

MAKERERE



UNIVERSITY

**RESISTANCE IN TROPICAL MAIZE TO THE MAIZE WEEVIL
AND LARGER GRAIN BORER**

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Declaration

This study is original and has not been submitted for any other degree award to any other University before and no part of the dissertation is plagiarized work.

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Dedication

To my family and friends for their moral support during my studies.

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Preface

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- **Mwololo J.K.** , S. Mugo, P. Okori., T. Tadele, M.Otim. and S.W. Munyiri 2012: Sources of Resistance to the Maize Weevil *Sitophilus zeamais*. Journal of Agricultural Science Vol 4: 206-215.
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- **Mwololo J.K.**, P. Okori., S. Kassa, S. Mugo, T. Tadele, M.Otim. and S.W. Munyiri 2012: Genetic Relationship in Tropical Maize Germplasm in Response to Postharvest Insect Pests. Draft Manuscript.
- **Mwololo J.K.**, P. Okori., S. Kassa, S. Mugo, T. Tadele, M.Otim. and S.W. Munyiri 2012: Quantitative Trait Loci Mapping in Maize for Resistance to the Maize weevil and Larger grain borer. Draft Manuscript.

Abstract

Storability of maize grain is constrained by two postharvest insect pests namely the maize weevil (*Sitophilus zeamais*) and larger grain borer (LGB) (*Prostephanus truncatus*). Host plant resistance is the most economic way to manage such pests especially for resource constrained farmers as a component of integrated pest management. Breeding for resistance to these pests in maize is dependent on good understanding of genetic mechanisms underlying the resistance. The objectives of this study were to (i) assess resistance in tropical maize to the maize weevil and larger grain borer; (ii) determine the genetic relationships in tropical maize germplasm in relation to resistance to the postharvest insect pests; and (iii) map quantitative trait loci (QTL) associated with postharvest insect pest resistance in tropical maize. Two hundred and ninety five (295) entries of maize genotypes including resistant and susceptible checks were evaluated for biophysical traits at two sites during 2010 and 2011 rain seasons. The germplasm was subsequently screened for resistance to the maize weevil and LGB. Data was collected on husk cover tip length, and grain texture in the field. Biochemical traits were analyzed on the maize grain. Harvested grain were evaluated for postharvest insect pest resistance namely grain damage, grain weight loss, and numbers of insects. Grain hardness was measured as a putative trait of resistance at harvest. Genetic relationship among accessions was determined using biophysical/bioassay and simple sequence repeat DNA (SSR) data. To map resistance loci using QTL approach, a mapping population of 203 F_{2:3} derived progenies was developed from a cross between susceptible and resistant inbred lines. The F_{2:3} progenies were crossed to a tester and the testcrosses were evaluated across six environments. The same data used for assessment of pest resistance were taken for the QTL generation. Multivariate and univariate analysis of variance for all the traits was done using the general linear model (GLM) of statistical analysis system (SAS). The insect-damage traits and protein content were correlated using a canonical correlation. The genetic relationship and distance were estimated using the

NTSYS and MEGA software. Genetic mapping was done using Joinmap 4, while QTL analysis was done using PLABQTL.

There were great variations in resistance levels among the genotypes to the maize weevil and LGB. The most resistant genotypes were CKPH08003, BRAZ 2451 and CKSPL10028, while the most susceptible were PH 3254, BRAZ 4 and CML 312, among the hybrids, landraces and inbred lines, respectively. Dual resistance to maize weevil and LGB was observed in the germplasm evaluated. Commercial varieties were the most susceptible among the hybrids evaluated. Percentage weight loss was the most important trait for grouping the genotypes into resistant and susceptible categories. Protein and oil contents were high in grain of resistant compared to susceptible genotypes. The resistant hybrids and inbred lines had flint texture, whereas the susceptible ones had dent kernel texture. Grain texture was positively correlated ($r = 0.7$) with grain hardness. High genetic divergence for resistance to maize weevil and LGB was observed among tropical maize germplasm. QTL for resistance to maize weevil and LGB were mapped to chromosomes 1, 2, 3, 5, 8, 9, that also contain loci for resistance to disease and lepidopteran insect pests, and enzymes involved in biosynthesis of cell wall and phenolic acid. The QTL for damage resistance traits were located in chromosomes 1, 5 and 9. Chromosome 1 had a common QTL linked to protein content, grain hardness and husk cover tip length. Additive genetic effects were prevalent in all detected QTL. Overall, these studies show that mechanisms of resistance to the maize weevil and LGB are similar. As such breeding for resistance to both pests is possible within a breeding programme. The results obtained in this study are helpful in understanding the genetic basis of resistance to the maize weevil, management options for the LGB and for fine mapping of QTL. There is potential for development of genotypes with dual resistance to the maize weevil and LGB.

Chapter One

Background and Introduction

1.1 Economic importance of maize

Maize (*Zea mays* L.) is an essential component of the global food security. In Africa, maize is primarily grown by small-scale farmers for human food and animal feed. Maize grain is consumed in different ways; it can be grilled, boiled, roasted or made into various products. Maize is also used to produce alcohol, starch, pulp abrasive, pharmaceutical oils and recently for fuel production (Acharya and Young, 2008). Maize serves as an important source of proteins, energy and some of the important vitamins and minerals to billions of people worldwide, particularly in Africa, South America and Asia (Pswarayi and Vivek, 2004). The per capita consumption of maize in Sub Saharan Africa (SSA) is 42 Kg. Globally, 140 million ha of land are under maize with approximately 96 million ha in the developing world, and SSA account for about 30 million ha. The global demand for maize is likely to increase and is expected to overtake the demand for rice and wheat by 2030. In developing countries, the demand is projected to increase from 282 million tonnes in 1995 to 504 million tonnes in 2020 (Pingali and Pandey, 2001). Despite the importance of maize in sub-Saharan Africa, farmers obtain low yields of 1.5 t/ha compared to global average of 6 t/ha due to a number of constraints.

