Are variations in kernel-related morphometric and chemical parameters correlated with differences in *Sitophilus oryzae* attack in maize?

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Abstract

Insect infestation during seed storage affects cereal productivity. *Sitophilus oryzae*, which is present in various climates, is one of the primary pests for maize (*Zea mays*) seeds. In this study, kernels of a set of eight inbred lines of importance for both maize breeding and research activities were characterised for morphological and biochemical parameters as well as for their susceptibility to *S. oryzae*. *S. oryzae* was reared in laboratory-controlled conditions and the susceptibility index (SI, range: 0-11), which depends on both number of emerging adults and their median developmental period, was measured. Maize kernels of each of the eight maize lines were put in contact with less than 3-day old males and females of *S. oryzae* for 13 days. Infested kernels were checked daily for new adult emergence. Four maize lines were susceptible to *S. oryzae* attack (SI = 7.5 to 9.7) while the others appeared to be moderately resistant (SI = 4.6-6.9). Our results highlighted the occurrence of variability for insect susceptibility among different maize genotypes, however, variations in the kernel traits analysed do not allow us to make predictions about the response to insect attack.

Keywords: coleoptera, maize inbred lines, pest susceptibility, rice weevil, seed traits, *Zea mays*

Introduction

The productivity of maize is at risk due to the incidence of different pests and pathogens (Balconi *et al.*, 2010, 2014; Torri *et al.*, 2015) attacking maize kernels both pre- and post-harvest. In particular, maize is one of the agricultural commodities susceptible to
infestation by storage insects, which may cause a huge loss of quality and germination of the seeds (Derera et al., 2014). Insect attack damages both the endosperm and the embryo. Maize endosperm is the largest domain in the kernel and represents a good source of feeding material since it accumulates starch and protein, while the embryo, which comprises root and leaf primordia, represents the succeeding plant generation (Consonni et al., 2005).

Three species of the genus *Sitophilus* Schoenherr are pests of cereal seeds. *S. granarius* (L.) was the first species described in 1758 by Linnaeus. *S. oryzae* (L.) was subsequently detected on rice and *S. zeamais* Motschulsky was described on maize, but each of the species can thrive on all cereal seeds. *S. oryzae* and *S. zeamais* are more widespread than *S. granarius* since the latter is wingless. *S. oryzae* (L.) is one of the primary pests of stored maize, found not only in warm and tropical areas but also in temperate climates (CABI, 2018). The female chews a hole with the mandibles and lays one egg in each kernel. These species develop over a wide temperature range, the lower and upper temperatures are 15°C (Nakakita et al., 1997) and 35°C (Mansoor et al., 2017), respectively. The feeding damages the seeds, which also allows the development of fungi, thus causing a reduction in germination (Muzemu et al., 2013; Zunjare et al., 2014). Crop yield losses due to these harmful organisms can be substantial and may be prevented, or reduced, by crop protection measures.

How and if nutritional properties, endosperm texture or tegument hardness may influence insect attacks has still to be elucidated. Several authors have considered how the physical characteristics of endosperm and embryo, such as hardness, thickness and seed size, influence resistance of genotypes to insect infestation (Ivbiljaro, 1981; Ashamo, 2001; Lale et al., 2013; Limonta et al., 2013; Akpodiete et al., 2015), while Zunjare et al. (2016) affirmed that “pericarp thickness and seed hardness did not impart resistance”.

In this work, kernel-related traits taken into consideration included morphometric parameters, endosperm texture and nutritional properties. Beside pericarp thickness, seed hardness can be related to the ratio between the vitreous or semi-vitreous component of the endosperm, which is the hard portion located at the periphery, and the white floury component, which is the soft portion present in the central part of the kernel. On this basis, dent genotypes, characterised by the presence of little vitreous endosperm, and flint genotypes showing a larger proportion of vitreous endosperm (Gayral et al., 2016), have been analysed.

As to kernel nutritional properties, lines differ in starch content, and proportion of two types of glucose homopolymers that accumulate in the maize endosperm, *i.e.* the linear amylose molecule and the branched amylopectin, which depends on the activity of enzymes involved in starch chain elongation and branching (Zeeman et al., 2010). Total protein content, anthocyanin presence and total antioxidant capacity (TAC), were also taken into consideration.

