Genetic Analyses of Resistance to Stored Grain Weevil (*Sitophilus oryzae* L.) in Maize

Rajkumar Zunjare[1], Firoz Hossain[1]*, Nepolean Thirunavukkarasu[1], Ashish K. Vishwakarma[1], Neha Pandey[1], Vignesh Muthusamy[1], Sunil K. Jha[1], Pradyumn Kumar[2], Satish K. Guleria[3], Narendra K. Singh[4] and Hari S. Gupta[1,5]

[1] Indian Agricultural Research Institute (IARI), New Delhi, India;
[2] Directorate of Maize Research (DMR), New Delhi, India;
[3] CSK-Himachal Pradesh Krishi Vishvavidyalaya (CSK-HPKV), HAREC, Bajaura, India;
[4] GB Pant University of Agriculture & Technology (GBPUAT), Pantnagar, India;
[5] Borlaug Institute for South Asia (BISA), New Delhi, India.
*Corresponding author; Email: fh_gpb@yahoo.com

Introduction

Maize (*Zea mays* L.) assumes world-wide significance due to its utilization as a human food and livestock feed, as well as a source for several hundred industrial products. The suitability of maize to diverse environments is unmatched by any other crop due to its ability to grow in diverse climates. Together with rice and wheat, maize provides at least 30 percent of calories consumed by more than 4.5 billion people in 94 developing countries (Shiferaw et al. 2011). In Asia, maize is cultivated on an area of 57.59 million hectares yielding about 288.84 million metric tons (FAOSTAT 2013). About 67 percent of the total maize production, in the developing world, comes from low- and lower-middle-income countries and plays an important role in the livelihoods of millions of poor farmers. By 2050, the demand for maize in the developing world is expected to double (Rosegrant et al. 2009).

Maize grain weevil, *Sitophilus spp*. (Coleoptera: Curculionidae) has emerged as one of the most damaging stored-grain, insect-pests in maize, especially in the tropical and sub-tropical region where high humidity and temperature occurs throughout the year (Makate 2010; Tefera et al. 2013; Derera et al. 2014). Among different species, *Sitophilus*, *S. zeamais* is prevalent in Latin America, Europe and Africa, whereas *S. oryzae* (rice weevil) causes significant loss in Asian countries. *S. oryzae* is a polyphagous insect that feeds on cereals including rice, wheat and maize (Tefera 2012; Mwololo et al. 2013). In developing countries such as India, maize grains are often traditionally stored in jute bags often stacked in open areas under tarps. Humidity from rainfall during the monsoon generated rainy season, combined with storage practices, create conducive conditions for weevil infestation (Hossain et al. 2007). This is unlike the situation in developed countries where grains are stored in commercial-grade metal silos with controlled moisture content and fumigation to guard against insects (Masasa et al. 2013). Both larvae and weevil adult insects penetrate the seeds internally and cause losses between 12 percent and 20 percent while the extent of damage may be up to 80 percent in the untreated kernels (Giga et al. 1991; Pingali and Pandey 2001). The weevil also affects the seed viability and germination under field conditions, by causing injury to the embryo and endosperm (Demissie et al. 2008). Further, the infested grains enable secondary infestation by other harmful organisms such as *Aspergillus flavus* and *Fusarium verticillioides*; which lead to production of harmful mycotoxins on food grains (Robens and Cardwell 2005; Dowd et al. 2005).

Though application of synthetic insecticides may address stored- grain pest problems, the high cost of insecticide, danger of resistance from the increased load of insect populations, chemical residues in food, side-effects to beneficial insects exposed to hazards of insecticide and potential harm to human and livestock are of serious concern (Paes et al. 2012; Kazozi 2013). Therefore, stimulating genetic resistance in plants holds promise as it is a sustainable and environmentally friendly approach and it does not involve extra-cost during cultivation (Abebe et al. 2009). Resistant genotypes offer a practical and economical approach to minimize losses from insect pests (Keba et al. 2013). Thus, screening of maize genotypes for weevil resistance and identification of a suitable donor genotype, is of prime importance for a resistance breeding program. Additionally, an understanding the nature of gene actions governing resistance to the stored- grain weevil and the genetics of resistance; will enable breeders to adopt a suitable strategy for developing a weevil-resistant maize cultivar.

