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Antibiosis Mechanism of Resistance to Larger Grain Borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in Maize

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ABSTRACT

Host plant resistance is a valuable component of integrated pest management in maize. Maize stored on-farm without controlled moisture content and insecticide treatment is highly susceptible to damage by Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). The aim of this study was to determine the resistance of Mozambican maize genotypes against *P. truncatus*. Seventeen maize genotypes composed of seven experimental hybrids, one released hybrid, two improved open pollinated varieties (OPV), three landraces from Mozambique and four checks (two resistant and two susceptible) from Kenya were screened for their resistance to LGB. The F_1 and F_2 hybrids were evaluated at Kiboko, Kenya in a completely randomized design trial, replicated four times in a post-harvest laboratory. A selection index computed from the number of LGB, grain weight loss (%), seed damage (%) and flour weight were used to categorize the materials as either resistance or susceptible. Fifty percent of the F_1 hybrids tested were resistant, 25% moderately resistant and 25% susceptible. Twenty five percent of F_2 hybrids evaluated were resistant and 75% susceptible. EV8430DMRSR, an OPV and Kandjerendjere, a landrace were the most resistant genotypes with less than 10% weight loss and less than 25% seed damage. This study showed that high protein content contributed towards resistance while high starch contributed to susceptibility. It was concluded that antibiosis mechanism could contribute to LGB resistance in maize. The identified resistant genotypes could be used as cultivar or as source of resistance in maize breeding programs for resistance to LGB.

Key words: Maize, *Zea mays*, post-harvest, *Prostephanus truncatus*, resistance

INTRODUCTION

Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn), is an important pest of maize, dried cassava and woody plants in the tropics. The LGB was introduced in Africa in 1970s from Mexico (Markham *et al.*, 1991). The pest is associated with 9-90% grain loss on stored maize depending on the periods of storage (Bett and Nguyo, 2007; Gueye *et al.*, 2008; Markham *et al.*, 1991; Schneider *et al.*, 2004; Tefera *et al.*, 2010). Treatment of maize for storage with insecticides has been recommended to protect the grain from LGB. However, pesticides pose health and environmental risks and are too expensive for small-holder farmers (Dhliwayo and Pixley, 2003; Golob, 2002). Biological control is a suitable strategy but takes too long to be effective. Developing resistant maize to LGB is a viable option to reduce the costs of production and storage. The objectives of this study were to: (1) Identify sources of resistance in Mozambique elite maize for resistance to LGB and (2) Determine the influence of biochemical and physical kernel properties on resistance to LGB in maize.

MATERIAL AND METHODS

Experimental material and site: Thirteen Mozambican maize genotypes, two susceptible and two resistant checks from Kenya were evaluated for resistance to LGB (Table 1 and 2).

Mozambican maize genotypes included one released and seven experimental hybrids, two Open Pollinated Varieties (OPVs) and three landraces. The experimental hybrids were developed from inbred lines with good combining ability. Mozambican improved genotypes were chosen based on their relative importance in the Mozambique Maize Breeding Program. Landraces were chosen based on popularity with farmers. All seeds were produced under irrigation at CIMMYT's research field at the Kenya Agricultural Research Institute (KARI) Kiboko Farm except the F_1 seed for commercial checks (PH4 and H513) which were obtained from commercial sources. Kiboko is located at 2°15' S and 37°75' E, at altitude of 950 m and receives an annual rainfall of 530 mm. The soils are sand clays while temperatures range between 14.3-35.1°C annually. The F_1 and F_2 hybrids, OPVs and landraces were produced by hand pollination between August and December 2010 and the F_2 hybrids produced between October 2010 and February 2011. Fertilizers were applied at the rate of 60 kg N and 60 kg P_2O_5 ha^{-1}. Nitrogen fertilizer was applied in two splits and irrigation was done when necessary. Fields were hand-weeded and the crop harvested manually at physiological maturity.

Laboratory rearing of LGB: LGB was reared on maize grain according to the methods described by Tefera *et al.* (2010). Four hundred grams of H513 grain (susceptible hybrid) with 11-12%

Table 1: List of genotypes evaluated for maize resistance to the larger grain borer at KARI Kiboko, Kenya

