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Mechanisms and levels of resistance in hybrids, open pollinated varieties and landraces to *Chilo partellus* maize stem borers

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Abstract

Reduction in maize grain losses could be enhanced through identification of existing resistant genotypes and pyramiding the resistance into elite materials. This study was carried out in two trials of 100 commercial hybrids and open pollinated varieties (OPVs), and 75 landraces to identify resistant genotypes and mechanisms of resistance to Chilo partellus maize stem borers. The trials were laid out in α-lattice designs, each replicated three times during the 2010/11 and 2012 rainy seasons. Each plant was artificially infested with five C. partellus neonates three weeks after planting. Data collected included leaf toughness, stem hardness, trichome density, stem sugar content, leaf damage, number of stem borer exit holes, tunnel length and grain yield. Data were analyzed using PROC GLM of SAS 2007 package and means separated using Fishers protected least significant difference test (LSD) at (P < 0.05). Canonical discriminant analysis was performed to discriminate the mechanisms evaluated. The most resistant genotypes were CIMMYT experimental resistant hybrid checks followed by landraces and the OPVs. Top 10 resistant commercial hybrids and OPVs were PH1, PH4, DHO1, DH04, DK8031, KDVI, KDV2, PH3253, ECA-Strigoff-VL and EEQPM-8-EA, while H629, H6212, KH 600-15A, H6213 and H6210 were the most resistant commercial hybrids. Canonical discriminant analysis identified percentage stem sugar content and trichome density as the most important resistance mechanisms for discriminating the genotypes. The resistant landraces and OPVs could be utilized in breeding for maize stem borer resistance, while the resistant commercial hybrids and OPVs could be recommended for production in the relevant ecologies in Kenya to curb maize stem borer related yield losses.

Key words: Exit holes, leaf toughness, resistance, spotted stem borer, sugar content, trichome density, tunnel length

INTRODUCTION

Globally, maize is the third important cereal crop after wheat and rice. Maize is a staple food to about 300 million people in sub-Saharan Africa (SSA) and it is grown on 29m hectares (Smale et al., 2011; FAOSTAT, 2012). In Kenya, maize is the most important food crop and it is grown on 2.2m ha and yields about 3.6m tons (FAOSTAT, 2012). Maize grain shortage is common in Kenya owing to various production constraints. In Kenya and many other SSA countries, maize yields are low at 1.5 t/ha compared to the world average of about 5.0 t/ha (FAOSTAT, 2012). Yield losses attributed to maize stem borers in the SSA region are about 13% (Smale et al., 2011). In Kenya, yield losses attributed to stem borers are estimated at between 13-15% and losses can be higher depending on the resistance level of the genotype (De Groote, 2002). Maize production has only increased slightly over the years since the 1970s compared to the unmatched increase in population growth (Mati, 2000). The low production coupled with a slow rate of increase in maize production in Kenya and other SSA countries has been associated with various biotic and abiotic stresses that include foliar diseases and insect pests, low

soil fertility, salinity, and drought stress. The important biotic factors associated with maize yield losses include maize stem borer insect pests. The major stem borer species in Kenya are *Chilo partellus* Swinhoe (spotted stem borers), *Busseola fusca* Fuller (African stem borers), *Sesamia calamistis* Hampson (pink stem borers) and *Eldana saccharina* Walker (African sugarcane borer) (Ong'amo *et al*., 2006). *Chilo partellus*, an exotic species introduced in Kenya around 1932 is the most economically important owing to its capacity to invade and occupy large areas previously predominantly occupied by other species (Mbapila *et al.,* 2002; Ong'amo *et al.,* 2006). The fact that it is most common on the low land areas which are almost always also moisture deficient tend to aggravate its effect on maize crop.

Every stage of the maize plant is susceptible to stem borer attack, however, stem borers spend most of their life cycle hidden within the stem and feeding on the pith leading to disruptions on water and nutrient uptake (Malvar *et al*., 2008). Control methods including cultural and use of chemical pesticides have been recommended for stem borer control. Cultural methods require technical knowledge from the farmer and are mostly associated with heavy labor investment while pesticides are costly and not entirely environmentally safe. The challenges associated with these methods have reduced their usage and success in SSA, giving rise to the need for host plant resistance as the most efficient and economical method for maize stem borer control.

Host plant resistance includes all those characteristics that enable a plant to tolerate, avoid or recover from the attack of insects under conditions that would cause greater injury to other plants of the same species. The effects of resistant plants on insects can be manifested as antibiosis, in which the biology of the pest is adversely affected; or as antixenosis, in which the plant acts as a poor host and the pest searches for an alternate host plant. When the inherent genetic qualities of the plant provide the ability to endure or recover from insect damage, it is said to express tolerance to the pest (Painter, 1951; Smith, 2005). The three systems of resistance are relevant to breeding for host resistance in plants and include morphological barriers to insects and presence of insect-repellant or toxic substances and toxins that have a repellent effect (Krattiger, 1997). The constitutive defense mechanisms in maize against stem borers originate from secondary metabolites that are synthesized by the plant during growth (Malvar *et al.,* 2008). To advance breeding for maize stem borer resistance, genotypes with different mechanisms of resistance can be identified and used for pyramiding of resistance genes for increased and durable resistance. Gene pyramiding has been a useful approach to maximize utilization of existing novel gene resources to confer multiple attributes of resistance in genotypes of interest as one way of delaying insect adaptation (Jiang *et al.,* 2004).