1.2 Maize production constraints

Maize production in SSA is limited by abiotic and biotic stresses as well as socio-economic factors (Simmons, 2003). The abiotic constraints include drought and soil infertility. The key biotic factors leading to low yields are diseases and pests, such as maize streak virus transmitted by leafhoppers (*Cicadulina mbila*), gray leaf spot caused by the fungus *Cercospora zeaemaydis*, turcicum leaf blight caused by *Exserohilum turcicum* and ear rot caused by *Stenocarpella maydis*. The key field insect pests

are maize stalk borer namely *Chilo partellus* (Swinhoe), *Busseola fusca* and *Sesamia calamistis*. The postharvest insect pests of importance are Angoumois grain moth (*Sitotroga cerealella*), maize weevil (*Sitophilus zeamais* Motschulsky) (Coleoptera: Curculionidae) and larger grain borer (*Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)) during storage (Pingali and Pandey, 2001).

1.3 Maize storage constraints

Postharvest insect pests of maize destroy kernels and pre-dispose stored grain to secondary attack by pathogens (Evans, 1987; Tefera *et al.*, 2010). This particularly happens in the tropics and sub tropics where temperatures and relative humidity are high. Earlier studies have reported grain losses as high as 80% in developing countries and 30 % on average due to postharvest insect pest infestation (Tapondjou *et al.*, 2000). Crop improvement for postharvest insect pest resistance is among the important strategies of mitigating biotic factors affecting productivity of maize in Sub-Saharan Africa. Resistant varieties that delay or prevent development of predominant postharvest insect pests contribute to a stable supply of food grain. Consequently, reduction in damage from insect pests and diseases that attack the maize crop and stored grain should be emphasized to increase the quantity and quality of food grain to meet the food demand for the increasing world population.

Maize weevil is an important pest of stored maize in the tropics, particularly in the lowland and mid-altitude hot and humid environments (Pingali and Pandey, 2001). The adults and larvae of maize weevils are internal feeders and cause great losses to grains, both quantitatively and qualitatively. Earlier studies have reported more than 20% losses caused by weevils and up to 80% loss in on-farm stores in tropical countries (Pingali and Pandey, 2001). Maize weevils cause direct damage to the stored grain and also affect viability of stored grain which is often used as seed in succeeding seasons. The maize weevil is an old pest of the crop and conceivably has co-evolved with the crop to carry

resistance. On the other hand, the larger grain borer (LGB) is a recent pest of maize in the region although in Central America also the home of maize it is a well known pest. As such, using the maize weevil as a known pest selected for in the region, indirect and direct assessment of resistance and mapping of the respective loci can be done. The pest was first sighted in East Africa in the 1970s and in West Africa in the early 1980s; and has now spread to more than 18 African countries (Markham *et al.* 1991; Omondi *et al.* 2009). Grain losses of between 9-45 % are caused by LGB infestation depending upon the period of storage and maize genotype (Kumar, 2002).

Fumigation and grain dressing using storage pesticides is recommended for maize weevil and LGB control, but resource-poor farmers cannot afford such a practice. Moreover, the use of such chemicals raises environmental concerns due to residual toxicity (Buglio and Wilkins, 2004). The development of resistance to pesticide among maize weevil and LGB populations also poses a challenge (Banwo and Adamu, 2003). Development of safe, cost effective and environmentally friendly strategy is required in order to provide efficient and sustainable control of postharvest insect pests of stored grain. Host plant resistance against postharvest insect pest damage is considered most preferable as part of integrated pest management to reduce losses and maintain grain quality. This is because it is economically viable, ecologically sound and compatible with other control approaches. Genetic diversity and variability for the trait in question is important for progress in the development of resistant genotypes in a breeding program. Genetic markers have enhanced genetic diversity studies (Melchinger *et al.*, 1999). This ensures success in the generation of improved genotypes which will contribute to meeting food demands in the coming decades.

1.4 Justification of the study

In Africa, maize is primarily grown by small-scale farmers for food and feed. Maize productivity is thus critical to raise rural income and stimulate economic growth (Byerlee *et al.*, 1997). Most of the improved maize varieties possess good agronomic performance and tolerance to abiotic and biotic stresses, but are susceptible to postharvest insect pests (Fornal *et al.*, 2007). This is particularly important in developing countries, where grain is mainly for domestic use but stored under conditions conducive for insect pest attack. During the vegetative and grain filling stages, stem borers and armyworms cause losses in the range of 5-50 % and additional losses are caused by post-harvest insect pests which can cause 100 % kernel damage and 30 % weight loss during six months of storage (Fornal *et al.*, 2007). The maize weevil and LGB are storage pests that are among the major pests responsible for serious losses of maize worldwide. Their damage results directly in lost food, and seed thus reducing food security and future maize production for farmers (Pingali and Pandey, 2001). The extensive use of pesticides by farmers in these regions is neither practicable nor desirable (Bugchio and Wilkins, 2004; Asawalam *et al.*, 2006). Host plant resistance through genetic improvements remains the most cost effective component of integrated pest management to minimize postharvest losses due to pests.

Genetic variation for resistance to postharvest storage pests exists in maize. Various biochemical and physical characteristics have been identified as mechanisms of kernel resistance to postharvest storage pests. Studies that have used germplasm with more diversity have confirmed high heritability of maize weevil resistance (Derera *et al.*, 2001). However, the relationship between physical and biochemical traits and molecular data in relation to weevil and LGB resistance has not been established. Breeders need new and better sources of resistance and increased knowledge of their genetics to design effective breeding methods. Determination of genetic diversity among maize genotypes for resistance to maize

weevil and LGB using molecular markers is, therefore, important. The combined use of biophysica/bioassay and molecular methods for the study of genetic variations among maize genotypes would provide useful information for improvement of this crop in relation to resistance to storage pests. The international maize and wheat improvement center (CIMMYT) in Kenya initiated a breeding program that has generated germplasm for quality protein maize (QPM), drought tolerance, resistance to stem borers, and resistance to postharvest insect pests (CIMMYT Annual Report, 2001). The genetic diversity among this germplasm and its response to weevil and LGB resistance has not been determined.