The aim of this study is to verify whether variation in kernel traits including morphometric parameters, endosperm texture, pericarp thickness, and nutritional properties, such as starch content, total protein content and total antioxidant capacity (TAC), can be correlated with variation in susceptibility to *S. oryzae* attack.
Materials and methods

Plant materials
All genetic materials (inbred lines) included in this work belong to the germplasm collection of CREA Bergamo, (www.ccpgr.cgiar.org/working-groups/maize/maize-wg). Inbred lines have been maintained through sib-mating at the experimental station of the CREA Institute “Azienda La Salvagna” (249 m., 45°68’N, 9°64’E) for 10 years. Uniformity and stability of traits among plants of each line have been observed across generations through: i) field evaluations of agronomical traits, ii) ear and seed morphometric records.

For this work, ears were dried at 40°C for a week to about 12-13% moisture content and stored at room temperature. Kernels were removed from ten mature ears using an electric sheller, and kernels from each line were mixed thoroughly.

The eight inbred lines have different origin and seed features (table 1; figure 1). B73 is a historic American line selected by the Iowa State University that dominated the world scene for over 30 years (Russell, 1972). Even today, a considerable portion of modern germplasm has genetic traits that can be traced back to B73. Its first genome sequence was released in 2009 (Schnable et al., 2009). In the B73Pl1 line, the introgression of a functional allele of the Purple plant1 (Pl1; Cone et al., 1993) gene leads to the

| Maize line | Origin | Flowering time | Seed phenotype | Utilisation |
|------------|--------|----------------|----------------|-------------|
| B73        | Selected from SSS (Stiff Stalk Synthetic) | Medium | Wild type | As female line to produce hybrids |
| B73Pl1     | Selected from SSS (Stiff Stalk Synthetic) | Medium | Wild-type, red pericarp due to introgression of the Pl allele | |
| Lo1411wx   | Cross between the Pioneer waxy hybrid P3394E and the inbred line Lo1067 | Medium | Waxy, due to homozygosity of the waxy (wx) allele | As female line to produce wx hybrids |
| Lo1096wx   | Cross between Pioneer P1540 and a waxy inbred line | Medium | Waxy, due to homozygosity of the waxy (wx) allele | As male line to produce wx hybrids |
| Lo1488     | Derived by PR33A46 Pioneer hybrid | Medium | Wild type | As male line to produce medium-late hybrids |
| Lo1496     | Cross between BP42 945A and P3730 lines | Early | Wild type | As male line to produce hybrids |
| Lo1521ae   | Cross between Lo1309ae and Lo1095ae inbred lines | Medium-late | Tarnished endosperm due to homozygosity of the amylose extender1 (ael) allele | As female line to produce ae hybrids |
| Lo1530     | Pioneer PR31G98 hybrid | Late | Wild type | As male line to produce hybrids |
accumulation of anthocyanin pigment in different plant organs, such as seed pericarp, anthers and leaves. The Lo1411wx and Lo1096wx lines carry defective alleles of the waxy gene encoding a granule-bound starch synthase. Mutations at the waxy locus eliminate amyllose synthesis, resulting in 100% amylopectin in the endosperm (Tsai, 1974; Wessler and Varagona, 1985).

Lo1521ae is homozygous for the amylose extender1 (ae1) mutant allele. The product of ae1 is a starch branching enzyme (Kim et al., 1988); mutants in the ae1 gene produce starch with a much higher amylose-content than amylopectin, showing significantly longer branch-chains than the normal maize starch (Liu et al., 2013).

**Morphometric analysis**

Maize kernels were imbibed for one hour in distilled water and longitudinally cut with a scalpel. Sectioned kernels were then observed with the Axio Zoom.V16 microscope; images were acquired as a series of Z-stack with the AxioCam 506 color camera and assembled with the ZEN 2 pro software (Zeiss Carl Zeiss Microscopy GmbH, Jena, Germany). To obtain fluorescence images, samples were exposed to UV light (100-400 nm) and the autofluorescence acquired through a GPF filter (wavelength from 395 to 475 nm). Images of whole kernels and pericarp were taken at 5× and 20× magnification, respectively. Measures of kernel length and wideness and pericarp thickness were elaborated by means of ImageJ software (https://imagej.nih.gov/ij/index.html).