Entry	Genotype	Type	Status	Origin
1	(P2×P4)	Single cross hybrid	Experimental hybrid	IIAM-Mozambique
2	(P3×P6)	Single cross hybrid	Experimental hybrid	IIAM-Mozambique
3	Hluvukane	Three way cross hybrid	Released hybrid	IIAM-Mozambique
4	(P4×P7)×P2	Three way cross hybrid	Experimental hybrid	IIAM-Mozambique
5	(P3×P7)×(P4×P6)	Double cross hybrid	Experimental hybrid	IIAM-Mozambique
6	(P4×P6)×(P3×P7)	Double cross hybrid (reciprocal)	Experimental hybrid	IIAM-Mozambique
7	(P2×P7)×(P4×P6)	Double cross hybrid	Experimental hybrid	IIAM-Mozambique
8	(P4×P6)×(P2×P7)	Double cross hybrid (reciprocal)	Experimental hybrid	IIAM-Mozambique
9	Djandza	Open pollinated variety (OPV)	Released OPV	IIAM-Mozambique
10	EV8430DMRSR	Open pollinated variety (OPV)	Released in 2011 as Dimba	IIAM-Mozambique
11	Kandjerendjere	Landrace	Landrace	Mozambique
12	Xidiwane	Landrace	Landrace	Mozambique
13	Sacana	Landrace	Landrace	Mozambique
Checks				
14	CKPH08020	Three way cross hybrid	Resistant check	CIMMYT-Kenya
15	CKPH08028	Three way cross hybrid	Resistant check	CIMMYT-Kenya
16	PH4	Top cross hydrid	Susceptible check	Kenya seed company
17	H513	Top cross hydrid	Susceptible check	Kenya seed company

Table 2: Pedigree of the S5 maize inbred lines used to form the hybrids evaluated in this study

Inbreed lines	Pedigree	Origin
Parent 2 (P2)	ZM521-15-1-1-1-3-B	IIAM-Mozambique
Parent 3 (P3)	ZM621-19-1-1-3-1-B	IIAM-Mozambique
Parent 4 (P4)	SUWAN8075DMR-79-2-1-2-2-B	IIAM-Mozambique
Parent 6 (P6)	MATUBASG-14-1-4-3-1-B	IIAM-Mozambique
Parent 7 (P7)	SYNSYNF1FS-4-6-1-2-1-B	IIAM-Mozambique

moisture content were placed in 1 L glass jars covered with perforated lids. Two hundred unsexed adult LGB were introduced into the jars. The jars were maintained at Controlled Temperature and Humidity (CTH) at KARI-Kiboko Post-harvest Laboratory at ambient temperatures (27±2°C), 65-70% relative humidity and 12:12 (light:Dark) photoperiod. After 35 days, newly emerged LGB adults were daily transferred to fresh grain in glass jars and kept in the CTH room until sufficient numbers of insects were obtained.

Screening for LGB: Experiments were set in KARI-Kiboko Post-harvest Laboratory. After harvest, the maize was sun dried for a week, then fumigated with Gastoxin™ phosphine fumigant for seven days in plastic drums to kill any insect that may have been present on the grain. Five cobs of each genotype were then hand-shelled and dried to 12-13% moisture content. Two separate sets of the laboratory experiments were evaluated. The first set composed of eight F_1 hybrids, two OPVs, three landraces, two resistant and susceptible checks while the second composed of F_2 seed of four hybrids, two OPVs, three landraces, two resistant and susceptible checks.

In each experiment, samples of 100±1 g of clean, undamaged maize grains of each genotype were weighed and placed in clean 250 cm^3 glass jars. The tops of the lids of these jars were cut out, leaving only the screw-top rings with fine wire gauze, to promote air circulation in the jar and avert the insects from escaping. The jars were left in a CTH room for one week for acclimatization at 28±2°C and 65±5% RH with 12:12 photoperiod to achieve uniform grain moisture content and grain temperature among all samples. After acclimatization, 30 active and unsexed 20-25 days old LGB adults were picked randomly from laboratory culture and introduced into each jar. The jars were laid in completely randomized design with four replications and kept undisturbed in the CTH room for 90 days. Temperature and relative humidity in CTH room was maintained at 27±2°C and 65-70%, respectively with 12:12 photoperiod.

Data collection: Data was collected on number of living and dead LGBs, number and weight of damaged and undamaged grain, weight of flour produced by the borers in individual jars after 90 days of incubation. Glass jars were opened and the content separated into damaged and undamaged grains, insects and flour using 4.7 mm and 1.0 mm sieves for each jar. Damaged grains had visible holes and/or tunnels. An electronic scale (Ohaus, 0.0001 g, 400 g) was used to weigh damaged and undamaged grains and flour weight was expressed as a percentage of the initial grain weight. Seed damage was expressed as a proportion of damaged seed over the total number of seeds sampled and the percentage of weight loss was estimated using the count and weigh method as described below by Boxall (1986):

$$\text{Weight loss (\%)} = \frac{\text{Wu}\times\text{Nd}-\text{Wd}\times\text{Nu}}{\text{Wu}\times(\text{Nd}+\text{Nu})}\times 100 \tag{1}$$

where, Wu is weight of undamaged grains, Wd is weight of insect damaged grains, Nu is the number of undamaged grains and Nd is the number of insect damaged grains.

The percentage reduction in grain weight among the genotypes relative to the susceptible check was computed using the genotype with the highest weight loss:

$$R = \frac{\text{Weight loss of entry (\%)} - \text{Weight loss of the most susceptible check (\%)}}{\text{Weight loss of the most susceptible check (\%)}} \times 100 \tag{2}$$

where, R is percentage reduction for each genotype compared to the most susceptible check the percentage reduction data was multiplied by negative 1 (-1), to present them as positive values for convenience.