Earlier studies have reported that insect resistance loci in maize are clustered in regions associated with biochemical resistance factors, such as flavonoids and cell wall components (Krakowsky *et al.*, 2007). Currently there is some information about loci associated with maize resistance to maize weevil. Marker assisted selection would enhance efficient and effective breeding for resistance to postharvest insect pests by accelerating the transfer of major loci contributing to maize weevil and LGB resistance in a modern breeding programme. Developing genetic maps for maize weevil and LGB resistance would enhance better understanding of the basis for resistance and development of molecular markers for use in marker assisted recurrent selection for resistance breeding.

1.5 Study hypotheses

The general hypothesis of the study was that the mechanisms of resistance to the maize weevil and LGB are similar. The study was based on the following specific hypotheses:

1. There is resistance to maize weevil and larger grain borer in tropical maize germplasm.
2. Genetic relationship in tropical maize germplasm for resistance to postharvest insect pests can be elucidated by use of simple sequence repeat markers (SSRs) and phenotypic traits.
3. Quantitative trait loci (QTLs) for resistance to maize weevil and LGB can be mapped using SNPs.

1.6 Objectives

1.6.1 Overall objective

To provide genetic information for reduction of post-harvest losses in maize through breeding for resistance to postharvest insect pests.

1.6.2 Specific objectives

1. Assess resistance in diverse maize genotypes to the maize weevil.
2. Assess resistance in diverse maize genotypes to the larger grain borer.
3. Determine the genetic relationship among tropical maize genotypes based on biophysical traits and SSR markers in relation to postharvest insect pest resistance.
4. Map quantitative trait loci (QTL) associated with postharvest insect pest resistance in tropical maize.

Chapter Two

Literature Review

2.1 Introduction

The maize weevil and LGB are among the most economically important post-harvest pests of maize. The maize weevil has co-evolved with the maize crop and is a serious storage pest of maize in the tropics (Longstaff, 1981). The LGB was introduced to Africa from Mesoamerica in the early 1980s and has spread in most parts of Africa, threatening maize production due to its aggressive nature and extent of damage caused within a short period (Boxall, 2002; Tefera *et al.* 2011). The two pests occur in the field and under storage (Caswell, 1962). Breeding for resistance is the most feasible solution to postharvest losses caused by the postharvest insect pests. Farmers need maize varieties with consumer preferred traits, therefore, breeding for resistance should be coupled with suitable adaptable agronomic traits.

2.2 The life cycle of maize weevil

The maize weevil belongs to the family Curculionidae and order Coleoptera, found in most of the tropical areas. Maize weevil co-evolved with most cereals and commonly attacks standing crops, in particular, maize before harvest, and is also associated with rice (Buckland, 1981; Dix and Al, 1985). The entire life cycle of the maize weevil takes 30 - 45 days to complete. The adult female weevil lays her eggs within the grain kernels at a rate of 25 per day spread over 100 days. The maize weevil bores into the grain with its long snout and lays a single egg. The eggs hatch in approximately 3 days, depending on the humidity and moisture content of the grain. The larvae, which are approximately 4 mm, white and legless, begin to feed on the internal contents of the maize while developing, which takes approximately 18-23 days. Subsequently, the larva pupates, and transform into adult weevils. The

weevil then emerges, by cutting a small circular hole in the grain and the process begins over again. The adult maize weevil will feed on the maize during its lifespan, which is approximately 5-8 months long, before dying. The optimum temperature, moisture content and relative humidity for growth of maize weevil populations on grain are 30°C, 13% and 75 % respectively (Throne, 1994). The weevils do not breed well at temperatures below 20°C or above 32°C, or in food with moisture content below 11%.

2.3 The life cycle of larger grain borer

The LGB belongs to the family Bostrichidae and order Coleoptera. This pest is exotic to Africa and is reported to breed only in maize and cassava (Nang'ayo *et al.*, 1993). The adults can live in and damage many stored products such as bulrush millet, sorghum, yam, and wheat, as well as structural wood and wooden utensils. The LGB also occurs in the wild and is able to breed on dead dry wood of a range of trees, as well as dried stems of cassava and maize plants (Nansen *et al.*, 2004). The optimum conditions for development of the larger grain borer on maize are 32°C and 70-80% relative humidity, and under these conditions, the life cycle can be completed in 24-25 days (Hodges, 1998). Adults bore into maize grain making neat round holes, generating large quantities of dust as they tunnel from grain to grain. After mating, adult females lay eggs within the grain in blind ended chambers bored at right angles to the main tunnel. Oviposition begins 5-10 days after adult emergence, reaching a peak at 15-20 days (Bell and Watters, 1982). The mean development period under optimum conditions for eggs is 3 days, 14 days for larvae (3 instars), 4 days for pre-pupae, and 3 days for pupae (Demianyk and Sinha, 1988). The potential level of infestation is high, with life time egg production in laboratory cultures varying from 50-400. The last larval instar of LGB constructs a pupal case from frass stuck together with a larval secretion either within the grain or in the surrounding dust (Demianyk and Sinha, 1988). Females tend to outlive the males, with a mean survival time of 61 days for females and 45 days for males (Bell

and Watters, 1982). The LGB is able to tolerate dry conditions, and develop in grain at low moisture in contrast to many other postharvest insect pests, which are unable to increase in number under low moisture conditions. Because it appeared recently as a world-wide pest, there is limited screening for resistance in grain and understanding of the mechanisms associated done.