Different types of resistance mechanisms to insect pest attack have been reported and include both morphological and biochemical traits and work individually or collectively. Trichome density, leaf and stem hardness are important forms of physical resistance mechanisms (antibiosis) against maize stem borer damage (Kfir *et al*., 2002). Conversely, higher levels of pith sugars have been proven to contribute to increased stem borer susceptibility in cereals (Grof *et al.,* 2007).

Availability of information on maize genotypes with appreciable levels of resistance to stem borer insect pest is needed for the diverse maize growing ecologies in Kenya. Knowledge on levels of stem borer resistance, and the mechanisms involved in commercial maize hybrids and open pollinated varieties (OPVs) in Kenya is scanty. Classifying the mechanisms of resistance in existing maize genotypes could improve efficiency of selection through pyramiding of different mechanisms into the preferred elite materials.

MATERIALS AND METHODS

Germplasm and testing sites

The trial was conducted at the Kenya Agricultural Research Institute (KARI) Kiboko Farm in the mid-altitude dry agro-ecological zone of Kenya. Kiboko lies at 950m above sea level, 37.75E, and 2.15S and receives about 530mm of rainfall per annum, coming in two short rainy seasons. Kiboko's maximum daily temperature is 35°C and a minimum of 14°C. The soils are sandy clays. Eighty five hybrids which included commercial hybrids and CIMMYT experimental insect resistant checks, 15 OPVs and 68 landraces of Caribbean origin which included seven resistant CIMMYT hybrid checks were grown in α-lattice designs replicated three times during the 2011/2012 short rains season.

Experimental design

Due to phenotypic differences, hybrids and OPVs were established together as one trial and landraces as a separate trial. The genotypes were planted in single row plots of seven meters i.e. 29 hills per row spaced at 75cm and 25cm between and within rows, respectively. Two seeds were sown per hill and later thinned to one plant per hill. The trial was designed to accommodate destructive sampling of five plants per row. The plots, were, therefore marked with five strings in such a way as to separate the first one border plant, the next five plants (sampling plants), one protected plant, 10 plants to be infested, 10 pesticide protected plants, and two border plants in each row plot (Figure 1). The first plant in each

←----------7.5m----------→

Number of plants	1	5	1	10	10	2
Treatment	Border plant	Destructive sampling plants	Border plant	Borer infested plants	Borer protected plants	Border plants

←----------29 hills----------→

Figure 1 Field plot layout

row was the border plant; the five consequent plants were used for destructive sampling data collection. The next 10 plants were each infested with five second instar *C. partellus* neonates three weeks after planting. All the other plants were protected from insect damage by applying an insecticide (Bulldock® granules-Beta-Cyfluthrin – AI) at the rate of 8kg/ha, concurrently during infestation. The crop was rain fed but supplemental irrigation was applied as needed. Fertilizers were applied at the rate of 60kg/ha N and 102 kg/ha P_2O_5 at planting, and top-dressed at the rate of 48kg N/ha 30 days after planting. Plowing and harrowing were carried out using tractor drawn implements, while planting, weeding, harvesting and shelling operations were performed manually.

Data collection

Data was collected on leaf damage score (LD) on visual rating two weeks after infestation on individual plant basis on each of the 10 infested plants using a scale of 1-9 (where, 1= no visible leaf damage and 9 = plants dying as a result of leaf damage) as described by (Tefera *et al.,* 2011). At the time of leaf damage scoring, leaf toughness in kilogram-force was taken on five randomly selected plants per row using a penetrometer (Model FT011, ALFOSINE-Italy) at infestation time. The youngest leaf with fully developed ligule was punched on the adaxial side 2-3 veins away from the mid-rib. Before the onset of flowering, 10 randomly selected leaf samples per plot were taken from the protected plants for trichome density count. The leaf below the first ear was cut at the center of the blade and a cork borer of 1 cm diameter was used to punch a maize leaf disk for which the number of trichome hairs was counted under a dissecting microscope (10X). At the silking stage, stem penetrometer resistance was measured using a Penetrometer (FHT-803 fruit firmness tester software) using a fabricated needle. Five plants per row were punctured at the center of the second internode below the primary ear. The force was recorded in maximum kg-force. Stem pith sugar content was taken using a Brix Refractometer (r2mini Handheld Refractometer) after silking. The second internode below the primary ear was cut into ten (10cm) pieces and a 1cm radius cork borer used to extract the pith. The pith tissue was then squeezed to extract about two drops of juice onto the Refractometer sensor, and the sugar content reading expressed as a percentage (%). At harvest, the numbers of stem borer exit holes (EH) were counted and the cumulative tunnel length (TL) in cm was measured after splitting the stems across the middle.

Data analysis

Analysis of variance was done using PROC GLM (SAS, 2007) and the means compared using Fishers protected least significant difference test (LSD) at ($P < 0.05$). Genotypes were considered fixed effects. A multivariate analysis of variance within a canonical variate analysis was performed using the SAS package (Canonical discriminant analysis tool) to determine the most variable mechanism and resistance trait among the genotypes. Correlation analysis coefficients were calculated for mechanisms of resistance (trichome density, leaf toughness, sugar content and stem penetrometer resistance) and the stem borer damage parameters (leaf damage, exit holes and cumulative tunnel length) using canonical correlations (secures error control) in order to establish the relationship between the two sets of variables. A selection index based on the significant damage parameters was computed by summing up the ratios among values and dividing by the number of parameters summed up, as described in Tefera *et al*. (2011). Genotypes with selection index values of less than 0.8 were considered highly resistant, those between 0.8-1.0 considered moderately resistant, 1.0-1.2 as moderately susceptible, and over 1.2, as highly susceptible.

