Role of Husk Cover and Physical Properties of Maize Grains Towards Resistance to *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae)

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Abstract

This study evaluated twenty maize varieties for their resistance to *Sitotroga cerealella* under both free and no-choice tests. Ear damage score, developmental period, total progeny emerged, susceptibility index, percent grain damage and grain weight loss were evaluated susceptibility parameters. Ear husk and grain biophysical characteristics which may influence infestation by *S. cerealella* were also investigated and correlated with susceptibility parameters. Majority of the varieties were resistant and moderately resistant under free choice test. However, under no-choice test, only three varieties, i.e., Pratap Makka-5, EH-2101 and EH-2253 were categorized as resistant and moderately resistant, while the remaining varieties showed susceptible to highly susceptible reaction. Under both free and no-choice tests Pratap Makka-5, EH-2101 and EH-2253 were grouped under the same category. We found an inverse relationship between husk extension and ear damage (-0.610*), husk leaf number and ear damage (-0.785**), and percent grain damage and grain hardness (-0.648**); while strong positive relationship between grain size and susceptibility index (r=0.673**) and ear damage score and grain texture (r = 0.698**) were observed. Additionally, a strong negative correlation was found between progeny emerged and grain hardness (-0.696**). Our study leads to the conclusion that of the different husk cover and grain physical variables used to assess *S. cerealella* behaviour and biology; husk extension, husk tightness, grain hardness, grain size, and grain texture were better indicators of resistance for practical application. Such information could be used to establish a baseline for developing agronomically and biophysically elite maize germplasm that confers resistance against *S. cerealella* in the field and storage.

Keywords: Grain physical characters, Husk covers, Maize weevil, Varietal susceptibility

Introduction

The cultivation of maize is becoming popular worldwide because of its higher yield potential. It is a staple food for most people of the Asian and African countries which is an important source of carbohydrate. Maize occupies the largest land area of all staples in Sub-Saharan Africa (SSA). More than 208 million people in SSA depend on maize for food security and economic well-being. Destruction of food grain by stored grain insect pests is a major factor responsible for food shortage and food self-insufficiency in many tropical countries. Low agricultural yields have been blamed for the food self-insufficiency of those tropical countries. Significant volumes of cereal grains in developing countries are lost after harvest. Minimizing cereal losses in the supply chain could be one resource-efficient way that can help in strengthening food security, sustainably combating hunger, and improving farmers' livelihood (Kumar and Kalit, 2017).

Among various insect pests that have been commonly reported infesting stored grains, the Angoumois grain moth, *Sitotroga cerealella* (Oliver) and rice weevil, *Sitophilus oryzae* L. are the most destructive primary pests of cereal grains such as maize, sorghum, wheat,etc. (Anonmous, 1979; Pathak and Jha, 2003). Infestation by *S. cerealella* starts in the field but most damage occurs during storage. These pre-harvest infestations could be conducive to post-harvest insect population build-up. Its damage makes kernels more susceptible to secondary insect pests. The losses caused by *S. cerealella* to stored maize have been estimated ranging from 13 to 21% (Anonmous, 1979). For long time the efficacy of a particular control measure was the only consideration in pest control, which led to the wide use of insecticides in maize stores around the world. However, due to unavailability and high cost, the majority of farmers in developing countries do not apply chemical insecticides against storage insect pests of cereals (Mendesil *et al.* 2007). Moreover, storage insects are increasingly becoming resistant to many synthetic chemical compounds (Guedes *et al.*, 1995). In general, the injudicious use of pesticides has generated a number of biological and environmental hazards in air, water, soil and food. These man-created problems have further resulted in environmental pollution, adverse effect on non-target organisms, resistance development, and food contamination with toxic residues (Dhuyo and Ahmed, 2007; Kumar et al., 2007). Keeping in view these biological threats, alternate and non-hazardous techniques which are cost-effective and eco-friendly insect pest management options are of paramount importance.

In the management of *S. cerealella*, the use of host plant resistance plays an important role. Transgenic varietal resistant, most often employing insecticidal proteins derived from *Bacillus thuringiensis* (Bt), has proven effective in early trials (Lynch *et al.*, 1999). However, transgenic crops have been the subject of worldwide controversy (Gura, 2001; Rector *et al.*, 2002) and may not be acceptable in all markets. In addition, the development of pest insect populations with resistance to Bt toxins is a concern (Palumbi, 2001).

Susceptibility to infestation by *S. cerealella* varies among maize genotypes, according to their husk cover qualities and grain physical characteristics. Seed hardness directly affected resistance to several stored insect-pests such as: *S. cerealella* in paddy (Aruna *et al.*, 2009), *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* Motschulsky in maize (Meikle *et al.*, 1998; Gudrups *et al.*, 2001). A significant correlation was observed between kernel size and hardness of different maize genotypes and number of

S. cerealella per kernel in a free and no-choice test (Khan *et al.*, 2005). The maize kernels with complete and tight husk cover were virtually non-infested with *S. cerealella* and *S. zeamais* (Weston *et al.*, 1993; Kossou, 2001; Demissie *et al.*, 2008). The characteristics of husk cover and grain physical properties in relation to maize resistance to moths and weevils have not been extensively studied as compared to biochemical characters that confer the resistance (Ashmo and Khanna, 2006; El-Sebai *et al.*, 2006; Shafique *et al.*, 2006; Shafique and Chaudry, 2007; Meena and Singh, 2007; Butron *et al.*, 2008; Demissie *et al.*, 2008). Knowledge of grain resistance based on husk cover qualities, grain physical characteristics and insect behavior would help in decreasing the post-harvest storage losses. The use of resistant or least susceptible varieties integrated with other sustainable pest control methods will provide a long-lasting solution. Therefore, the objectives of this study were to evaluate the susceptibility of twenty maize varieties against *S. cerealella* under free and no-choice tests and to associate the husk cover and grain physical characteristics with resistance/susceptibility parameters.

Materials and Methods

Maize varieties used for the study

Twenty improved maize varieties used for the study were obtained from all India Coordinated Maize Improvement Project, Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur, India. The maize varieties evaluated against *S. cerealella* are indicated in Table 1.

