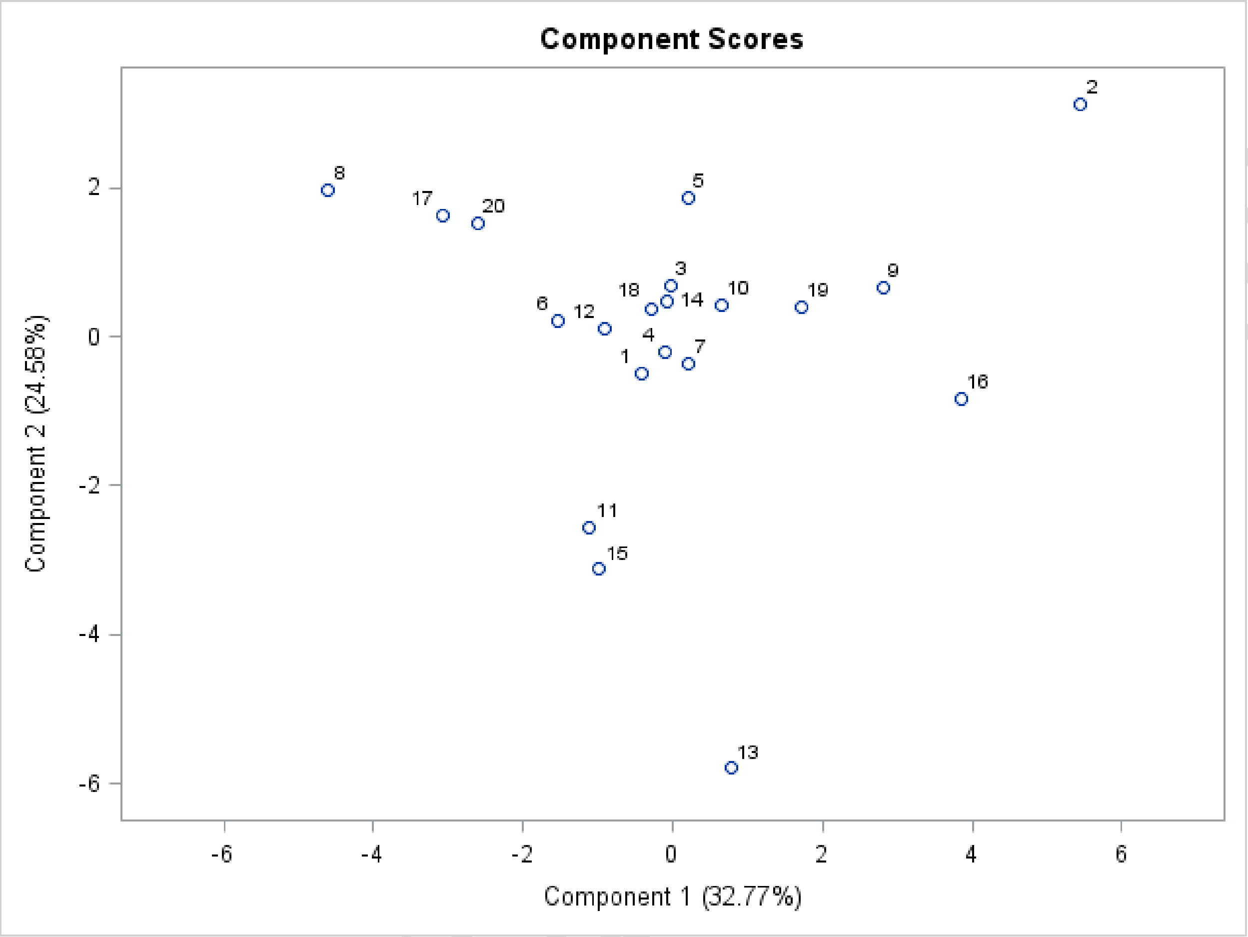


Fig. 2. Components scores plot of 21 maize genotypes subjected to infestation by *S. zeamais*

