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BREEDING FOR WEEVIL (*Sitophilus Zeamais* Motschulsky) RESISTANCE IN MAIZE (*Zea mays* L)

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ABSTRACT

A study was conducted with an overall objective of establishing the determinants of weevil resistance in maize. Field experiments were done at GART using a North Carolina Design II with three replications and also at Nanga research in Mazabuka. Laboratory experiments were conducted at Zambia Agriculture Research Institute (ZARI) Entomology laboratory and University of Zambia, Food Science Department where insect bioassay and the biochemical tests were done respectively. In the insect bioassay grain hardness, grain weight loss, median development period, F1 progeny emergence and the Dobie index of susceptibility indices were measured. Protein and the Phenolic content were determined under the biochemical tests among genotypes. They were highly significant differences in all the twenty seven genotypes evaluated. Results showed that Parental survival accounted for 78.5 % of the total variation, Phenolic content was strongly and positively correlated (r = 0.423***) with grain hardness providing a good measure of resistance accounting for the 10.9 % of the total variation. The study showed the possibility of breeding maize genotypes with an increased resistance and also susceptible lines had a decreasing Phenolic content but increasing Phenolic content resulted in increased resistance. It was therefore concluded that Phenolic content and parental survival can be used as an indirect selection criteria for weevil resistance.

Contribution/Originality: This study is one of very few studies which have investigated the development of the Host-plant resistance as a pest control method is environmentally safe, economically cheaper method to farmers and most compatible with other components in the Integrated Pest Management initiatives.

1. INTRODUCTION

Maize, *Zea mays* L., is now one of the principal cereal food crops in the tropics and sub tropics (Makate, 2010). Maize is an essential component of the global food security and forms a major part of the diet of millions of people including Zambia (Kamanula et al., 2011). It grows under a wider range of ecological conditions depending on the variety (ASARECA–TUUSI, 2009). The crop is versatile in its use, environmental adaptation and it is also consumed all over the world by both humans and animals (Keba & Waktole, 2013).Attempts have been made through breeding programmes for high yielding, as well field pests and disease resistant varieties which has resulted into many hybrids and composite varieties made available to farmers. Despite these high yielding varieties, farmers do not gain the maximum possible benefits from maize production if the quantity and the nutritional quality reduce (FAO, 1991). This is due to the various constraints including pest infestations at different levels of

the production cycle particularly during storage phase. Storage losses due to pests threaten livelihoods of farmers across Africa (Kamanula et al., 2011). The maize weevil, *S. zeamais* is the most serious storage pest of maize in the tropics (Bosque-Perez & Buddenhagen, 1992). The maize weevil affects the crop before harvest and multiplies further after storage (Caswell, 1962). Therefore development of the host-plant resistance as a pest control method is environmentally safe, economically cheaper to farmers and most compatible with other components in the integrated pest management (IPM) initiatives (Chapman, 2000). The overall objective of this study was to establish mechanisms of weevil resistance in maize, whose specific objectives were;

1. To characterize genotypes for traits related to weevil resistance in maize.
2. To estimate the genetic basis of the mechanisms of weevil resistance in maize.

2. MATERIALS AND METHODS

2.1. Study Area

A field experiment was set up at Golden Valley Agricultural Research Trust (GART) 80km north of Lusaka, Zambia during the 2011 – 2012 growing season in which hybridization was done. The field experiment was laid out using a North Carolina design II in which eight female lines from Zambia Agriculture Research Institute (ZARI) and nine male lines from CIMMYT were crossed. Plant emergence in some lines was poor due to poor synchronization and also due to the breakdown of the pump at Nanga during the critical periods of production; only twenty seven lines were successfully used for evaluation of weevil resistance.

2.2. Laboratory Analysis

Laboratory analysis was done in two ways: weevil bioassay and through biochemical bioassay. The laboratory analysis were done according to the procedures of Dobie (1977) and also (Makkar, 2003).

2.3. Weevil Bioassay

Laboratory analysis based on the weevil bioassay was done using the modified Dobie's method (Dobie, 1974; Serratos, Blanco-Labra, Arnason, & Mihm, 1997). The freshly harvested seed of each variety were first sun dried to about 12-13 % moisture content. Five cobs of each genotype were hand shelled and the grain was packed into 5 x 8 polythene bags and then they were closed by using the rubber bands and were then stored in a deep freezer at the temperature of -16°C for one week to kill any previous infestation by the insects which included the adults, larva or eggs (Kossou, Mareck, & Bosque-Perez, 1993).

Fifty (50) grams of the grains, from each plot was placed into new 350 ml plastic jars, measuring 11.7 cm in height and about 5.2 cm in diameter at the mouth. The tops of the lids of these jars were cut out leaving only the screw top rings. The polythene screen lids allowing ventilation and preventing the escape of insects were placed on jars. Forty unsexed weevils of mixed age, in no choice experiments (Serratos et al., 1997) were initially counted into vials with the help of pairs of tweezers and a denominator Multiple - Tally counter were put into each jar.

