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Evaluation of biophysical, anatomical and biochemical traits of resistance to *Sitophilus oryzae* L (Coleoptera: Curculionidae) in stored maize

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Abstract

Sitophilus oryzae L is the most destructive insect pest of stored maize and is widely distributed in tropical regions. In the present study, eighteen maize genotypes were screened for several susceptibility parameters against *S. oryzae* by using «No Choice method». Biophysical traits (test weight, thousand kernel weight, kernel hardness), anatomical fractions (tip cap, germ, pericarp, endosperm), biochemical variants (starch, protein, oil, sugar) were correlated with insect susceptibility parameters. There is significant relationship among test weight, kernel hardness, and insect susceptibility parameters. Pericarp was positively correlated while endosperm, starch content were negatively correlated with median development period but were non-significant. Majority of the maize genotypes containing harder kernels and thick pericarp showed less susceptibility to *S. oryzae*. The results indicated that the biophysical, anatomical and biochemical traits are responsible for varying levels of resistance to *S. oryzae*.

Keywords: biophysical, anatomical, chemical traits, susceptibility, maize, *Sitophilus oryzae*

Introduction

In India, maize is the third most important cereal crop next to rice and wheat and has the highest yield potential among all the cereals. According to latest available data, it is being cultivated on 9.3 million ha across varied agro-ecological regions with a total production of 24.26 million tonnes (DES, 2015). Around 75-80 per cent of the total maize produced is consumed internally by several industries viz., feed (60%), food (20%), starch (12%), ethanol (1%), etc. In order to run the industries without interruption all through the year, procurement and storage is the common practice in India. However, insect pest infestation is one of the major constraints during storage which causes 10% losses in tropical regions. Rice weevil, *Sitophilus oryzae* L (Coleoptera: Curculionidae) is the primary pest causing both quantitative and qualitative losses during storage. Infestation commences in the field when maize cob turns yellow (Haines, 1991) and completes its lifecycle inside the seed and produce larger populations in short time. Heavy infestation of this pest leads to fungal attack and making the grain unfit for germination. Excessive use of chemical insecticides result in the development of resistance and leads to serious health and environmental hazards. Resistance breeding is the most promising alternative as it provides economical means to minimize post harvest losses (Mwololo et al, 2013). However, the strategy of host plant resistance has been underutilized in maize particularly on storage pests (Pingali and Pandey, 2001). Further, limited studies have been undertaken on resistance mechanisms of maize grains, against storage pests infestation with special reference to *S. oryzae* (Chavan, 2008). However, efforts have been made to improve the resistance levels to storage pests by manipulating grain composition through recurrent selection (Scott et al, 2008). The phenolic acids (PAs), a major cell wall components of maize grain, have been explored as broad base resistance mechanism against storage insect pests by various workers (Garcia Lara et al, 2004; 2010); which are particularly useful as antioxidants causing health benefits in humans (Del Pozo-Insfran et al, 2006). In addition, Garcia Lara and Bergvinson (2014) identified quantitative trait loci in maize grain by conducting in depth genetic studies associated with biochemical basis of resistance. Nwosu et al (2015) reported resistant maize genotypes 2000SYNEE-WSTR and TZBRELD3C5 and identified antixenosis and antibiosis as mechanisms of resistance to *S. zeamais* infestation. In light of these findings, the present work was designed to understand the level of tolerance of different maize grains, which were released in recent years in India, with respect to *S. oryae* infestation and to compare the relationship between biophysical (test weight, kernel hardness, true density), anatomical (endosperm, pericarp thickness, germ) and biochemical parameters (starch, protein, oil and sugar contents) of maize kernel with resistance/susceptibility to *S. oryzae* under laboratory conditions.

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Materials and Methods

Eighteen recently released, highly productive public-bred maize hybrids were selectively chosen by considering several factors viz., genetic background, grain colour, texture, type [quality protein maize (QPM), and non-QPM], origin etc to know relative susceptibility of kernels to *S. oryzae* during storage. Kernel samples were, not previously treated with insecticides, adjusted to moisture content of 12% by using the formula ([(100 - Initial moisture %) / (100 - Desired moisture %) - 1] × sample weight) and allowed to equilibrate for one week at 26 ± 1°C, 65 ± 5 % RH.