RESULTS

i) Landraces ANOVA, canonical discriminant analysis and correlation coefficients

The germplasm differed significantly in variables evaluated for stem borer resistance and mechanisms of resistance (Table 1). The sugar percentage content showed high and significant differences (<.0001) and ranged from 5.32 % in GUAN, to 10.83 % in CHIS 114 which were both highly resistant landraces, the trial mean was 9.09. Trichome density similarly showed high and significant differences (<.0001) and ranged from 0.86 in GUAT 280 which was moderately resistant to 12.26 in

Table 1: Analysis of variance for mechanisms of resistance and stem borer damage evaluation traits on the hybrids, OPVs and landraces

		Leaf damage		No. of exit holes		Tunnel length (cm)		Leaf toughness		Stem hardness		Sugar content %		Trichome density	
Source	DF	F-Value	Pr. > F	F-Value	Pr. > F	F-Value	Pr. > F	F-Value	Pr. > F	F-Value	Pr. > F	F-Value	Pr. > F	F-Value	Pr. > F
Hybrids & OPVs	99	1.24	0.108	1.47	0.014	1.97	<.0001	1.35	0.043	1.13	0.2473	2.02	<.0001	1.62	0.003
Landraces	74	1.51	0.026	1.68	0.007	1.73	0.0048	1.62	0.011	1.13	0.277	2.39	<.0001	2.53	<.0001

CIMMYT hybrid experimental check CKPH09001 which was highly resistant, the trial mean was 6.46. Leaf toughness showed significant differences (Pr>0.05) and ranged from 0.07 kg-force in BRAZ 1470 which was highly susceptible to 0.23kg-force in CKIR06009 which was highly resistant, the trial mean was 0.17kg-force (Table 2 below). The susceptibility index categorized the landraces into 17 highly resistant, 22 moderately resistant, 8 moderately resistant and 21 highly susceptible. The topmost 15 highly resistant landraces had a mean susceptibility index of 0.67 while the most highly susceptible had a mean index of 1.39. The most resistant was GUAT 1093 while the most highly susceptible was BRAZ 1736. Most GUAT accessions were mostly resistant while BRAZ accessions were mostly susceptible. Among the most highly resistant genotypes were three of CIMMYT's experimental resistant checks CKIR07008, CKIR06009 and CKIR09001.

The Anova's univariate statistics showed significant differences among the landraces for leaf damage, number of borer exit holes and cumulative tunnel length, sugar content, and trichome density, i.e. the variables class means were, therefore, not equal to zero at P<0.05. The canonical analysis indicated that the most important resistance mechanism in discriminating the landraces was the percentage stem sugar content followed by trichome density and leaf toughness in that order with canonical coefficient loadings in CAN1 of 0.95, CAN2 of 0.97, and CAN3 of 0.97, respectively (Table 3 below).The landraces R^2 values for sugar content, trichome density and leaf toughness were 0.55, 0.46 and 0.37, respectively. Number of stem exit holes explained the highest variation among resistance parameters (0.47). The R^2 values for resistance traits were 0.46 for leaf damage, 0.48 for exit holes and 0.47 for tunnel length.

The canonical loadings further showed that number of stem borer exit holes was the most important parameter in evaluating resistance with canonical coefficient loadings of 0.86 in CAN1, followed by cumulative stem tunnelling in CAN2 with 0.75 and leaf damage in CAN3 with 0.41.Canonical correlations indicated that the mechanisms of resistance and damage parameters were not correlated (Table 4below). 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 not significant. Accordingly, the Wilks' Lambda, one of the four multivariate statistics that tests the null hypothesis that the canonical correlations and all the smaller ones are zero was also not significant (Pr>0.2635), and so were the Pillai's Trace (Pr>0. 2634) and Hotelling - Lawley Trace (0. 2644).

ii) Hybrids and OPVs ANOVA, canonical discriminant analysis and correlations

There were significant differences in the hybrids and OPVs for number of stem borer exit holes, tunnel length, leaf toughness, stem sugar content, and trichome density (P<0.05) (Table 1). The susceptibility index categorized all the hybrids and OPVs into 18 highly resistant, 36 moderately resistant, 32 moderately susceptible and 14 highly susceptible. Five commercial hybrids were highly resistant, eight moderately resistant, 10 moderately susceptible and six highly susceptible. Five OPVs were highly resistant, six moderately resistant, four moderately susceptible and none was highly susceptible. The five highly resistant CIMMYT experimental insect resistant hybrid checks were CKIR09005, CKIR09001, CKIR09004 and CKIR09006 and CKIR06006 with an average selection index of 0.63 while the five highly resistant commercial hybrids and OPVs were KDV1-3-#, EEQPM-8-EA-#, DH02, KDV1-2-# and DKC8053. The most highly susceptible hybrids were H6210, 531A, H6213, H629, KH 600-15A with an average selection index of 1.78 (Table 5).