Weevils were kept for ten (10) days for the *S. zeamais* to oviposit as described by Derera, Pixley, and Giga (2001). After 10 days sieving was done to remove adult weevils. Live and dead weevils were counted. Tweezers were used to probe immobile weevils to establish whether they were dead or alive. Progeny emergence counts were made every two days beginning 25 days after the removal of the parent insects and ending when all progeny (F_1) had emerged. Emerged progenies were removed from the jars at each count (Siwale, Mbata, McRobert, & Lungu, 2007).

Seeds of each variety without the *S. zeamais* were kept under similar conditions and served as controls. The treatments were arranged in a Completely Randomized Design replicated three times. The jars were placed in the controlled temperature and the relative humidity room at 27- 30°C temperature and 43 to 60% relative humidity. The relative humidity was provided by water placed in four troughs (Bekele & Hassanali, 2001). The genotypes

were kept undisturbed (except times of sieving the F_1 emergency) in the controlled temperature and relative humidity room for ninety days (90) days then assessment was done on physical parameters of seed as shown below.

2.4. Grain Hardness

Fifty grammes of each sample were weighed. Each sample was ground in a laboratory mill under the brand name of Retsh ™, Type ZM 1000 (GmbH & Co. KG 5657 HAAN 1, Germany). The speed and the time setting were at 10000 revolutions per minute. The collected meal was put back in labelled plastic bags. The meal was then hand sifted. The collected flour and retained grit were emptied in separate labelled 5 x 8 cm white plastics and these were subsequently weighed and data recorded.

The weight of the grit and flour were added together for each genotype to get the total weight, which was about the same weight as the original weight of grain from where flour and grit samples were derived. Grain hardness was expressed as percent grit of total weight of sample (grit plus flour after sieving a 50g ground maize sample). Therefore grit percentage was used as a proxy for grain hardness.

2.5. Seed Damage and Weight Loss

Ninety (90) days after incubation, the glass jars were opened. The content in each jar was separated into grains, insects and dust using 4.7 and 1.0mm sieves to assess each genotype's seed damage. The weight of dust produced was recorded. The damaged seeds (holed seeds) and the undamaged seed by the *S.zeamais* were counted. Seed damage was expressed as a proportion of the total number of seed sampled (Abebe, Tefera, Mugo, Beyene, & Vidal, 2009). Seed weight loss was determined using the count and weight method (Gwinner, Harnisch, & Muck, 1996).

Weight loss (%) = (Wu x Nd) – (Wd x Nu) x 100 / Wu x (Nd + Nu);

Where Wu = Weight of undamaged seed.

Nu = Number of undamaged seed.

Wd = Weight of damaged seed.

Nd = Number of damaged seed.

2.6. Dobie's Susceptibility Index

The Dobie index was used as a criterion to separate varieties into different resistance groups. The Dobie Index of susceptibility was calculated using the formula

I = nlog (no of adult weevil progeny emerged)/MDP

Where I = Dobies's susceptibility index

MDP = median development period

The Dobies' index was used to separate maize genotypes into resistance or susceptibility groups following the scales used by CIMMYT (Pixley, 1997) which is as follows:

Dobie relative index of less than or equal to 4 was classified as resistant.

Dobie relative index of 4.1 to 6.0 classified as moderately resistant.

Dobie relative index of 6.1 to 8.0 classified as moderately susceptible.

Dobie relative index of 8.1 to 10 classified as susceptible.

Dobie relative index of more than 10 was classified as highly susceptible.

2.7. Biochemical Bioassay

2.7.1. Protein Content

The Kjeldahl procedure (Barbano, Lynch, & Fleming, 1991) was used to determine the crude protein content. Twenty grams sample of the whole maize kernels were ground in a laboratory mill for each genotype. Three replications for each sample were used.

2.8. Phenolic Acid Content Determination

This was determined by using the Folin – Ciocalteu method (Makkar, 2003) using the UV- Spectrophotometer. Tannic acid was used as a standard and this was done in three replications.

2.9. Statistical Analysis

The analysis of variance (ANOVA) for all the measured parameters was done using the GENSTAT Thirteen Edition and SPSS 16.0. Heritability was estimated using the North Carolina design II (Hallauer & Miranda, 1988).

3. RESULTS.

3.1. Protein Content

Genotypes were significantly different ($p < 0.05$) from each other for crude protein with an overall mean content of 8.8% as shown in Table 2. Among genotypes crude protein was in the range of 7.2 % to 11.4%. Genotypes 60N and 24N had high significant crude protein whose means were 11.4% and 10.6% respectively Table 2. Lower levels of crude protein were found in genotypes 95U (7.8%), 38N (7.7%) and with the lowest in 78N (7.4%) when compared to other genotypes Table 2.