Materials and methods

Genetic materials

A set of 230 diverse inbred lines containing 183 national and 47 exotic inbreds, were screened to evaluate their responses to weevil infestation. The Indian inbred lines were developed by various maize breeding centers, while the exotic inbred lines were developed at CIMMYT, Mexico; CIMMYT-

HarvestPlus Program and Kasetsart University, Thailand. A set of 50 inbreds were evaluated in the first experiment (I), while in the second experiment (II), 112 inbreds were analyzed for their response against rice weevil infestation. In the third experiment (III), 68 specialty corn genotypes (popcorn, sweet corn and QPM) were evaluated. Further, 63 hybrid combinations generated from a 9 line × 7 tester set were evaluated for weevil response at three locations: (i) IARI Experimental Farm, New Delhi; (ii) CSK-HPKV, HAREC, Bajaura, India; and (iii) The Crop Research Centre, GBPUAT, Pantnagar, India.

Screening method for weevil infestation

Twenty-five kernels of each inbred and 100 randomly-selected kernels from the self-fertilized ears of experimental hybrids were analyzed for their responses against weevil infestation. Maize kernels were cleaned and brought to a moisture level ~15 percent. The conditioned kernels were weighed and transferred to a plastic petri-plate with a ventilated lid. Screening involved the use of newly-emerged un-sexed insects and eight pairs of inbreds and fifteen pairs of hybrids. The insects were kept for seven days for oviposition in each petri-plate (Hossain et al. 2007; Lara et al. 2009; Muzemu et al. 2013; Masasa et al. 2013). After seven days, the released insect pairs were removed and the petri-plates, and the plates with their seed, were kept in an incubator at 28 ± 2^0 C and 70±5 percent relative humidity (RH) for a 30-day incubation period. The petri-plates were monitored regularly for the emergence of insect progenies. After the appearance of the first weevil, progenies were counted and removed from each petri-plate on every alternate day for a period of 40 days. Finally, grain-weight-loss and total number-of-insect-progeny-emerged were recorded. The characteristics such as pericarp- thickness and grain hardness were recorded through the use of an ocular- micrometer-calibrated-compound microscope and texture analyzer, respectively. Germination tests for the inbreds were carried out after the completion of infestation experiments. All inbred evaluations were carried out in three replications for performing statistical analysis.

Statistical analysis

The data generated for grain-weight-loss, number of insect-progeny emerged, germination percentage, pericarp thickness and grain hardness, were analyzed for the analysis of variance (ANOVA); and LSD was carried out to rank the genotypes based on the level of resistance, using SAS Version 6.12. Pearson's simple correlation coefficients were estimated using Office-Excel 2007. The analyses for line x tester mating design were carried out as per the procedure described by Kempthorne (1957) and combining-ability analysis used the WINDOSTAT 8.5 software tool.

Results and discussion

Assessment of genetic variability for stored grain weevil resistance

ANOVA revealed a wide, significant, genetic variation for grain-weight-loss, number-of-insect-progeny-emerged, germination percentage, pericarp thickness and grain hardness across the three experiments (Table 1). This suggests that the existence of ample genetic variation would be conducive to genetic improvement of resistance. A variation of 3.40 percent to 42.32 percent grain-weight-loss was observed across the three experiments (Figure 1). A total of seven inbreds recorded grain-weight-loss of <5 percent, while, 56 inbreds had a grain-weight-loss of 5 percent to 10 percent. Regarding the number of insect progeny emerged, the study found broad genetic variation from 5 to 76.33 across the experiments. A wide variation also occurred with germination percentage (0.00 to 81.33 percent) across the experiments (Figure 2). Among the inbreds studied, only 23 inbreds showed germination of >70 percent and a large number of the 173 inbreds had <50 percent germination, showing the higher susceptibility of this trait upon weevil infestation. Weevils cause internal seed damage affecting the seed morpho-physiological quality thereby reducing germination (Canepelle et al. 2003). A variation of 36.18 µm to 194.83 µm was observed for pericarp thickness (Figure 3), while the grain hardness ranged between 62.33 to 1171.67 Newton (Figure 4).

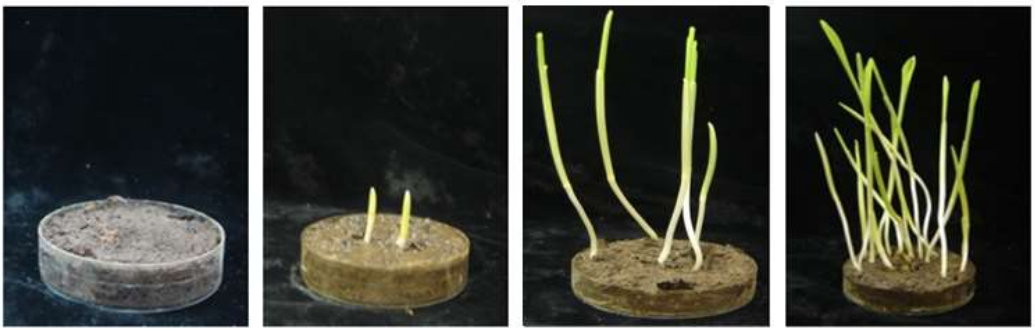
Table 1. Genetic variability for various morphological characters among inbred lines