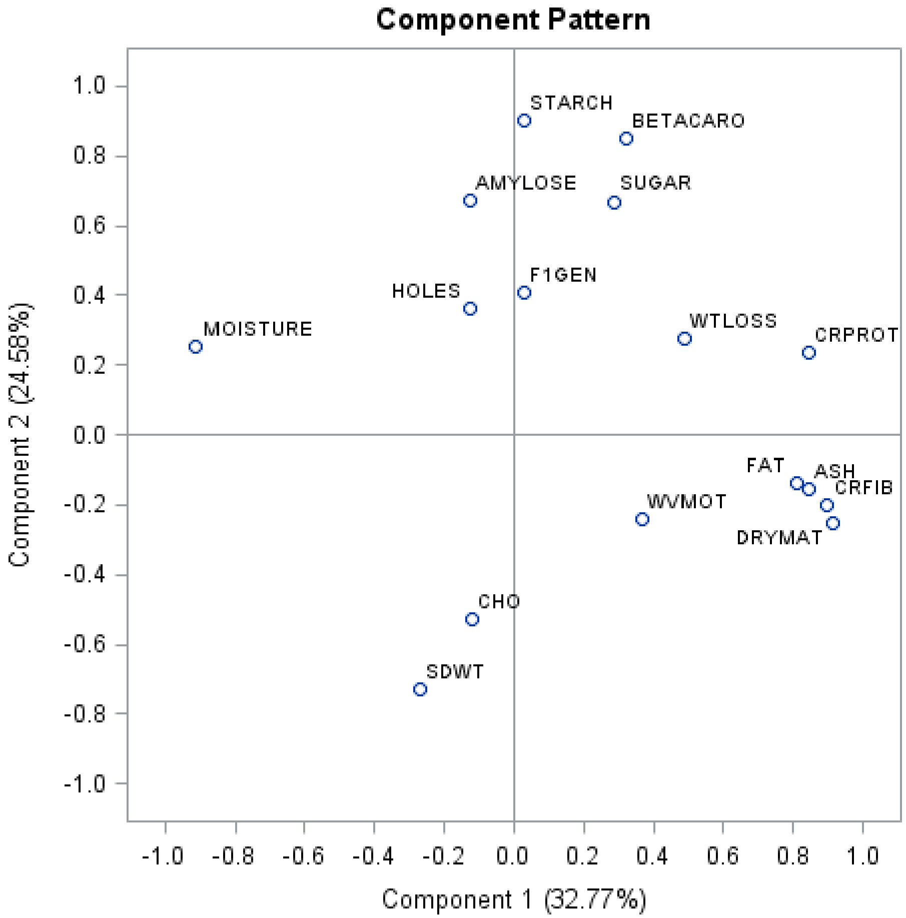
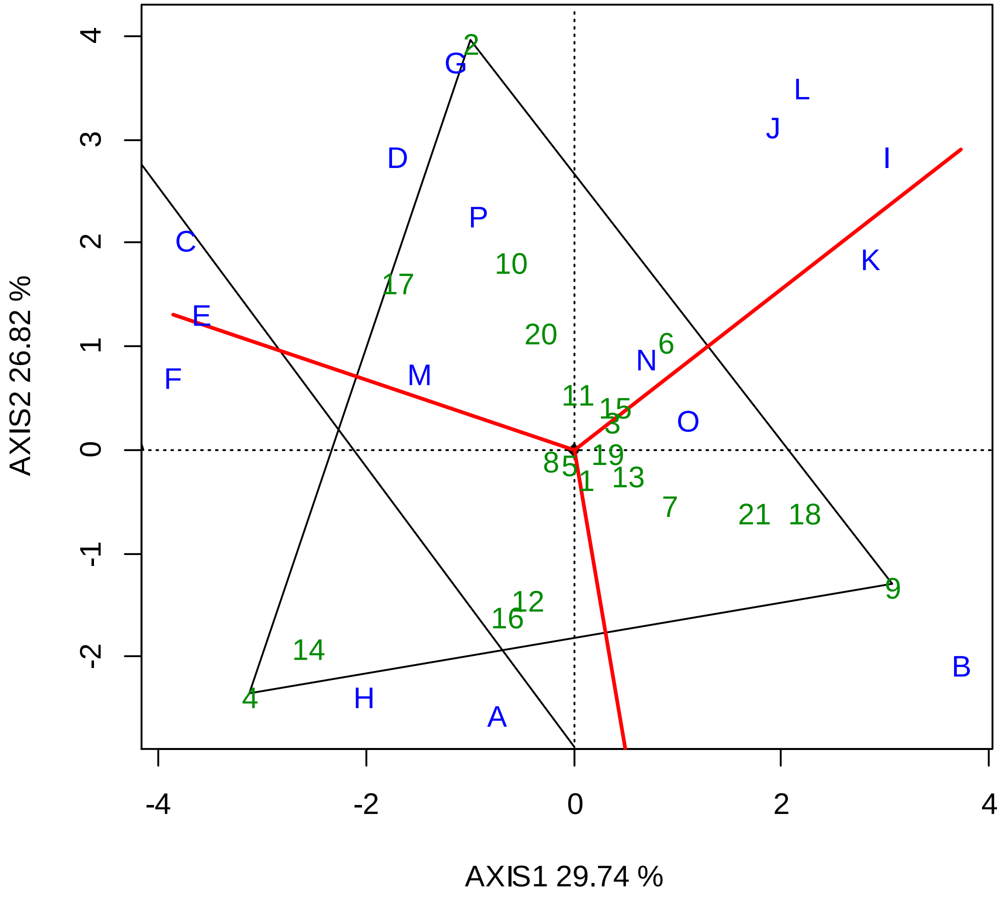