Physical and biochemical parameters: Physical and biochemical maize kernel parameters have been reported to confer resistance to maize weevil (Arnason *et al*., 1997). Parameters measured included grain hardness, protein content, starch and oil contents. Sample tested consisted of 10 kernels for each genotype replicated thrice. Grain hardness was determined by the force to break the maize kernel using the displacement force test station model 921A. The average force of 10 kernels was used as the force to break the kernel of that genotype. Protein, starch and oil contents in the maize kernel were measured using the Infratec™ 1241 Grain Analyzer (Graintech, 2011).

Data analysis: Data on percentages was angular-transformed and count data was log transformed before analysis of variance using GenStat 12th edition statistical software (GenStat, 2010). The analysis of variance and correlation analyses for grain weight loss, flour weight, seed damage, number of dead and living borers, protein content, oil content, starch and force to break maize kernels were calculated. Tukey's range test at ($p<0.05$) was used to compare genotype means. Selection index based on susceptibility parameters was computed to classify the genotypes into resistant or susceptible by summing the ratios between values and overall mean and dividing the number of parameters considered. Susceptibility parameters considered were number of living adults, weight loss (%), flour weight (%) and seed damage (%). Classification of genotypes into susceptibility and resistant groups was based on Bergvinson *et al*. (2002) method where, ≤ 0.6 = Highly resistant; 0.61-0.8 = Moderately resistant; 0.81 to 1.0 = Moderately susceptible and >1.0 = Highly susceptible.

RESULTS

Grain weight loss, seed damage, flour weight and number of live and dead LGB for the F_1 generation set: There were highly significant differences ($p<0.001$) among genotypes for all the above traits in the F_1 generation. This set had a mean weight loss of 12.2% with H513 (susceptible check) recording the highest weight loss of 26.6% and double cross (P4×P6)×(P2×P7) recording the lowest weight loss of 6.18% . The individual means of seed damage in the F_1 set ranged between 16.4 and 62.9% where Xidiwane and (P4×P6)×(P2×P7) showed the highest and the lowest weight losses respectively with a grand mean weight loss of 32.4% (Table 3). The genotypes in this set showed a grand mean of 12.78% for flour weight produced from LGB damage with individual means ranging between 5.2 and 40.9% (Table 3).

Table 3: Means of parameters for maize resistance to the LGB in the F1 generation set

Genotype	Weight loss (%)	Seed damage (%)	Flour weight (%)	No. of LGB dead	No. of LGB alive
(P2×P4)	7.87 ± 1.15^{ab}	22.47 ± 3.51^{ab}	5.65 ± 0.38^{ab}	26 ± 7^{a}	25 ± 11^{ab}
(P3×P6)	15.89 ± 0.86^{e}	31.56 ± 1.39^{bcde}	9.17 ± 0.85^{d}	56 ± 15^{bcdef}	45 ± 26^{abc}
Hluvukane	6.71 ± 0.19^{a}	18.69 ± 3.06^{a}	5.72 ± 0.71^{ab}	31 ± 16^{ab}	30 ± 8^{ab}
(P4×P7)×P2	8.81 ± 1.49^{abc}	27.29 ± 5.39^{abcd}	7.34 ± 0.96^{bcd}	73 ± 16^{def}	38 ± 18^{ab}
(P3×P7)×(P4×P6)	15.25 ± 1.28^{de}	43.13 ± 7.38^{e}	15.21 ± 0.68^{e}	75 ± 16^{ef}	116 ± 17^{cde}
(P4×P6)×(P3×P7)	8.31 ± 0.35^{ab}	23.81 ± 5.03^{ab}	7.18 ± 0.59^{bcd}	35 ± 10^{abc}	46 ± 21^{abc}
(P2×P7)×(P4×P6)	7.36 ± 1.03^{ab}	20.92 ± 3.60^{ab}	7.81 ± 0.48^{cd}	34 ± 11^{ab}	45 ± 37^{ab}
(P4×P6)×(P2×P7)	6.18 ± 1.98^{a}	16.38 ± 5.63^{a}	5.24 ± 1.01^{a}	35 ± 9^{abc}	35 ± 14^{ab}
Djandza	11.71 ± 1.00^{cd}	39.56 ± 8.15^{de}	20.96 ± 1.18^{f}	93 ± 14^{f}	169 ± 15^{de}
EV8430DMRSR	7.89 ± 1.43^{ab}	20.30 ± 6.60^{ab}	5.83 ± 0.97^{abc}	50 ± 14^{abcdef}	23 ± 11^{a}
Kandjerendjere	9.91 ± 0.35^{bc}	24.30 ± 5.30^{abc}	7.76 ± 1.09^{cd}	36 ± 7^{abcd}	35 ± 30^{ab}
Xidiwane	21.49 ± 2.19^{g}	62.94 ± 0.80^{g}	34.58 ± 1.21^{g}	91 ± 15^{f}	337 ± 16^{e}
Sacana	16.98 ± 1.33^{ef}	43.72 ± 6.71^{e}	12.85 ± 1.40^{e}	50 ± 16^{abcdef}	73 ± 29^{bcd}
CKPH08020	9.73 ± 0.11^{bc}	36.92 ± 3.30^{cde}	8.85 ± 0.71^{d}	67 ± 5^{cdef}	44 ± 9^{abc}
CKPH08028	8.75 ± 1.63^{abc}	22.47 ± 2.74^{ab}	6.23 ± 0.82^{abc}	29 ± 14^{a}	25 ± 4^{ab}
PH4	20.44 ± 1.03^{fg}	45.04 ± 1.02^{ef}	20.53 ± 0.94^{f}	39 ± 9^{abcde}	143 ± 29^{de}
H513	26.62 ± 1.69^{h}	58.24 ± 3.20^{fg}	40.91 ± 1.40^{h}	59 ± 14^{bcdef}	255 ± 21^{e}
Mean	12.16	32.37	12.78	51	84
Max	26.62	62.94	40.91	93	337
Min	6.18	16.38	5.24	26	23

