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## Resistance of tropical maize genotypes to the larger grain borer

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**Abstract** Post-harvest losses caused by the larger grain borer (LGB, *Prostephanus truncatus*) aggravate food insecurity among small-scale farmers. Host plant resistance is a method of LGB control which should be prioritized in order to reduce these losses. The objective of this study was to assess maize resistance to the larger grain borer and recognize some potential causes of resistance. One hundred and sixty-three (163) genotypes were tested; these included 85 hybrids, 2 checks, 6 open pollinate varieties, and 70 landraces, among them gene bank accessions. Grain biochemical content, (protein, oil and starch) and insect resistance parameters, (percentage grain damage, weight loss, flour weight, and number of emerged insects) were measured. There were significant differences ( $P < 0.001$ ) among the genotypes for all the traits measured except

number of insects. The most resistant hybrids were CKPH08024, CKPH08009, CKPH08012, CKPH08014, CKPH08033, CKPH08026, CKPH08014, and CKPH08003. The most resistant landrace accessions were BRAZ 2451, GUAT 1162, BRAZ 2100, and GUAN 36. The percentage weight loss was found to be the most important resistance trait for discriminating among genotypes for it had the largest canonical coefficient. Protein content had higher contribution to variation in resistance to the larger grain borer and this probably contributed to the grain hardness which is a putative trait of resistance to storage pests. The LGB-resistant germplasm could be used for the development of an integrated pest-management program against the LGB.

**Keywords** Biochemical traits · Insect resistance · Protein content · Post-harvest pests  
*Prostephanus truncatus*

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### Introduction

The larger grain borer (LGB) (*Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)) is among the major storage pests responsible for losses of maize worldwide (Tefera et al. 2010). This pest is exotic to Africa; it was first sighted in East Africa in the 1970s and in West Africa in the early 1980s (Markham et al. 1991). It has now spread to more than 18 African countries (Omondi et al. 2009; Tefera et al. 2010). The LGB causes qualitative and quantitative grain losses by feeding on the kernels and burrowing into them for oviposition. The damaged kernels also provide easy entry for secondary infection by mycotoxin-producing fungi. Mycotoxin is toxic to human beings and can be fatal (Tefera et al. 2010). Losses due to the

LGB are estimated to range from 9 to 45 %, depending on the duration of storage (Kumar 2002; Gueye et al. 2008).

Host plant resistance is the most economically and environmentally viable option for the management of the LGB, especially among resource-constrained farmers. Host plant resistance can easily be combined with other mechanisms of pest control in an integrated pest-management strategy. Because the LGB is exotic to Africa, sources of resistance to this pest are limited. However, preliminary studies indicate that there is genetic variability in resistance to the LGB and other coleopterans among tropical maize germplasm accessions (Arnason et al. 1994; Mwololo et al. 2010; Tefera et al. 2011).

Kernel resistance to the maize weevil is conditioned by biochemical and physical characteristics (Arnason et al. 1994). High concentrations of simple phenolics, namely hydroxycinnamic acids, have been reported in the pericarp of resistant genotypes (Arnason et al. 1997). These phenolics act against the maize weevil by offering mechanical resistance and antibiosis in the pericarp and aleurone layer, respectively. In other resistant genotypes, high concentrations of dehydrodiferulic acids (DiFAs) and the cross-linking of polysaccharides by DiFAs fortify the pericarp cell wall, thereby increasing resistance to attack by weevils and presumably other coleopteran pests (Arnason et al. 1997; Bergvinson and Garcia-Lara 2004; García-Lara et al. 2004). In addition, the cross-linking of plant cell wall polymers by dehydrodiferulates contributes to the defense mechanisms of the maize against pests and diseases. These cross-links are involved in the defense mechanisms against insects such as the European corn borer (*Ostrinia nubilalis*), and the Mediterranean corn borer (*Sesamia nonagrioides*), the southwestern corn borer (*Diatraea grandiosella*), the sugarcane borer (*Diatraea saccharalis* (Fabricius)), and the maize weevil (*Sitophilus zeamais*) (Santiago and Malvar 2010).