3.2. Phenolic Content

Genotypes were highly significantly different ($p < 0.05$) for Phenolic content Table 1. The Phenolic content among genotypes was found to be between 24 and 80.7 mg per 100g of maize grain. The mean Phenolic content among genotypes was 55.8mg/100g of maize grain. Genotype 60N had the highest Phenolic content of 80.7 mg per every 100g of grain. The lowest level of phenolics was found in genotype 78N (24mg per 100g of grain).

3.3. Parental Survival

Parental survival of *S. zeamais* among the genotypes was significantly different ($p < 0.05$). Table 2 presents the number of live adult weevils obtained after the ten days oviposition period. The overall mean survival number for parent weevils at the end of the oviposition period was 12.6. The number of parental survival among the entries was in the range of 3.0 to 32.7. The highest parental survival was recorded from genotype 78 N (32.7) which was not statistically different from 67N (28.0). Least parental survival numbers were also found among the 31N (3.0), 60N (3.0) and 74N (3.0) genotypes.

3.4. Progeny Emergence

The F_1 progeny emergency among the genotypes was significantly different ($p < 0.05$) among genotypes Table 1. The grand mean for progeny emergence was 15.5 and the range of emergence among entries was 2.3 to 98 weevils. Among all the maize genotypes evaluated, significantly higher numbers of F_1 progenies (98) emerged from genotype 78N. Lowest numbers of F_1 progenies emergence were found in genotypes 60N (2.3), 74N (3.0), 4N (3.0) and 31N (4.0).

3.5. Median Development Period

The median development period among the genotypes was significantly different among the genotypes ($p < 0.05$). The median development period ranged from 26 to 79.8 days with the grand mean median development period of 50.3 days Table 2. Genotype 1N had a statistically higher median development period of 79.8 days. Genotype 78N had the least development period of 26.0 days.

Table-1. Summary of the combined analysis of the analysis of variance of the measured traits in the maize genotypes.

Source	DF	SW	GH	PS	F_1E	MDP	MC	GWL	PR	PH	SI
Entry	26	0.006***	21.82***	21.2***	1080.9***	806.155***	1.0635**	25.43***	6.323***	6979.6***	8.98***
CV (%)		2.4	6.3	7.4	5.5	1.6	4.7	3.5	3.1	4.2	8.4
S.E.D		0.0073	2.171	0.748	0.6789	0.754	0.6719	0.1592	0.2167	2.187	0.1414

Note: *** = significant at 5% level

Key: SW = 100seed weight, GH = grain hardness, PS = Parental survival, F_1E = progeny emergency, MDP = median development period, MC = moisture content, GWL = grain weight loss, PR = protein, PH = Phenolic, SI = susceptibility index.

Table-2. Summary of means for the genotypes.

Entry	MDP	PS	F_1	PH	GWL	MC	PR	SI	GH	100SW
1N	79.84	4.00	4.00	80.00	4.53	11.60	9.23	0.75	47.83	35.24
4N	78.10	8.67	3.00	78.67	4.60	12.57	9.57	0.61	45.84	35.44
77N	63.10	10.00	9.00	58.00	4.67	13.23	9.30	1.65	39.71	30.25
60N	59.45	3.00	2.33	80.67	4.27	12.40	11.37	0.37	51.67	35.44
8N	52.30	18.33	29.00	68.33	6.93	11.77	9.33	2.14	39.24	36.02
6N	56.41	11.00	15.00	73.00	4.80	12.60	10.23	1.61	43.29	36.27
74N	78.14	3.00	3.00	58.00	2.47	13.43	9.20	0.82	43.07	36.66
24N	62.72	4.33	4.00	75.33	4.93	12.83	10.60	0.80	47.49	33.83
26N	31.00	29.00	27.00	31.00	6.73	12.20	7.57	4.62	34.50	30.87
73U	52.45	21.00	10.33	66.33	4.67	12.70	9.60	1.53	38.04	38.30
31N	69.72	3.00	4.00	70.67	4.13	12.60	7.87	0.85	45.28	38.47
67N	29.04	28.00	31.00	29.00	7.13	12.93	8.70	5.14	38.17	42.87
19N	71.43	4.00	5.00	51.00	5.13	11.77	9.00	1.37	39.13	36.66
56N	53.65	4.33	4.67	58.00	4.13	11.80	9.23	1.15	37.67	45.54
13N	29.00	12.33	21.33	62.00	4.67	11.60	7.17	2.14	40.82	35.40
45U	41.35	9.67	8.00	73.67	4.33	13.47	8.47	1.23	37.80	43.10
80U	46.35	18.33	30.00	47.00	7.00	12.67	8.20	3.14	43.22	38.50
66U	45.18	13.33	10.67	66.33	6.47	13.30	9.00	1.55	43.07	39.65
46U	45.90	8.67	6.00	67.33	4.87	12.47	8.80	1.16	41.67	43.60
63U	57.14	8.00	14.67	46.00	4.73	12.77	9.23	2.54	43.17	38.67
91U	48.55	7.00	7.00	45.33	4.93	13.87	8.37	1.86	38.50	38.47
12N	26.89	22.00	20.00	32.00	7.40	12.77	7.83	4.07	38.60	34.87
38N	37.34	20.67	5.33	28.00	5.60	12.87	7.73	4.20	39.13	35.25
78N	26.00	32.67	98.00	24.00	19.07	12.10	7.40	8.30	33.78	41.10
95U	33.28	19.67	23.67	33.00	4.60	13.60	7.80	4.16	40.37	40.87
80N	40.11	10.33	9.00	53.00	4.47	12.50	8.40	1.80	38.50	32.00
74U	56.03	5.33	5.00	50.00	5.20	12.00	9.10	1.40	41.13	38.63
Mean	50.26	12.58	15.18	55.76	5.65	12.61	8.82	2.26	41.14	37.4
LSD	1.482	1.513	1.848	7.85	0.316	0.133	0.433	0.276	1.748	0.014