Fig. 3. Component loadings of sixteen measured traits of maize genotypes subjected to infestation by *S. zeamais*

3.4 Genotype × trait biplot analysis

Figure 4 is a polygon view of the GT biplot for a visual display of the trait profiles of the 21 maize genotypes. Three distinct sectors were revealed by the polygon view. Genotype EVDT-Y2008 STR was at the vertex of sector 1 which was composed of IFE-TZEE-WSTR, NA/SA/07/018, NG/SA/07/022, NG/SA/07/140, NG/SA/07/184, TZM 212, and TZB-SR B. Sector 2 had NG/SA/07/122 as the vertex genotype while other genotypes in the sector were 06CHTTW19, NG/SA/07/029, TZM-1296, OBA SUPER 11 F2, TZM 1311, and TZM 144. In the same vein, sector 3 was composed of genotypes NA/SA/07/018, NG/SA/07/044, NG/SA/07/72, TB87/97/15, TZB-SR A with Igbogbo local at the vertex. Genotypes in sector 1 were associated with dry matter, fat, ash, crude protein, starch, sugar and beta carotene contents as well as weevil mortality, F_1 insect generation and seed weight loss. Sector 2 was associated with moisture and amylose contents, and number of holes on seeds while sector 3 was associated with carbohydrate content and seed weight. The GT biplot is an effective tool for multivariate evaluation of crop genotypes. It permits a visual ranking of entries' performance and associates individual genotypes with related traits. Similarly in application to the GGE and GYT biplots, the GT polygon divides genotypes into sectors each with its associated closely-related traits, a vertex and other member genotypes (Oyetunde et al., 2021; Yan and Fregeau-Reid, 2018). Genotypes EVDT-Y2008 STR, IFE-TZEE-WSTR, NA/SA/07/018, NG/SA/07/022, NG/SA/07/140, NG/SA/07/184, TZM 212, and TZB-SR B thus showed superior trait profile for dry matter, fat, ash, crude protein, starch, sugar and beta carotene contents, weevil mortality, F_1 insect generation and seed weight loss. This is an indication that the genotypes would be resourceful in improving the associated traits, and consequently, reaction to infestation by *S. zeamais*. The genotypes delineated to sectors 2 and 3 would also be useful in schemes focused on developing maize for the associated traits. The vertex genotype in each sector would likely be the most effective in improving the associated traits.



KEY				
ID	**Description**		**ID**	**Description**
1	06CHTTW19		20	TZM 212
2	EVDT-Y2008 STR		21	TZM-1296
3	IFE-TZEE-WSTR		A	Seed weight
4	IGBOGBO LOCAL		B	Moisture
5	NA/SA/07/018		C	Dry matter
6	NG/SA/07/022		D	Fat
7	NG/SA/07/029		E	Ash
8	NG/SA/07/044		F	Crude fiber
9	NG/SA/07/122		G	Crude protein
10	NG/SA/07/140		H	Carbohydrate
11	NG/SA/07/184		I	Starch
12	NG/SA/07/72		J	Sugar
13	OBA SUPER 11 F2		K	Amylose
14	TB87/97/15		L	Beta Carotene
15	TSL COMPOSITE 4		M	Weevil mortality
16	TZB-SR A		N	F_1 insect generation
17	TZB-SR B		O	Number of holes on seeds
18	TZM 1311		P	Seed weight loss
19	TZM 144			

Fig. 4. The polygon view of the genotype × yield-trait biplot of 21 maize genotypes evaluated in the study.