Traits	Experiment I		Experiment II		Experiment III	
	Mean	Range	Mean	Range	Mean	Range
Grain-weight-loss (%)	19.49	4.50 - 42.32	16.28	4.90 - 42.19	16.51	3.40 - 41.21
No. of progenies emerged	26.03	5.67 - 69.33	24.21	6.00 - 75.67	17.05	5.00 - 76.33
Germination (%)	24.80	0.0 - 81.33	30.17	0.0 - 77.33	24.80	0.0 - 81.33
Pericarp thickness (µm)	104.41	58.45 - 189.27	92.02	47.32 - 194.83	92.02	36.18 - 178.13
Grain hardness (Newton)	301.57	113.0 - 834.67	335.59	136.00 - 1171.67	253.93	62.33 - 600.33

CML394 LM16 MGQSO-370

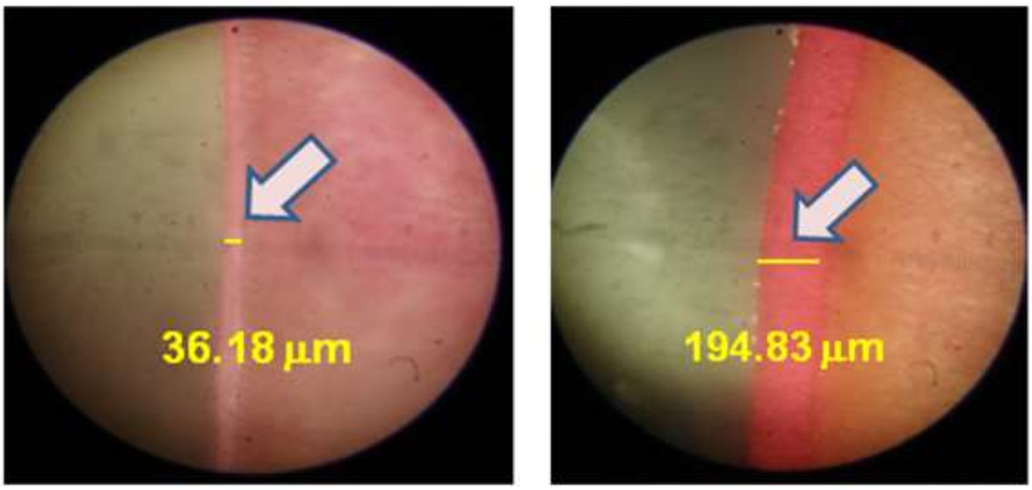
MGSH12 MGSU20 V401

Figure 1. Variation in grain-weight-loss among the inbreds after weevil infestation



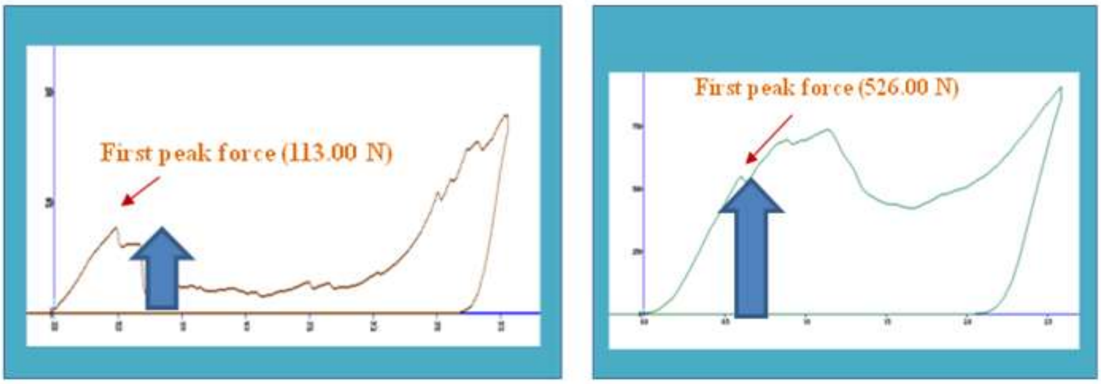
V401 V390 EI116 CML502

Figure 2. Variation in germination percentage among the inbreds after weevil infestation