Key:100sw = one hundred seed weight, GH =grain hardness, PS = parental survival, F1= f1 progeny emergence, MC =moisture content, GWL=grain weight loss, PR = Protein, PH = Phenolic, SI =susceptibility index, MDP = Median Development.

3.6. Grain Hardness

Grain hardness showed discrimination among genotypes. They were significant differences among the genotypes ($p < 0.05$). Grand mean hardness value of 41.1% was observed among genotypes. Genotype 60N was statistically higher than the other genotypes with 51.7% and genotype 1N had similarly a higher grain hardness value of 47.8%. Genotypes 26N (34.5%) and 78N (33.8%) had statistically lower grain hardness values.

Table-3. Correlation coefficients of *S. zeamais* infestation of the maize genotypes.

PAR	SI	F_1E	MDP	GWL	GH	100SW	PS	PH	PR
SI	1								
F_1E	0.197	1							
MDP	-0.312	-0.082	1						
GWL	-0.308	0.083	0.189	1					
GH	-0.361***	0.085	-0.355***	-0.131	1				
100SW	-0.473***	0.093	0.171	0.167	-0.138	1			
PS	0.612***	-0.316	-0.504***	0.248	-0.122	0.052	1		
PH	-0.213	-0.048	-0.27	0.09	0.423***	0.14	-0.225	1	
PR	-0.155	0.172	-0.289	-0.151	-0.042	0.06	-0.095	-0.144	1

Key: SI =susceptibility index, F1 E = F1 emergency, MDP = median development period, GWL = grain weight loss, 100SW = 100 seed weight, PS = parental survival, PH = Phenolic, PR = proteins, PAR = parameter.

3.7. Dobie Index of Susceptibility (SI)

Significant differences ($p < 0.05$) were observed on index of susceptibility among the genotypes evaluated Table 1. The SI in this study ranged from 0.4 to 8.3 for genotypes 60N and 78N respectively. According to the

CIMMYT classification, out of the twenty seven maize genotypes evaluated against *S. zeamais* for resistance, twenty three genotypes were found to be relatively resistant; three genotypes 26N, 12N and 67N were moderately resistant. Only one genotype (78N) was moderately susceptible.

The results Table 3 shows an inverse relationship between the susceptibility index (SI) and median development period, grain weight loss (%), grain hardness, 100 seed weight, Phenolic and protein. The parental survival ($r = 0.612^{***}$) and F_1 progeny emergencies ($r = 0.197$) were positively correlated to susceptibility index. The inter component correlations among traits showed that median development period was positively correlated with grain weight loss ($r = 0.189$), 100 seed weight ($r = 0.171$) though they were not significant ($p > 0.05$). Seed weight was also positively correlated to grain weight loss ($r = 0.167$) but it was not significant($p > 0.0$.

3.8. Stepwise Multiple Regression

Significant contributions to the total variation were observed from the four traits namely parental survival of adult weevils, Phenolic content, F_1 progeny emergency and grain hardness. Parental survival had a most significant influence on susceptibility index of 78.5% of the total variation Table 4. Additional of other variables such as Phenolic, F_1 progeny emergency, and grain hardness also showed significant influence of 10.9%, 8% and 0.5% respectively.

Table-4. Stepwise correlation of susceptibility index and other traits.

Variable	Partial square	R-model square	R-F value	Pr >F
Parental survival	0.785	0.785	91.452	0.000
Phenolics	0.109	0.894	24.589	0.000
F_1 emergency	0.08	0.974	71.874	0.000
Grain hardness	0.005	0.979	5.584	0.000

Table-5. Classification of Maize weevil resistance among genotypes using Dobie Index.