Table 1. List of maize varieties tested and their type, grain color, and maturity group.

S.N.	Name of variety	Type	Grain color	Maturity group
1	PMH-1	Hybrid	Yellow	Late
2	Seed tech2324	Hybrid	White	Early
3	Pratap makka-5	Composite	Yellow	Medium
4	Navjot	Composite	Yellow	Medium
5	PE HM-2	Composite	Yellow	Early
6	Aravali makka-1	Composite	White	Early
7	Pratap chari makka-6	Composite	White	Late
8	Super 9220	Hybrid	Yellow	Late
9	KH-101	Hybrid	Yellow	Late
10	PAC-790	Hybrid	Yellow	Late
11	NK-30	Hybrid	Yellow	Early
12	HM – 10	Hybrid	Yellow	Late
13	GK – 3090	Hybrid	Yellow	Medium
14	Viveк Hybrid-9	Hybrid	Yellow	Late
15	HQPM-1	Hybrid	Yellow	Late
16	EHQ-16	Hybrid	White	Medium
17	HQPM-7	Hybrid	Yellow	Late
18	EH-2101	Hybrid	Yellow	Early
19	EH-2253	Hybrid	Yellow	Medium
20	EHQ-63	Hybrid	Yellow	Medium

Investigation on husk quality traits

Twenty maize varieties were grown at the experimental field of the Rajasthan College of Agriculture, Udaipur during 2012 and 2013 main growing seasons. The varieties were planted using a randomized complete block design with three replications. At harvest ten ears from the middle rows of each plot were randomly selected and harvested. For each sampled ear, harvest husk cover qualities were characterized as both bare tipped or complete husk cover, and loose or tight husk cover. Besides, data on husk leaf number and 100 seed weight were also recorded. Ear damage by *S. cerealella* at harvest was also assessed based on natural infestations. Ear damage was assessed by visual damage rating scale of 0–5. Husk tip extension was measured as the length (cm) of the husk extending beyond the tip of the ear by using a ruler. Husk tightness rating was also done for the sampled ears on a scale of 1–4 based on a visual assessment of cob sheath looseness or tightness (Giles and Ashman, 1971), where: 1 = very tight husk (all husks are strongly attached to the ear), 2 = slightly tight (half of the upper husk leaves are detached from ear), 3 = loose (all except the last sheath are detached from the ear) and 4 = very loose husk (ears are totally protruded from the sheath). Data on husk leaf number was recorded by dehusking and counting number of husk leaves separately.

Ear damage assessment under free choice test

After ear damage score at harvest the ears were placed in labelled nylon cloth bags and brought to entomology laboratory. The bags were arranged in CRD with three replications. The bags were tightly closed to avoid new infestation and then kept in the laboratory at ambient temperature for one month in order to investigate latent infestation. A storage time of one month was chosen because it was sufficient time to allow larvae to complete their development but short enough to prevent eggs laid by emerging adults to complete development to adults (Weston *et al.*, 1993). After a month, the ears were investigated for latent infestation and then transferred into perforated seed bag to allow new infestation and kept at the same condition to evaluate ear damage under free choice test. After three months of the storage period, insect damage rating (0–5 scale) for each ear was also visually evaluated by rotating the ear in fingers at least twice to estimate ear damage (Compton and Sherington, 1999; Kumar, 2002), where; 0–1 = slight damage (highly resistant), 1.1–2 = light damage (resistant), 2.1–3 = moderate damage (moderately resistant), 3.1–4 = heavy damage (susceptible) and 4.1–5 = extremely heavy damage (highly susceptible).

Susceptibility of shelled grain under no-choice test method

Rearing of S. cerealella

The pure culture of *S. cerealella* was obtained from Entomology laboratory, Indian Agricultural Research Institute, New Delhi. The culture was subsequently reared on disinfested and conditioned maize grains of Pratap maize variety in 4 L plastic jars in the laboratory. The moth culture was maintained by continuously releasing the insects on fresh disinfested and conditioned grains. The culture was maintained at a temperature of 28 ± 2.0°C and relative humidity of 65 ± 5% and 12 h light: 12 h dark photoperiod in biological oxygen demand (B.O.D) incubator.

Bio-assay procedures and trail design

Samples of 200 g of the disinfested and conditioned maize grains were taken from each batch of the selected maize varieties for the experiment and were put in a 350 cm^3-

glass jar covered with muslin cloth and tightened with rubber band to permit adequate ventilation. Each treatment was replicated three times in a Completely Randomized Design (CRD). Fifty unsexed one to two day old moths of *S. cerealella* were collected from stock culture and released in the glass jar from top for no-choice oviposition on maize grains. After 10 days of oviposition, the moths (dead/live) were removed and the jars were then kept at the same experimental condition for F_1 progeny emergence. Dobie index of susceptibility was used to categorize the varieties into different resistant/susceptibility group. Twenty-eight days after moth introduction, the containers were checked every other day for adult emergence and data was recorded for first generation adult emergence. Examination of each jar and collection of emergent moths was continued until no further emergence had been noted for 14 days. The numbers of days from middle of oviposition period to fifty percent F_1 progeny emergence were recorded for each replicate to get median development time. Based on the number of moths emerged in each test variety and median developmental period; Dobie index of susceptibility was calculated by the following formula (Dobie, 1978).

$$\text{Susceptibility Index (SI)} = \frac{\text{Natural } \log F_1}{D} \times 100;$$

Where, F_1 is the total number of first generation emerging adults and D is the median developmental period. The susceptibility index, ranging from 0 to 11, was used to classify the maize varieties: 0–4 = resistant, 4.1–7.0 = moderately resistant, 7.1–10 = susceptible and $\geq$10.1 = highly susceptible.