Biophysical, anatomical and biochemical characterization of maize kernels

The biophysical properties like test weight, kernel hardness and true density of maize kernels were estimated by using standard procedures. The test weight was determined according to AACC Method 55-10, i.e weight of 100 ml grain was recorded and expressed in kilogram hectoliter^{-1}, while thousand kernel weight by weighing 1000 randomly selected whole kernels. The kernel hardness was evaluated by randomly selecting ten maize kernels of each genotype with the help of TA.HD texture meter and the force required to break the kernels was expressed in Newtons (Mwololo et al, 2013). True density was determined by filling a 200 ml cylinder with kernels from a set height, tapping twice (to obtain uniform packing and to minimize wall effect, if any) and then weighing the contents (Barnwal et al, 2012).

The anatomical properties of tip cap, germ, pericarp and endosperm were obtained from dissected kernels (hand dissection) previously soaked for 30 minutes in water according to the procedure described by Gutierrez-Uribe et al (2010). Biochemical analysis of kernel composition of macro molecules viz., starch, protein, sugar and oil were determined by using Anthrone reagent, Micro-Kjeldahl method of AOAC (1970), Nelson Somogyi method (Nelson, 1944), and AOAC method respectively. The kernels were grinded to fine powder and then defatted by using petroleum ether and finally kept in desiccators before performing biochemical analysis.

Susceptibility tests

100 gm maize kernels were infested with 30 unsexed six days old *S. oryzae* adult weevil, reared on whole maize kernels at 26 ± 1°C, 65 ± 5 % RH. The experiments were carried out in triplicate in plastic jars with ventilated lids. The adult insects were removed after one week and samples were kept under the same experimental conditions for 45 days. Newly emerged adult progeny removed every alternate day from 46th day onward and counted for fifteen days (up to 60th day). Weight loss and Dobie index were calculated (Dobie, 1974).

$$\text{weight loss } (\%) = \frac{(Wu \cdot Nd) - (Wd \cdot Nu)}{Wu \cdot (Nd + Nu)} \cdot 100$$

Wu = weight of undamaged grains; Nu = number of undamaged grains; Wd = weight of damaged grains; Nd = number of damaged grains.

Dobie index was calculated with the formula:

DI = [log e x Number of adults emerged] / [MDP] x 100

where, MDP is median development period (calculated time from middle of the oviposition period to the emergence of 50% of emerged progeny).

The susceptibility index, ranging from 0 to 11, was used to classify the maize genotypes; where; 0 - 3 = least susceptible, 4 - 7 = moderately susceptible, 8 - 10 = susceptible and ≥ 11 = highly susceptible.

Statistical Analysis

Analysis of variance (ANOVA), Pearson's correlation coefficients and multivariate analysis for biophysical, anatomical, biochemical and susceptibility parameters were performed with SAS 9.3 version by using general linear model (GLM), PROC CORR and PRINCOMP procedure respectively. Prior to ANOVA, the per cent and number data were transformed to

Table 1 - Biophysical characterization and susceptible parameters obtained from index of susceptibility test under artificialinfestation against *S. oryzae*.