Entry	Dobie Index	Classification
1N	0.75	Resistant
4N	0.59	Resistant
77N	1.28	Resistant
60N	0.48	Resistant
8N	2.14	Resistant
6N	1.61	Resistant
74N	0.8	Resistant
24N	0.8	Resistant
73U	1.53	Resistant
31N	0.84	Resistant
67N	5.14	Moderately resistant
19N	1.37	Resistant
56N	1.14	Resistant
13N	2.14	Resistant
45U	1.23	Resistant
80U	3.14	Resistant
66U	1.55	Resistant
46U	1.16	Resistant
63U	1.71	Resistant
91U	1.86	Resistant
12N	4.07	Moderately resistant
38N	1.4	Resistant
78N	8.34	Moderately susceptible
95U	2.88	Resistant
80N	1.8	Resistant
74U	1.29	Resistant
26N	4.62	Moderately resistant

3.9. Genetic Parameters

It was observed that the non additive variation controls all the traits that were measured in this study Table 7. The calculated narrow sense heritability was low in most traits ranging from 5.9 to 22.2% Table 6. Heritability analysis according to Derera et al. (2001) on genetic analyses can be performed only for the index of susceptibility because it incorporates all resistance parameters. Therefore in this study it was estimated that heritability of the traits was 20.9% since this was the heritability based on susceptibility index in this study Table 6. The heritability estimate was considered low since it was below 30 %.

Table-6. Estimated genetic parameters for some traits in the maize genotypes.

Variance Component	F1E	MDP	Grain weight loss	Hardness	Kernel weight	Phenols	Proteins	Susceptibility Index
σ_{Am}^2	157.36	542.2	2.69	38.76	0.000025	107.2	0.156	3.756
σ_{Af}^2	-23.52	-174.8	0.296	13.72	0.00114	-40	-0.4753	-0.648
σ_T^2	66.9	183.68	1.496	26.24	0.000582	33.6	0.3318	1.554
σ_D^2	239	812.2	3.752	68.68	0.006	39.76	5.53	4.3
h^2 (%)	17.9	15.6	22.2	21.65	8.12	8.3	5.9	20.9

Table-7. Summary for the average degree of dominance for the traits.

Trait	Degree of dominance
F_1 emergence	1.74
Median development period	1.73
Grain weight loss	1.67
Grain hardness	1.88
Mortality (%)	1.69
Parental survival	2.80
Phenolic content	6.33
Protein content	8.67

Table-8. Mean squares for susceptibility index and the other agronomic traits among the parental lines.

Source of Variation	df	MDP	PS	F1	PH	GWL	PR	GH	SI
Rep	2	0.79	0.3	0.28	4.12	10.76	9.16	3.26	0.16
Crosses	15	0.21*	0.28*	1.87	22.65**	8.7*	4.61	2.87**	0.41**
GCA males	3	0.68	0.23	0.89	25.46**	6.8*	3.55	1.51*	0.58 *
GCA females	3	0.26	0.17	0.55	11.71**	3.9**	3.33	2.86**	0.56*
SCA	9	0.09	3.55	0.08	12.41*	4.1**	1.81	2.14	0.35
Error	30	0.55	0.99	3.61	3.29	0.34	4.61	4.12	1.4
CV %		1.5	2.2	3.4	4.6	3.2	2.6	3.8	3.6

Note: *, ** significant at 5% and 1% probability level respectively.

Table-9. SCA effects.

Entry	CROSS	MDP	PS	F1	PH	GWL	PR	SI	GH	100SW
1N	151 x10075	29.58**	-8.58	-11.18**	24.24**	-1.12	0.7	-1.51	6.69**	-2.16*
67N	151 x 10096	-34.45	15.42**	15.82**	-26.76**	1.48*	0.17	2.88**	-2.97**	5.47**
74U	151 x 10111	5.77*	-7.25	-10.18**	-5.76	-0.45	0.57	-0.86	-0.01	1.23
60N	151 x 10112	9.19**	-9.58	-12.85**	24.91**	-1.38	2.84**	-1.89**	10.53**	-1.96
74N	152 x 10075	27.88**	-9.58	-12.18**	2.24	-3.18	0.67	-1.44	1.93	-0.74
56N	152 x 10096	3.39	-8.25	-10.51**	2.24	-1.52	0.7	-1.11	-3.47	8.14*
63U	152 x 10111	6.88*	-4.58	-0.51	-9.76	-0.92	0.7	0.28	2.03	1.27
38N	152 x 10112	-12.92	8.09**	-9.85**	-27.76**	-0.05	-2.8**	1.94	-2.01	-2.15
24N	1212 x 10075	12.46**	-8.25**	-11.18**	19.57**	-0.72	2.07**	-1.46	6.35	-3.57**
12N	1212 x 10096	-23.37**	9.42**	4.82	-23.76**	1.75	-0.7	1.81	-2.54	-2.53*
77U	1212 x 10111	12.84**	-2.58	-6.18**	2.24	-0.98	0.77	-0.61	-1.43	-7.15*
91U	1212 x 10112	-1.71	-5.58*	-8.18**	-10.43	-0.72	- 0.16	-0.4	-2.64	1.07
6N	917 x 10075	6.15**	-1.58	-0.18	17.24	-0.85*	1.7	-0.65	2.15	-1.13
80U	917 x 10096	-3.91	5.75**	14.82**	-8.76	1.35	-0.33	0.88	2.08	1.1
4N	917 x 10111	27.84**	-3.91	-12.18**	22.91**	-1.05	1.04	-1.65	4.7	-1.96*
80N	917 x 10112	-10.15**	-2.25	-6.18**	-2.76*	-1.18	- 0.13	-0.46	- 2.64	-5.4**
s.e.d		0.56	0.399	0.861	0.319	0.2232	0.1361	0.1211	0.67	0

Note: *, **, significant at 5% and 1% probability level respectively.