At the termination of the experiment and after the record of the final weight, the grains containing holes were separated from the sound grains and both damaged and sound grains were counted. Grain damage was expressed as a proportion of the total number of grains. The percent weight loss was calculated according to the following formulae:

$$\text{Grainweightloss}(\%) = \frac{(\text{Initial Weight} - \text{Final Weight})}{\text{Initial Weight}} \times 100$$

Physical grain characterization

Whole kernels were used for physical characterization. At harvest grain texture was rated visually using a scale of 1–5 as described by Kim and Kossou (2003); (1 = flint; 2 = semi flint; 3 = semi dent; 4 = dent; 5 = extremely dent). The grain size (number of grains/50 g) was determined by counting the number of grains per 50 g of each test variety. Ten kernels were randomly picked from each maize variety to determine kernel length and breadth by using digital calipers. Hundred seed weight was determined by counting hundred seeds in three replicates per maize variety and then weighed in the laboratory using a sensitive weighing balance. The number of kernels contained in a 50 ± 0.1 g grain sample of each variety was determined and this number was divided into 50 g to obtain the weight per kernel. The seed hardness was measured by using a manually operated hardness tester. It is expressed as kg force applied. The crushing strength was calculated with respect to the projected area of the indenter used (πr^2). The diameter of the indenter was 3 mm.

$$\text{Crushingstrength}\left(\frac{\text{kg}}{\text{cm}^2}\right) = \frac{\text{Weight required for crushing the seed}}{\text{Projected area of seed under load}}$$

The bulk densities were measured by using a measuring cylinder (1000 ml) and a digital balance. The weight of the seeds (*mgA*) filled in the cylinder divided by the volume of the cylinder (*Vc*) gave us the bulk density (ρb). It was calculated as shown by Ahmed and Raza (2010a).

$$Bulk\ density\ (Kg/m^3) = \frac{m_{gA}}{V_c}$$

Grain samples for the specific gravity estimation were drawn by volume and averaged 20 g. These samples were weighed first in air and again when suspended in a mesh basket submerged in tap water plus a wetting agent to maximize contact of seeds with water. Specific gravity was calculated according to the formula given by Ahmed and Raza, 2010b:

$$\text{Specificgravity(SG)} = \frac{\text{Weightinair}}{(\text{Weightinair} - \text{Weightinwater})}$$

Statistical data analysis

All non-categorical data were subjected to analysis of variance (ANOVA) by using PROC GLM procedure (SAS institute, 2004) and differences among means were compared by the Student-Newman-Keuls test at 5% level of significance. Husk cover characters, ear damage score at harvest, 100 seed weight, and grain texture data were analyzed separately for each season. Data on percentage grain damage was analysed after angular transformation (arcsine$\sqrt{proportion}$*180/π). Percentage of grain weight loss was square root transformed ($\sqrt{X+0.5}$). Total progeny emergence and measurement of husk tip extension (cm) data were log transformed before subjected to ANOVA. All transformation was performed as per the statistical rule of data transformation given by Steel and Torri (1984). Analysis of variance was performed on the transformed and untransformed data. The husk tightness, ear damage and grain texture score data were categorical variables, hence were subjected to Kruskal-Wallis non-parametric test. Means of untransformed data are presented in the result tables. The correlation between damage by the grain moth, husk quality traits, and kernel physical characters were examined using Pearson's correlation coefficient using PROC CORR procedure of the SAS software (SAS Institute 2004). All data used in the correlation procedure were the combined results of the 2-years study.

Results

Husk covers qualities and other damage and agronomic traits

There were significant differences among varieties in husk tip extension, husk tightness, number of husk leaves, ear damage score at harvest, hundred seed weight and grain texture in both 2012 and 2013 growing seasons (Tables 2 and 3). Results revealed that entries 2, 3, 4, 9, and 13 had significantly better husk tip extension as measured by the length of the extension of the husk beyond the ear tip in both seasons; whereas, entries 5, 7, 10, 14, 17, 19, and 20 had significantly poor husk tip extension. Entries 3, 4, 7, 9, 10, 11, and 15 had significantly tight husk cover as compared to others; whereas, for number of husk leaves, entries 1, 3, 10, and 13 had significantly higher husk leaf number as measured by the husk leaf count during dehusking in both growing seasons. It was observed that at harvest the overall infestation levels in the field during the study period were quite low to clearly differentiate the varieties. In general, in both 2012 and 2013 the entries, 2, 3, 11, and 13 that had good husk characteristics (extended tip and tight husk) resulted in lower ear damaged score. Those varieties with poor husk characteristics (bare tipped and loose husk cover), harboured relatively a greater number of moths and suffered ear damage (Tables 2 and 3). Relatively higher ear damage was observed in the year 2013. Hundred seed weight ranged from 15 to 26 g. The grain weight of 100 seeds was lowest in entry 15 followed by entry 10. Highest weight of 100 seeds was recorded for entries 17, 18, and 19 followed by entries 3, 20, and 7 in both seasons compared to the rest of the entries. Results on grain texture revealed that among the 20 maize varieties 12 of them were categorized as flint and semi-flint, while the remaining eight varieties were identified as dent and semi-dent.

Table 2. Husk cover, ear damage, hundred seed weight and grain texture rating for twenty maize varieties in 2012.