Pericarp thickness measures were taken in the abaxial region of the kernel in the position indicated by arrows in figure 2 (B73 fluorescence image). For each parameter, 10 kernels were analysed for each genotype, with three technical replicates.

**Chemical analyses of seeds**

Subsamples were taken and seeds milled with a ZM 200 Retsch Ultra-Centrifugal mill equipped with a DR 100 vibratory feeder (Retsch GmbH, Haan, Germany) to a 1 mm sieve size and stored at 4°C.

Flour samples were scanned in duplicate in the visible and NIR regions of the electromagnetic spectrum in reflectance (400-2500 nm) at 2 nm intervals using a scanning monochromator NIRS 6500 (NIRSystems) (Foss Italia). Crude protein, crude lipid and starch (% on a dry matter basis) were calculated using NIRS prediction equations by means of the MATLAB program. MATLAB- Spectra were exported as csv files and
processed using Matlab7.9 (The Math Works Inc., Natick, MA, USA) and PLS Toolbox7.9 (Eigenvector Research Inc., Manson, WA, USA).

Total antioxidant capacity (TAC) was expressed as mmol of Trolox equivalent (TE) for a kg of dry matter and determined by a specific calibration equation (Redaelli et al., 2016).

**Insect rearing**

*S. oryzae* was laboratory-reared in the Department of Food, Environmental and Nutritional Sciences, Università degli Studi of Milan, on maize kernels at 27 ± 1°C and 70 ± 5% RH. To obtain newly emerged adults, 200 adults (mixed population) were collected from the mass rearing and put in a glass jar (1 L) with 400 g of maize; the adults were removed after 13 days. The jar with the maize kernels was kept in the thermostatic cell to allow the development of laid eggs. The new adults emerged in about 28 days and were selected for the tests.

**Set up of screening for susceptibility to S. oryzae**

The first step was to determine the minimum amount of kernels to use in the experiments. Three trials were set up in which insects were grown in 156 mL glass jars with 100, 200 and 300 kernels respectively, each replicated five times. Five male/female couples of *S. oryzae*, three days old, were put in each jar. Sex was determined by examining the thickness and length of the rostrum and the 6° abdominal sternite aspect (Dinuta et al., 2009; Manivannan et al., 2017). Couples were left for 13 days to allow mating and then removed. The number of F₁ adults in the different groups was daily recorded. In the trial with 100 kernels, 36.4 ± 8.95 (± s.e.) adults were recorded, a number that is significantly different from those recorded in the experiments with 200 and 300 kernels, 114.4 ± 9.94 and 124.2 ± 13.36, respectively. The number of adults recorded on 200 and 300 kernels was not statistically different (one-way ANOVA F2, 12 = 19.427, P ≤ 0.001, LSD range test).

Taking account of the previous results, experiments with the different maize lines were carried out in 156 mL glass jars with 200 kernels. Tests with each of the eight lines were replicated three times. Five males and five females of *S. oryzae*, less than three days old, were put in each jar. Adults were held in the glass jar to allow mating and oviposition for 13 days and then removed. Infested kernels were kept into the glass jar and checked daily for new adult emergence. The Susceptibility Index (SI) was calculated according to Dobie (1974):

\[ SI = \frac{\ln F_1}{MDP} \times 100 \]

Where F₁ = first-generation emerging adult total number; MDP = median developmental period. SI ranged from 0 to 11; when the value was from 0 to 4 the line was considered resistant (R), from 4.1 to 7 moderately resistant (MR), from 7.1 to 10 susceptible (S) and more than 10.1 very susceptible (VS).