Table-10. GCA effects for the parental lines.

Males	F1	GWL	SI	MDP	PH	PR	PS
10075	-9.09**	-6.38**	-2.57**	41.54**	27.19**	1.93**	-16.16**
10096	-7.75**	-6.88**	-2.63**	24.54**	29.69**	1.06**	-6.50**
10111	-2.75**	-6.05**	-2.96**	40.54**	18.53**	-0.30*	-14.16**
10112	3.91**	-3.95**	-1.62**	9.54**	40.79**	-0.30*	-14.16**
s.e.d	0.84	0.21	0.08	0.5	0.28	0.126	0.38
Females							
151	2.25**	4.62**	2.17**	-28.79**	-29.74**	-0.47*	12.90**
152	3.91**	6.12**	2.51**	-30.46**	-27.51**	-0.87**	7.37**
917	5.91**	5.75**	2.20**	-27.46**	-28.11**	-0.77*	18.44**
1212	3.58**	6.75**	2.89**	-29.46**	-30.84**	-0.87*	7.37**
s.e.d	0.861	0.2232	0.1211	0.56	0.319	0.1361	0.3991

Note: *, ** indicates significance at the 0.05 and 0.01 levels of probability respectively.

3.10. Combining Ability

The mean squares due to general combining ability were significant for all variables measured. However, the mean squares due to the specific combining ability were not significant for the same traits Table 8.

Estimates of the Specific Combining Ability (SCA) and the General Combining Ability (GCA) effects for the various traits are presented in Table 9 and 10 respectively.

4. DISCUSSION

The results were discussed in two parts.

4.1. Factors Related to Weevil Resistance in Maize

Protein content was negatively correlated with the susceptibility index of maize genotypes. This was consistent with findings reported by Dobie (1977); Keba and Waktole (2013). Furthermore, genotypes in this study with higher protein content were classified to be resistant based on CIMMYT (2001) classification. This was evident in genotype 60N which had the highest protein content (11.3%) and the number of adult weevils surviving at the end of the experiment on this genotype was only 3.0. The lowest genotypes in terms of protein content were genotypes 95U (7.8%) and 78N (7.4%) which had parental survival numbers of 19.7 and 32.7 respectively. This was also consistent with what other researchers found out (Derera et al., 2001; García-Lara et al., 2004).

Further analysis with the stepwise regression analysis which is a stronger tool than correlation for use in indirect selection showed that protein content was not significant in the observed susceptibility index. This suggests that none of the maize varieties tested was completely resistant for proteins. These findings were consistent with Siwale et al. (2007); Tongjura, Amuga, and Mafuyai (2010). Although protein may seem to have some antibiosis effect, lack of a definite relationship with physical resistance parameters in this study may indicate other resistance factors in maize studied.

Arnason et al. (1997) also reported on the presence of biochemical compounds, Phenolics especially the ferulic acid in the maize grain in conferring resistance. The level of the Phenolic compounds was negatively correlated with susceptibility index. This was in agreement with the findings reported by Dobie (1977). It was also noticed that genotypes with the highest amount of the phenols like genotype 60N (80.676mg/100g) had less grain weight loss (4.3%) since weevil attack may have been prevented by the amounts of Phenolic compounds particularly ferulic acid component. Genotypes with lower amounts of Phenolic, 78N, had 24mg/100g of grain had a higher grain weight loss of 19%. This was also consistent with other authors Derera et al. (2001); Classen et al. (1990); Sen, Mukhopadhyay, Wetzel, and Biswas (1994); Arnason et al. (1997). These authors reported that phenolic compounds particularly ferulic acid had an influence on the hardness of the grain such that it was able to make the cell walls hard and limit the biodegradability of the cell wall polysaccharides by insects. The Phenolic acids were able to cause

adverse effects to weevil feeding behavior and survival. Therefore, biochemical screening of the maize grain may be used as a first step towards selection of genotypes for resistance.

In terms of grain weight loss, resistant maize varieties had a minimum grain damage and small quantity of powder formed. Grain weight loss was highest in genotype 78N in which there was a 19 % grain weight loss. The median development period among the genotypes had an average of 50.3 days. The range of the median development period was wider (26 to 79.8 days). The period was longer in the resistant genotype (60N) in which the median development period was 79.8 days but median development period was shorter in the susceptible genotype 78N with median development period of 26 days. For susceptible genotypes the development period of weevils was shorter and vice versa.