Means followed by same letters within a column are not significantly different at 5% level of Tukey's range test, P2: ZM521-15-1-1-1-3-B, P3:= ZM621-19-1-1-3-1-B, P4: SUWAN8075DMR-79-2-1-2-2-B, P6: MATUBASG-14-1-4-3-1-B, P7: SYNSYNF1FS-4-6-1-2-1-B

Individual means of live LGB on this set ranged between 23 and 337 insects with a grand mean of 84. The lowest number of live insects was observed on EV8430DMRSR and highest observed on Xidiwane.

The F_1 set had a grand mean of 51 dead LGB with individual means ranging between 26 and 93 insects. The lowest number of dead LGB was recorded on (P2×P4) while the highest was observed on Djandza.

Grain hardness, protein, starch and oil contents for the F_1 generation set: There were highly significant differences ($p<0.001$) among genotypes for grain hardness, protein, starch and oil contents in the F_1 generation.

The force to break the kernels in F_1 set ranged between 128.3 and 216.7 Newton (N) with a mean of 195.07 N. The highest force was recorded on the resistant check CKPH08028 while the lowest force was recorded on Hluvukane (Table 4). The grand mean of the F_1 set for protein content was 12.8% with (P3×P6) having the highest protein content of 15.2% and H513 (susceptible check) having the lowest protein content of 8.6% (Table 4).

In the F_1 sets, H513 recorded the highest starch content of 69.9% while (P3×P6) recorded the lowest of 66.3%. The grand mean was 67.2% (Table 5). The F_1 individual means for oil content ranged between 4.9 and 5.9% with EV8430DMRSR and H513 recording the highest and the lowest oil contents with a mean of 5.5% (Table 4).

Grain weight loss, seed damage, flour weight and number of live and dead LGB for the F_2 generation set: There were highly significant differences ($p<0.001$) among genotypes for grain weight loss, seed damage and flour weight and number of live and dead LGB in the F_2 generation.

Table 4: Means for the seed biochemical parameters related to resistance to the LGB in F1 generation set

Genotype	Protein (%)	Starch (%)	Oil (%)	Force (N)
(P2×P4)	14.30±0.17^{j}	66.70±0.36^{abc}	5.20±0.10^{ab}	215.00±1.44^{hi}
(P3×P6)	15.17±0.15^{k}	66.30±0.20^{a}	5.33±0.06^{bc}	212.00±0.86^{hi}
Hluvukane	12.53±0.06^{de}	67.67±0.12^{def}	5.40±0.17^{bcd}	128.30±1.34^{a}
(P4×P7)×P2	13.33±0.06^{hi}	66.77±0.15^{abc}	5.63±0.12^{defg}	198.20±1.94^{f}
(P3×P7)×(P4×P6)	12.73±0.21^{ef}	67.33±0.25^{bcdef}	5.50±0.10^{cde}	206.50±1.62^{g}
(P4×P6)×(P3×P7)	13.67±0.15^{i}	66.67±0.12^{ab}	5.53±0.12^{cdef}	213.50±1.17^{hi}
(P2×P7)×(P4×P6)	12.83±0.12^{efg}	66.57±0.51^{ab}	5.77±0.06^{efg}	203.90±0.16^{fg}
(P4×P6)×(P2×P7)	13.23±0.31^{ghi}	66.97±0.25^{abcde}	5.57±0.06^{cdef}	182.30±1.91^{cd}
Djandza	12.23±0.15^{cd}	67.90±0.00^{f}	5.37±0.06^{bcd}	181.40±0.90^{c}
EV8430DMRSR	13.13±0.12^{fgh}	66.90±0.20^{abcde}	5.90±0.00^{g}	214.70±0.51^{hi}
Kandjerendjere	13.30±0.06^{hi}	66.87±0.06^{abcd}	5.53±0.06^{cdef}	198.10±1.76^{f}
Xidiwane	11.80±0.14^{bc}	67.77±0.38^{ef}	5.47±0.12^{bcd}	172.40±2.16^{b}
Sacana	12.73±0.12^{ef}	66.77±0.42^{abc}	5.80±0.10^{fg}	210.00±1.43^{gh}
CKPH08020	12.73±0.06^{ef}	67.17±0.06^{abcdef}	5.63±0.06^{defg}	191.30±1.07^{e}
CKPH08028	13.20±0.00^{gh}	67.17±0.21^{abcdef}	5.57±0.06^{cdef}	216.70±0.66^{i}
PH4	11.57±0.21^{b}	67.57±0.46^{cdef}	5.80±0.10^{fg}	188.20±2.53^{de}
H513	8.60±0.20^{a}	69.90±0.50^{g}	4.93±0.12^{a}	182.80±1.34^{cd}
Mean	12.77	67.23	5.53	195.07
Max	15.17	69.90	5.90	216.70
Min	8.60	66.30	4.93	128.30