**Statistical analyses**

Data, normally distributed, were submitted to one-way ANOVA and least significance difference test (α = 0.05) and Pearson product-moment correlation (IBM SPSS Statistics 25).
Results

**Kernel related parameters**

The proportion of vitreous and floury endosperm varies among lines (figure 2). Vitreous endosperm was greater than floury endosperm in Lo1496, equal in B73, B73Pl1, Lo1411wx and Lo1521ae, and less in Lo1488 and Lo1530. Variation among lines was also observed for kernel length and width, as well as for pericarp thickness (table 2) as measured from fluorescence images. Pericarp thickness showed the highest and the lowest values, respectively, in Lo1488 and B73Pl1. Lo1411wx showed the highest 1000-kernel weight (306.5 g). The genotype with the lowest weight was Lo1530 (175.0 g).

Figure 2. Representative images and fluorescence (green) images of sectioned kernels of the eight maize inbred lines. Floury (F) and vitreous (V) portions of the endosperm are indicated in the B73, while semi-vitreous (SV) is indicated in the Lo1096wx seed sections. The arrow indicates the position in which pericarp thickness was measured.
The starch content ranged from 64.9 to 72.2% dry weight in Lo1096wx and Lo1496, respectively, with an average of 69.35% and the lipid content ranged from 2.9 to 4.9% dry weight in Lo1488 and Lo 1096 wx, respectively with an average of 3.71% (table 3). The level of proteins observed in different inbred lines was the qualitative parameter that showed the lowest variation; it varied from 11.7 to 13.3% dry weight with an average of 12.38%. The highest protein content was measured in Lo1096 wx and the lowest in Lo1530.

One-way ANOVA for 1000-kernel weight (g) $F_{7, 24} = 194.25$, $P < 0.00$; length $F_{7, 72} = 11.739$, $P < 0.00$; width $F_{7, 72} = 8.636$, $P < 0.00$; pericarp $F_{7, 72} = 149.152$, $P < 0.00$. Means followed by a different letter in a column are significantly different ($\alpha = 0.05$, LSD multiple range test). s: semi-vitreous.

The starch content ranged from 64.9 to 72.2% dry weight in Lo1096wx and Lo1496, respectively, with an average of 69.35% and the lipid content ranged from 2.9 to 4.9% dry weight in Lo1488 and Lo 1096wx, respectively with an average of 3.71% (table 3). The level of proteins observed in different inbred lines was the qualitative parameter that showed the lowest variation; it varied from 11.7 to 13.3% dry weight with an average of 12.38%. The highest protein content was measured in Lo1096wx and the lowest in Lo1530.

Table 3. Seed chemical composition and total antioxidant capacity (TAC) in the eight maize inbred lines.

| Maize line | Starch (% dry weight) | Lipid (% dry weight) | Protein (% dry weight) | TAC (mmol TE kg$^{-1}$ dry weight) |
|------------|-----------------------|----------------------|------------------------|-----------------------------------|
|            | mean ± SE             | mean ± SE            | mean ± SE              | mean ± SE                         |
| B73        | 70.49 ± 0.99 ab       | 4.41 ± 0.04 b        | 12.29 ± 0.11 cd       | 14.18 ± 0.22 cd                   |
| B73P/II    | 66.85 ± 1.03 bc       | 3.08 ± 0.06 e        | 12.25 ± 0.09 cd       | 13.78 ± 0.16 d                    |
| Lo1096wx   | 64.86 ± 0.77 bc       | 4.88 ± 0.12 a        | 13.29 ± 0.03 a        | 15.96 ± 0.33 a                    |
| Lo1411wx   | 70.01 ± 0.57 ab       | 3.54 ± 0.05 d        | 12.03 ± 0.09 cd       | 15.10 ± 0.00 bc                   |
| Lo1521ae   | 68.11 ± 0.97 b        | 4.03 ± 0.17 c        | 12.33 ± 0.10 cd       | 15.31 ± 0.05 ab                   |
| Lo1488     | 71.83 ± 0.54 a        | 2.88 ± 0.00 e        | 12.62 ± 0.01 bc       | 13.15 ± 0.14 de                   |
| Lo1496     | 72.25 ± 0.73 a        | 3.47 ± 0.01 d        | 12.70 ± 0.20 b        | 13.05 ± 0.06 e                    |
| Lo1530     | 71.71 ± 0.58 a        | 3.43 ± 0.00 d        | 11.68 ± 0.06 e        | 14.47 ± 0.33 c                    |

Means followed by a different letter in a column are significantly different ($\alpha = 0.05$, LSD multiple range tests).
The antioxidant capacity varied from 13.0 to 16.0 mmol TE kg\(^{-1}\) dry weight with an average of 14.37 mmol TE kg\(^{-1}\) (table 3). The highest antioxidant capacity was measured in Lo1096\(_{wx}\) while the lowest was detected in Lo1496.