Higher grain weight loss values may have been expected in this study if the young weevils of same age particularly 0 to 3 weeks old were used. This was demonstrated by Dobie (1977) in which fecundity and the feeding of maize was highest when weevils were in the range of 0 to 3 weeks old after which there was a steady decline. In this study, weevils which were used were of unknown age such that it was possible that some of the weevils used may have been past the 0 to 3 weeks old. Parental survival was negatively and significantly ($p < 0.05$) correlated to the median developmental period ($r = -0.504^{***}$). Through stepwise multiple regression analysis, it was observed that parental survival in terms of explaining total variation had a highest contribution of 78.5%. This means that the number of parent weevils that were alive or dead in given genotypes gave an indication of susceptibility or resistance. There was a negative correlation ($r = -0.355$) between grain hardness and the median development period of the weevils which was significant ($p < 0.05$). This means that genotypes with a harder testa took more time for the weevils to develop on the grain as was evident in the low susceptibility index value indicating resistant genotypes Table 5. Susceptible genotype like genotype 78N with low grain hardness (33.8%) had 98 progenies Table 2 emerging indicating a high possibility of higher damage by weevils.

Grain hardness was further found to be significantly ($p < 0.05$) and positively associated with Phenolic compounds ($r = 0.423^{***}$). Grain hardness contributed 0.5% to the total variation of susceptibility index. Increased Phenolic compounds increased hardness as well which may have contributed to the resistance of genotypes. This was consistent with the study reported by Arnason et al. (1997) in which increased Phenolic content was observed to be concentrated on the cell walls of the grain and then makes the grain harder depending on the concentration of Phenolic content.

The range of susceptibility index values obtained in this study ranged from 0.4 for genotype 60N to 8.4 for genotype 78N. Most genotypes in this study were resistant.

4.2. Inheritance of Weevil Resistance in Maize

The study showed that narrow sense heritability calculated was low (20.9%), indicating a very low gain to selection Table 6. To breed for higher Phenolic content among genotypes in a breeding programme it would be necessary so that to do the population improvement through recurrent selection since the trait has the heterotic response. This means that inbred lines will have to be developed in order to come up with hybrids. These hybrids will express heterosis in terms of high weevil resistance. The negative GCA effects of the female inbred lines indicate reduced Phenolic content in the maize results into the increased positive significant susceptibility values. This indicates that female lines are likely to contribute an increased weevil attack in their crosses. Through indirect selection of some of these traits like Phenolic, it is possible to improve maize varieties to weevil attack. It is possible that the contribution of the Phenolic compounds could have been more than 10.9% Table 4 if parental lines used would have had a higher Phenolic content than the mean Phenolic content of 36.8 mg/100g of grain Table 2. Female line 151 and male line 10112 could be used as parents in making synthetic populations for recurrent selection. While doing the SCA effects among genotypes for yield, testing for weevil resistance among hybrids can

be done because this trait is showing some heterotic response in this study. This is in agreement with the study that was conducted by Serratos et al. (1993).

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REFERENCES

Abebe, F., Tefera, T., Mugo, S., Beyene, Y., & Vidal, S. (2009). Resistance of maize varieties to the maize weevil Sitophilus zeamais (Motsch.)(Coleoptera: Curculionidae). *African Journal of Biotechnology*, *8*(21), 5937-5943.Available at: https://doi.org/10.5897/ajb09.821.

Arnason, T., Conilth de Beyssac, B., Philogene, B. J. R., Bervinson, D., Serratos, J. A., & Mihm, J. A. (1997). *Mechanisms of resistance in maize grain to the maize weevil and the larger grain borer. In Mihm, J.A (Ed.), Insect Resistant maize - recent advances and utilization.* Paper presented at the International Symposium., Mexico City. 27 Dec -3 Dec 1994. CIMMYT, Mexico City.

ASARECA–TUUSI. (2009). Drought and low-N torelant maize germplasm and varieties– inbred lines, populations, OPVs, Hybrids.

Barbano, D. M., Lynch, J. M., & Fleming, J. R. (1991). Direct and indirect determination of true protein content of milk by Kjeldahl analysis: Collaborative study. *Journal of the Association of Official Analytical Chemists*, *74*(2), 281-288.Available at: https://doi.org/10.1093/jaoac/74.2.281.

Bekele, J., & Hassanali, A. (2001). Blend effects in the toxicity of the essential oil constituents of ocimum kilimandscharicum and Ocimum kenyense (Labiateae) on two post-harvest insect pests. *Phytochemistry*, *57*(3), 385-391.Available at: https://doi.org/10.1016/s0031-9422(01)00067-x.

Bosque-Perez, N. A., & Buddenhagen, I. W. (1992). *The development of host- plant resistance to insect pests: Outlook for the tropics. In: Menken, S.B.J., et al. (Ed.).* Paper presented at the 8th International Symposium. Insect-pest Relationships. Kluwer Academic Publishers, Dordrech.

Caswell, G. H. (1962). Agricultural entomology in the tropics (pp. 40 -76). London: Edward Arnold.

Chapman, R. (2000). Entomology in the twentieth century. *Annual Review of Entomology*, *45*(1), 261-285.