MGSU-5 CML465

Figure 3. Variation for pericarp thickness among inbreds



MGQSO-370 **BAJIM-06-3**

Figure 4. Variation for first peak force required to break the grains, an indicator of grain hardness

Reponses of specialty corn inbreds to weevil infestation

The mean grain-weight-loss of 26.60 percent and higher number-of-insect-progeny-emerged in quality protein maize (QPM) genotypes, indicated a higher susceptibility of the QPM genotypes when compared with the sweet corn and popcorn inbreds (Figure 5). The popcorn inbreds recorded a mean-germination percentage of 78.30 percent, whereas shrunken (*sh2sh2*), sugary (*su1su1*) and QPM type recorded a germination of 21.40 percent, 21.90 percent and 13.30 percent, respectively. Even though the sweet corn showed lower or similar grain-weight-loss to popcorn type, they had a lower germination percentage than the popcorn. This is may be due to the fact that sweet corn inbreds contain less starch in the endosperm and even less damage to the endosperm by the weevil causes a greater reduction in germination capacity of the seed.

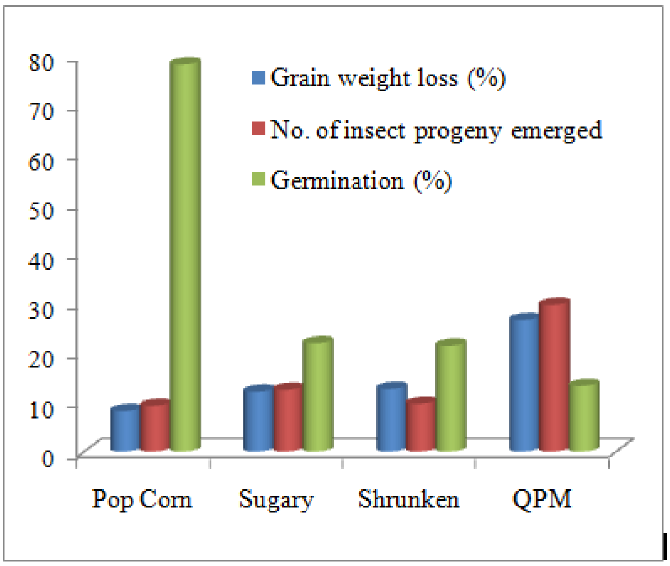


Figure 5. Comparison of specialty corn inbreds for various morphological characters

Association among traits

A positive correlation between grain-weight-loss and number-of-insect-progeny-emerged was observed across the experiments (Hossain et al. 2007; Lara et al. 2009; Dari et al. 2010; Temesgen and Waktole 2013; Derera et al. 2014). The study also revealed a negative correlation between germination percentage with both grain-weight-loss and progeny emergence. Okiwelu et al. (1987) observed a decrease in germination with enhanced levels of weevil infestation. Interestingly, no association was observed among pericarp thickness with either grain-weight-loss or insect progeny emerged (Gomez et al. 1983). Lack of correlation between grain hardness with grain-weight-loss and insect progeny emergence, as observed in the study, as was reported by Lara et al. (2009). Ruswandi et al. (2009) reported a negative correlation between grain hardness and grain damage, possibly due to snout penetration by the weevils into the maize grain, which may depend on hardness of the grain.

Identification of resistant inbreds

The study found that grain-weight-loss, number-of-progeny-emerged and germination-percentage were associated with resistance. Thus, these three traits were considered for generating a cumulative resistance index (CRI) using LSD ranks in accordance with the procedure reported by Arunachalam and Bandopadhyay (1984) for identification of resistant lines. Due to the lack of correlation of pericarp thickness and grain hardness with weevil infestation, these traits were not considered. CRI values varied from 0.10 to 2.93, 0.05 to 2.89 and 0.22 to 2.64 among experiments I, II and III, respectively. From the experiments I and II, 10 inbreds (CML394, SKV21, LM13, Pant109, Pant124, CML442, MGB1, CML207, HKI209 and HUZM185) were identified with >2.75 CRI, showing their higher-level of resistance to weevil infestation. These inbreds will serve as potential donors in the weevil resistance breeding program. CML394 and CML442 were also reported resistant to grain weevil in an earlier study (Dhliwayo and Pixley 2003). The mean CRI for the popcorn inbreds was 1.84, while sugary and shrunken types had a mean cumulative resistance index of 1.26 and 1.52, respectively. QPM genotypes were susceptible with a CRI of 0.76 (Figure 6).