Entry	Varieties	Husk tip extension (cm)	Husk tightness rating (1–4)	Husk leaves (No.)	Ear damage score at harvest (0–5)	100 seed weight (gm)	Grain texture (1–5)	Grain texture (dent/flint)
1	PMH-1	1.90±0.86d-g	2.00±0.00a	11.00±1.15bc	0.16±0.12bc	23.49±0.45e	4.53±0.33ab	Dent
2	Seed tech2324	5.26±1.12ab	2.00±0.00a	10.00±0.57bcd	0.15±0.04bc	21.16±0.18i	4.00±0.57abc	Dent
3	Pratap makka-5	2.93±0.58a-f	1.67±0.33ab	13.66±1.20a	0.00±0.00c	25.70±0.79ab	3.33±1.20a-e	Semi-flint
4	Navjot	4.43±1.29a-d	1.66±0.33ab	9.33±0.33b-e	0.16±0.07bc	22.80±0.10ef	1.00±0.00h	Flint
5	PE HM-2	1.23±0.62fg	2.00±0.00a	10.00±1.00bcd	0.35±0.16a	19.64±0.58jk	1.33±0.33gh	Flint
6	Aravali makka-1	5.00±1.44abc	2.00±0.00a	10.00±0.57bcd	0.00±0.00c	22.35±0.17fgh	1.66±0.67fgh	Flint
7	Pratap chari makka-6	1.83±1.20efg	1.66±0.33ab	9.00±0.57c-f	0.26±0.17ab	24.84±0.17cd	2.00±0.00e-h	Semi-flint
8	Super 9220	4.33±1.58a-e	2.00±0.00a	7.33±0.88ef	0.00±0.00c	22.65±0.26fg	2.33±0.33d-h	Semi-flint
9	KH-101	5.56±1.78ab	1.66±0.33ab	9.33±0.33b-e	0.16±0.06bc	25.42±0.34bc	2.67±1.20c-g	Semi-flint
10	PAC-790	2.66±0.24a-g	1.66±0.33ab	11.33±1.33b	0.17±0.11bc	18.97±0.56k	2.00±0.00e-h	Semi-flint
11	NK-30	4.60±0.60abc	1.00±0.00c	7.00±1.00f	0.00±0.00c	19.93±0.16j	3.67±0.33a-d	Semi-dent
12	HM – 10	3.66±0.48a-g	2.00±0.00a	7.66±0.33ef	0.08±0.02c	19.70±0.38jk	3.00±0.00b-f	Semi-dent
13	GK – 3090	6.00±0.28a	2.00±0.00a	10.67±0.33bc	0.00±0.00c	21.59±0.16hi	4.67±0.33a	Dent
14	Vivek Hybrid-9	2.06±0.93c-g	2.00±0.00a	7.33±0.33ef	0.24±0.00ab	21.22±0.48i	1.00±0.00h	Flint
15	HQPM-1	4.10±0.55a-d	1.33±0.33bc	9.33±0.33b-e	0.34±0.03a	15.89±0.28k	4.66±0.33a	Dent
16	EHQ-16	3.20±0.64a-f	2.00±0.00a	9.00±0.57c-f	0.17±0.06bc	21.8±0.11ghi	2.66±0.33c-g	Semi-flint
17	HQPM-7	1.20±0.41fg	2.00±0.00a	7.66±0.33ef	0.18±0.02bc	26.39±0.21a	1.33±0.33h	Flint
18	EH-2101	5.30±0.43ab	2.00±0.00a	7.33±0.33ef	0.00±0.00c	26.02±0.43ab	2.67±0.88c-g	Semi-flint
19	EH-2253	0.83±0.20g	2.00±0.00a	10.33±0.66bcd	0.24±0.19ab	25.84±0.21ab	4.33±0.33ab	Dent
20	EHQ-63	2.56±1.19b-g	2.00±0.00a	8.33±0.66def	0.33±0.00a	24.41±0.22d	3.00±0.57b-f	Semi-dent
Mean		3.44	1.84	9.28	0.16	22.50	2.80	
$F_{(19,40)}$		2.69	2.18	5.33	1.37	60.23	5.18	
P		0.0047	0.0199	<0.0001	0.0491	<0.0001	<0.0001	

Table 3. Husk cover, ear damage, hundred seed weight and grain texture rating for twenty maize varieties in 2013.

Entry	Varieties	Husk tip extension (cm)	Husk tightness rating (1–4)	Husk leaves (No.)	Ear damage score at harvest (0–5)	100 seed weight (gm)	Grain texture (1–5)	Grain texture (dent/flint)
1	PMH-1	1.90±0.49ij	2.00±0.00a	13.76±1.58bc	0.19±0.17c	23.37±0.30e	4.33±0.08ab	Dent
2	Seed tech2324	5.26±0.62abc	2.00±0.00a	12.50±1.44de	0.17±0.09c	21.07±0.11i	4.00±0.28abc	Dent
3	Pratap makka-5	2.93±0.26fg	1.60±0.20b	17.10±1.77a	0.00±0.00c	25.37±0.51c	3.33±0.66cde	Semi-flint
4	Navjot	4.43±0.29bcd	1.60±0.20b	11.66±1.30ef	2.18±0.66a	22.83±0.03f	1.00±0.00j	Flint
5	PE HM-2	1.23±0.13jk	2.00±0.00a	12.50±1.25de	0.98±0.70b	19.43±0.39k	1.33±0.08ij	Flint
6	Aravali makka-1	5.00±0.72abc	2.00±0.00a	12.50±1.60de	0.00±0.00c	22.27±0.11g	1.67±0.20h-j	Flint
7	Pratap chari makka-6	1.83±0.23ij	1.83±0.16a	11.26±1.17fg	0.27±0.08c	24.86±0.07d	2.00±0.00ghi	Semi-flint
8	Super 9220	4.33±0.81b-e	2.00±0.00a	9.16±1.36i	0.00±0.00c	22.71±0.07f	2.33±0.08fgh	Semi-flint
9	KH-101	5.56±0.55ab	1.60±0.10b	11.66±1.30ef	0.18±0.09c	25.36±0.21c	2.66±0.49efg	Semi-flint
10	PAC-790	2.66±0.03gh	1.60±0.10b	14.16±1.48b	0.20±0.04c	18.70±0.34l	2.00±0.00ghi	Semi-flint
11	NK-30	4.60±0.34a-d	1.00±0.00c	8.76±1.18i	0.00±0.00c	19.98±0.03j	3.67±0.08bcd	Semi-dent
12	HM – 10	3.67±0.19def	2.00±0.00a	9.60±1.02hi	0.10±0.06c	19.69±0.20jk	3.00±0.00def	Semi-dent
13	GK – 3090	6.00±0.14a	2.00±0.00a	13.33±1.58bcd	0.00±0.00c	21.56±0.05h	4.67±0.20a	Dent
14	Vivek Hybrid-9	2.06±0.09hi	2.00±0.00a	9.16±1.01i	0.26±0.05c	21.10±0.31i	1.00±0.00j	Flint
15	HQPM-1	4.10±0.31cde	1.43±0.07b	11.67±1.30ef	0.75±0.10b	15.79±0.19m	4.67±0.20a	Dent
16	EHQ-16	3.20±0.28efg	2.00±0.00a	11.26±1.29fg	0.16±0.00c	21.80±0.07h	2.66±0.08efg	Semi-flint
17	HQPM-7	1.20±0.17k	2.00±0.00a	9.60±1.15hi	0.20±0.03c	26.29±0.13a	1.33±0.08ij	Flint
18	EH-2101	5.30±0.02abc	2.00±0.00a	9.16±1.01i	0.00±0.00c	25.83±0.12b	2.66±0.37efg	Semi-flint
19	EH-2253	0.83±0.01k	2.00±0.00a	12.93±1.33cd	0.96±0.70b	25.86±0.09ab	4.33±0.08ab	Dent
20	EHQ-63	2.57±0.41ghi	2.00±0.00a	10.43±1.31gh	0.36±0.02c	24.47±0.04d	3.00±0.28def	Semi-dent
Mean		3.44	1.83	11.61	0.61	22.42	2.78	
$F_{(19,40)}$		18.47	10.31	3.46	4.32	87.06	25.09	
P		<0.0001	<0.0001	0.008	<0.0001	<0.0001	<0.0001	