Different types of proteins, such as the hydroxyproline-rich glycoproteins (HRGPs) or extensins, protease and trypsin inhibitors, storage proteins and ribosomal inhibitors on grains, have been associated with resistance to fungal diseases and insect invasion (Bergvinson and Garcia-Lara 2004). The extensins are involved in cell wall organization, wound healing, and plant defense mechanisms. These can also be cross-linked within the cell wall, and their presence has been associated with its tensile strength (Cassab 1998; García-Lara et al. 2004). These cross-linkages provide a biochemical mechanism for controlling the mechanical properties of the cell wall, and it is likely that this complex contributes to insect resistance by fortifying the pericarp cell wall, thereby increasing the physical strength of this structure and the overall kernel hardness (García-Lara et al. 2004).

Breeders need new and better sources of resistance and increased knowledge of their mechanisms of resistance. Biochemical components that have specific and localized

activity against storage pests and diseases, but that are not toxic to humans, are ways of enhancing resistance while maintaining the desired nutritional and processing qualities of the grain (Arnason et al. 1997). The objective of this study was, therefore, to assess maize resistance to the larger grain borer and recognize some potential causes of resistance.

## Materials and methods

### Germplasm

One hundred and sixty-three (163) genotypes were chosen for the study. These included 87 hybrids comprising of commercial, drought tolerant and quality protein maize genotypes from the international maize and wheat improvement center (CIMMYT) breeding program, 6 open pollinated varieties (OPVS) and 70 landrace accessions from the CIMMYT germplasm bank in Mexico. Resistant (CKPH09001) and susceptible (PH4) checks were included in the study. Field trials were established in two locations, Kiboko and Embu, which are Kenya Agricultural Research Institute (KARI) centers. Kiboko is a dry mid-altitude agro-ecological zone, situated at about 975 m above sea level, while Embu is a moist, mid-altitude zone at 1,350 m above sea level. The experimental materials were planted in a  $20 \times 5$  and  $15 \times 5$  alpha lattice design, in  $2 \times 5$  m rows for the hybrids with the OPVS and landraces, respectively, each replicated three times. The inter-row spacing was 75 cm, while the intra-row spacings was 25 cm. Two seeds were sown per hill and later thinned to one plant per hill, giving a final plant density of 53,333 plants per hectare. To insure a healthy crop, agronomic practices including weeding, fertilizer application, stem borer control and supplemental irrigation were done by hand when necessary. At harvest grains of the maize was shelled and dried to obtain grains for insect resistance bioassay.

### Evaluation of maize germplasm for resistance to the LGB

The insects used in the experiment were obtained from Kiboko Maize Entomology Laboratory where they were reared on grains of the maize cultivar PH3253 under controlled conditions (28 °C and 75 % relative humidity (RH)). The maize grains were sun-dried and disinfested by fumigation with phostoxin tablets for 7 days to eliminate infestation from the field. Samples of 100 g were taken from each plot among the three replications, to obtain a final three replications of samples per genotype. The kernels were placed in 250 ml jars, infested with 50 unsexed, newly reared LGB insects, and incubated for 90 days at 26–28 °C and 70–75 % RH in the screening laboratory at

KARI, Kiboko. The experimental design in the laboratory was a randomized complete design with three replications.

The contents of each jar were sieved with mesh sieves (Endecotts Ltd., UK<sup>1</sup>) after 90 days to separate grains, insects and flour. The flour produced was measured with a weighing balance whereas the number of damaged kernels and adult insect progeny were counted. The grain weight loss was computed by subtracting the final from the initial weight of the grain sample and expressing it as a percentage. The damaged kernels were separated from the undamaged ones using tunneling as the criterion. These were counted and the grain damage percentage computed. The parameters used for the LGB resistance assessment were the weight loss percentage, the grain damage percentage, the flour weight and the number of insects recovered (Tefera et al. 2011).

### Biochemical analysis

The protein, oil, and starch content percentages were determined from whole grains of each genotype using a Foss Infratec<sup>2</sup> 1241 Grain Analyzer machine. Five hundred gram (500 g) grain samples of each genotype were obtained from each of the three replications per genotype and used to estimate these traits. The machine was set to divide the sample into five sub-samples to spread the analysis, and then run to give the readings of the different biochemical components in the grain.