Means followed by same letters within a column are not significantly different at 5% level of Tukey's range test, P2: ZM521-15-1-1-1-3-B, P3: ZM621-19-1-1-3-1-B , P4: SUWAN8075DMR-79-2-1-2-2-B, P6: MATUBASG-14-1-4-3-1-B and P7: SYNSYNF1FS-4-6-1-2-1-B

The mean weight loss of F_2 sets was 18.1% with Xidiwane and EV8430DMRSR showing highest and lowest weight loss of 34.9 and 6.8%, respectively (Table 5). The F_2 set recorded a grand mean of 40.7% for seed damage where the individual means ranged between 16.3 and 63.8%. Xidiwane and EV8430DMRSR recorded the highest and lowest seed damage of 63.8 and 16.3%, respectively The F_2 set individual means for produced flour weight due to LGB damage ranged between 5.5% and 44.0% with a mean of 16.8%. Xidiwane recorded the highest flour weight while EV8430DMRSR, CKPH08020 and CKPH08028 recorded the lowest flour weight (Table 5).

The mean for dead LGB in F_2 set was 53 with individual means ranging between 21 and 135. Xidiwane recorded the highest number of dead LGBs while CKPH08028 and (P2×P4) recorded the lowest (Table 5). The individual F_2 means for living LGBs ranged from 24 to 356 with a general mean of 127 insects (Table 5). Xidiwane had the highest number of living insects and (P3×P7)×(P4×P6) the lowest.

Grain hardness, protein, starch and oil contents for the F_2 generation set: There were highly significant differences ($p<0.001$) among genotypes for grain hardness, protein, starch and oil contents in the F_2 generation. The F_2 individual means of the force to break the kernels ranged from 170.8 to 214.10 N with a grand mean of 191.55 N. Xidiwane showed the lowest force and EV8430DMRSR the highest. For protein content, the individual means ranged from 11.7 to 14.1% with a grand mean of 13.0%. EV8430DMRSR and Xidiwane recorded the highest and lowest protein content, respectively (Table 6).

The individual means for endosperm starch in the F_2 set ranged from 66.9 to 68.0% with Hluvukane and EV8430DMRSR recording the highest and the lowest starch contents, respectively.

Table 5: Means of parameters for maize resistance to the LGB in F_2 generation set

Variety	Weight loss (%)	Seed damage (%)	Flour weight (%)	No. of LGB dead	No.of LGB alive
(P2×P4)	21.22±3.77^{cdef}	44.37±5.63^{cdef}	18.61±2.26^{cd}	21±09^{ab}	128±25^{bcde}
Hluvukane	16.00±3.50^{abcde}	39.82±10.06^{bcde}	16.64±4.47^{bcd}	47±24^{abc}	78±35^{abcd}
(P4×P7)×P2	14.78±5.23^{abcd}	36.11±11.66^{abcd}	12.98±4.40^{abc}	41±21^{abc}	82±30^{bcd}
(P3×P7)×(P4×P6)	10.99±4.83^{abc}	25.69±9.67^{abc}	9.13±4.46^{ab}	51±27^{abc}	24±014^{a}
Djandza	18.45±4.98^{bcde}	51.41±1.12^{def}	19.39±3.69^{cd}	37±33^{abc}	153±47^{cde}
EV8430DMRSR	6.84±3.98^{a}	16.29±7.84^{a}	5.53±1.83^{a}	29±18^{ab}	38±28^{ab}
Kandjerenjere	9.53±4.35^{ab}	25.19±13.80^{abc}	10.62±4.69^{abc}	35±10^{abc}	69±40^{abcd}
Xidiwane	34.91±5.07^{f}	63.79±3.57^{f}	43.98±1.87^{e}	135±45^{c}	356±36^{e}
Sacana	18.09±3.19^{bcde}	41.19±4.82^{bcdef}	17.65±3.59^{bcd}	82±21^{abc}	125±42^{bcde}
CKPH08020	10.87±2.98^{abc}	26.43±4.98^{abc}	7.50±4.05^{a}	36±15^{abc}	64±39^{abc}
CKPH08028	9.42±4.37^{ab}	21.82±8.75^{ab}	6.32±4.15^{a}	21±13^{a}	49±43^{ab}
PH4 25.45±2.80^{def}	49.07±4.91^{def}	19.95±3.48^{cd}	41±34^{abc}	190±33^{cde}	
H513 28.70±5.27^{ef}	61.08±9.82^{ef}	26.69±5.14^{d}	109±23^{bc}	216±34de	
Mean 18.07	40.71	16.75	53	127	
Max 34.91	63.79	43.98	135	356	
Min 6.84	16.29	5.53	21	24	