The means for number of exit holes and tunnel length were 1.44 and 7.78cm, respectively. The numbers of exit holes were lowest in CKIR09005 which was highly resistant at 0.44 and highest in

Table 2 Fifteen most resistant and 15 most susceptible landraces reaction to *Chilo partellus* maize stem borer and the resistance mechanisms

Pedigree	Entry	Indices	Leaf damage	Exit holes (#)	Tunnel length	Leaf toughness	Stem hardness	Sugar content	Trichome density
Resistant									
CHIS 114	22	0.43	3.2	1.17	1.16	0.17	2.75	10.83	10.39
GUAT 1093	13	0.57	2.99	1.35	3.39	0.19	3.11	9.83	11.71
CKIR07008	73	0.57	2.42	1.91	3.38	0.22	2.51	9.26	7.02
CKIR06009	72	0.6	2.51	1.32	6.11	0.23	2.69	9.27	7.52
PARA 151	24	0.6	2.69	1.46	5	0.22	2.79	9.31	5.63
GUAT 1034	7	0.68	3.43	0.93	7.08	0.19	2.47	7.91	7.89
GUAT 1100	5	0.68	2.65	1.68	7.1	0.21	3.23	9.57	8.32
OAXA 553	16	0.71	2.91	1.34	8.25	0.17	3.07	9.9	8.56
CKPH09001	69	0.71	2.46	2.2	6.46	0.22	2.74	9.27	12.26
VERA GP24	25	0.71	3.29	1.83	5.1	0.17	2.62	8.84	10.23
BRAZ 1371	47	0.71	2.89	1.67	7.32	0.19	2.08	9.3	3.45
GUAT 1081	1	0.75	2.85	2.22	6.4	0.15	2.42	7.11	7.01
GUAT 1155	10	0.76	3.17	1.91	6.82	0.17	2.93	9.13	5.54
BRAZ 1832	35	0.77	3.32	1.52	8.2	0.16	2.61	9.47	6.79
GUAT 1010	3	0.78	3.11	2.03	7.25	0.21	2.8	10.53	6.85
Mean		**0.67**	**2.93**	**1.63**	**5.93**	**0.18**	**2.72**	**9.3**	**7.94**
Susceptible									
NAYA 130	36	1.2	4.24	3.58	11.29	0.17	2.47	9.19	6.6
BRAZ 1797	40	1.2	3.21	4.86	10.06	0.17	2.75	8.59	4.2
BRAZ 2017	29	1.21	3.32	4.46	11.6	0.12	2.9	8.11	1.17
BRAZ 2149	31	1.27	3.27	4.23	14.34	0.18	2.64	8.81	8.11
SAOP GP11	60	1.33	2.81	3.81	19.51	0.13	2.33	8.74	3.9
GUAN 34	56	1.33	3.74	4.26	14.85	0.19	2.67	6.33	5.06
CHIS 94	58	1.34	4.45	4.53	11.6	0.16	2.65	8.5	3.39
BRAZ 1486	27	1.34	3.64	3.5	18.36	0.23	2.8	9.81	3.42
BRAZ 4	26	1.38	3.43	3.69	19.45	0.16	1.84	8.83	3.35
VALL 380	57	1.41	3.75	4.62	15.79	0.18	2.38	8.47	4.23
BRAZ 2179	32	1.45	3.34	4.57	18.74	0.15	2.31	10.23	3.41
NAYA 129	61	1.48	2.92	5.76	16.84	0.14	2.92	9.77	3.36
VALL 385	46	1.51	3.99	4.76	17.72	0.15	2.7	9.47	4.63
BRAZ 1364	34	1.68	4.33	4.84	22.14	0.21	3.04	10.34	6.59
BRAZ 1736	66	1.76	4.07	5.5	23.12	0.16	2.81	8.88	3.85
Mean		**1.39**	**3.63**	**4.46**	**16.36**	**0.17**	**2.61**	**8.94**	**4.35**
Trial mean			**3.24**	**2.85**	**10.93**	**0.174**	**2.73**	**9.09**	**6.468**
CV			**18.17**	**42.2**	**48.17**	**24.62**	**17.38**	**11.03**	**40.19**
P<value			**0.026**	**0.01**	**0.004**	**0.011**	**0.2769**	**<.0001**	**<.0001**

H629 at 4.22 which was highly susceptible. The tunnel length was lowest in CKIR09004 which was highly resistant at 1.96 and highest in H629 at 18.40cm which was highly susceptible. The mean for leaf toughness, trichome density and sugar content were 0.16, 7.39 and 7.88, respectively. Leaf toughness ranged from 0.12 in CKPH08028 which was among the most highly susceptible to 0.24 in DHO4 which was among the highly resistant. Trichome density count ranged from 13.46 in H628 to 0.00 in 614D which were both moderately

Table 3 Canonical discriminant analyses for mechanisms of resistance to maize stem borer and damage traits in tropical landraces

Mechanisms Traits	Total SD	Pooled SD	Between SD	R^2 –Value	Pr>F	CAN1	CAN2	CAN3	Attributed Variation
Leaf toughness	0.0505	0.049	0.0309	0.37	0.1787	0.2320	0.0614	**0.9706**	0.17
Stem hardness	0.527	0.5263	0.3051	0.33	0.4748	0.0480	0.3201	-0.0202	0.14
Sugar content	1.34	1.0949	1.0008	0.55	<.0001	0.9544	0.0930	-0.2838	0.42
Trichome density	3.5865	3.2172	2.4465	0.46	0.0023	0.0860	0.9770	-0.1044	0.27
Damage traits									
Leaf damage	0.659	0.592	0.448	0.46	0.0026	0.730	0.542	0.416	0.17
No. of exit holes	1.574	1.385	1.097	0.48	0.0006	0.859	0.365	0.360	0.47
Tunnel length	6.920	6.172	4.752	0.47	0.0016	0.615	0.747	0.250	0.36