Genotype	Test weight (kg hl^{-1})	Thousand kernel weight	Kernel Hardness	True density	Dobie index	Adults emerged	MDP	Weight loss
CMH-08-292	78.0±0.6abc	312.1±6.3cde	535.07±19.8bcd	0.62	9.1±0.1d	87.0±4.8hi	49±0.1b	3.8±0.4e
HQPM5	78.4±0.2ab	286.2±6.0ef	621.38±27.9abc	0.66	11.1±0.1ab	217.3±10.8de	48.3±0.2b	8.4±0.5cd
HQPM7	80.1±0.8a	276.1±5.8fg	359.93±14.3def	0.64	8.4±0.1d	67.0±4.1i	49.3±0.2ab	5.3±1.0d
CMH-08-287	73.5±1.6bcdef	350.6±5.9ab	838.63±58.6a	0.67	10.7±0.1bc	209.0±6.1def	49.3±0.1ab	11.4±0.8bc
Prakash	77.8±0.7abc	285.0±4.5ef	538.29±9.3bcd	0.69	8.9±0.1d	92.7±3.9hi	50.3±0.1a	3.3±0.1e
HM12	73.8±0.6bcdef	213.1±1.0i	425.77±18.8cdef	0.67	10.8±0.1ab	230.0±3.8d	49±0.1b	12.6±1.5ab
DHM117	76.0±1.2abcde	294.9±4.9def	438.92±14.0cdef	0.71	9.3±0.1cd	115.3±2.7h	50.3±0.2a	6.2±0.2d
PMH4	72.0±0.4def	332.5±6.2bc	504.88±14.0cd	0.67	12.3±0.1a	345.3±2.3a	47.3±0.2b	8.8±2.6cd
HM8	78.0±1.0abc	293.3±7.1def	382.59±1.62def	0.69	8.5±0.1d	69.3±2.3i	49.3±0.2ab	6.9±0.9d
PMH3	74.1±0.3bcdef	284.7±5.4ef	385.41±10.6def	0.51	10.5±0.1bc	186.0±2.6gf	49±0.1ab	6.9±0.2d
DHM119	73.5±0.3bcdef	335.7±6.0bc	465.60±16.7cde	0.71	10.7±0.1bc	196.0±8.6efg	49.3±0.2ab	7.0±1.4d
CMH-08-282	69.2±0.8gf	335.0±5.8bc	418.80±10.8cdef	0.61	11.8±0.1ab	309.7±4.7b	48.7±0.1b	15.6±0.1a
PMH1	73.5±0.3cdef	323.2±6.5bc	789.55±46.6ab	0.67	11.6±0.1ab	280.7±2.3bc	48.3±0.1b	13.6±0.4ab
HM5	64.6±0.9g	165.5±1.6j	266.76±21.3f	0.67	10.6±0.1bc	191.3±3.5efg	49.3±0.2ab	7.0±0.8d
DHM113	74.7±0.9bcde	372.6±2.4a	485.28±9.0cde	0.69	10.6±0.1bc	171.3±3.2g	48.3±0.1b	9.0±1.4cd
VivekQPM9	76.8±0.1abcd	246±2.6h	293.21±15.9ef	0.65	8.9±0.2d	83.7±7.8i	49.3±0.1ab	6.6±0.8d
VMH43	71.2±2.4ef	313.9±6.5cd	566.07±20.8abcd	0.69	11.6±0.1ab	271.3±7.0c	48±0.2b	14.1±0.4ab
HQPM1	74.1±0.9bcdef	255.8±5.4gh	344.72±4.38def	0.68	10.7±0.1bc	188.7±10.9efg	49±0.2b	6.6±0.4d

Each value represents the mean ± SEm of 3 replications. Means within a column followed by different letters are significantly different (Tukeys' Test; p = 0.05).

Table 2 - Anatomical fractions of different maize genotypes.

Genotype	Tipcap (%)	Germ (%)	Pericarp (%)	Endosperm (%)
CMH-08-292	3.0 ± 0.1 cde	13.4 ± 1.2 ab	9.1 ± 0.7 ab	74.5 ± 1.0 cd
HQPM 5	3.6 ± 0.1 ab	13.5 ± 0.1 ab	8.3 ± 0.5 ab	74.6 ± 1.0 cd
HQPM 7	3.2 ± 0.3 de	12.1 ± 0.1 abc	5.7 ± 0.1 b	79.0 ± 0.3 abc
CMH-08-287	2.5 ± 0.1 e	12.7 ± 0.1 abc	7.3 ± 0.7 ab	77.5 ± 0.7 abcd
Prakash	3.3 ± 0.2 de	12.7 ± 0.1 abc	7.3 ± 0.7 ab	76.7 ± 1.4 bcd
HM 12	4.0 ± 0.1 ab	12.5 ± 0.7 abc	7.4 ± 0.8 ab	76.1 ± 0.6 bcd
DHM 117	3.7 ± 0.4 cde	12.7 ± 0.5 abc	6.8 ± 1.3 ab	76.8 ± 1.6 bcd
PMH 4	2.7 ± 0.1 e	10.7 ± 0.4 bc	4.4 ± 0.1 b	82.2 ± 0.5 a
HM 8	3.4 ± 0.5 de	10.9 ± 0.1 bc	7.0 ± 0.7 ab	78.7 ± 0.6 abcd
PMH 3	4.2 ± 0.2 a	12.5 ± 0.1 abc	5.8 ± 0.1 b	77.5 ± 0.7 abcd
DHM 119	2.7 ± 0.2 e	9.4 ± 0.1 c	8.7 ± 1.1 ab	79.2 ± 0.5 abc
CMH-08-282	2.5 ± 0.3 e	13.5 ± 1.2 ab	8.3 ± 0.1 ab	75.7 ± 1.6 bcd
PMH 1	3.6 ± 0.1 bcd	12.1 ± 0.1 abc	7.7 ± 0.9 ab	76.6 ± 1.5 bcd
HM 5	4.8 ± 0.1 abc	15.3 ± 0.4 a	7.6 ± 0.1 a	68.5 ± 1.4 e
DHM 113	3.7 ± 0.2 cde	14.0 ± 1.1 ab	6.1 ± 0.7 ab	73.7 ± 1.1 d
Vivek QPM 9	3.5 ± 0.1 ab	12.5 ± 0.7 abc	7.0 ± 0.6 ab	77.0 ± 0.6 bcd
VMH 43	3.8 ± 0.1 ab	11.1 ± 1.4 bc	5.3 ± 0.6 b	79.8 ± 0.4 ab
HQPM 1	2.7 ± 0.2 e	14.2 ± 1.0 ab	5.6 ± 0.3 b	77.5 ± 0.6 abcd