From this qualitative analysis, it emerged that Lo1096\(_{wx}\) showed a low starch content (64.9% dm) accompanied by the highest level of protein (13.3% dry weight), lipid content (4.9% dry weight) and antioxidant level (16 mmol TE kg\(^{-1}\) dry weight).

**Insect susceptibility**

The highest numbers of adults were recorded on B73\(_{Pl1}\) and Lo1096\(_{wx}\), the lowest on Lo1411\(_{wx}\) (table 4.) The shortest development period was on B73\(_{Pl1}\) and the longest on Lo1496. Pearson product-moment correlation indicates a strong significant negative association between the number of emerged adults and the length of the development period \((r = -0.61, N = 24, P < 0.001)\). The lines B73, B73\(_{Pl1}\), Lo1096 and Lo1530 were susceptible, since they showed a SI value greater than 9, while the others appeared to be moderately resistant (table 4).

### Table 4. Mean number (± S.E.) of adults (F1) emerged, mean development period (MDP days) (± S.E.) of *Sitophilus oryzae*, susceptibility index (SI) and SI value recorded on the tested maize lines.

| Maize line     | F1 Progeny emergence mean ± SE | MDP (days) mean ± SE | SI (%) | SI value |
|---------------|--------------------------------|----------------------|--------|----------|
| B73           | 31.66 ± 3.71 ab                | 49.15 ± 1.01 bc      | 9.1    | S        |
| B73\(_{Pl1}\) | 43.00 ± 1.00 a                 | 46.27 ± 0.62 d       | 9.7    | S        |
| Lo1096\(_{wx}\)| 37.66 ± 8.57 a                | 47.96 ± 0.99 bcd     | 9.2    | S        |
| Lo1411\(_{wx}\)| 7.33 ± 2.85 d                 | 51.77 ± 1.09 ab      | 4.6    | MR       |
| Lo1488        | 15.00 ± 1.15 cd                | 50.91 ± 1.02 ab      | 6.4    | MR       |
| Lo1496        | 14.00 ± 4.16 cd                | 52.26 ± 1.13 a       | 5.9    | MR       |
| Lo1521\(_{ae}\)| 16.00 ± 2.52 cd               | 48.71 ± 1.15 bcd     | 6.9    | MR       |
| Lo1530        | 23.66 ± 0.88 bc                | 51.53 ± 0.95 ab      | 7.5    | S        |

One-way ANOVA: F1 progeny emergence \(F_{7,16} = 10.555, P < 0.00\); MDP \(F_{7,558} = 4.6, P < 0.00\). Means followed by a different letter in a column are significantly different (\(a = 0.05\), LSD multiple range test). Susceptibility index (SI): 0 to 4 = resistant (R); 4.1 to 7 = moderately resistant (MR); 7.1 to 10 susceptible (S).

To explore the correlation between the different values observed for the kernel morphogenetic parameters and susceptibility to *S. oryzae*, radar charts were constructed (figure 3) that report the number of adults together with kernel length and width measures (A) and pericarp thickness, (B) in the different inbred lines. The number of adults did not correlate with the length and the width of the kernels.

Although it appeared that B73\(_{Pl1}\) had the highest number of adults and the thinnest pericarp, while Lo1411 had the lowest number of adults and the thickest pericarp, these correlations were not supported by the statistical analysis. Pearson product-moment correlation only indicated a moderate negative correlation between the number of emerged...
adults and the pericarp thickness ($r = -0.45$, $N = 8$, n.s.) (figure 3). Moreover, the number of emerged adults varied among different lines that showed similar chemical composition. The number of adults on the different lines was not correlated to starch content ($r = 0.21$, $N = 8$, n.s.), lipid content ($r = -0.16$, $N = 8$, n.s.), protein content ($r = 0.60$, $N = 8$, n.s.), and TAC ($r = 0.06$, $N = 8$, n.s.). TAC and SI were also not correlated ($r = -0.1$, $N = 8$, n.s.).