Means followed by same letters within a column are not significantly different at 5% level of Tukey's range test, P2 = ZM521-15-1-1-1-3-B, P3 = ZM621-19-1-1-3-1-B, P4 = SUWAN8075 DMR-79-2-1-2-2-B, P6 = MATUBASG-14-1-4-3-1-B and P7= SYNSYNF1FS-4-6-1-2-1-B

Table 6: Means for the seed biochemical parameters related to resistance to the LGB in F_2 generation set

Genotype	Protein (%)	Starch (%)	Oil (%)	Force (N)
(P2×P4)	12.83±0.12^{cd}	67.83±0.15^{cd}	5.40±0.10^{abcde}	188.8±2.34^{de}
(P4×P7)×P2	13.50±0.10^{fg}	67.33±0.06^{abcd}	5.53±0.06^{bcdef}	190.1±2.12^{de}
Hluvukane	12.43±0.12^{bc}	68.03±0.55^{d}	5.27±0.15^{ab}	187.1±3.81^{cd}
(P3×P7)×(P4×P6)	13.80±0.26^{gh}	67.07±0.40^{ab}	5.37±0.15^{abcd}	194.0±2.34^{def}
EV8430DMRSR	14.10±0.10^{h}	66.90±0.17^{a}	5.63±0.06^{def}	214.1±1.58^{g}
Djandza	12.93±0.06^{de}	67.90±0.26^{d}	5.23±0.06^{a}	181.3±2.05^{bc}
Kandjerenjere	13.93±0.15^{h}	67.33±0.15^{abcd}	5.20±0.00^{a}	195.7±3.10^{ef}
Sacana	13.27±0.06^{ef}	67.43±0.06^{abcd}	5.63±0.06^{def}	209.7±1.21^{g}
Xidiwane	11.67±0.15^{a}	67.70±0.10^{bcd}	5.67±0.12^{ef}	170.8±3.78^{a}
CKPH08020	12.93±0.06^{de}	67.43±0.15^{abcd}	5.80±0.10^{f}	194.2±1.03^{ef}
CKPH08028	13.43±0.06^{fg}	67.13±0.06^{abc}	5.80±0.00^{f}	197.4±2.51^{f}
PH4	12.17±0.21^{b}	67.67±0.32^{bcd}	5.60±0.10^{cdef}	175.2±1.45^{ab}
H513	12.10±0.17^{b}	67.87±0.12^{d}	5.33±0.06^{abc}	191.7±1.24^{def}
Mean	13.01	67.51	5.50	191.55
Max	14.10	68.03	5.80	214.10
Min	11.67	66.90	5.20	170.80

Means followed by same letter(s) within a column are not significantly different at 5% level of Tukey's range test, P2: ZM521-15-1-1-1-3-B, P3: ZM621-19-1-1-3-1-B, P4: SUWAN8075DMR-79-2-1-2-2-B, P6: MATUBASG-14-1-4-3-1-B and P7: SYNSYNF1FS-4-6-1-2-1-B

The grand mean of starch content among the F_2 hybrids was 67.5%. The highest oil content of 5.8% was recorded on CKPH08020 and CKPH08028 while the lowest content of 5.2% was recorded on Kandjerendjere and Djandza. The mean of the oil content in this set was 5.2%.

Reduction in grain weight loss: The F_1 hybrid (P4×P6)×(P2×P7) showed the highest percentage reduction of 76.8% in weight loss due to LGB damage while Xidiwane recorded the lowest with

Table 7: Percentage of reduction in weight loss over susceptible check against the LGB

	Percentage of reduction over check	
Genotype	F1 generation set (H513)	F2 generation set (H513)
(P2×P4)	70.45	26.06
Hluvukane	74.79	44.26
(P4×P7)×P2	66.92	48.48
(P3×P7)×(P4×P6)	42.56	61.72
Djandza	56.00	35.72
EV8430DMRSR	70.36	76.18
Kindjerendjere	62.78	66.78
Xidiwane	19.27	-21.65
Sacana	36.21	36.97
CKPH08020	63.45	62.14
CKPH08028	67.12	67.16
PH4	23.24	11.34
(P3×P6)	40.32	-
(P4×P6)×(P3×P7)	68.77	-
(P2×P7)×(P4×P6)	72.37	-
(P4×P6)×(P2×P7)	76.79	-
H513	-	-
Mean	56.96	42.93
Max	76.79	76.18
Min	19.27	-21.65

P2 = ZM521-15-1-1-1-3-B, P3 = ZM621-19-1-1-3-1-B , P4 = SUWAN8075DMR-79-2-1-2-2-B, P6 = MATUBASG-14-1-4-3-1-B and P7 = SYNSYNF1FS-4-6-1-2-1-B

19.3% (Table 7). The overall mean for the reduction in weight loss was 57.0%. The grand mean of F_2 hybrids weight loss reduction due to LGB damage was 42.9% with individual means ranging between (-21.7) and 76.2%. Xidiwane and EV8430DMRSR showed the lowest and the highest reduction in weight loss respectively.