Table 4: Summary statistics of the canonical correlations between the two sets of variables (damage parameters and mechanisms of resistance) in the 100 hybrids and OPVs and 75 landraces

	Canonical correlation	Squared Can Correlation	Eigen Values	Likelihood Ratio	Approx. F Value	Num DF	Den DF	Pr>F
Hybrids & OPVs								
CAN1	0.207223	0.04294	0.0449	0.94891	1.29	12	775.5	0.2169ns
CAN2	0.084651	0.00717	0.0072	0.99149	0.42	6	588	0.8663ns
CAN3	0.036709	0.00135	0.0013	0.99865	0.2	2	295	0.8196ns
Landraces								
CAN1	0.244847	0.06	0.0638	0.92971	1.34	12	577.07	0.1897ns
CAN2	0.102971	0.0106	0.0107	0.989	0.4	6	438	0.8759ns
CAN3	0.019998	0.0004	0.0004	0.9996	0.04	2	220	0.9570ns

ns = not significant at P>0.05

susceptible. Sugar content was highest in CKPH08038 at 15.17% and lowest in EEQPM-9-EA-#-#-# at 5.82% which were both moderately resistant. The average sugar content for the trial was 7.88 (Table 5 below).

The Anova's univariate statistics showed significant differences among the hybrids and OPVs for cumulative tunnel length, sugar content and trichome density i.e. the variables class means were therefore not equal to zero at P<0.05 (Table 6 below). The canonical analysis indicated that the most important resistance mechanism in discriminating the genotypes was the percentage stem sugar content followed by trichome density (similar to landrace accessions) and stem penetrometer resistance in that order, with canonical coefficient loadings in CAN1 of 0.95, CAN2 of 0.85, and CAN3 of 0.83, respectively. The R^2 values for resistance traits were 0.63 for sugar content, 0.44 for trichome density, 0.35 for stem penetrometer resistance and 0.33 for leaf toughness (Table 3 above).

The most important damage parameter in indicating resistance in hybrids and OPVs was the tunnel length in CAN1 with canonical coefficient loadings of 0.95, followed by number of stem borer exit holes in CAN2 of 0.84 and leaf damage in CAN3 of 0.58. The R^2 values for resistance traits were 0.47 for tunnel length, 0.39 for number of exit holes and 0.38 for leaf damage. Similar to the landrace accessions, the canonical correlations indicated no linear correlations between stem borer damage parameters and the mechanisms of resistance involved (Table 4 above). The Wilk's lambda multivariate statistic that tests the first canonical correlation which shows the greatest correlation was Pr>0.2060. The smaller multivariate statistic canonical correlations were also not significant for Pillai's Trace Pr>0.2090) and Hotelling-Lawley Trace (Pr>0.2035).

DISCUSSIONS

Mechanisms of resistance

Canonical discriminant analysis indicated that percentage

Table 5: Fifteen most resistant and 15 most susceptible hybrids and OPVs reaction to *Chilo partellus* and the resistance mechanisms

Pedigree	Entry	Indices	Leaf damage (score)	Exit holes (#)	Tunnel length (cm)	Leaf toughness	Trichome density	Sugar content (%)	Stem hardness
Resistant									
CKIR09005	42	0.51	2.5	0.44	2.02	0.17	7.02	7.47	2.1
CKIR09001	38	0.61	2.41	0.88	4.16	0.16	10.63	8.05	3.11
CKIR09004	41	0.66	3.09	1.13	1.96	0.18	8.81	7.07	2.2
CKPH08025	76	0.68	2.9	1.39	2.49	0.2	10.81	9.28	2.87
PH1	19	0.69	2.83	1.15	3.27	0.16	7.36	7.83	2.28
CKIR06006	48	0.69	2.47	1.11	4.73	0.16	8.75	6.9	2.4
CKIR09006	43	0.69	2.67	1.74	3.18	0.2	7.94	6.78	1.75
CKIR06001	46	0.71	2.63	1.28	4.39	0.14	6.21	8.05	1.85
CKIR04003	99	0.74	2.37	1.42	4.97	0.21	8.48	9	2.13
ECA STRIGOFF-VL-125-#-#	93	0.75	3.1	1.28	3.99	0.16	8.37	8.37	2.26
PH1	9	0.76	2.76	1.59	4.29	0.16	9.64	8.28	1.82
PH4	8	0.77	2.68	1.85	4.34	0.14	5.73	9.16	2.35
DH04	11	0.77	3.48	1.37	3.21	0.24	9.36	8.9	2.17
CKIR06007	49	0.78	2.31	1.73	5.56	0.17	3.77	8.15	1.91
CKIR06009	51	0.78	2.76	2.06	3.98	0.15	8.05	7.7	2.16
Mean		**0.72**	**2.73**	**1.36**	**3.77**	**0.17**	**8.06**	**8.07**	**2.22**
Susceptible									
631Q	22	1.21	3.78	2.73	9.8	0.19	10.57	6.16	2.02
CKPH08037	59	1.22	3.41	2.62	11.23	0.16	7.31	7.64	2.68
CKPH09003	56	1.23	3.06	3.08	11.51	0.15	8.88	8.02	2.05
CKPH08002	66	1.23	3.21	2.28	12.57	0.18	6.3	7.83	1.98
WH403	12	1.23	3.52	3.76	9.6	0.17	5.66	8.23	2.28
H6213	1	1.24	3.14	3.5	11.09	0.14	10.47	5.31	2.3
CKPH08004	68	1.24	2.87	2.72	13.3	0.13	4.73	8.4	2.1
CKPH09001	52	1.24	3.63	3.02	10.62	0.16	4.24	6.99	2.44
DK8031	24	1.26	2.65	3.58	12.67	0.19	11.18	7.46	2.38
KH 600-15A	18	1.29	3.46	2.57	13.11	0.15	5.04	7.12	2.67
H6210	3	1.29	3.89	3.17	10.94	0.17	9.19	7.41	1.99
CKPH08041	63	1.5	2.71	3.39	18.28	0.16	8.9	8.19	2.45
H6212	2	1.54	3.41	3.82	16.93	0.14	8.52	8.08	2.41
H629	4	1.63	3.6	4.14	18.4	0.13	7.49	6.61	1.54
Mean			**3.37**	**3.24**	**12.86**	**0.16**	**7.17**	**7.42**	**2.24**
Trial means			**3.11**	**1.44**	**7.78**	**0.16**	**7.39**	**7.88**	**2.18**
CV			**19.1**	**44.65**	**47.32**	**16.99**	**46.51**	**16.96**	**22.04**
P<value			**0.108**	**0.014**	**<.0001**	**0.043**	**0.0031**	**<.0001**	**0.247**