2.4 Resistance of maize grain to maize weevil and larger grain borer

The postharvest insect pests are a major threat to food security and production of the next crop. Given that maize weevil and LGB are endemic in Africa and coupled with the fact that use of pesticides lead to pest resistance and environmental hazards, host plant resistance is the most economically viable solution for postharvest pest management (Tefera *et al.* 2011). Resistant varieties that delay or prevent development of the postharvest pests contribute to a stable supply of food grains and market prices during the dry season. Public and private sector maize breeders should, therefore, consider breeding for resistance to maize weevil and LGB to better meet demand, and increase adoption and purchase of improved seed by farmers. Breeding for resistance as a management strategy is underpinned by genetic studies. Earlier studies on the maize weevil have provided information which forms the foundation for breeding for resistance to postharvest insect pests. Given that there is genetic variation for maize weevil resistance and that host species specialization is a possibility, the LGB being a Coleopteran would have the same mechanisms of resistance as the weevil. Breeding improved varieties to meet the market demand need screening for adaptable agronomic traits.

There are two components of resistance in plants namely antibiosis and non-preference to insect pest, studied and form important basis of resistance to postharvest insect pests (Derera *et al.*, 2001). Non-preference is the heritable feature of the grain, which discourages insects from feeding, colonising and oviposition or a combination of the three. Grain texture is the basis of non-preference resistance,

because the smooth pericarp prevents weevils from feeding and oviposition (Derera *et al.*, 2001). Antibiosis denotes toxic grain characters that result in adverse effects on the insect's life when the insect uses a resistant plant for food. Tolerance denotes resistance whereby the plant shows an ability to grow and reproduce or to repair injury in spite of supporting a population equal to that damaging a susceptible host (Agrios, 2005). Evaluation of resistance to stored-grain pests should focus on measuring antibiosis and or non-preference because tolerance does not function in stored grain (Schoonhoven *et al.*, 1975; Derera *et al.*, 2001).

2.5 Mechanisms of resistance in cereals to postharvest insect pests

There is significant variation for resistance to maize weevil and LGB. There are two approaches that are acceptable from a food-safety perspective in breeding for resistance to postharvest insect pests. First, physical barriers that do not adversely affect processing such as fiber and waxes and second, biochemicals that have specific and localized activity against pests and diseases but not toxic to humans or livestock (Russin *et al.*, 1997). Biochemical components are proteins or metabolites of grain which are products of genes reported to confer resistance to insects or diseases. They include phenolic acids, hydroxyproline-rich glycoproteins, sugars, soluble peroxidase and protein inhibitors (García-Lara *et al.*, 2004). Most phenolic acids implicated in resistance are ester-linked to cell wall polysaccharides, as well as phenolic dimers, such as dimeric phenolic acids (DiFA). Recent studies demonstrate that maize weevil resistant genotypes have higher concentrations of total DiFAs. Cross-linking of polysaccharides by DiFAs is also considered as important in fortification of the pericarp cell wall. Other biochemical components of the cell wall which need further exploration include the role of hydroxyproline rich proteins (extensins) that are known to accumulate in the pericarp of maize. Grain hardness is a physical factor associated with resistance to grain storage pests (García-Lara *et al.*, 2004).

2.6 Towards breeding for resistance in crop plants

2.6.1 Genetic diversity and its role in breeding

Knowledge on the genetic diversity among maize lines is useful for several purposes, including understanding the genetic variability available for use in prediction of heterosis, selection for conservation and estimation of any possible loss of genetic diversity (Hartl and Clark, 1997). Genetic diversity for postharvest insect-pest resistance is an entry point for understanding the genetic basis of host-plant resistance which is essential to ensure that traits being used for selection meet consumer demands. Genetic diversity studies have been enhanced through the use of genetic markers (Bergvinson and Garcia-Lara, 2004). Genetic diversity can be exploited to characterize resistance mechanisms that are associated with different crops and to incorporate resistance into improved varieties using conventional and genetic engineering approaches (Dhliwayo and Pixley, 2003). The traditional methods for estimating genetic diversity have been the use of morphological markers. However, they are less preferred when assessing diversity because they are biased, mainly indicating the effects of recent selection and genetic drift on the population. Development and use of molecular marker technologies have facilitated analysis of plant genomes, genetic structure and variations among crop accessions (Wang *et al.*, 2004), the use of which is discussed here.

2.6.2 Tools and approaches for breeding for resistance

Several studies have reported quantitative inheritance of maize weevil resistance. Inheritance of resistance to maize weevil is conditioned by additive and, to a lesser extent, non-additive gene action (Derera *et al.*, 2001). Other studies with diverse germplasm have also confirmed the polygenic nature of weevil resistance, the importance of maternal effects, additive and non-additive gene action (Dhliwayo *et al.*, 2005). The progress in conventional breeding is limited by low heritability of quantitative traits; since they are conditioned by simultaneous interactions of many genes and with the

environment (Barton and Turelli, 1989). In addition the breeding cycle to advance generations is long, therefore it takes time to deliver an improved variety. Identification of molecular markers for postharvest insect pest resistance could facilitate transfer and pyramiding of the resistance genes through marker-assisted recurrent selection (MARS).

Rapid advances have recently been made in developing powerful molecular genetic tools for use in the life sciences. These are widely used to support the selection of loci linked to important traits such as yield, abiotic and biotic stress resistance, and quality traits of crop species. The development of these biotechnological tools coincides with increased focus on the use of landraces and wild relatives as sources of resistance to insect pests, pathogens and traits of agronomic value. Introgression of such loci into the cultivated crop germplasm is critical, because it provides economically beneficial traits and increases the genetic diversity of related cultivated crops. Such advancements in crop improvement are possible if the use of molecular markers is fully exploited.