### Statistical analyses

A multivariate analysis of variance within a canonical variate analysis was performed using SAS (Canonical Discriminant Analysis) to determine the difference between genotypes and the most variable insect resistance trait among the genotypes. A univariate analysis of variance (ANOVA) complemented with Tukey's HSD multiple range test for the individual resistance and biochemical traits was done using Genstat software. The number of insects was log transformed ( $\log_{10}$ ), while flour produced, grain damage and weight loss were angular transformed ( $\arcsin\sqrt{\text{proportion}}$ ) to normalize their distribution before subjecting them to ANOVA. The insect resistant traits were correlated with the grain biochemical contents using a canonical correlation to secure error control and recognize the strongest correlation.

## Results

### Canonical discriminant analysis

The canonical discriminant analysis derives a linear combination of the variables that has the highest possible

multiple correlation with the groups given two or more groups of observations with measurements on several quantitative variables. The ANOVA option specifies testing of the hypothesis that the class means are equal using univariate statistics. The resulting  $R^2$  values range from 0.12 for the number of insects to 0.29 for percentage weight loss and all the variables except the number of insects ( $P = 0.165$ ) were significant ( $P < 0.0001$ ). The multivariate test for differences between the classes was also significant ( $P < 0.0001$ ) thus the multivariate tests of the hypothesis that the class mean vectors are equal indicate that not all of the mean vectors are equal ( $P < 0.001$ ). The Wilks Lambda, Pillais trace, Hotelling-Lawley Trace, and Roys Greatest root values were 0.3750, 0.8281, 1.1860, and 0.6788, respectively.

The first canonical correlation which is the greatest possible multiple correlation with the classes that can be achieved using a linear combination of the quantitative variables was 0.6358 (Table 2). The likelihood ratio test for the hypothesis that the current canonical correlation and all the smaller ones are zero show that none of them was zero and as expected the first line was equivalent to Wilk's Lambda multivariate test. The  $R^2$  between the first canonical variable (CAN1) and the class variable, 0.4043, was much larger than the corresponding  $R^2$  for the rest three (Table 2).

The CAN1 shows that the linear combination of the centered variables CAN1 were  $0.7308 \times \text{flour weight} - 0.681978 \times \text{Number of insects} - 0.6442 \text{ grain damage} + 1.04668 \text{ weight loss}$  separates the entries most effectively (Table 1). In the first canonical variate, weight loss is undoubtedly the most important variable for discriminating among genotypes for it had the largest coefficient (loading value). The first canonical variable is the linear combination of the variables flour weight (%), number of insects, grain damage (%), and weight loss (%) that provides the greatest difference (in terms of a univariate  $F$  test) between the class means and it accounted for most of the variation in the data set (65.23) (Table 1). The second canonical variable provides the greatest difference between class means while being uncorrelated with the first canonical variable. The third canonical variate is much less important in classifying individuals since it only explained 10 % of the total variation.

There were significant differences ( $P < 0.001$ ) among the hybrids and landraces for all the traits measured except for the number of insects. The genotypes were classified as resistant ( $WL < 26\%$ ), moderately resistant ( $WL = 26.1\text{--}30\%$ ) and susceptible ( $WL \geq 30$ ) based on the weight loss which was shown as the best variable for grouping the genotypes as indicated by the canonical discriminant and correlation analysis (Table 2). The resistant genotypes had low percentages of weight loss, flour weight, and grain damage, and fewer insects.

**Table 1** The canonical loadings of the variables and standardized coefficients from the variate analysis

Variable	Canonical loading structure				Canonical coefficients								
	Can corr	SE	Squared can corr	Eigen value	Cum variance	LR	F value	Num DF	Den DF	Pr > F	Can 1	Can 2	Can 3
Flour weight (%)	0.6358	0.016	0.4043	0.68	0.57	0.38	2.23	648	5193.80	<.0001	0.7308	0.1096	-1.5404
No. insects	0.4633	0.021	0.2146	0.27	0.83	0.63	1.35	483	3897.60	<.0001	-0.6820	0.0509	0.4294
Grain damage (%)	0.3391	0.023	0.1150	0.13	0.91	0.80	0.95	320	2600.00	0.7280	-0.6442	0.9284	-0.199
Weight loss (%)	0.3067	0.024	0.0940	0.10	1.00	0.91	0.85	159	1301.00	0.9050	1.0467	0.1662	1.2641
Latent roots											0.68	0.27	0.13
Percentage variation											65.23	16.05	10.96