Each value represents the mean ± SEm of 3 replications. Means within a column followed by different letters are significantly different (Tukeys' Test; $p = 0.05$).

angular and square root to correct for heterogeneity of variance. The differences among treatment means were compared using TUKEY test. In addition, cluster analysis was performed using Centroid Hierarchial system to separate the different classes of susceptibility using PROC CLUSTER.

Results

Insect Susceptibility Parameters

Significant differences were observed among the tested genotypes with respect to per cent weight loss (Table 1). The maximum per cent weight loss was observed in genotypes VMH 43 (14.1), PMH 1 (13.6) and HM 12 (12.6) while it was minimum in Prakash (3.3) and CMH-08-292 (3.8). There were significant differences in the number of F_1 progeny emergence among the maize genotypes. Progeny emergence tended to be higher in PMH 4 (345.3), CMH-08-282 (309.7) and PMH1 (280.7) while it was lowest in HQPM 7 (67.0) and HM 8 (69.3). The Dobie index, a measure of susceptibility, was in the range of 8.50 to 12.3 (Table 1) among the genotypes.

Biophysical traits

The biophysical parameters were given in Table 1. The test weight (TW) values of different maize genotypes ranged from 64.6 kg/hl (HM 5) to 80.1 kg hl^{-1} (HQPM 7). The true density values ranged from 0.51 (PMH 3) to 0.71 (DHM117 and DHM 119). The thousand kernel weight ranges from 165.5 (HM 5) to 372.6 g (DHM 113). The force to break the kernels (Kernel hardnes) was high in genotype CMH-08-287 (838.63 N) followed by PMH 1 (789.55 N) while the lowest levels were observed in HQPM 1 (344.72 N), HM 5 (266.76 N) and Vivek QPM 9 (293.21 N). In general the kernel hardness values of QPM hybrids are lowest, which substantiate the fact that the QPM endosperm is relatively soft as compared to non-QPM hybrids. Similarly, in the present study, the average kernel hardness of QPM hybrids is 404.8 N while it was 502.97 N for non QPM hybrids but was non significant by t test ($p > 0.05$). HM5 has lowest kernel hardness whereas PMH 1 has shown highest kernel hardness.

Anatomical fractions

The proportion of major anatomical parts were shown in Table 2. Typical percentage values of germ, pericarp and endosperm values in maize were 10-12, 5-6, 75-85. The values obtained were more or less similar to those depicted in Table 3 being PMH 1 (LM 13 X LM 14) with endosperm percentage less than 75%. The genotype with highest pericarp percentage was observed in CMH-08-292 (UMI 1201 X UMI 1230) whereas the lowest was recorded in PMH 4 (LM 5 X LM 16), VMH 43 (V-373 X V-341) and PMH 3 (LM 17 X LM 14). The percentage of germ varies from 9.4 in DHM 119(BML 2 X BML 15) to 15.3 in HQPM 1(HKI 193-1 X HKI 163).

Biochemical Variants

Biochemical composition of maize genotypes was depicted on Table 3. The highest percent starch content was observed in PMH 1 (LM 13 X LM 14) whereas the lowest in HQPM 7 (HK1-193-1 X HKI-161). The present finding showed that genotypes with highest starch content have soft kernels and contributed towards susceptibility to *S. oryzae* infestation with index of susceptibility 11.6 and maximum weight loss (13.6%). HM 5 (HKI 1344 X HKI 1348-6-2) and HQPM 1 (HKI 193-1 X HKI 163) contained highest protein percentages with 13.2 and 13.0, respectively whereas in DHM 119 (BML 2 X BML 15) only 10.9%. Similarly, the same genotype HQPM 1 (HKI 193-1 X HKI 163) contained highest oil content with 4.9% where as the lowest (2.3%) was observed in DHM 117 (BML 6 X BML 7). Highest sugar content (4.9%)

Table 3 - Chemical composition for different maize genotypes.