Molecular markers are based on differences in the DNA nucleotide sequences largely as a product of mutations. These differences are referred to as DNA polymorphisms, and arise as a result of mutations which include insertions, deletions, duplications, and substitutions of nucleotides (Pinto *et al.*, 2003). The DNA polymorphisms can be visualized using a wide variety of molecular marker methodologies. A wide range of molecular markers have been used in maize for various purposes. The most common molecular markers used earlier were restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites (Gupta and Varshney, 2000). Simple sequence repeats (SSRs), also known as microsatellites, are simple repetitive DNA sequences consisting of two, three or four nucleotides (di-, tri-, and tetra-nucleotide repeats, respectively (Pinto *et al.*, 2003). The SSR markers are codominant,

multi-allelic, highly polymorphic, require low amount of DNA, easily automated for high throughput screening, and are highly transferable among populations (Gupta and Varshney, 2000). In maize, microsatellites have been used for genome mapping, marker-assisted selection and heterotic grouping (Enoki *et al.*, 2002). The single nucleotide polymorphism (SNPs) markers have gained preference in the scientific community. These are currently the markers of choice in molecular genetics as they are the most abundant source of genetic polymorphism (Slate *et al.*, 2009). The development of single nucleotide polymorphism (SNPs) genotyping has led to increase in the number of data-points generated in commercial breeding programme (Bernardo, 2008).

These genetic markers can complement morphological markers in selection of improved crop varieties. Selection based on genotypic or DNA-based differences rather than phenotypic differences would be enhanced, and therefore the potential of increasing selection efficiency (Krakowsky *et al.*, 2007). These markers can be used for genetic diversity studies and QTL mapping for resistance to postharvest insect pests. The identification of molecular markers for postharvest insect pest resistance can facilitate and speed up development of resistant genotypes. Molecular markers are essential in marker-assisted selection (MAS) and facilitate mapping and tagging of agriculturally important genes. The use of molecular techniques can support more efficient transfer of desirable genes between different varieties and the introgression of novel genes from wild species (Mohan *et al.*, 1997). Knowing the causal gene underlying a quantitative trait locus (QTL) is important since it allows for more precise selection of recombinants and reduction of linkage drag (Xu *et al.*, 2006). As more genes are cloned, valuable information on the molecular mechanisms controlling agronomic traits of interest are gained, providing foundation for more precise methods of further breeding. This advancement in breeding has led to provision of information on the genetic architecture of complex traits (Holland, 2007).

Linkage analysis and association mapping are the two most commonly used methods for QTL mapping. Association mapping requires collections of germplasm instead of biparental populations and is a modern approach to genetic mapping to find genes underlying quantitative traits (Yu *et al.*, 2006). This method relies on linkage disequilibrium to study the relationship between phenotypic variation and genetic polymorphisms (Breseghello and Sorrells, 2006). Genetic association mapping takes into account thousands of polymorphisms to evaluate for QTL effect, but it is only capable of identifying phenotypic effects of alleles with reasonably high frequency in the population under investigation. Rare alleles cannot be evaluated because of low detection power, therefore, classical biparental mapping is more appropriate. Genetic linkage analysis is more useful for genome wide scan for QTL, while association mapping gives more precise location of an individual QTL. Consequently, linkage analysis is critical for preliminary location of QTLs and association mapping for precise location (Mackay, 2001; Glazier *et al.*, 2002).

The simplest method of detecting linkage between a DNA marker and a QTL is by using single marker analysis (Holland, 2007). This involves testing a DNA marker in a mapping population and determining if significant differences exist between trait means based on the genotype of a marker. Linear regression is most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL linked to the marker. Composite interval mapping is a more powerful QTL detection method that utilise flanking markers and other co-factors, and is based on maximum likelihood multiple regression (Frisch and Melchinger, 2001). Once tightly-linked markers that reliably predict a trait phenotype are identified, they may be used in MARS (Haussmann *et al.*, 2004; Collard *et al.*, 2006). The fundamental advantages of MAS as a compliment to conventional phenotypic selection are that it may save time, effort, resources and money by carrying out selection at the seedling stage. Therefore, undesirable plant genotypes can be quickly eliminated

and single plants may be selected (Zwart *et al.*, 2006). These advantages can be exploited by breeders to accelerate an efficient and effective breeding process (Collard *et al.*, 2005; Collard and Mackill, 2008). Target genotypes can be more effectively selected which may enable certain traits to be ‘fast-tracked’, potentially resulting in quicker variety release. Marker assisted selection would be a compliment for phenotyping, allowing selection in off-season nurseries and making it more cost effective by eliminating undesirable lines at early generations (Ribaut and Hoisington, 1998; Collard and Mackill, 2008).

2.7 Sectional conclusion

Reducing post-harvest losses due to insect pests through genetic improvement is needed to meet demand for food. The most feasible way to control postharvest losses is development of resistant varieties through conventional breeding coupled with MAS when possible. Through co-evolution of insect pests and plants, the plant-hosts naturally develop protective mechanisms to survive insect pest attack. Numerous studies on resistance to weevil have been done and that information could be used to underpin breeding for resistance to LGB. Since LGB is also a coleopteran, it is possible that the mechanisms of resistance are similar for the two insect pests. There is, therefore, need to identify the underlying mechanisms of resistance to LGB. Plant breeders need new and better sources of resistance and increased knowledge for effective breeding against LGB. The genetic diversity among tropical maize germplasm in relation to postharvest pest insect resistance using biophysical traits and molecular markers should be documented. Identifying QTLs in maize populations for postharvest insect pest resistance is crucial in understanding the basis for resistance and development of stable loci (markers) for use in MARS for resistant genotypes. The focus of this study was to investigate the genetic basis of resistance to the LGB using the maize weevil as a model.