**Discussion**

Various authors have argued that the morphometric characteristics of kernels are not related to the susceptibility to the insect (Gomez *et al.*, 1982; Zunjare *et al.*, 2014, 2016; Rahardjo *et al.*, 2017), while others assert that colour, hardness, thickness and kernel size may influence the resistance of cereals to insect storage pests (Ivbiljaro, 1981; Ashamo, 2001; Lale *et al.*, 2013; Akpodiete *et al.*, 2015). In our study, differences in kernel length and width, and in pericarp thickness, were not related to the number of
S. oryzae offspring (figure 3). For example, in the Lo1411wx and Lo1530 lines, which are characterised by the greatest width of the caryopsis, the least and the greatest number of adults respectively were observed. Moreover, the two most susceptible lines, B73Pl1 and Lo1096wx, showed both the lowest (49.9 µm) and one of the highest values of pericarp thickness (91.4 µm), respectively. Our results also suggest that there is no correlation between the protein and starch content (table 3) and the numbers of the progeny of S. oryzae (table 4), as stated by Tongjura et al. (2010) regarding S. zeamais. Nwosu (2016), instead, observed the influence of protein in maize lines resistant to the same species. The lines considered by Nwosu presented a protein content ranging from 4 to 14%, while the protein content of the lines observed in this research ranged from 11.68 to 13.29, too small a range to determine differences in the biology of this insect. Moreover, this pest species feeds on the seed starch, which is not a limiting factor when the insect is fed on corn seeds, while amino acids are supplied to the insect by endosymbionts (Heddi et al., 2003). Seed lipid content, that ranged from 2.88 to 4.88 (table 3), does not affect insect behaviour; finally, no evident preference for the type of endosperm was highlighted by storage pest development. Similarly, no correlation was detected between the number of adults (table 4) and the TAC values (table 3) of the eight lines. Total antioxidant capacity (TAC) refers to the overall activity of different compounds, generally present in the maize seed, that contribute to its antioxidant properties, such as carotenoids, polyphenols, flavonoids and anthocyanins (Serpen et al., 2007; Brewer, 2011). This parameter, which is generally understood to be related to the in vivo defence mechanisms of the plant, which produces more antioxidant molecules to combat the pathogen attack, seemed not to affect the responses to S. oryzae attack in stored materials in this study.

The results obtained in the present work indicate the existence of variability for the susceptibility to S. oryzae in the sample of eight maize inbred lines analysed. Lo1411wx, Lo1488, Lo1496 and Lo1521ae were identified as moderately resistant genotypes, while B73, B73Pl1 and Lo1530 were shown to be more susceptible. However, data obtained from the morphometric and chemical analysis of the kernels of the tested lines are not associated with susceptibility to S. oryzae attack. In other words, variations in the parameters taken into consideration are not predictive of the type of response to S. oryzae attack.

To our knowledge, this type of correlations up to now is not clearly understood, as there are multiple parameters involved and it is difficult to find a key parameter. We may speculate, in agreement with a previous study, that susceptibility to storage insect attack could be attributed to quantitative more than to single gene traits (Locatelli et al., 2019). A wider collection of genotypes will allow to investigate the molecular mechanisms at the basis of the kernel-insect interaction and eventually to analyse the genetic variability at the basis of insect susceptibility in different maize lines (Gafishi et al., 2012; Kasozi et al., 2016; Sodedji et al., 2018).

Considering that cereals could be stored for long periods before being distributed for sowing, and that S. oryzae is among the main pests that cause economically significant damage to seeds, when selecting new corn lines in breeding programmes, in addition to the agronomic characteristics, assessing the susceptibility to the attack of this species by biological tests must also be considered (Abebe et al., 2009).
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References

Akpodiete, O.N., Lale, N.E.S., Umeozor, O.C. and Zakka, U. (2015). Role of physical characteristics of the seed on the stability of resistance of maize varieties to maize weevil (Sitophilus zeamais Motschulsky). IOSR Journal Environmental Science Toxicology and Food Technology, 9, 60-66. <https://doi.org/10.9790/2402-09226066>

Ashamo, M.O. (2001). Varietal resistance of maize to the maize weevil, Sitophilus zeamais Motsch. (Coleoptera: Curculionidae). Zeit Pflanzenkr Pflanzenschutz, 108, 314-319.