Determination of resistance based on selection index: The F_1 hybrids were highly and moderately highly resistant compared to F_2 generation (Table 8). The individual mean selection indices ranged between 0.45 and 2.54. The lowest and highest selection index was observed on (P4×P6)×(P2×P7) and Xidiwane, respectively. The F_2 individual mean selection indices ranged from 0.37 to 2.32. EV8030SRDMR and Xidiwane recorded the highest and lowest selection indices, respectively.

Correlations among important traits: The number of LGB, flour weight, grain weight loss and seed damage showed strong and significant correlations among them. In the F_1 set, LGB alive (r = 0.8766), WL (r = 0.8694) and SD (r = 0.8502) were positive and significantly correlated with flour weight (Table 9). Starch content showed significant negative correlation with protein (r = -0.9006). In the F_2 generation, positive and significant correlations were observed among FW with LGB alive (r = 0.8340), WL (r = 0.9172) and SD (r = 0.9141) (Table 10). Positive and significant correlation were also observed among WL with LGB alive (r = 0.8428) and SD (r = 0.9489). Starch content presented negative and significant correlation with protein content (-0.7041).

Table 8: Selection index (SI) and reaction of the genotypes in the F1 and F2 generation against the larger grain borer (LGB)

	F1 generation		F2 generation	
Genotype	SI	Reaction	SI	Reaction
(P2×P4)	0.51	Highly resistant	1.14	Highly susceptible
Hluvukane	0.47	Highly resistant	0.90	Moderately susceptible
(P4×P7)×P2	0.63	Moderately Resistant	0.81	Moderately susceptible
(P3×P7)×(P4×P6)	1.26	Highly susceptible	0.51	Highly resistant
Djandza	1.42	Highly susceptible	1.21	Highly susceptible
EV8430DMRSR	0.49	Highly resistant	0.37	Highly resistant
Kandjerendjere	0.64	Moderately Resistant	0.60	Highly resistant
Xidiwane	2.54	Highly susceptible	2.32	Highly susceptible
Sacana	1.13	Highly susceptible	1.05	Highly susceptible
CKPH08020	0.77	Moderately Resistant	0.57	Highly resistant
CKPH08028	0.54	Highly resistant	0.47	Highly resistant
PH4	1.56	Highly susceptible	1.38	Highly susceptible
H513	2.50	Highly susceptible	1.66	Highly susceptible
(P3×P6)	0.87	Moderately susceptible		
(P4×P6)×(P3×P7)	0.62	Moderately Resistant		
(P2×P7)×(P4×P6)	0.59	Highly resistant		
(P4×P6)×(P2×P7)	0.45	Highly resistant		
Mean	1.00		1.00	
Max	2.54		2.32	
Min	0.45		0.37	

P2: ZM521-15-1-1-1-3-B, P3: ZM621-19-1-1-3-1-B, P4: SUWAN8075DMR-79-2-1-2-2-B, P6: MATUBASG 14-1-4-3-1-B and P7: SYNSYNF1FS-4-6-1-2-1-B

Table 9: Correlation coefficients among parameters for maize resistance to the LGB in the F1 generation set

Parameters	LGB_alive (#)	SD (%)	WL (%)	FW (%)	Protein (%)	Starch (%)
SD (%)	0.5910***					
WL (%)	0.7653***	0.5732***				
FW	0.8766***	0.8502***	0.8694***			
Protein (%)	- 0.6100***	- 0.2812*	- 0.5880***	-0. 7913***		
Starch (%)	0.6127***	-0.5862***	0.5620***	0.7788***	-0. 9006***	
Force (N)	-0.2088ns	- 0.103ns	- 0.0225ns	- 0.2203ns	0.4147**	-0.4586***

*,**,***Significant at 5, 1, 0.1% level, ns: Non significant

Table 10: Correlation coefficients among parameters for maize resistance to the LGB in the F2 generation set

Parameters	LGB_alive (#)	SD (%)	WL (%)	FW (%)	Protein (%)	Starch (%)
SD (%)	0.8405***					
WL (%)	0.8428***	0.9489***				
FW (%)	0.8340***	0.9141***	0.9172***			
Protein (%)	-0.7026***	0.7503***	-0.7737***	-0.8067***		
Starch (%)	0.5061***	0.3780*	0.3805*	0.5932***	-0.7041***	
Force (N)	-0.5158***	-0.5759***	-0.5467***	-0.6434***	0.7303***	-0.4039*