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Chapter Three

Resistance in Tropical Maize Genotypes to the Maize Weevil *Sitophilus zeamais*

3.1 Introduction

Small-scale farmers in Africa, store maize grain to ensure household food supplies and seed (Adetunji, 2007). Stored grain may be attacked by pests (insects, rodents and birds) and pathogens (bacteria and fungi) when not protected, especially under poor storage. Insect pests are thus a major threat, causing losses of up to 30 % of food produce (Pimentel, 2007). The damage caused by postharvest insect pests is higher than that caused by rodents and micro-organisms. The maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae), the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) are the most important pests of stored cereal grains (Abate *et al.*, 2000). In addition to direct destruction of grain through feeding and reproduction activities, the presence of insects has direct influence on grains, causing an increase in grain temperature and moisture content (Odogola, 1994), that in turn increases attack by pathogens. Postharvest losses due to *Sitophilus zeamais* range from 20% to 90% (Dereral *et al.*, 2001; Tefera *et al.*, 2010). The damage caused is irreversible and affects both farmers and other grain handlers and dealers. The adults and larvae of weevils feed on maize grain affecting seed viability. Meeting the ever-increasing demand for sufficient quantity and acceptable quality of food, therefore, requires a reduction in damage from insects that attack the stored maize grain and seed.

Different technologies have proved effective in controlling postharvest insect pests, including environmental manipulations to hinder growth, maturation and reproduction. Such environmental manipulations can be attained by employing a number of control measures, including the use of pesticides, cultural and physical control methods (Pereira *et al.*, 2009). Pesticides are effectively used

against postharvest insect pests but are often associated with a number of drawbacks including high costs, environmental pollution, food safety, and resistance by insect pests (Asawalam *et al.*, 2006). Host plant resistance through genetic improvements remains a cornerstone of integrated pest-management to minimize storage losses and impact on grain quality. Breeding for resistance to storage insect pests was initially ignored, probably due to the long duration from crop establishment to postharvest screening for resistance and the high cost involved. Genetic variation and heritability of resistance to maize weevil and other storage pests have been reported; however, effective breeding for resistance requires identifying resistant materials in the maize gene pool and understanding the mechanisms of resistance (Derera *et al.*, 2001; Mwololo *et al.*, 2010).

Progress has been made in developing maize germplasm resistant to postharvest insect pests, but the current resistance levels are based on few sources from an unimproved gene bank accession. The success of any breeding programme depends on the presence of genetic variability. The higher the variability for a given trait, the higher the genetic gain that can be expected through selection. Plant scientists, therefore, should search for more sources of resistance among adapted genotypes within the tropical germplasm. The objective of the study was, therefore, to assess the reaction of maize genotypes to maize weevil infestation and damage in order to identify sources of resistance for use in breeding programmes and for deployment to farmers.

3.2 Materials and Methods

3.2.1 Maize germplasm

A total of 295 genotypes were used in the study, including hybrids, open-pollinated varieties (OPVs) landraces and inbred lines (Appendix 1-3). The hybrids used comprised of commercial, drought-tolerant, insect resistant and quality protein maize (QPM) genotypes from CIMMYT maize breeding

programme. Thirteen (13) OPVs, 75 landrace accessions and 120 inbred lines used in this study were obtained from the CIMMYT different breeding programs. Two hybrid checks, CKPH09001 (resistant to maize weevil and larger grain borer and H513, a susceptible check were included in the study.

3.2.2 Experimental sites and design

Field trials were established at the Kiboko and Embu centers of the Kenya Agricultural Research Institute (KARI). Kiboko is a dry, mid-altitude agro-ecological zone, located at 37° 75'E and 2° 15'S, situated at about 975 masl. Average rainfall in Kiboko is 530 mm, with mean monthly maximum temperature of 35.1°C and a minimum of 14.3 °C and has sandy clay soils. Embu is a moist, mid-altitude zone at 1350 masl, located at 37° 42'E and 0° 49'S. The average rainfall in Embu is 1,200 mm with a mean monthly maximum and minimum temperature of 25°C and 14.1°C and has clay loam soils. The hybrids and OPVs were planted in one trial due to their similarity in architecture, while the landraces and inbred lines were each planted in separate trials. The experiments were replicated three times in 20 x 5; 15 x 5 and 20 x 5 α -lattice design in 2 x 5 m row plots, for the hybrids and OPVs, landraces and inbred lines trials, respectively. Two seeds were planted per hill and later thinned to one, giving a total plant density of 53,333 plants per hectare. In order to ensure a healthy crop, agronomic practices including weeding, fertilizer application, stem borer control and supplemental irrigation were done when necessary. At harvest, the maize was shelled and dried to obtain grain for insect pest-resistance bioassays.

3.2.3 Evaluation of maize germplasm for resistance to maize weevil

The insects used in the experiment were obtained from the KARI/CIMMYT Kiboko Maize Entomology Laboratory where they were reared on the grains of maize cultivar PH3253 under controlled conditions (28°C and 75% relative humidity). The maize grains were sun-dried to a moisture

content of 12-13 % and disinfested by fumigating with phostoxin tablets for seven days to eliminate field infestation. Samples of 100 grams of grain were taken from each plot of the three replications. The kernels were placed in 250 ml jars, infested with 50 unsexed 7-10 day old maize weevils, and stored for 90 days at 26-28°C and 70-75% relative humidity in the Entomology Laboratory at KARI/CIMMYT Kiboko. Experiments were set-up following a completely randomized design with 3 replications. The contents of each jar were sieved with mesh sieves (Endecotts Ltd, UK) 90 days after infestation to separate grains, insects and flour. The flour produced was weighed while the number of damaged kernels and adult insect progeny were counted. The grain weight loss was computed by subtracting the final from the initial weight of the grain sample and expressed as a percentage (Tefera *et al.*, 2011a). Damaged kernels were separated from the undamaged based on grain tunneling and holes. These were counted and the percentage of damaged grain computed. The weight of the damaged and undamaged grain was measured.

3.2.4 Statistical analyses

A multivariate analysis of variance within a canonical variate analysis (Canonical Discriminant Analysis) was performed using statistical analysis system software (SAS) to determine the difference between genotypes and the most variable insect resistance trait among the genotypes. A univariate analysis of variance (ANOVA) for the individual resistance traits was done using the general linear model (GLM) procedure of SAS statistical package (SAS Institute, 2003). The number of insects was log transformed ($\log_{10}$), while flour produced, grain damage, weight of damaged and undamaged grains and weight loss were angular transformed ($\arcsin\sqrt{\text{proportion}}$) to normalize their distribution before analysis. Insect-damage traits were correlated using a canonical correlation to secure error control and recognize the strongest correlation.