Balconi, C., Motto, M., Mazzinelli, G. and Berardo, N. (2010). Ear secondary traits related to aflatoxin accumulation in commercial maize hybrids under artificial field inoculation. World Mycotoxin Journal, 3, 239-250. https://doi.org/10.3920/WMJ2010.1205

Balconi, C., Berardo, N., Locatelli, S., Lanzanova, C., Torri, A. and Redaelli, R. (2014). Evaluation of Fusarium verticillioides ear rot resistance and fumonisin accumulation in Italian maize inbred lines. Phytopathologia Mediterranea, 53, 14-26. <https://doi.org/10.14601/Phytopathol_Mediterr-11776>

Brewer, S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Comprehensive Reviews in Food Science and Food Safety, 10, 221-247. <https://doi.org/10.1111/j.1541-4337.2011.00156.x>

CABI (2018). Sitophilus oryzae (lesser grain weevil). <https://www.cabi.org/isc/datasheet/10887>

Cone, K.C., Cocciolone, S.M., Moehlenkamp, C.A., Weber, T., Drummond, B.J., Tagliani, L.A., Bowen, B.A. and Perrot G.H. (1993). Role of the regulatory gene Pl in the photocontrol of maize anthocyanin pigmentation. The Plant Cell, 5, 1807-1816. <https://doi.org/10.1105/tpc.5.12.1807>

Consonni, G., Gavazzi, G. and Dolfini, S. (2005). Genetic analysis as a tool to investigate the molecular mechanism underlying seed development in maize. Annals of Botany, 96, 353-362. <https://doi.org/10.1093/aob/mci187>

Derera, J., Pixley, K.V. and Makanda I. (2014). Resistance of maize to the maize weevil: III. Grain weight loss assessment and implications for breeding. Journal of Stored Products Research, 5, 24-35. <https://doi.org/10.1016/j.jspr.2014.04.004>

Dinuta A., Bunescu, H. and Bodis, I. (2009). Contributions to the knowledge of morphology of the granary weevil (Sitophilus granarius L.), major pest of the stored cereals. Bulletin of University Agriculture Sciences and Veterinary, 66, 59-66. <https://doi.org/10.15835/BUAVMCN-AGR%3A3731>

Dobie, P. (1974). The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestation by Sitophilus zeamais Motsch. (Coleoptera, Curculionidae). Journal of Stored Products Research, 10, 183-197. <https://doi.org/10.1016/0022-474X(74)90006-X>

Gafishi, K.M., Karungi, J., Asea, G. and Gibson, P. (2012). Determination of the heterotic groups of maize inbred lines and the inheritance of their resistance to maize weevil. African Crop Science Journal, 20 (Supplement S1), 99-104.

Gayral, M., Gaillard, C., Bakan, B., Dalgalarrondo, M., Kilmorjani, K., Delluc, C., Brunet, S., Linossier, L., Morel, M.H. and Marion, D. (2016). Transition from vitreous to floury endosperm in maize (Zea mays L.) kernels is related to protein and starch. Journal of Cereal Science, 68, 148-154. <https://doi.org/10.1016/j.jcs.2016.01.013>

Gomez, L.A., Rodriguez, I.G., Poneleit, C.G. and Blake, D.F. (1982). Preference and utilization of maize endosperm variants by the rice weevil. Journal of Economic Entomology, 75, 363-367. <https://doi.org/10.1093/jee/75.2.363>

Heddi, A. (2003). Endosymbiosis in the weevil of the genus Sitophilus: genetic, physiological, and molecular interactions among associated genomes. In Insect Symbiosis, (Eds. K. Bourtzis and A. Miller), pp. 67-82, CRC Press LLC, New York.
Ivbiljaro, M.F. (1981). The resistance of new varieties of maize to post-harvest infestation by *Sitophilus zeamais* Motsch and *Sitophilus oryzae* (L.). *Journal of Agricultural Science*, 96, 479-481. <https://doi.org/10.1017/S0021859600066260>