Genotype	Starch (%)	Protein (%)	Oil (%)	Sugar (%)
CMH-08-292	70.8 ± 0.1 abc	12.4 ± 0.2 abcd	4.1 ± 0.02 bcd	4.1 ±0.03 d
HQPM 5	68.3 ± 0.1 ef	11.5 ± 0.1 efg	3.4 ± 0.06 efg	3.2 ± 0.02 h
HQPM 7	67.5 ± 0.5 f	12.9 ± 0.2 ab	2.7 ± 0.2 hi	3.6 ± 0.05 fg
CMH-08-287	70.4 ± 0.4 abcd	12.1 ± 0.1 bcde	3.7 ± 0.03 cdef	3.6 ± 0.01 efg
Prakash	71.1 ± 0.1abc	12 ± 0.1 cdef	4.0 ± 0.06 bcd	4.1 ± 0.01 d
HM 12	69.4 ± 0.1 de	11.2 ± 0.1 g	3.7 ± 0.05 cdef	3.9 ± 0.1 ed
DHM 117	71.1 ± 0.7 ab	12 ± 0.1 cde	2.3 ± 0.10 i	4.4 ± 0.01 bc
PMH 4	69.7 ± 0.2 abc	12.2 ± 0.2 bcde	3.6 ± 0.20 def	3.5 ± 0.05 fgh
HM 8	70.3 ± 0.1 cd	11.1 ± 0.2 g	3.8 ± 0.07 cde	3.3 ± 0.02 gh
PMH 3	70.7 ± 0.1 abcd	11.6 ± 0.1 efg	2.8 ± 0.05 gh	3.4 ± 0.01 fgh
DHM 119	69.7 ± 0.1 abcd	10.9 ± 0.1 g	3.4 ± 0.02 efg	3.7 ± 0.01 ef
CMH-08-282	71.0 ± 0.1 abc	12.7 ± 0.3 abc	4.2 ± 0.05 bc	4.0 ± 0.03 d
PMH 1	71.6 ± 0.3 a	11.7 ± 0.1 defg	3.4 ± 0.08 efg	3.9 ± 0.10 ed
HM 5	70.8 ± 0.1 abcd	13.2 ± 0.2 a	3.3 ± 0.08 efg	3.9 ± 0.02 ed
DHM 113	71.1 ± 0.2 abc	12.8 ± 0.1abc	4.2 ± 0.05 bc	4.2 ± 0.05 cd
Vivek QPM 9	70 ± 0.2 bcd	11.6 ± 0.1 efg	3.2 ± 0.05 fgh	3.2 ± 0.05 h
VMH 43	70.3 ± 0.1 abcd	11.3 ± 0.1 fg	4.5 ± 0.03 ab	4.5 ± 0.03 b
HQPM 1	70.2 ± 0.2 abcd	13 ± 0.10 a	4.9 ± 0.01 a	4.9 ± 0.01 a

Each value represents the mean ± SEm of 3 replications. Means within a column followed by different letters are significantly different (Tukeys' Test; p = 0.05).

was again observed in HQPM 1 while it was lowest in HQPM 5 (HKI 163 X HKI 161) and Vivek QPM 9 (VQL 1 X VQL 2) with 3.2%.

Correlation of susceptible parameters with biophysical, anatomical and biochemical traits

Table 4 represents the analysis of correlation matrix among biophysical, anatomical, biochemical and insect susceptibility parameters of maize genotypes. Kernel hardness appeared to be positively and significantly correlated with median development period (r = 0.50) while it was negatively and significantly correlated with thousand kernel weight (r = -0.68) and weight loss (r = -0.51). The correlation between test weight and Dobie index (r = -0.63) and weight loss (r = -0.53) was negative. Per cent weight loss and Dobie index are important parameters considered for kernel susceptibility. Besides significant relationship between biophysical and susceptibility parameters, anatomical parts of maize were also correlated. The most significant negative correlation (r = -0.82) was found between germ and endosperm content followed by endosperm and tipcap (r= -0.54), pericarp (r = -0.49). The association between endosperm and thousand kernel weight was significant and positively correlated (r = 0.45). Chemical composition of maize was also correlated with susceptibility parameters. Starch percentage was positively correlated with all the susceptibility parameters. The most significant positive correlation (r = 0.78) was observed between germ and protein content. The highest correlations were observed between weight loss and Dobie index (r = 0.76), adult emergence (0.79). The strongest association was found between adult emergence and median development period (r = -0.72), Dobie index (r = 0.98). A significant negative correlation was found between median development period and Dobie index (r = -0.73). In our laboratory, a parallel assay was done with least susceptible control CML394 in which weight loss and Dobie index was found to be 0.02 % and 1.39, respectively. None of the genotypes were found to be least susceptible except CML 394.