sugar content was the best resistance mechanism that discriminated the landrace accessions followed by trichome density and leaf toughness in that order. Susceptibility to *C. partellus* stem borer, therefore, increased with incresing stem pith sugar content which contributed the highest variation amongst the mechanisms evaluated. This was consistence with the reports of Santiago *et al.* (2003) and Grof *et al*. (2007) who reported that increased levels of pith sugars contributed to increased stem borer susceptibility in maize and rice cereals. However, in a few of germplasm evaluated that principle did not hold true, for example CHIS 114 which was the most resistant landrace had a high sugar content at 10.83%, while GUAN 34 had 6.33% sugar content and was highly susceptible This finding supports the report that there are several sugar analogs in cereals and each may have different effects towards the resistance or the susceptibility of a genotype (Juma, 2010). The contribution of each resistance mechanism trait may differ between germplasm and, therefore, higher

Table 6: Canonical discriminant analyses for mechanisms of resistance to maize stem borer and damage traits in hybrids and OPVs

Mechanisms Traits	Total SD	Pooled SD	Between SD	R^2 –Value	Pr>F	CAN1	CAN2	CAN3	Attributed Variation
Leaf toughness	0.0339	0.0338	0.0196	0.33	0.4643ns	0.158197	0.270934	0.389304	0.16
Stem hardness	0.5095	0.4999	0.3051	0.36	0.254ns	0.123365	0.014374	0.839971	0.12
Sugar content	1.1729	0.8748	0.9326	0.63	<.0001	0.953734	0.270608	0.111107	0.51
Trichome density	3.8452	3.5062	2.5703	0.44	0.0024	0.349382	0.85654	0.154494	0.21
Damage traits									
Leaf damage	0.6179	0.5964	0.3806	0.38	0.1182ns	0.270456	0.842122	-0.46657	0.23
No. of exit holes	1.1422	1.0894	0.7171	0.39	0.0611ns	0.541028	0.605852	0.583294	0.31
Tunnel length	4.4093	3.9366	3.0228	0.47	0.0004	0.950974	0.17018	0.258238	0.46

ns = not significant at P>0.05

resistance levels could be achieved by a combination of these traits.

Similar to landrace accessions, in hybrids and OPVs, canonical discriminant analysis indicated that the percentage sugar content was the best resistance mechanism for discriminating the hybrids and OPVs followed by trichome density. Resistance levels increased with decreasing sugar content, and increasing trichomes density. Sugar content contributed the greatest variation (0.51) while trichome density contributed 0.21 variation in the resistance in genotypes evaluated. The hybrids and OPVs exhibited lower mean sugar content (7.88%) than the landraces (9.09%) which supported the higher resistance levels in the landraces than the hybrids as indicated by the lower susceptibility index values.

Canonical discriminant analysis ranked trichome density as the second best mechanism in discriminating the landrace accessions after sugar content. Resistance increased with increasing trichome density in the majority of the landraces. Kumar (1997), Rao and Panwar (2000) and Dalin *et al*. (2008) showed that leaf trichome density was a main contributing factor towards stem borer resistance in maize. Increased trichome density may contribute to non-preference for egg-laying on that host negatively affecting larvae feeding and thus development. Kumar (1997) reported that non-preference types of resistance mechanisms were highly operational within tropical maize germplasm against insect pest herbivores. Handley *et al.* (2005) similarly reported that in the model plant *Arabidopsis thaliana's* resistance to a specialist herbivore (white fly) was positively related to the density of leaf trichomes, and oviposition preference by the female moths was positively correlated to larval performance on different populations. Studies in Africa indicated that ovipositional nonpreference on maize genotypes was attributed to the presence of trichomes and surface waxes which inhibited the insect's growth and development (Kumar, 1997).