*Test of H0* the canonical correlations in the current row and all that follow are zero, *Can corr* canonical correlation, *SE* standard error, *Cum* cumulative, *LR* likelihood ratio, *Num* numerator, *Den* denominator, *DF* degrees of freedom, *Can* canonical variable coefficient

The most resistant genotype among the hybrids was CKPH08024 with a percentage weight loss of 16.66 %, which was a third of the most susceptible hybrid, H628 which had a percentage weight loss of 48.62 %. The resistant check (CKPH9001) had a percentage weight loss of 25.94 %, which was higher than that of the most resistant hybrids and landraces. More than half of the resistant genotypes were CIMMYT hybrids which had been bred for maize weevil resistance in an earlier breeding program. Four among the resistant and moderately resistant hybrids (CKIR06009, CKIR06007, CKIR07018, and CKIR04003) were CIMMYT hybrids developed for stem borer resistance. This indicates that they have multiple levels of resistance, and/or that the genes controlling resistance to storage and field pests in maize are similar. The most susceptible genotypes had a percentage weight loss of 50.58 % which was 1.4 times that of PH4, the susceptible check. The LGB reproduction was adversely affected on resistant grains, as indicated by the low average number of emerged insects post-incubation: 164 among the resistant genotypes compared to 247 among the susceptible ones (Table 2).

The top five most resistant hybrids were CKPH08024, CKPH08012, CKPH08009, CKPH08003, and CKPH08014, and the most susceptible were H628, H6213, 16210, and H626. The most resistant landraces were BRAZ 2451, GUAT 1162, BRAZ 2100, and GUAN 36 and the most susceptible were RIGSGP10, BRAZ 1470, BRAZ 1384, BRAZ 4, and NAYA 129.

### Canonical correlations

The probability level for the null hypothesis that all the canonical correlations are zero (0) among the variables was significant ( $P < 0.0001$ ) as indicated by the multivariate statistics, so firm conclusions can be drawn from these results. The Wilks Lambda, Pillais trace, Hotelling-Lawley Trace, and Roys Greatest root values were 0.6288, 0.4035, 0.53903, and 0.4183, respectively. There were great variations in the correlation between grain damage traits and biochemical components (Table 3). From the canonical correlation it is clear that percentage protein content and grain damage accounted for a substantial amount of shared variance between the two sets of variables. As the output shows, the first canonical correlation is 0.5431, which is substantially larger than any of the between-set correlations (Table 3). The eigenvalue corresponding to the first canonical correlations is also high, thereby indicating that it accounted for the most of the variation between the respective canonical variates. The canonical redundancy analysis shows that the first pair of canonical variables is a good overall predictor of the opposite set of variables, the proportions of variance explained being 77.61 % and the cumulative proportions for all the three canonical variables

**Table 2** Resistance parameters of the most resistant and the most susceptible among the maize genotypes evaluated (mean  $\pm$  SE)