The results of principal component analysis (PCA) of the parameters measured were presented in Table 5. From the results, PC1, PC2, PC3, PC4, and PC5 accounted for 29%, 18%, 12%, 10%, and 9.7% of the total variation individually and 78.7% combined. Figure 1 depicts the hierarchial clustering of the description of dendrogram which has identified three groups of maize genotypes. Group 1 was composed of genotypes CMH-08-292, Prakash, VMH 43, HQPM 5, DHM 117, CMH-08-282, PMH 4, DHM 119, DHM 113 while group 2 contained HQPM 7, HQPM 1, HM 8, PMH 3, Vivek QPM9, PMH 4, HM 5,HM 12 and group 3 comprised of CMH-08-287, PMH 1. Group 1 comprised of nine genotypes which were characterized by high oil and sugar content but a low germ content. Group 2 was made up of seven genotypes and characterized by high tip cap, germ and protein content but low thousand kernel weight, true density, kernel hardness, pericarp, endosperm, starch, oil, and sugar contents. As starch and endosperm content was lower in group 2, minimum number of adults emerged (144) and low grain weight loss (6.4%) was recorded compared to the remaining groups. However, both the groups 1 and 2 can be considered as susceptible. Group 3 comprised of two genotypes which were characterized by high thousand kernel weight, kernel hardness, pericarp, endosperm, and starch content and low test weight, tipcap, and protein contents. It can be considered that this group contains genotypes that were highly susceptible. Presence of high endosperm and starch contents favours the multiplication of *S. oryzae* to a greater extent. In contrary, even though kernel hardness levels were high in group 3, more number of adults emerged (244) and maximum weight loss (12.5%) was recorded.

Table 4 - Pearson correlation coefficients (P = 0.05) for biophysical, anatomical, biochemical and susceptibility parameters of different maize genotypes.

	TW	TKW	KH	TD	TC	G	PC	END	ST	PR	OI	SG	DI	AE	MDP	WL
TW	1															
TKW	0.20	1														
KH	0.01	-0.68**	1													
TD	0.09	0.06	0.04	1												
TC	-0.22	-0.60**	0.18	-0.13	1											
G	-0.23	-0.39	0.37	-0.19	0.32	1										
PC	0.03	-0.03	0.20	0.02	-0.06	0.15	1									
END	0.34	0.45*	-0.39	0.06	-0.54*	-0.82**	-0.49*	1								
ST	-0.39	0.20	-0.11	0.06	0.11	0.22	0.15	-0.28	1							
PR	-0.25	-0.12	0.35	-0.10	-0.06	0.71**	-0.17	-0.44	0.09	1						
OI	-0.19	0.19	-0.04	0.19	-0.34	0.15	-0.02	0.005	0.23	0.15	1					
SG	-0.24	0.02	0.02	0.32	-0.05	0.30	-0.15	-0.11	0.41	0.39	0.50*	1				
DI	-0.63**	0.24	-0.42	-0.03	-0.09	-0.02	-0.14	0.06	0.14	-0.01	0.31	0.14	1			
AE	-0.62**	0.24	-0.37	-0.04	-0.13	-0.10	-0.16	0.15	0.12	-0.03	0.27	0.10	0.98**	1		
MDP	0.27	-0.32	0.50*	0.11	0.09	0.14	0.34	-0.22	0.14	0.01	-0.34	0.09	-0.73**	-0.72**	1	
WL	-0.53**	-0.51	-0.51*	0.02	-0.04	-0.06	0.02	0.05	0.24	-0.20	0.31	0.10	0.76**	0.79**	-0.52*	1

*correlation is significant at the 0.05 level (2- tailed); **correlation is significant at the 0.01 level (2-tailed). TW = Test weight; TKW = Thousand kernel weight; KH = Kernel Hardness; TD = True Density; TC = Tip Cap; G = Germ; PC = Pericarp; End = Endosperm; ST = Starch; PR = Protein; OI = Oil; SG = Sugar; DI = Dobie index; AE = Adult emergence; MDP = Median Development Period; WL= Weight loss.

Discussion

The results of the present study showed that several factors viz., biophysical, anatomical and biochemical traits were responsible for *S. oryzae* infestation (Chandrasekhar and Satyanarayana, 2006). Dobie index indicates that all genotypes were either susceptible (Dobie index between 8 to11) or highly susceptible (Dobie index > 11). The present finding is in agreement with Arnason et al (1994) who obtained dobie index > 11 in highly susceptible genotypes. The weevil prefers to lay more eggs on some genotypes which leads to maximum progeny emergence. The differences in the number of weevils emerged showed that there exists variation in susceptibility to weevil attack. Chavan (2008) observed maize lines MPQ-13, CML 469, CM 119 as least susceptible while CML 334 and CML 339 were highly susceptible to *S. oryzae* infestation on the basis of per cent infestation and adult emergence while studying maize inbred lines.