3.3 Results

3.3.1 Canonical discriminant analysis

The analysis of variance option specifies testing the hypothesis that the class means are equal using univariate statistics. The R^2 values ranged from 0.4 for the grain damage to 0.54 for the percentage flour weight and all the variables were significant (Table 1). The multivariate test for differences between the classes was also significant ($P \leq 0.001$) (Table 1). The linear combination of the centered variables were (CAN1) $0.5438 \times \text{No. of insects} + 0.7323 \times \text{Flour weight} + 0.6272 \times \text{weight of damaged grains} - 0.8385 \times \text{weight of undamaged grains} + 0.7805 \times \text{weight loss} + 0.5863 \times \text{grain damage}$ which separates the entries most effectively (Table 1).

Table 1. Canonical discriminant analysis parameters for the number of emerged weevils, flour and grain weight, weight loss and grain damage in the 175 genotypes

Variable	Pooled	Between	R-Square	F Value	Pr > F	Can 1	Can 2	Can 3
	SD	SD						
Number of insects	125.97	85.60	0.41	1.39	0.0273	0.5438	0.4247	0.1310
Flour weight (%)	1.47	1.32	0.54	2.42	<.0001	0.7323	0.5994	-0.1637
Weight-damaged grains (%)	15.46	11.95	0.47	1.79	0.0003	0.6272	0.3225	0.5808
Weight-undamaged grains (%)	20.87	17.97	0.52	2.23	<.0001	-0.8385	-0.1525	-0.3018
Weight loss (%)	10.03	8.08	0.49	1.94	<.0001	0.7805	-0.0894	-0.2350
Grain damage (%)	14.87	9.96	0.40	1.35	0.0401	0.5863	-0.1465	0.3596

Key: Can = Canonical variable coefficient; SD=Standard deviation.

Based on the first canonical variate, weight loss and/or flour weight were the most important variables for discriminating genotypes due to large coefficients (loading value). These were thus used to calculate the susceptibility indices by summing the ratio between the genotype mean values and grand means of the traits in question and dividing by 2 (the number of variables) (Tefera *et al.*, 2011b). The genotypes with susceptibility index values less than 0.7 were regarded as highly resistant, 0.7- 0.8 as resistant, 0.8- 1.2 as moderately susceptible and more than 1.2 as highly susceptible. Canonical coefficient for the

weight of the undamaged grains was also high but negatively correlated to the other variables, therefore not suitable for discrimination of the genotypes in relation to resistance.

3.3.2 Reaction of maize hybrids and OPVs to maize weevil infestation

There were significant differences ($P \leq 0.01$) among the hybrids for grain damage, weight loss, flour weight, number of adult insects, weight of damaged and undamaged grains (Table 2). The genotype $\times$ location interaction was not significant for all the traits evaluated, therefore, means from the combined sites were computed (Table 2). The genotypes were categorised as resistant, moderately resistant, moderately susceptible and highly susceptible based on the susceptibility index derived from flour weight and weight loss. The resistant genotypes had low percentage of flour weight, number of insects and high weight of undamaged grain.

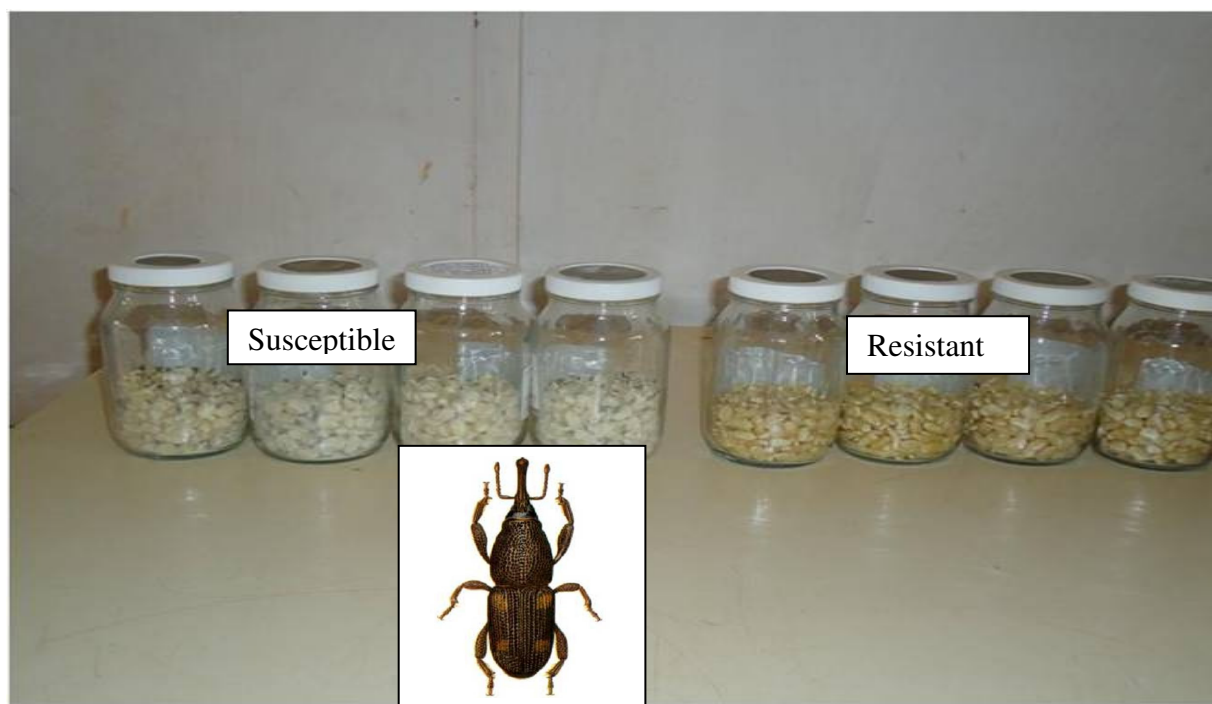


Plate 1. Maize weevil effect on susceptible and resistant genotype

Table 2. Weevil damage indices for 20 most resistant and 20 most susceptible maize hybrids and open pollinated varieties (OPVs) among the 175 genotypes evaluated for resistance to maize weevil