*,**,***Significant at 5, 1, 0.1% level, ns: Non significant, LGB_alive: No. of living LGB, SD: Seed damage (%), WL: Grain weight loss (%), FW: Flour weight (%), Protein: Protein content in kernel (%) and Starch: Starch content in the kernel (%)

DISCUSSION

Low numbers of living LGBs indicated resistance. This is due to the fact that the insects could not feed and reproduce. Abraham (1991) reported that damage severity during storage depended on the number of emerging adults and the duration of each generation. Grains of the resistant maize genotypes hindered LGB feeding and reproduction suggesting antibiosis mechanism of resistance. The high number of dead LGB observed in the susceptible genotypes could be attributed to biological process such as aging and high density. The number of living LGB that caused damage on the grain was high in the F_2 than in the F_1. This observation supports previous reports that F_2 hybrids tend to be more susceptible than the F_1 due to segregation. Segregation suggests a mixture of resistant, semi-resistant and susceptible in the F_2 population. Resistant materials are likely to be lower in the segregating population than in the non-segregating population. The observation of low progeny numbers in the resistant materials is supported by Kumar (2002), who reported that susceptible maize genotypes showed high LGB progeny numbers.

LGB susceptible maize showed high flour weight, seed damage and weight loss. This could be attributed to the high number of living LGB insects on the susceptible genotypes. Genotypes that allowed more LGB development were more damaged, leading to high flour, weight loss and seed damage. Damage by LGB converted grain into powder within a short period of time by extensive tunneling maize grain. The flour produced during the insects' feeding consists of insect eggs, endosperm flour and excreta unfit for both livestock and human consumption (Tefera *et al.*, 2011). This study showed that resistant genotypes produced less flour, suffered low seed damage and little grain weight loss. This observation is in agreement with (Kumar, 2002; Likhayo *et al.*, 2010; Mugo *et al.*, 2010; Tefera *et al.*, 2011).

Most maize genotypes with low starch and high protein contents in the grain showed resistance to LGB, except (P3×P6) in the F_1 and (P4×P7)×P2 in the F_2 generation. This observation suggests that the influence of starch and protein contents may not be effective indicators for LGB resistance. Genotypes with high starch content had soft kernels, thus more susceptible compared to the genotypes with lower starch levels. Proteins are composed of amino acids and some amino acids, including lysine and tryptophan and some types of protein have been reported to confer resistance to the maize weevil (Abebe *et al.*, 2009). Proteins with antibiosis effects have been reported in maize among field pests (Pechan *et al.*, 2002). In this study, data was collected on the total amount of protein thus further studies are needed to determine the protein type favorable to the LGB. Maize genotypes with high protein content tend to be more resistant to maize weevil (Derera *et al.*, 2001; Dhliwayo and Pixley, 2003; Garcia-Lara *et al.*, 2004; Siwale *et al.*, 2009). Resistance to storage insect is strongly correlated to physical factors such as tight husk covers, kernel hardness and low moisture content (Mugo *et al.*, 2010). Phenolic content, particularly ferulic acid in the kernels which is linked to grain hardness is associated with resistance (Arnason *et al.*, 1992, 1993, 1997; Tepping *et al.*, 1988) Chemical factors such as amylase and sugar contents have also been reported as factors for weevils resistance (Singh and McCain, 1963). Kernel hardness is unlikely to be an important factor for resistance to LGB, since the insect pest is also a wood pest.

From the selection index computed from key traits including the number of emerged LGBs, flour weight, seed damage and grain weight loss, one improved Mozambican OPV, EV8430DMRSR and the landrace Kandjerendjere showed resistance. Hybrid (P4×P7)×P2 had moderate resistance at F_1 but moderately susceptible at the F_2 generation. This result suggests that grain characteristics of the evaluated genotype contributed to resistance and there are differences in response between F_1 and F_2 for resistance to LGB. This findings are in agreement with Derera *et al.* (2001) who reported that there was no relationship between performance of F_1 and F_2 generations of maize for resistance to the maize weevil. Lack of correlation between the two generations is due to the fact that F_1 are not segregating unlike the F_2.

Positive and highly significant correlation among resistance traits such as grain weight loss, number of live LGBs, seed damage (%) and flour produced (%) in F_1 and F_2 hybrids was observed. The number of live LGB could be considered as a primary parameter since it influences all the other parameters. This observation has also been reported by others (Kumar, 2002; Mugo *et al.*, 2010; Mwololo *et al.*, 2010; Tefera *et al.*, 2011). Among the biochemical properties collected on the seed, only protein and starch showed consistent results in F_1 and F_2 maize hybrids. Negative and significant correlation was observed on protein content with respect to the number of live LGB, flour weight and weight loss. High protein content was associated with resistance to LGB while high starch levels contributed towards susceptibility. High starch content was associated with maize grain softness which contributes to vulnerability to grain damage by insects.

CONCLUSION

This study found out that high protein content contributed towards resistance while high starch contributed to susceptibility. It was concluded that antibiosis mechanism could contribute to LGB resistance in maize. The resistant genotypes identified could be used as cultivars by farmers and as sources of resistance in maize breeding programs for resistance to LGB.

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