There were, however, a few variations in the landraces whereby higher trichome density did not correlate to higher resistance levels in the germplasm. For instance, PARA 151 with a trichome density of 5.63 was among the top most resistant while BRAZ 2149 with a higher trichome density of 8.47 was highly susceptible. The type of trichomes, size, position and chemical composition may have an impact on the extent and role of the trichomes on different maize germplasm. Previous research has reported that the number of leaf trichomes, type, and the relative position to the stem terminus were best predictors of oviposition (Heinz and Zalom, 1995). In Handley *et al.* (2005) the different types of trichomes are reported to have diverse effects on herbivore pests depending on the secondary compound they contained. In maize, *C. partellus* lays its eggs on the upper and underside leaf surfaces, mainly near the midribs on young maize seedlings, therefore, the presence of trichomes would interfere with oviposition.

Similarly, canonical discriminant analysis ranked trichome density as the second best in discriminating the hybrids and OPVs into resistant and susceptible categories. Leaf trichomes have been reported to hinder/limit oviposition, larvae movement and feeding thereby affecting the pest's growth and development on the plant host (Handley *et al.,* 2005; Kfir *et al*., 2002). Dalin (2008) and Maes and Goossens (2010) similarly reported that resistance to insect pests in many plant species increased with increasing trichome density. Selection of plants with higher densities of trichomes can, therefore, be an efficient criterion to improve levels of resistance to maize stem borers.

Leaf toughness (leaf penetrometer resistance) was ranked third best in discriminating all the germplasm i.e. the landraces, hybrids and OPVs. Leaf toughness is one of the antibiosis resistance mechanisms that could limit stem borer feeding on maize leaves. Sarwar (2012) reported significant negative correlations between leaf toughness and leaf feeding which is important in conferring resistance in cereals. Further, Bergvinson *et al*. (1995), Smith (2005), Afzal *et al.* (2009) all reported that leaf toughness contributed an important defense

mechanism in maize plants against stem borers. These differences could, however, be attributed to the genetic compositions of the different germplasm evaluated. Resistance levels in the germplasm evaluated could have relied heavily on a combination of other mechanisms, such as pith antibiosis (stem sugar content), high trichome density etc and less on leaf toughness. Increased leaf toughness can contribute to reduced grain yield losses through reduced leaf damage that usually leads to loss in photosynthetic area. The fact that resistance increased with increasing leaf toughness was an indication that it act as a deterrent to larvae feeding.

Damage parameters

Leaf damage score, number of borer exit holes and cumulative stem tunnelling showed high and significant differences ($P>0.05$), and were important in indicating resistance. Number of stem exit holes contributed the highest variation followed by stem tunnelling length and leaf damage in that order, respectively. From canonical analysis, number of exit holes and tunnel length were the most important in discriminating the genotypes in all germplasm evaluated, while leaf damage score was an important parameter in landrace accessions only. Number of stem exit holes and cumulative tunnel length were highly correlated in both hybrids and OPVs ($r=0.75$) and landraces ($r=0.67$), a suggestion that any of the two traits could be used to evaluate germplasm for resistance to the stem borer, and as a result contribute to reducing phenotyping costs involved in the extensive data collection. Canonical correlations between the damage parameters and the mechanisms of resistance were not significant and the lack of correlation suggested the possible presence of other mechanisms of resistance most likely not assessed in this study. The resistance mechanisms could also have inherently differed between germplasm. Different germplasm may carry different forms of resistance genes that may act together to confer resistance. Beck (1965) reported that host plant resistance which included all heritable characteristics by which a plant reduced the possibility of successful attack as a host by an insect pest was an important pest control method. When several resistance mechanisms are involved, this could increase resistance levels in the genotypes and, therefore, contribute to reduction in yield losses through gene pyramiding. Levels of resistance in both the hybrids, OPVs and the landraces increased with decreasing number of exit holes, stem tunnel length and leaf damage score indicating that each contributed to the resistance. Arabjafari and Jalali (2007) and Afzal *et al.* (2009) reported comparable importance in reduced number of stem exit holes, stem tunnelling and leaf damage in reducing genotype susceptibility to maize stem borer.

CONCLUSIONS

Resistant genotypes were identified among the different tropical maize germplasm categories evaluated. Landraces and OPVs, however, exhibited the highest levels of resistance than the hybrid varieties. The percentage sugar content and the trichome density were identified as the best resistance mechanisms that discriminated all the genotypes evaluated. The two traits showed consistency throughout the different germplasm and contributed the highest variation towards the resistance. All the damage parameters evaluated were important in determining the resistance levels. The number of exit holes and tunnel length, however, consistently contributed more variation than leaf damage across the different genotypes. The results strongly suggested that different genes contributed towards the overall resistance exhibited, and each trait contributed a certain fraction. This was an indication that breeders have the opportunity to use the different forms of resistance mechanisms from different genotypes to pyramid such genes in elite genotypes for improved resistance levels. The lack of a strong linear canonical correlation between damage parameters and resistance mechanisms in the hybrids, OPVs and landraces suggested that other mechanisms of resistance beyond those that were evaluated in this study could have been involved in conferring resistance against the maize stem borer damage.

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REFERENCES

Afzal M, Nazir Z, Bashir M, Khan B (2009). Analysis of host plant resistance in some genotypes of maize against *chilo partellus* (swinhoe) (pyralidae: lepidoptera). Pak. J. of Bot. 41:421-428.

Arabjafari KH, Jalali SK (2007). Identification and Analysis of Host Plant Resistance in Leading Maize Genotypes against Spotted Stem Borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). Pak. J. of Biol. Sci. 10:1885-1895.