Genotype	Description	Weight loss (%)	Flour weight (%)	Grain damage (%)	No. of insects
CKPH08024	Hybrid	16.66 $\pm$ 2.21 a	13.14 $\pm$ 1.99 a	27.48 $\pm$ 2.98 a	125.0 $\pm$ 91.82 a
CKPH08009	Hybrid	20.84 $\pm$ 3.19 b	16.78 $\pm$ 2.04 c	31.47 $\pm$ 4.49 b	178.9 $\pm$ 27.42 a
CKPH08012	Hybrid	21.20 $\pm$ 2.67 b	14.20 $\pm$ 1.76 b	33.01 $\pm$ 3.14 b	159.6 $\pm$ 22.85 a
CKPH08003	Hybrid	21.87 $\pm$ 2.98 b	15.32 $\pm$ 1.91 b	33.55 $\pm$ 2.52 b	139.6 $\pm$ 26.89 a
CKPH08014	Hybrid	22.07 $\pm$ 2.58 c	15.40 $\pm$ 2.49 b	39.03 $\pm$ 3.91 d	160.6 $\pm$ 22.84 a
CKPH08033	Hybrid	22.17 $\pm$ 3.25 c	14.98 $\pm$ 2.49 a	37.50 $\pm$ 4.41 c	155.8 $\pm$ 29.86 a
BRAZ 2451	Landrace	22.53 $\pm$ 3.30 c	13.71 $\pm$ 2.025 a	24.59 $\pm$ 2.96 a	132.9 $\pm$ 18.35 a
BRAZ 2100	Landrace	23.30 $\pm$ 3.93 c	16.52 $\pm$ 2.76 c	37.77 $\pm$ 5.19 c	173.6 $\pm$ 31.21 a
CKPH08002	Hybrid	24.25 $\pm$ 4.73 d	19.22 $\pm$ 3.67 d	34.85 $\pm$ 4.55 b	193.8 $\pm$ 46.60 a
CKPH08041	Hybrid	24.32 $\pm$ 3.60 d	16.92 $\pm$ 2.96 c	33.76 $\pm$ 5.71 b	151.7 $\pm$ 27.55 a
CKIR09007	Hybrid	24.34 $\pm$ 1.86 d	18.47 $\pm$ 1.85 d	38.62 $\pm$ 3.21 c	171.7 $\pm$ 24.53 a
ECA-QPOPE	OPV	25.05 $\pm$ 4.76 d	21.02 $\pm$ 3.32 d	34.59 $\pm$ 4.29 b	194.0 $\pm$ 30.06 a
CKPH08028	Hybrid	25.40 $\pm$ 4.35 d	18.52 $\pm$ 2.75 d	36.03 $\pm$ 4.69 c	193.7 $\pm$ 38.29 a
GUAN 36	Landrace	25.55 $\pm$ 3.32 d	21.01 $\pm$ 2.74 d	35.73 $\pm$ 5.85 b	159.6 $\pm$ 16.65 a
CKPH09001-resistant check	Hybrid	25.94 $\pm$ 4.97 d	23.11 $\pm$ 3.22 d	33.96 $\pm$ 5.72 b	246.0 $\pm$ 31.67 a
ECA-STRIGOFF-VL-125-##	OPV	26.15 $\pm$ 3.48 d	15.77 $\pm$ 2.93 b	37.81 $\pm$ 4.95 c	156.1 $\pm$ 29.36 a
EEQPM-8-EA-##-##	OPV	26.25 $\pm$ 2.44 d	20.01 $\pm$ 1.91 d	40.50 $\pm$ 2.68 d	174.8 $\pm$ 21.25 a
BRAZ 1797	Landrace	26.79 $\pm$ 1.88 e	17.75 $\pm$ 1.63 d	32.24 $\pm$ 4.86 b	154.1 $\pm$ 14.49 a
KDV1-2-#	Landrace	27.19 $\pm$ 3.04 e	17.89 $\pm$ 2.24 d	43.21 $\pm$ 6.15 d	146.8 $\pm$ 31.22 a
VENE 897	Landrace	27.22 $\pm$ 2.33 e	17.69 $\pm$ 1.66 d	28.03 $\pm$ 4.05 a	136.3 $\pm$ 11.07 a
PH4-susceptible check	Landrace	35.82 $\pm$ 2.33 f	23.59 $\pm$ 1.66 d	43.84 $\pm$ 4.05 d	229.3 $\pm$ 11.07 a
PH 3253	Hybrid	42.32 $\pm$ 3.48 g	27.69 $\pm$ 2.32 e	50.75 $\pm$ 3.54 e	236.7 $\pm$ 32.84 a
BRAZ 1371	Landrace	44.23 $\pm$ 5.29 g	32.29 $\pm$ 3.82 f	39.35 $\pm$ 5.82 d	203.4 $\pm$ 32.45 a
H6210	Hybrid	44.77 $\pm$ 4.55 g	25.32 $\pm$ 3.28 e	59.08 $\pm$ 4.77 f	284.3 $\pm$ 38.65 a
BRAZ 222	Landrace	44.83 $\pm$ 3.74 g	34.30 $\pm$ 2.37 f	34.90 $\pm$ 7.27 b	218.2 $\pm$ 22.19 a
BRAZ 1486	Landrace	45.64 $\pm$ 3.39 g	44.32 $\pm$ 3.20 g	40.90 $\pm$ 7.83 d	241.4 $\pm$ 25.75 a
H626	Hybrid	45.98 $\pm$ 2.23 g	28.61 $\pm$ 2.51 e	52.12 $\pm$ 3.69 e	229.6 $\pm$ 26.73 a
NAYA 129	Hybrid	46.27 $\pm$ 4.14 h	31.74 $\pm$ 3.87 e	33.09 $\pm$ 4.16 b	221.6 $\pm$ 39.78 a
H6213	Hybrid	46.85 $\pm$ 5.08 h	31.62 $\pm$ 4.44 e	55.50 $\pm$ 3.73 e	280.6 $\pm$ 34.75 a
H628	OPV	48.62 $\pm$ 4.52 h	32.06 $\pm$ 3.23 e	54.05 $\pm$ 4.29 e	257.8 $\pm$ 32.19 a
BRAZ 1470	Landrace	50.57 $\pm$ 3.26 i	34.65 $\pm$ 3.86 f	45.31 $\pm$ 6.81 d	230.7 $\pm$ 42.82 a
BRAZ 4	Landrace	50.58 $\pm$ 6.68 i	33.89 $\pm$ 1.79 e	34.13 $\pm$ 6.12 b	220.4 $\pm$ 20.12 a
<i>F</i> value		3.32	2.75	2.28	5.78
<i>P</i> value		<.001	<.001	<.001	0.19