Table 5. Combined analysis of susceptibility parameters in twenty maize varieties tested against *S. cerealella*.

Entry	Varieties	Total F_1 progeny emerged (No.)	Median development time (Days)	Grain damaged (%)	Weight loss (%)	Susceptibility index	Resistance category
1	PMH-1	61.00 ± 10.81ab	35.83 ± 2.85a-d	31.12 ± 2.57ab	3.10 ± 0.48bcd	10.19 ± 0.32abc	HS
2	Seed tech2324	34.33 ± 8.38cd	33.17 ± 1.79abc	20.56 ± 4.73b-e	3.68 ± 1.03ab	8.00 ± 1.26de	S
3	Pratap makka-5	3.500 ± 0.67g	38.36 ± 1.94bcd	7.21 ± 1.38fg	1.45 ± 0.55b-e	2.88 ± 0.67g	R
4	Navjot	50.83 ± 10.39a-d	34.00 ± 0.45bcd	30.50 ± 7.22abc	2.33 ± 0.73b-e	10.02 ± 0.44a-d	S
5	PE HM-2	35.50 ± 7.47a-d	31.83 ± 1.27d	15.60 ± 2.81def	2.04 ± 0.15b-e	9.61 ± 0.96a-d	S
6	Aravali makka-1	25.17 ± 3.81de	34.17 ± 1.47bcd	15.27 ± 0.87def	2.31 ± 0.46b-e	8.16 ± 0.35cde	S
7	Pratap chari makka-6	69.84 ± 13.94a	40.00 ± 1.41a	33.02 ± 5.81a	5.49 ± 1.41a	9.43 ± 0.16a-d	S
8	Super 9220	35.33 ± 9.23cd	35.83 ± 1.89a-d	15.53 ± 4.50efg	1.65 ± 0.24de	8.25 ± 1.28cde	S
9	KH-101	57.00 ± 7.49abc	34.33 ± 1.93b-d	25.71 ± 3.42a-d	1.91 ± 0.23b-e	10.43 ± 0.24ab	HS
10	PAC-790	41.17 ± 6.92a-d	34.33 ± 1.92bcd	18.22 ± 1.34cde	2.58 ± 0.56b-e	9.56 ± 0.53a-d	S
11	NK-30	35.33 ± 8.48bcd	31.50 ± 1.56d	15.41 ± 2.96d-g	2.71 ± 0.45b-e	9.37 ± 0.64a-d	S
12	HM – 10	56.00 ± 14.85a-d	37.33 ± 2.67ab	22.97 ± 4.48a-e	3.11 ± 0.66bcd	9.15 ± 1.02a-d	S
13	GK – 3090	48.33 ± 17.57a-d	37.17 ± 2.71ab	22.19 ± 6.16a-e	3.24 ± 0.57bcd	8.27 ± 0.62cde	S
14	Vivek Hybrid-9	35.83 ± 1.54a-d	34.50 ± 2.91bcd	22.22 ± 2.35a-e	1.78 ± 0.09c-e	9.54 ± 0.69a-d	S
15	HQPM-1	53.83 ± 5.92abc	32.67 ± 0.67bcd	20.68 ± 1.21a-e	2.98 ± 0.65bcd	10.80 ± 0.42a	HS
16	EHQ-16	41.67 ± 15.03bcd	34.83 ± 3.13bcd	20.65 ± 6.48b-e	3.88 ± 1.60abc	8.46 ± 0.79b-e	S
17	HQPM-7	37.83 ± 9.38a-d	32.83 ± 0.65bcd	21.84 ± 2.82a-e	2.04 ± 0.15b-e	9.39 ± 0.67a-d	S
18	EH-2101	12.17 ± 2.70ef	32.00 ± 2.13cd	6.52 ± 1.14g	1.27 ± 0.21e	6.88 ± 0.98ef	MR
19	EH-2253	10.67 ± 2.51f	34.17 ± 1.99bcd	9.01 ± 2.07fg	1.27 ± 0.22e	5.84 ± 0.29f	MR
20	EHQ-63	54.17 ± 7.38abc	37.33 ± 3.15ab	28.04 ± 3.56abc	2.46 ± 0.69b-e	9.81 ± 0.76a-d	S
Mean		39.98	34.74	20.11	2.61	8.71	
$F_{(19,100)}$		4.83	1.53	4.14	2.28	6.26	
P		<0.0001	0.0417	<0.0001	0.0048	0.0002	

R = resistant; MR = moderately resistant; S = susceptible; and HS = highly susceptible.

Physical grain characterization and their correlation with susceptibility parameters

The physical parameters observed in the test varieties are presented in Table 6. The result showed that, all determined properties were significantly different among the studied maize varieties. Data given in Table 6 revealed that the size of the grains varied considerably among the varieties. The data showed the entries 17, 18, 19, 3, 7 and 9 had significantly large grain size (ranged from 188.50 to 198.33 number of grains/50 grams) whereas, significantly smaller grain size (252.67 to 317.00 number of grains/50 grams) were observed in entries 12, 10, 5, and 15.