Kasozzi, L.C., Derera, J., Tongoona, P., Tukamuhabwa, P., Muwonge, A. and Asea, G. (2016). Genotypic variation for maize weevil resistance in Eastern and Southern Africa maize inbred lines. *Uganda Journal of Agricultural Science*, 17, 83-97. <http://dx.doi.org/10.4314/ujas.v17i1.8>

Kim, S.K., Brewbaker, J.L. and Hallauer, A.R. (1988). Insect and disease resistance from tropical maize for use in temperate zone hybrids. In *Proceedings of the 43rd Annual Corn/Sorghum Research Conference*, American Seed Trade Association, pp. 194-226, Washington DC, USA.

Lale, N.E.S., Zakka, U., Atijegbe, S.R. and Chukwu, O. (2013). The response of different maize varieties to post-harvest infestation by *Sitophilus oryzae* (L.). *Agricultural Science*, 3, 17-22. <http://dx.doi.org/10.5923/j.plant.20130303.01>

Limonta, L., Locatelli D.P., Sangiorgio, S. and Consonni, G. (2013). Susceptibility of maize variants to *Plodia interpunctella*. *Italian Journal of Food Science*, 25, 283-288.

Liu, D., Parker, M.L., Wellner, N., Kirbyb, A.R., Cross, K., Morris, V.J. and Cheng, F. (2013). Structural variability between starch granules in wild type and in *ae* high-amylose mutant maize kernels. *Carbohydrate Polymers*, 97, 458-468. <https://doi.org/10.1016/j.carbpol.2013.05.013>

Locatelli, D.P., Castorina, G., Sangiorgio, S., Consonni, G. and Limonta, L. (2019). Susceptibility of maize genotypes to *Rhyzopertha dominica* (F.). *Journal of Plant Disease and Protection*, 126, 509-515. <https://doi.org/10.1007/s13488-019-0000-9>

Mansoor-ul-Hasan, A.A., Muhammad, J., Muhammad, W.J., Muhammad, S., Muhammad, Z.C. and Muhammad, L. (2013). The function of the waxy locus in starch synthesis in maize endosperm. *Biochemical Genetics*, 11, 83-96. <https://doi.org/10.1007/BF00485766>

---

Sodedji, F.A.K., Kwemoi, D.B., Kasozi, C.L., Asea, G. and Kyamanywa, S. (2013). Genetic analysis for resistance to *Sitophilus zeamais* (Motschulsky) among provitamin-A maize germplasm. *Maydica*, 63, 256-260.

Tsai, C.Y. (1974). The function of the waxy locus in starch synthesis in maize endosperm. *Talanta*, 24, 109-114.
Wessler, S.R. and Varagona, M.J. (1985). Molecular basis of mutations at the waxy locus of maize: correlation with the fine structure genetic map. *Proceedings of National Academy of Science USA*, **82**, 4177-4181. <https://doi.org/10.1073/pnas.82.12.4177>

Zeeman, S.C., Kossmann J. and Smith, A.M. (2010). Starch: its metabolism, evolution, and biotechnological modification in plants. *Annual Reviews of Plant Biology*, **61**, 209-234. <https://doi.org/10.1146/annurev-arpplant-042809-112301>

Zunjare, R., Hossain, F., Thirunavukkarasu, N., Muthusamy, V., Jha, S.K., Kumar, P. and Gupta, H.S. (2014). Evaluation of specialty corn inbreds for responses to stored grain weevil (*Sitophilus oryzae* L.) infestation. *Indian Journal of Genetics and Plant Breeding*, **74**, 564-567. <https://doi.org/10.1080/23311932.2015.1075934>

Zunjare R., Hossain, F., Muthusamy, V., Jha, S.K., Kumar, P., Sekhar, J.C., Thirunavukkarasu, N. and Gupta, H.S. (2016). Genetic variability among exotic and indigenous maize inbreds for resistance to stored grain weevil (*Sitophilus oryzae* L.) infestation. *Soil and Crop Sciences*, **2**, 1137156. <https://doi.org/10.1080/23311932.2015.1137156>