It is quite interesting that even though both HM 5 and PMH 1 were highly susceptible to *S. oryzae* infestation, they showed contrasting results with respect to kernel hardness. This can be explained by the fact that grain resistance depends not only on physical traits but also on chemical parameters such as type of hydroxycinnamic acids (phenols), E-ferulic acid, anthocyanin content etc. in maize (Serratos et al, 1987), which cause damage to midgut cells of insects (Kevin, 2002). Some genotypes with highest pericarp percentage viz., Prakash (CM 139 X CM 140), CMH-08-292- (UMI 1201 X UMI 1230) recorded minimum weight loss (3.3 - 3.8%) which could be due to the location of phenolic compounds within the aleurone layer (Serratos et al, 1993) and presence of higher antioxidant capacity on pericarp of the kernel (Garcia Lara and Bergvinson, 2014). Genotypes containing high endosperm content were found to be more susceptible to *S. oryzae*. Maximum weight loss was observed in PMH 1 and HM 5 which might be due to the presence of soft endosperm resulted in tunneling by more number of weevils (Siwale et al, 2009).

High starch content is also responsible for maximum weight loss in some genotypes. This observation corroborates the findings of Ichiro et al (2009) who stated that insects consume starch and protein from grains to grow and for laying eggs. In addition, maximum number of adults were emerged from CMH-08-282, PMH-1 containing high starch content. Similar result on the role of starch content as susceptible factor to storage pests were reported by Chijindu and Boateng (2008) and Osipitan and Odebiyi (2007). It was also observed that genotypes with highest protein content were found to be susceptible to *S. oryzae* and observed 6.6 to 7.0% weight loss with index of susceptibility >10. However, the genotypes containing highest protein content (HM 5 and HQPM 1) have lowest kernel hardness levels. These findings are consistent with the work of Arnason et al (1994) who reported that protein content was non significant for resistance correlations of storage pests. In contrast, Siwale et al (2009) reported presence of high protein content imparts resistance to maize weevil.

Kernel hardness plays an important role in providing tolerance to *S. oryzae* infestation as it has significant correlation with weight loss. With regards to biophysical traits, positive associations were found between thousand kernel weight and Dobie index, adult emergence and weight loss indicating kernels with high endosperm content are usually bigger and contain more starch. Hence more number of adults emerged and significant weight loss was observed. The present result is in consistent with previous report on the association of biophysical properties with susceptible parameters (Garcia Lara and Bergvinson, 2014). A negative relationship was observed between grain hardness and susceptibility to the maize weevil attack. A similar observation was reported by many authors (Garcia Lara et al, 2004; Krishna and Lakshmi, 2008) on correlation among kernel hardness

Table 5 - Latent vectors for the first five principal components for eighteen maize genotypes.

Traits	PC1	PC2	PC3	PC4	PC5
Test weight (kg hl^{-1})	-0.25628	-0.1215	0.207104	0.241392	0.334285
Thousand kernel weight	-0.31853	0.397151	-0.17893	-0.18585	0.257313
Kernel hardness	0.29133	-0.2368	0.305495	0.436995	0.130526
True density	-0.05048	0.236189	0.11718	0.552497	-0.46212
Tipcap (%)	0.275378	-0.33897	-0.20358	-0.1644	-0.465
Germ (%)	0.476038	0.097104	0.108806	-0.1674	0.195448
Pericarp (%)	0.130907	0.027755	-0.46288	0.528738	0.397305
Endosperm (%)	-0.48458	0.020246	0.21182	-0.06503	-0.09091
Starch (%)	0.199914	0.313171	-0.52186	-0.05902	-0.02526
Protein (%)	0.332873	0.152091	0.409423	-0.23579	0.312729
Oil (%)	0.054833	0.509007	0.147367	0.093649	-0.05078
Sugar (%)	0.193037	0.458085	0.209621	0.065429	-0.2644

and susceptibility index, adult emergence and weight loss. In contrary, no significant relationship between kernel hardness and weevil susceptibility in maize was found by Gudrups et al (2001) and also indicated variation in kernel hardness is due to size of kernel.