The higher degrees of hardness were observed in entries, 14, 6, 3, 1, and 10 and the lowest in entries 9, 11, 4, 12, 20, and 15. The same trend was observed for crushing strength, where the values ranged from 200.80 to 257.12 kg/cm^3, with entry 10 having the highest while entry 9 having the lowest crushing forces (Table 6). The grain hardness of highly susceptible entries *viz.*, entry 9 (14.19 kg), entry 4 (14.34 kg) and entry 15 (14.87 kg) was significantly lowest and statistically at par, while it was highest in resistant and moderately resistant entries *viz.*, entry 3 (17.33 kg) and entry 19 (16.33 kg) which was at par with entries 6, 14, 18 and 2. Exceptionally one variety, entry 1, which was highly susceptible, had significantly highest grain hardness (17.87 kg).

Table 6 also showed difference in varieties with respect to grain bulk density and specific gravity. Test weight (bulk density) of tested maize varieties typically ranges from 0.793 to 0.888 g/cc or 792.5 to 888.1 kg/m^3 and specific gravity ranged from 7 to 14. Entry 5 had significantly highest bulk density followed by entries 6, 8 and 17 which were statistically at par. Entry 2 had significant lowest bulk density followed by entries 11, 4 and 9. Entries 17 and 6 had highest and significantly differed from the rest of entries for specific gravity while entries 9, 13, 4 and 11 had lowest specific gravity and had non-significant difference among each other.

Minimum length of the maize grains (<9.00 mm) was recorded in entries 11, 15, 10 and 12, which were at par with each other. Maximum length of the grains was recorded in entry 13 (10.59 mm), entry 9 (10.51 mm), entry 18 (10.27 mm), entry 20 (10.24 mm) and entry 19 (10.23 mm), which were at par with each other. Lowest kernel breadth was for entry 16 (7.73 mm) followed by entry 15 (7.78 mm), which were at par with entry 5 (8.01 mm) and entry 6 (8.03 mm). It was highest) in entry 1 (8.89 mm) and entry 3 (8.84 mm) followed by entries 2 and 18 (8.58 and 8.55 mm, respectively) which were statistically at par with each other. In other test varieties it ranged from 8.05 to 8.51 mm.

Table 6. Combined analysis of physical grain characteristics for twenty maize varieties.

Entry	Varieties	Grain size No./50g	Grain hardness (kg force applied)	Crushing strength (kg/cm^2)	Grain bulk density (kg/m^3)	Specific gravity	Kernel length (mm)	Kernel breadth (mm)
1	PMH-1	216.67±1.14gh	17.87±0.65ab	252.88±9.31ab	839.38±3.62g	9.71±0.33f-i	9.78±0.09de	8.89±0.07a
2	Seed tech2324	243.33±1.67cd	16.38±0.43c-f	231.79±6.15c-f	792.50±2.04j	9.07±0.33g-i	9.44±0.13ef	8.58±0.07a-c
3	Pratap makka-5	196.00±1.73jk	17.33±0.23a-c	245.26±3.38a-c	856.25±1.79e	12.29±0.80b-d	10.17±0.19bc	8.84±0.18ab
4	Navjot	220. 67±1.05fg	14.34±0.31i	203.02±4.43i	812.50±1.70h	8.79±0.60h-j	9.68±0.09de	8.05±0.06g-i
5	PE HM-2	264.17±0.87b	15.92±0.33e-h	225.29±4.75e-h	888.13±0.89a	10.26±0.60e-h	9.17±0.17fg	8.01±0.06g-i
6	Aravali makka-1	229.67±1.25ef	17.24±0.52a-c	243.99±7.43a-c	876.25±1.54b	12.69±1.06ab	9.66±0.18de	8.03±0.18g-i
7	Pratap chari makka-6	198.00±0.00i-k	15.17±0.43g-i	214.69±6.10g-i	855.00±1.44e	11.04±0.56c-f	9.55±0.15de	8.51±0.12b-e
8	Super 9220	226.33±1.11e-g	16.03±0.43d-g	226.94±6.14d-g	875.00±1.44bc	10.60±0.37e-g	9.47±0.03ef	8.35±0.08c-g
9	KH-101	198.33±0.88i-k	14.19±0.42i	200.80±6.07i	813.75±1.79h	7.510±0.26j	10.51±0.19ab	8.35±0.16c-g
10	PAC-790	261.33±2.24b	18.17±0.38a	257.12±5.40a	868.13±0.89d	10.93±0.42c-f	8.78±0.03h	8.05±0.14g-i
11	NK-30	208.33±16.83hi	14.32±0.15i	202.75±2.18i	803.75±1.54i	8.93±0.06h-j	8.67±0.10h	8.24±0.08c-g
12	HM – 10	252.67±1.62bc	14.55±0.27i	205.89±3.93i	845.00±0.00f	9.74±0.42f-i	8.93±0.08gh	8.18±0.18c-g
13	GK – 3090	230.33±0.76ef	16.14±0.41d-g	228.47±5.93d-g	846.88±0.77f	8.59±0.57ij	10.59±0.11a	8.41±0.09c-f
14	Vivek Hybrid-9	236.33±2.20de	17.09±0.26a-d	241.93±3.90a-d	847.50±1.70f	10.81±0.44d-f	9.50±0.17d-f	8.11±0.17f-h
15	HQPM-1	317.00±3.71a	14.87±0.30hi	210.45±4.31hi	866.88±4.08d	10.05±0.97e-i	8.74±0.14h	7.78±0.06hi
16	EHQ-16	228.83±1.92ef	15.70±0.32f-h	222.21±4.60f-h	856.25±1.54e	11.21±0.41b-f	9.53±0.07d-f	7.73±0.14i
17	HQPM-7	188.50±1.02k	15.83±0.31e-h	224.05±4.35e-h	874.38±2.69bc	14.05±0.72a	9.85±0.09cd	8.33±0.14c-g
18	EH-2101	192.00±0.68k	16.87±0.27b-e	238.74±3.82b-e	848.13±2.27f	10.20±0.50e-h	10.27±0.13ab	8.55±0.15a-d
19	EH-2253	193.33±1.08k	16.33±0.22c-f	231.12±3.24c-f	870.63±1.36cd	11.55±0.18b-e	10.24±0.10ab	8.47±0.12c-e
20	EHQ-63	206.33±1.92h-j	14.85±0.42hi	210.16±6.23hi	858.13±0.77e	12.38±0.04bc	10.24±0.10ab	8.21±0.08d-g
Mean		225.41	15.96	225.88	849.72	10.52	9.64	8.28
$F_{(19,100)}$		57.50	10.06	10.00	180.91	8.27	20.14	5.96
P		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Grain size had highly significant positive relationship with susceptibility index (r=0.673**) and significant positive relationship with total progeny emergence (r=0.495*), while it was negative and significant with weight of flour (r=-0.518*). The grain hardness of maize varieties recorded highly significant and negatively correlated with total progeny emergence (r=-0.696**), percent grain damage (r=-648**), and with susceptibility index (r=-0.522*). Negative and significant correlation was also observed between grain bulk density and percent damaged grains (r=-0.494*), while its relation was non-significant with other insect damage parameters. All insect damage variables showed non-significant relation with specific gravity of the grains.