Statistics at *df*: rep = 2; *df* entries = 162, residual = 1299; total 1463

\* Values followed by the same letter within a column are not significantly different at  $P = 0.05$  (Tukey's HSD test) and the genotypes were grouped together as per that trait

being 1.00 (Table 3). Because the variables were measured in the same units, the standardized coefficients rather than the raw coefficients were presented and interpreted (Table 3).

The squared multiple correlation indicate that the first canonical variable of the resistance traits has some predictive power for protein content ( $r = -0.90$ ) and very little for oil content and starch content (Table 3). The first canonical variable of the biochemical content is a good predictor of percentage grain damage ( $r = 0.97$ ) and weight loss, a fair predictor of total number of insects

( $r = 0.29$ ) and poor one for the percentage flour weight ( $r = 0.15$ ). The first canonical variable for the resistance variables show positive signs thus indicating that among them there is no suppressor variable, with the most weight being on grain damage. The general interpretation of the first canonical correlation is that protein and oil content act as suppressor variables in relation to the resistance traits (grain damage, weight loss, and number of insects) whereas starch content is an activator variable. In other words, the starch content stimulates susceptibility to the larger grain borer damage. This canonical correlation is strong enough

**Table 3** Canonical correlation between the resistance traits and the biochemical content traits

Can	Correlation coefficients for the variables															
	Can corr	Squared can corr	Eigen value	Prop value	Cum	F value	Num DF	Den DF	Pr > F	Correlation coefficients for the variables						
										Protein	Oil	Starch	Weight loss	Flour weight	Grain damage	No. insects
Can 1	0.5431	0.2949	0.42	0.78	0.78	6.39	12	399.8	<0001	-0.90	-0.13	0.12	0.51	0.15	0.97	0.20
Can 2	0.3226	0.1041	0.12	0.22	0.99	2.98	6	304	0.0076	-0.62	1.07	-0.14	-0.87	-0.47	-0.29	0.72
Can 3	0.0674	0.0045	0.00	0.01	1.00	0.35	2	153	0.7062	-0.28	-0.21	-1.02	-0.83	0.64	-0.35	1.08

Can corr canonical correlation, Prop proportion of variance, Cum cumulative variance, Num numerator, Den denominator, DF degrees of freedom, Can canonical variable coefficient

to be of practical interest since the sample size was large enough to draw definite conclusions.

There were significant (<0.001) differences among the hybrids and landraces for protein and oil content percentages (Table 4). Based on the canonical correlation, the protein content (%) was indicated as the best variable which can be used to group the genotypes in relation to resistance to the larger grain borer. The most resistant genotypes had high levels of protein and oil. The protein content of the most resistant genotypes was above 11 %, whereas that of most of the susceptible ones ranged from 85.5 to 10 %. The oil content of the most resistant hybrids was above 5.6 %, while that of the most susceptible ones was below 5.5 %. The starch content was of little importance in relation to resistance.