Beck SD (1965). Resistance of plants to insects. Annual Rev. of Entomol. 10:207−232.

Bergvinson DJ, Hamilton RI, Arnason JT (1995). Leaf profile of maize resistance factors to European corn borer, *Ostrinia nubilalis.* J. of Chem. Ecology 21:343-354.

Dalin P, Agren J, Bjorkman C, Huttunen P, Karkkainen K (2008). Leaf Trichome Formation and Plant Resistance to Herbivory., p. 89-105, *In* A. Schaller, ed. Induced Plant Resistance to Herbivory. Springer Science+Business Media.

De Groote H (2002). Maize yield losses from stem borers in Kenya. Insect Sci. Applic. 22:89-96.

FAOSTAT (2012). FAOSTAT Statistics Division: Agricultural Data Retrieved from http://faostat.fao.org/site /567/Desktop Default.aspx? PageID=567#ancor. Food and Agriculture Organization of the United Nations, Rome, Italy.

Grof CPL, Albertson PL, Bursle J, Perroux JM, Bonnett GD, Manners JM (2007). Sucrose-Phosphate Synthase, a Biochemical Marker of High Sucrose Accumulation in Sugarcane. Crop Science 47:1530-1539.

Handley R, Ekbom B, Agren J (2005). Variation in trichome density and resistance against a specialist insect herbivore in natural populations of Arabidopsis thaliana. Ecological Entomology 30:284-292.

Heinz KM, Zalom FG (1995). Variation in Trichome-Based Resistance to Bemisia argentifolii (Homoptera: Aleyrodidae) Oviposition on Tomato. J. of Econ. Entomology 88:1494-1502.

Jiang GH, Xu CG, Tu JM, Li XH, He YQ, Zhang QF (2004). Pyramiding of insect- and disease-resistance genes into an elite indica, cytoplasm male sterile restorer line of rice. Plant Breeding 123:112-116.

Juma G (2010). Basis of host plant recognition and acceptance by *Busseola fusca* (fuller) (Lepidoptera: noctuidae) larvae, Jomo Kenyatta University of Agriculture and Technology, Nairobi.

Kfir R, Overholt WA, Khan ZR, Polaszek A (2002). Biology and management of economically important lepidopteran cereal stem borers in Africa. Annual Rev. Entomology 47:701-731.

Krattiger AF (1997). Insect Resistance in Crops: A Case Study of Bacillus thuringiensis (Bt) and its Transfer to Developing Countries. ISAAA Briefs No. 2, Ithaca, NY.

Kumar H (1997). Resistance in maize in *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae): an overview. Crop Protection 16:243-250.

Maes L, Goossens A (2010). Hormone-mediated promotion of trichome initiation in plants is conserved but utilizes species- and trichome-specific regulatory mechanisms. Plant Signaling and Behavior 5: 205–207.

Malvar RA, Butron A, Ordas B, Santiago B (2008). Causes of natural resistance to stem borers in maize. *In* E. N. Burton and P. V. William, eds. Crop protection research advances. Nova Science Publishers Inc., New York.

Mati BM (2000). The influences of climate change on maize production in the semi-humid-semi arid areas of Kenya. J. of Arid Environ. 46:333-344.

Mbapila JC, Overholt WA, Kayumbo HY (2002). Comparative development and population growth of an exotic stem borer, *Chilo partellus* (Swinhoe), and an ecologically similar congener, *Chilo orichalcociliellus* (Strand) (Lepidoptera: Crambidae). Insect Science Application 22:21-27.

Ong'amo GO, Le Ru BP, Dupas S, Moyal P, Calatayud PA, Silvain JF (2006). Distribution, pest status and agro-climatic preferences of lepidopteran stem borers of maize in Kenya. Annales de la Societe Entomologique de France 42:171-177.

Painter RH (1951). Insect resistance in crop plants. Macmillan, New York.

Pressoir G, Berthaud J (2004). Population structure and strong divergent selection shape phenotypic diversification in maize landraces. Heredity 92:95-101.

Rao CN, Panwar VP (2000). Morphological plant characters effecting resistance to Chilo partellus in maize. Annals of Plant Protection Science 8:145-149.

Santiago R, Souto XC, Sotelo J, Butrón A, Malvar RA (2003). Relationship between maize stem structural characteristics and resistance to pink stem borer (Lepidoptera: Noctuidae) attack. J. of Econ. Entomology 96:1563-1570.

Sarwar M (2012). Management of rice stem borers (Lepidoptera: Pyralidae) through host plant resistance in early, medium and late plantings of rice (*Oryza sativa* L.). J. of Cereals and Oil seeds 3:10-14.

SAS (2007). Proceedings of the Twenty-Eighth Annual SAS®Users Group International Conference. SAS Institute Inc, Cary, NC, USA.

Smale M, Byerlee D, Jayne T (2011). Maize Revolutions in Sub-Saharan Africa. Policy Research Working Paper 5659. World Bank Development Research Group: Agriculture and Rural Development Team.

Smith MC (2005). Plant Resistance to Arthropods: Molecular and Conventional Aproaches. Springer, Dordrecht, Netherlands.

Tefera T, Mugo S, Tende R, Likhayo P (2011). Methods of Screening Maize for Resistance to Stem Borers and Post-harvest Insect Pests. International Maize and Wheat Improvement Center (CIMMYT), Nairobi, Kenya.