Correlation coefficient between seed weight and insect damage parameters in different varieties of maize showed that the varieties had positive as well as negative correlation between 100 seed weight with the damage parameters of *S. cerealella*. Negative and significant correlation was observed between 100 seed weight and total emergence of moths (r=-0.589*). It had negative and highly significant correlation with susceptibility index (r=-0.729**) and positive and highly significant correlation with weight of flour (r=0.663**).

Kernel length had negative and highly significant correlation with susceptibility index (r=-0.806**), while it was non-significant with other susceptibility parameters. The breadth of the maize kernels had negative and significant correlation with susceptibility index (r=-0.542*). The thickness of the kernel had negative and highly significant correlation with total moth emergence (r=-0.801**) and median development time (r=-0.724**). The number of progeny emerged, median development time, susceptibility index, percent grain damage and grain weight loss were highly correlated. The correlation between moth progeny and median development time (r=0.827**), moth progeny and susceptibility index (r=0.985**), moth progeny and percent grain damage (r=0.898**), moth progeny and grain weight loss (r=0.697**), median development time and percent grain damage (r=0.818**), susceptibility index and percent grain damage (r=0.971**), weight of flour and grain weight loss (r=0.679**) and percent grain damage and grain weight loss (r=0.645) were positive and highly significant.

Correlation coefficients among the 20 maize varieties for nine traits (husk tip extension, husk tightness, husk leaf number, grain texture, grain hardness, 100 seed weight, ear damage, grain damage and susceptibility index) revealed a highly significant positive relationship between husk tip extension and grain texture (r = 0.673**), husk leaf number and grain hardness (r = 0.659**), ear damage score and grain texture (r = 0.698**), and percent grain damage and susceptibility index (r = 0.658**) while a highly significant inverse relationship between husk tightness and 100 seed weight (r = -0.884**), husk leaf number and ear damage score (r = -0.785**), ear damage score and grain hardness (r = -0.740**), susceptibility index and grain hardness (r = -0.695**), and susceptibility index and 100 seed weight (r = -0.758**) were observed. There was a significant relationship between husk tip extension and moisture content (r = 0.567*), husk tightness rating and grain hardness (r = 0.609*), husk leaf number and grain texture (r = 0.582*), and ear damage score and percent grain damage (r = 0.603*). Husk tip extension and ear damage (r = -0.610*) and grain texture and grain hardness (r = -0.521*) had a significant inverse relationship.

Discussion

This study demonstrated considerable variation in resistance levels among the maize varieties with respect to ear damage score, number of insects, seed damage, seed weight loss and the susceptibility index. These differences in the susceptibility of the varieties indicate the inherent ability of a particular trait to resist *S. cerealella* attack. Evaluation of varieties under free-choice test indicated that the higher the initial infestation at harvest the higher the subsequent infestation in the store. The presence of adult moth on stored ears after 30 days of storage showed that *S. cerealella* would mate and reproduce in the field on ears before harvest. The differences recorded among the varieties in rating ear damage during the free-choice test and susceptibility index during no-choice test indicated that *S. cerealella* preferred some varieties relative to others. Garcia-Lara *et al.* (2004) and Derera *et al.* (2001) reported that non-preference was based on the lack of feeding stimulants in the resistant grains. Relatively longer developmental time was required on the resistant variety Pratap makka-5 and minimum number of F_1 insects emerged, while the reverse was true for the susceptible varieties. Lengthening of development periods will also result in reduction of number of generations in a season. According to Horber (1988), the index of susceptibility is based on the assumption that the greater numbers of F_1 generation insects and the shorter the duration of the development, the more susceptible the seeds would be. Our result on median development time was in agreement with Hany *et al.* (2013), who found that the median developmental period from egg to adult reared on maize was between 34.03 and 39.33 days.

There existed differences and similarities between the two ways of grouping (visual scoring and susceptibility index) the varieties into resistant and susceptible categories. For instance, entries 3, 18, and 19 were grouped under the same category in the two tests. Similar results were obtained by Tefera *et al.* (2011) who evaluated different maize hybrids against larger grain borer and maize weevil under both no-choice and free-choice tests. Since the two methods complement each other, we recommend using those varieties that are consistently resistant in both tests. Although the free-choice test is relatively simple and cost-effective, the distribution of the insects might not be uniform and this could probably lead to escape by some varieties. The no-choice test represents a rigorous exposure, in which the insects have no choice other than subsisting on a given variety. In no-choice tests, confining insects with lower ranked hosts induces deprivation. Deprivation can induce some insects to accept a variety it may reject if not deprived (Withers, 1997). Therefore, complete or partial acceptance of varieties by an insect in no-choice tests does not necessarily reflect a lack of field specificity.