### Discussion

This study showed that there were differences in resistance levels among maize hybrids, OPVs and landrace accessions as indicated by grain weight loss, flour weight, grain damage, and number of insects. The percentage weight loss is the most important variable for grouping the genotypes into resistant and susceptible. The low percentage of grain weight loss among the resistant genotypes indicates that they have genes that confer resistance to the LGB. Grain resistance to storage pests has been attributed to physical factors such as grain hardness, and to biochemical traits such as phenolic compounds (García-Lara et al. 2004). The presence of resistant genotypes among the wide germplasm screened suggests that sources of resistance exist among the landrace accessions and OPVs, and that it is possible to develop improved maize hybrids that are resistant to the LGB. This would lead to an increased base of sources of resistance to the LGB. The resistant hybrids can be made available to farmers and/or improved further through breeding for additional farmer-preferred traits. The canonical discriminate analysis and correlation coefficients gives invaluable information that maize resistance against the LGB can be evaluated based on the percentage weight loss followed by grain damage. The level of damage and weight loss during storage is strongly correlated with the number of adult insects (Tefera et al. 2011).

The results of these studies showed that protein and oil content were high in resistant genotypes compared to susceptible genotypes. However, it was further indicated that the protein content was the most important variable based on the canonical loading values/coefficients. It is probable that these two are among other biochemical components associated with resistance, either directly or indirectly, within the resistant genotypes. However, some genotypes were susceptible to the LGB but had



**Table 4** Biochemical contents among the maize genotypes evaluated ranked based on the protein content (mean  $\pm$  SE)

Genotype	Protein content (%)	Oil content (%)	Starch content (%)
PH 3253	8.50 $\pm$ 0.75 a	5.37 $\pm$ 0.13 b	70.55 $\pm$ 0.61 b
DH04	8.80 $\pm$ 0.01 a	5.60 $\pm$ 0.00 c	69.60 $\pm$ 0.01 a
PH4-susceptible check	8.85 $\pm$ 0.38 a	5.25 $\pm$ 0.03 b	69.50 $\pm$ 0.12 a
611D	8.90 $\pm$ 0.06 a	5.45 $\pm$ 0.09 b	69.10 $\pm$ 0.23 a
H6213	8.95 $\pm$ 0.2 a	4.85 $\pm$ 0.03 a	70.20 $\pm$ 0.46 b
H628	9.00 $\pm$ 0.01 b	4.70 $\pm$ 0.01 a	70.30 $\pm$ 0.01 b
SC Duma 43	9.00 $\pm$ 0.4 b	4.75 $\pm$ 0.09 a	71.10 $\pm$ 0.12 b
SUSUMA	9.00 $\pm$ 0.57 b	5.32 $\pm$ 0.28 b	69.45 $\pm$ 0.49 a
DKC8053	9.15 $\pm$ 0.61 b	5.55 $\pm$ 0.03 c	69.15 $\pm$ 0.43 a
KH 600-15A	9.15 $\pm$ 0.61 b	5.10 $\pm$ 0.17 a	70.3 $\pm$ 0.69 b
CKIR06001	9.20 $\pm$ 0.01 b	5.03 $\pm$ 0.09 a	70.4 $\pm$ 0.01 b
CKIR06006	9.20 $\pm$ 0.29 b	5.05 $\pm$ 0.03 a	70.65 $\pm$ 0.09 b
CKIR09007	9.25 $\pm$ 0.09 b	4.85 $\pm$ 0.03 a	70.25 $\pm$ 0.38 b
CML395/CML444	9.25 $\pm$ 0.09 b	5.15 $\pm$ 0.03 b	70.25 $\pm$ 0.43 b
PH1	9.30 $\pm$ 0.01 b	5.90 $\pm$ 0.00 c	69.50 $\pm$ 0.01 a
GUAN 34	11.5 $\pm$ 0.01 c	6.60 $\pm$ 0.00 e	66.90 $\pm$ 0.01 a
CKPH08009	11.65 $\pm$ 0.03 c	6.50 $\pm$ 0.01 e	67.75 $\pm$ 0.03 a
CKPH09002	11.67 $\pm$ 0.35 c	6.90 $\pm$ 0.12 e	67.20 $\pm$ 0.14 a
CKPH09001-resistant check	12.00 $\pm$ 0.09 c	6.55 $\pm$ 0.15 e	67.60 $\pm$ 0.55 a
CKPH08004	12.03 $\pm$ 0.42 c	6.20 $\pm$ 0.01 d	68.20 $\pm$ 0.01 a
CKPH08020	12.03 $\pm$ 0.46 c	6.20 $\pm$ 0.06 d	69.30 $\pm$ 0.29 a
CKPH09003	12.35 $\pm$ 0.22 d	6.05 $\pm$ 0.15 d	69.70 $\pm$ 0.27 a
CKPH08035	12.65 $\pm$ 0.03 d	5.65 $\pm$ 0.14 c	67.40 $\pm$ 0.01 a
PARA GP3	12.65 $\pm$ 0.09 d	6.60 $\pm$ 0.23 e	66.05 $\pm$ 0.32 a
VERA 197	12.65 $\pm$ 0.14 d	5.60 $\pm$ 0.17 c	67.55 $\pm$ 0.2 a
CKPH08028	12.7 $\pm$ 0.12 d	6.40 $\pm$ 0.23 e	68.55 $\pm$ 0.03 a
<i>F</i> value	24.45	11.3	3.44
<i>P</i> value	<0.01	<0.01	<0.01