The positive correlation of starch to susceptibility parameters showed preference of weevil to high starch content. Correlation coefficients reveal negative relationship between protein content and susceptibility index which is in accordance with Dobie (1977) who studied correlations of maize grains with *S. zeamais*. Sugar content is negatively correlated to adult emergence which is in agreement with Classen et al (1990) who reported positive correlation between sugar content and mortality of larger grain borer *Prostephanus truncatus*. Chemical parameter sugar content is also an important factor for contributing grain resistance to weevil attack on maize (Siwale et al, 2009; Singh and Mc Cain, 1963). There was positive correlation between weight loss and susceptibility index which is in agreement with Torres et al (1996) who also reported positive association between the two parameters on sorghum. Similarly, Dushyant et al (2009) reported positive correlation between weight loss and adult emergence of *S. oryzae*. The most significant positive correlation was found between Dobie index and adult emergence. The results on correlation coefficients from the present study were found to be similar to Abebe et al (2009) who reported positive association between the susceptibility parameters.

Result of the PCA analysis indicates that, not one factor but the combination of many factors contribute for resistance against storage pests during storage. Thus, the complexity for breeding for resistance to storage pests increases tremendously. PCA of maize genotypes revealed that the first principal axis had high positive contributing factor from germ, protein and kernel hardness. The second principal axis had high positive contributing factor from oil, sugar, thousand kernel weight, and starch while protein content and thousand kernel weight recorded high loadings in third principal component. The fourth axis had high loadings of true density and pericarp while it was pericarp, test weight and protein content recorded high loadings in fifth principal component. The results of the present study has shown that grain resistance

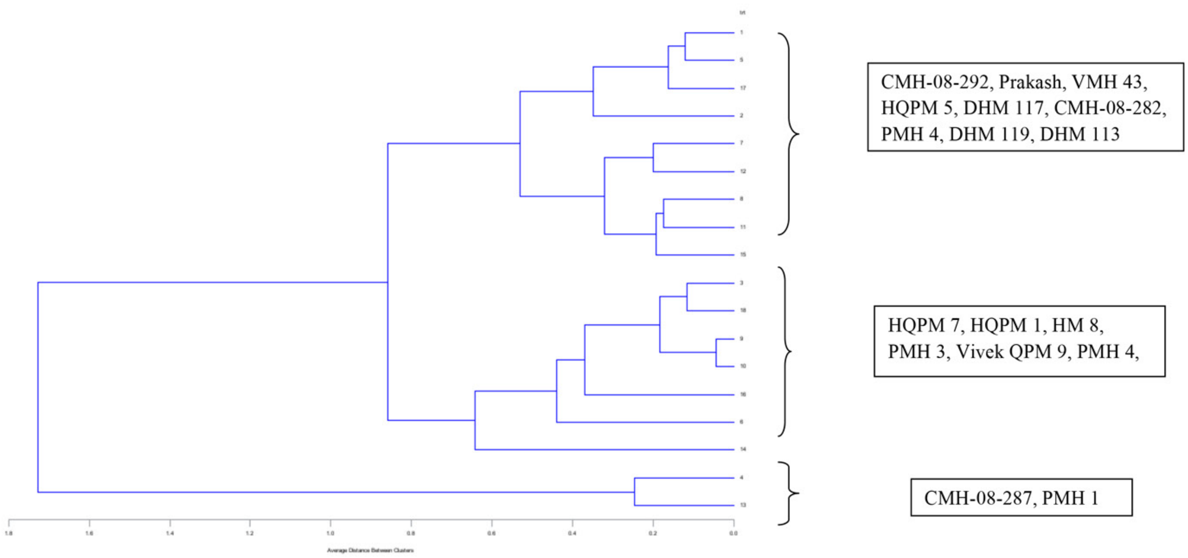

Figure 1 - Ascending hierarchial classification of the description of dendrogram of maize genotypes.

to *S. oryzae* attack depends not only on kernel hardness, thickness of pericarp, starch, protein content but also on biochemical factors. The present result is in agreement with Mebarkia et al (2010) who reported that biochemical constituents play vital role for the development of *Sitophilus granarius* in wheat varieties. The probable reason might be grain resistance does not depend purely upon physical traits but also on chemical constituents such as presence of phenolics, anthocyanin content etc. The present finding is consistent with the work of Zakka et al (2013) who reported physical factors alone are not responsible for grain resistance to Sitophilus sp in maize.

Conclusion

It was observed that there were significant differences among the maize genotypes for the traits evaluated. Since susceptibility was correlated to biophysical grain trait kernel hardness; anatomical fractions such as pericarp, endosperm, and biochemical variants viz., starch, protein, and sugar contents, all the parameters to be considered while formulating the breeding programme to develop maize genotypes with higher levels of resistance to *S. oryzae*.

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