We observed increased susceptibility of ear to *S. cerealella* infestation after removal of the husk. This result indicates that the contribution of husk was negligible once the cob is de-husked. Demissie *et al.* (2008) stated that an exposed cob is more vulnerable to maize weevil than one enclosed in the husk, and good husk cover is considered key to protecting the cob from insect and fungi attack in the field. The husk cover characteristics have been reported to be under the control of additive gene action with non-additive playing a minor role (Brewbaker and Kim, 1979). To establish practical criteria for husk cover evaluation, all three husk characteristics i.e., husk tip extension, husk tightness and number of husk leaves in relation to moth as well as weevil penetration would probably give a better definition of a "good husk cover" in maize. Following the wide variability observed in the husk cover characteristics among the varieties evaluated, it is, therefore, possible to develop maize varieties with desirable husk cover characteristics through conventional breeding. Besides, resistance of stored maize to insect attack has been attributed to physical factors such as grain hardiness, pericarp surface texture and grain size (Dobie, 1974; Tipping *et al*., 1988).

Of course, kernel characteristics being hard, dent or flint are an important factor for their reaction to weevils and moths, as it has also been confirmed in the current study. Kim *et al*. (1988) reported that dent maize populations are known to be highly susceptible to weevil attack in West Africa. Moreover, Kim *et al*. (1988) and Kossou *et al*. (1992) reported that cultivars having flint grains are known to be less prone to weevil damage and also had higher numbers of husk leaves and subsequently had tighter husk cover than dent ones. Regarding seed size, Aruna *et al*. (2009) reported that the varieties with large grain size were found to be significantly more susceptible to *S. cerealella* than the varieties with smaller grain size. But inconsistent results were obtained in the present study with regard to grain size. It appears that variation in the susceptibility is most probably due to other physical nature of the grains. These results are in conformity with those of Ahmed and Raza (2010a, b) who reported that maize variety with small and lighter grains had highest moth emergence and lower development time. However, fecundity was maximum on larger grain than smaller one since heavier and larger grain provide better habitat for *S. cerealella* as compared to light and small grains. Based on these results, it is thus reported that grain size is not responsible towards susceptibility/resistance to *S. cerealella* rather as number of grain increases probability for the newly emerged larvae to get more options for entrance might increase if not influenced by grain hardness or other chemical stimuli.

The hardness of grains has been regarded as a resistance factor in maize and sorghum (Gudrups *et al*. 2001), but hardness alone is not sufficient to impart resistance in grains to *S. cerealella*. It may be true for other insect species such as *Sitophilus* spp., which pierce the outer of grains to insert eggs and then plug it. It might be due to non-preference based on lack of feeding stimulus in the resistant kernels (Khattak *et al*., 1987). Bergvinson (2002) reported that various physical characteristics such as kernel hardness and pericarp traits were identified as mechanisms of kernel resistance against the maize weevil. In general, our findings were similar to that of Fouad *et al*. (2013) who studied relationship between physico-chemical characteristics of corn kernels and susceptibility to *Sitotroga cerealella*. The differences recorded among the varieties in ear damage rating during the natural free-choice test indicated that *S. cerealella* preferred some varieties relative to others. According to the current investigation this might be due to differences in husk cover qualities before de-husking and biophysical and biochemical properties of the grains after de-husking the cob.

The significant correlation among the number of insects, susceptibility index, per cent grain damage and grain weight loss indicate that resistance in maize against *S. cerealella* can be expressed in terms of any of the mentioned damage parameters. The present results corroborate with Shafique & Chaudry (2007) and Soujanya *et al*. (2013) who reported the correlation between adult progeny of *S. cerealella* and weight loss in maize genotypes was significantly positive. There was significant positive correlation between grain size and susceptibility index. These findings were comparable with those of Prakash *et al*. (1979); Hamed and Khan (1994) and Nirmala *et al*., (2009) who reported that the grain size had significant effect on the development of stored grain insects.

Negative and significant correlations found between ear damage score & husk extension and ear damage score & husk leave number reflect that ear with husks extending far beyond the tip of the ear and possess more husk leaves should reduce moth penetration, damaged kernels and therefore increase insect mortality as no grain could be reached by the insect. Besides, moth penetration through the husk leaves may be reduced and resulted in lower ear damage as the cob husk is too tight leading to higher pressure on the cob. These results are in agreement with Anuradha *et al.* (1989) who observed that the thickness of the husk of rice varieties was negatively and significantly correlated (-0.80**) with susceptibility index of *S. cerealella*. Prakash *et al.* (1979) reported thickness of husk is negatively correlated with weight loss in rice varieties against rice weevil. Bergvinson (2004) revealed that maize with tighter husks or a harder kernel was insect resistant.

Conclusion

From this study it can be concluded that significant differences in susceptibility to *S. cerealella* were observed among the maize varieties. Susceptibility was correlated to maize husk cover and grain physical characteristics. The most resistant maize can be described as a variety with long and tight husks leaves possessing a hard, large and flinty grain texture. The presence of adult moth on stored ears after one month of storage showed that *S.cerealella* would mate and reproduce in the field on ears before harvest. Evaluation under no-choice test gave better and reliable results. So, when designing a selection strategy for developing high-yielding varieties which have acceptable levels of resistance to *S. cerealella*, one should recognize that germplasms must go through both field and laboratory selections. Our study leads to the conclusion that different husk cover and grain physical variables used to assess *S. cerealella* behaviour and biology, husk extension and tightness, grain hardness, grain size, and grain texture in combination with other biochemical factors were better indicators of resistance for practical application. The correlations between grain size, texture, grain hardness and husk cover characteristics suggests that it is possible to minimize field infestation and storage damage by *S.cerealella* in one breeding program. Therefore, the resistant and moderately resistant varieties can be utilized in integration with other non-chemical techniques as an eco-friendly way to reduce damage by *S.cerealella*. Further studies on biochemical composition of the varieties also help in better understanding of the resistance mechanisms against *S. cerealella*.

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