The most resistant and the most susceptible genotypes were presented to reduce the size of the tables and for clarity in both Tables 2 and 4. Statistics at *df*: rep = 2; *df* genotypes = 162; residual = 1,299; total = 1,463

\* Values followed by the same letter within a column are not significantly different at  $P = 0.05$  (Tukey's HSD test) and the genotypes were grouped together as per that trait

high-protein content (>11 %). This could be attributed to the amino acid composition in the maize grains, since the nature of the amino acid which makes up the protein structure in a particular grain of a specific genotype is a key factor in resistance to storage pests. For instance, earlier studies on quality protein maize (QPM), which had lysine and tryptophan as the building blocks of protein, found it to be susceptible to the maize weevil due to the soft endosperm conferred by these two amino acids (Firoz et al. 2007). (Siwale et al. (2009) reported that weevil-resistant improved maize hybrids and open pollinated varieties (OPV) of genotypes had a high-protein content compared to susceptible ones. Arnason et al. (1997) indicated that protein content is negatively correlated with the susceptibility of maize to the maize weevil. This is also consistent with other studies, which found that the hydroxyproline-

rich proteins (extensins), that are known to accumulate in the pericarp, play a role in resistance to storage pests (Dhliwayo and Pixley 2003; García-Lara et al. 2004).

The current results based on the large number of and diverse germplasm evaluated and the canonical correlation confirm that protein content in combination with other factors in the maize grain contributes to LGB resistance. The mechanisms operating could either be antibiosis by the protein alone, or in combination with other factors for possible synergistic effect. For instance, the protein content could be contributing to kernel hardness, which is a putative trait of grain resistance to storage pests. It is clear that there are key genes responsible for the synthesis of key proteins associated with LGB resistance, but there is a need to investigate further the amino acid composition in the resistant and susceptible genotypes which have

high-protein content. There is a window for further exploration at the molecular level of the genes which are involved in conferring a high content of specific proteins correlated with resistance to the LGB, through molecular characterization of the germplasm used.

The oil content in most of the resistant genotypes was higher than that of the susceptible genotypes, despite the low canonical correlation coefficients among the traits measured. It is probable that lipids play a role in contributing to maize resistance to the LGB as part of the anti-biosis components of resistance. This is in agreement with earlier studies (Jood et al. 1996) who reported a low proliferation of khapra beetles (*Trogoderma granarium*) on wheat, maize, and sorghum which had a high-lipid content. The basis of resistance due to the presence of lipids in maize kernels could be that oxidization over time leads to the production of volatiles that influence the movement of storage pest insects away from the grain (Nawrot et al. 1985).

The first canonical correlation showed that protein and oil content act as suppressor variables in relation to the insect grain damage traits (grain damage, weight loss, flour weight, and number of insects) indicating that they were associated with resistance to the LGB. Moreover, the correlation coefficients further indicated that the protein content had on average a higher contribution to variation (−0.90) in resistance than the oil content (−0.13). As a result, protein content would be more important as a trait of resistance, subject to further studies.

## Conclusions

The use of resistant genotypes is key in developing a sustainable integrated pest-management program against the LGB. Percentage weight loss was observed as the most important trait for discriminating genotypes for resistance against LGB. The resistant hybrids were CKPH08024, CKPH08009, CKPH08012, and CKPH 08014. The most resistant landrace accessions (BRAZ 2451, GUAT 1162, BRAZ 2100, GUAN 36, and GUAT 1162) can be used as sources of resistance in breeding programs to address the problem of post-harvest losses due to the LGB by developing effective management strategy for LGB. Protein content is an important trait conferring resistance to maize grains against the LGB subject to further studies.

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