4. CONCLUSIONS

There was evidence of adequate genetic variability among the maize genotypes, which permitted grouping into clusters. Thus, heterosis can be exploited through parents selected from different clusters. Four of the six most important traits to the observed variation namely dry matter, crude protein, ash, and fat contents had strong positive association with weevil mortality, F1 insect generation, and seed weight loss. This implies that maize reaction to the weevil, *S. zeamais*, can be influenced through biofortification, with genotypes in sector 1 as target genotypes. Further research should focus on determining possible jettisoning of quality to improve resistance to *S. zeamais*.

REFERENCES

1. FAO. http://www.fao.org. 2013. Internet Information Accessed on 16 February; 2021.

2. Mustafa HSB, Ahsan M, Aslam M, Ali Q, Ejaz-ul-Hasan Bibi T, Mehmood T. Genetic variability and traits associationin maize (*Zea mays* L.) accessions under drought stress. Journal of Agricultural Research. 2013;51(3):231-238.

3. Chaudhry AR. Maize in Pakistan. Punjab Agri. Res. Coordination Board, Uni. Agri. Faisalabad, Pakistan. 1983.

4. Ali Q, Ahsan M, Kanwal N, Ali F, Ali A, Ahmed W, Ishfaq M, Saleem M. Screening for drought tolerance: comparison of maize hybrids under water deficit condition. Advancements in Life Sciences. 2016;3:51-58.

5. Obok E, Ittah M, Udo I, Ojubi E, Ulafor J, Umoetok S.. Genetic variability and seasonal influence of fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) damage to early vegetative growth of African maize accessions in the humid tropics of south-eastern Nigeria. Journal of Animal & Plant Sciences. 2021;31(1):143-155.

6. Megan N, Hope R, Cairns V, Aldred D. Postharvest fungal ecology: Impact of fungal growth and myco-toxin accumulation in stored grain. European Journal of Plant Pathology. 2003;109:723-730.

7. Rees D. Insects of stored products. CSIRO Publishing, Collingwood, Victoria, Australia. 2004.

8. Pereira C, Perereira E, Cordeiro E, Lucia TD, Tótola M, Guedes R. Organophosphates resistance in the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae): magnitude and behavior. Crop Protection. 2009;28:168-173.

9. Mwololo JK, Mugo S, Okori P, Tefera T, Otim M, Munyiri, SW. Sources of resistancet o the maize weevil *Sitophilus zeamais* in tropical maize. Journal of Agricultural Science. 2012;4(11):206-215.

10. Asish K, Manivannan N, Varman PV. Character association and path analysis in sunflower. *Madras Agricultural Journal.* 2008;95(7-12):425-428.

11. SAS Institute. The SAS System for Windows. 2014;Version 9.3. Cary, NC: SAS Inst.

12. Choukan R, Hossainzadeh A, Ghannadha MR, Warburton ML, Talei AR, Mohammadi SA. Use of SSR data to determine relationships and potential heterotic groupings within medium to late maturing Iranian maize inbred lines. Field Crops Res. 2006;95:212-222.

13. Oyetunde OA, Olayiwola MO, Osho BT. Genotypic diversity and trait profiles of some *Amaranthus* accessions. Adv. Hort. Sci. 2021;35(3):277-284.

14. Shrestha J. Cluster Analysis of Maize Inbred Lines. Journal of Nepal Agricultural Research Council. 2016;2:33-36.

15. Magudeeswari P, Divakara Sastry EV, Devi ThR. Principal component (PCA) and cluster analyses for plant nutrient traits in baby corn (*Zea mays* L.), Indian J. Agric. Res. 2019;53(3):353-357.

16. Semassa AJ, Anihouvi VB, Padonou SW, Adjanohoun A, Aly D, Gbenou J, Baba-Moussa L. Nutritional Properties Assessment of Endogenous and Improved Varieties of Maize (Zea mays L.) Grown in Southern Benin. Pakistan Journal of Biological Sciences. 2017;20:267-277.

17. Yan W, Fregeau-Reid J. Genotype by yield × trait (GYT) biplot: a novel approach for genotype selection based on multiple traits. Scientific Report. 2018;8:8242.