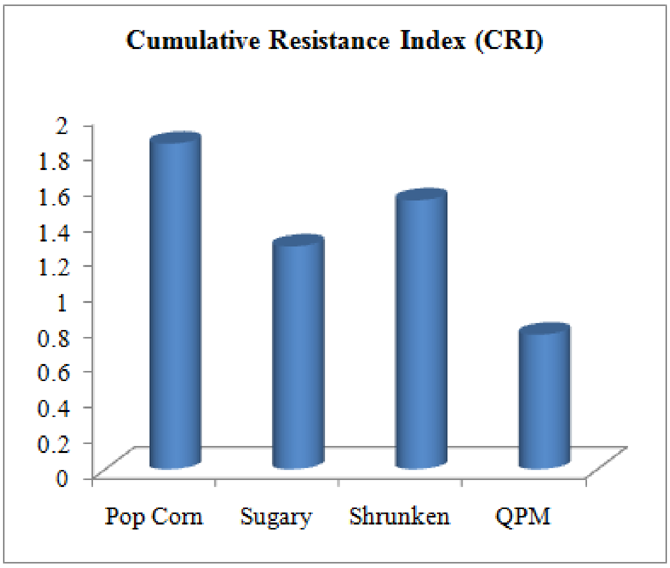


Figure 6. Mean CRI values among different specialty corn inbreds

Combining ability analyses of stored grain weevil resistance

Significant differences for grain-weight-loss and number-of-insect-progeny-emerged were observed among the genotypes, parents, crosses and parents compared to crosses at the three locations *viz.* Delhi, Bajaura and Pantnagar. This reveals parents and crosses significantly differed for the target traits. Parents were distinctly different from the cross combinations generated in the mating design indicating scope for genetic improvement. ANOVA-pooled analyses determined a significant relationship between the influence of location and the extent to which weevil infestation occurred (as mean square due to location, location × genotype, location × parents, location × crosses and location × parents vs. crosses target traits). This finding is in congruence with the results of Kim and Kossou (2003) and Lara et al. (2009).

ANOVA (for the combining ability analyses, variance due to lines; testers and line × tester) was significant for the target traits. The results thus indicate that both additive and non-additive gene action play an important role in determining the extent of grain-weight-loss and number-of-insect-progeny-emerged after infestation by the weevils. Degree of dominance also indicated that both additive and non-additive gene actions were of almost equal magnitude, with a slight preponderance of non-additive gene action compared with the additive gene action. Several researchers (Kim and Kossou 2003; Dhliwayo et al. 2005; Dari et al. 2010; Kazozi 2013; Derera et al. 2014) reported similar results against *S. zeamais* infestation. However, Dhliwayo et al. (2005) demonstrated that SCA was more important than GCA for weevil number-of- progeny-emerged from F_2 grains whereas, Lara et al. (2009) showed that genetic effects were mainly of a dominant type for both grain-weight-loss and adult-progeny-emerged. However, Tipping et al. (1989) reported GCA is more important than SCA for number of eggs laid in seed. Similarly, Dhliwayo and Pixley (2003) and Kanyamasoro et al. (2012) reported that additive gene action was more important than non-additive gene action in determining resistance to maize weevil. The narrow sense heritability for grain-weight-loss and number-of-insect-progeny-emerged was 29.41 percent and 32.55 percent, respectively. Lara et al. (2009), while working with a QTL mapping experiment for weevil tolerance in maize, mentioned 48.0 percent and 45.0 percent of narrow sense heritability similar traits, respectively. Dari et al. (2010) also reported broad sense heritability of 67.0 percent and 55.0 percent for grain-weight-loss and 62.0 percent and 50.0 percent for number-of-weevils-emerged among lines and hybrids, respectively. Based on GCA and mean performance of both the traits found that Pant110 and CM135 (among the lines) and (MGP462) among the testers were found be promising inbreds for weevil resistance.

Conclusions

The investigation deals with comprehensive genetic analyses of Indian and exotic maize inbred lines response to stored-grain weevil (*S. oryzae*) infestation. Wide-genetic-variability exists among maize germplasm for grain-weight-loss, number-of-progeny-emerged and germination-percentage that were associated with resistance to weevil infestation. Pericarp thickness and grain hardness did not show any correlation with weevil resistance. Resistant inbreds identified in the study, therefore, have potential to be used as donors in the resistance breeding program. The combining- ability study revealed that stored-grain weevil resistance in maize is governed by both additive and non-additive gene actions. This experiment also confirmed the role of the environment in determining weevil resistance.

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