CIMMYT. (2001). Maize research Highlights 1999-2000. International maize and wheat improvement centre. Mexico: CIMMYT.

Classen, D., Arnason, J., Serratos, J., Lambert, J., Nozzolillo, C., & Philogene, B. (1990). Correlation of phenolic acid content of maize to resistance to Sitophilus zeamais, the maize weevil, in CIMMYT'S collections. *Journal of Chemical Ecology*, *16*(2), 301-315.

Derera, J., Pixley, K., & Giga, D. (2001). Resistance of maize to the maize weevil: I. Antibiosis. *African Crop Science Journal*, *9*(2), 431-440.

Dobie, P. (1974). The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by Sitophilus zeamais Motsch. (Coleoptera, Curculionidae). *Journal of Stored Products Research*, *10*(3-4), 183-197.Available at: https://doi.org/10.1016/0022-474x(74)90006-x.

Dobie, P. (1977). The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by sitophilus zeamais motsch. Coleoptera, Curculionidae. *Journal of Stored Products Research*, *10*(3-4), 183-197.Available at: https://doi.org/10.1016/0022-474x(74)90006-x.

FAO. (1991). *Maize post-harvest operations.* Rome: FAO.

García-Lara, S., Bergvinson, D. J., Burt, A. J., Ramputh, A. I., Díaz-Pontones, D. M., & Arnason, J. T. (2004). The role of pericarp cell wall components in maize weevil resistance. *Crop Science*, *44*(5), 1546-1552.Available at: https://doi.org/10.2135/cropsci2004.1546.

Gwinner, J., Harnisch, R., & Muck, O. (1996). Manual on the prevention of post-harvest seed losses, post-harvest project (pp. 294-295). Hamburg, FRG: GTZ, D-2000.

Hallauer, A. R., & Miranda, J. B. (1988). Quantitative genetics in maize breeding Iowa state (pp. 49-52). Ames: University Press.

Kamanula, J., Sileshi, G. W., Belmain, S. R., Sola, P., Mvumi, B. M., Nyirenda, G. K., . . . Stevenson, P. C. (2011). Farmers' insect pest management practices and pesticidal plant use in the protection of stored maize and beans in Southern Africa. *International Journal of Pest Management*, *57*(1), 41-49.Available at: https://doi.org/10.1080/09670874.2010.522264.

Keba, T., & Waktole, S. (2013). Differential resistance of maize varieties to maize weevil (Sitophilus zeamais Motschulsky) (Coleoptera: Curculionidae) under laboratory conditions. *Journal of Entomology*, *10*(1), 1-12.Available at: https://doi.org/10.3923/je.2013.1.12.

Kossou, D. K., Mareck, J. H., & Bosque-Perez, N. (1993). Comparison of improved and local maize varieties in the republic of Benin with emphasis on susceptibility to sitophilus zeamais motschulsky. *Journal of Stored Products Research*, *29*(4), 333-343.

Makate, N. (2010). The susceptibility of different maize varieties to post harvest infestation by Sitophilus zeamais (Motsch) (Coleoptera: Curculionidae). *Scientific Research Essay*, 5030-5034.

Makkar, H. P. S. (2003). *Quantification of Tannis in tree and shrub foliage, laboratory manual*. Vienna, Austria: Kluwer Academic Publishers.

Pixley, K. (1997). CIMMYT Mid-altitude maize breeding programme (Vol. 97, pp. 7-13). CIMMYT-Zimbabwe Annual Research Report, 1996/97.

Sen, S., Mukhopadhyay, S., Wetzel, J., & Biswas, T. K. (1994). Characterization of the mitochondrial DNA polymerase from Saccharomyces cerevisiae. *Acta Biochimica Polonica*, *41*(1), 79-86.

Serratos, J. A., Blanco-Labra, A., Arnason, J. T., & Mihm, J. A. (1997). *Genetics of maize grain resistance to maize weevil. In Mihm J.A. Insect resistant maize: Recent advances and utilization*. Paper presented at the Proceedings of an International Symposium, 27 Nov – 3 Dec, 1994.

Serratos, J., Arnason., J., Baum, B., Gale, J., Lambert, J., Bergvinson, D., . . . Jewell, D. (1993). Variation in resistance of mexican landraces of maize to maize weevil Sitophilus zeamais, in relation to taxonomic and biochemical parameters. *Euphytica*, *74*(3), 227-236.

Siwale, J., Mbata, K., McRobert, J., & Lungu, D. (2007). Comparative resistance of improved maize genotypes and landraces to maize weevil. *African Crop Science Journal*, *17*, 1-16.

Tongjura, J. D. C., Amuga, G. A., & Mafuyai, H. B. (2010). Laboratory assessment of the susceptability of some varieties of Zea mays infested with Sitophilus zeamais, Motsch. *Coleoptera, Curculionidae in Jos, Plateau State, Nigeria. Science World Journal*, *5*(2).Available at: https://doi.org/10.4314/swj.v5i2.61514.