Resistant genotypes			Damage indices					Susceptibility Index
Entry	Genotype	No. of Insects	Flour weight (%)	Weight D. grains (%)	Weight UD grains (%)	Weight loss (%)	Grain damage (%)	
67	CKPH08003	68.92	0.48	14.28	79.48	6.24	23.50	0.40
69	CKPH09004	73.83	0.55	19.91	73.70	6.38	28.56	0.42
70	CKPH08009	83.00	0.55	15.91	77.25	6.84	25.89	0.44
71	CKPH08010	60.00	0.61	15.29	78.20	6.51	25.08	0.45
78	CKPH08028	84.67	0.68	14.25	79.73	6.02	26.48	0.46
77	CKPH08026	80.75	0.68	18.46	74.74	6.80	19.71	0.48
57	CKPH08035	83.83	0.62	20.39	71.82	7.79	32.29	0.50
73	CKPH08014	72.08	0.73	15.96	77.11	6.92	19.92	0.51
59	CKPH08037	83.17	0.66	15.93	76.27	7.80	29.62	0.52
72	CKPH08012	66.92	0.68	17.53	74.72	7.75	25.79	0.52
75	CKPH08024	62.17	0.79	14.73	78.39	6.88	20.73	0.52
76	CKPH08025	81.00	0.70	16.75	74.73	8.52	28.28	0.56
62	CKPH08040	71.42	0.80	20.93	70.24	8.83	29.84	0.60
23	500Q	92.50	0.89	17.69	74.16	8.21	33.63	0.61
54	CKPH09002	114.42	0.81	26.70	63.62	9.68	44.02	0.64
68	CKPH08004	96.17	0.90	17.65	73.31	9.04	23.76	0.64
49	CKIR06007	89.58	0.94	21.36	69.84	8.80	30.47	0.65
55	CKPH08033	103.08	0.92	25.68	65.12	9.21	42.54	0.66
53	CKPH08032	100.08	0.87	26.58	63.73	9.70	40.08	0.66
66	CKPH08002	101.25	0.95	23.84	67.01	9.15	26.34	0.66
Resistant check	CKPH09001	78.08	0.72	24.21	66.26	9.53	38.56	0.60
Mean		84.52	0.75	19.01	73.10	7.89	28.69	0.55
Susceptible genotypes								
29	CKIR07017	155.42	2.42	41.54	44.17	14.29	45.48	1.35
38	CKIR09001	234.67	2.33	39.39	43.62	16.99	53.88	1.42
46	CKIR06001	194.25	2.49	35.04	49.04	15.92	34.80	1.44
11	DH04	230.83	2.26	39.69	42.06	18.25	72.79	1.45
21	531A	222.08	2.50	50.12	33.44	16.44	60.63	1.46
14	SC Duma 43	205.08	2.52	50.95	31.33	17.72	59.03	1.51
44	CKIR09007	211.17	2.56	38.57	44.01	17.42	48.93	1.52
36	CKIR07008	190.42	2.32	43.39	35.23	21.38	66.35	1.58
18	KH 600-15A	192.25	2.22	42.40	35.27	22.33	67.27	1.59
6	H626	230.83	2.63	50.57	29.73	19.70	64.99	1.63
33	CKIR07003	198.00	2.76	35.41	45.41	19.18	54.92	1.65
4	H629	210.75	2.22	48.15	27.75	24.11	71.84	1.66
3	H6210	226.92	2.58	53.88	23.82	22.31	81.29	1.71
24	DK8031	194.67	3.29	42.04	39.41	18.55	61.74	1.80
5	H628	196.67	2.72	48.68	22.38	27.08	69.91	1.94
2	H6212	268.25	2.80	50.21	22.16	27.63	78.41	1.98
13	SC Duma 41	284.33	3.28	52.29	24.18	23.53	67.96	1.99
17	611D	285.00	3.20	49.14	26.54	24.32	69.58	1.99
1	H6213	261.67	3.44	56.41	19.92	23.67	80.00	2.05
16	PH 3253	255.75	3.96	47.43	29.76	22.80	73.35	2.19
Susceptible Check	H513	190.08	2.17	46.26	36.73	17.01	66.88	1.40
Mean		222.45	2.72	45.76	33.46	20.68	64.16	
CV		19.09	32.79	29.74	24.18	27.49	34.80	
P Value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Key: D-damaged; UD-Undamaged; R-resistant; CV-coefficient of variation; P-probability level

The highly resistant and moderately resistant represented 39% of the genotypes evaluated, with 36% being moderately susceptible and 25% highly susceptible. The most resistant genotype among the hybrids was CKPH08003 with susceptibility index of 0.40 which was five times lower than that of PH 3252, the most susceptible hybrid. Percentage weight loss and flour weight of the most resistant was three fold lower than that of the most susceptible genotype and twice lower than the mean of all the genotypes (Table 2). The numbers of insects were higher in the most susceptible and lower among the resistant genotypes. The resistant check, CKPH9001, had a susceptibility index of 0.60 which was higher than that of the 11 genotypes categorized as the most resistant. Most of the resistant material were CIMMYT hybrids which had been bred for postharvest insect pest resistance. The most susceptible genotypes had a susceptibility index of 2.19 which was 1.9 times higher than that of the susceptible check (H513). The genotypes were classified into four categories based on the susceptibility index. The classification shows that the genotypes were not normally distributed probably due to the fact that they were deliberately selected (Figure 1).

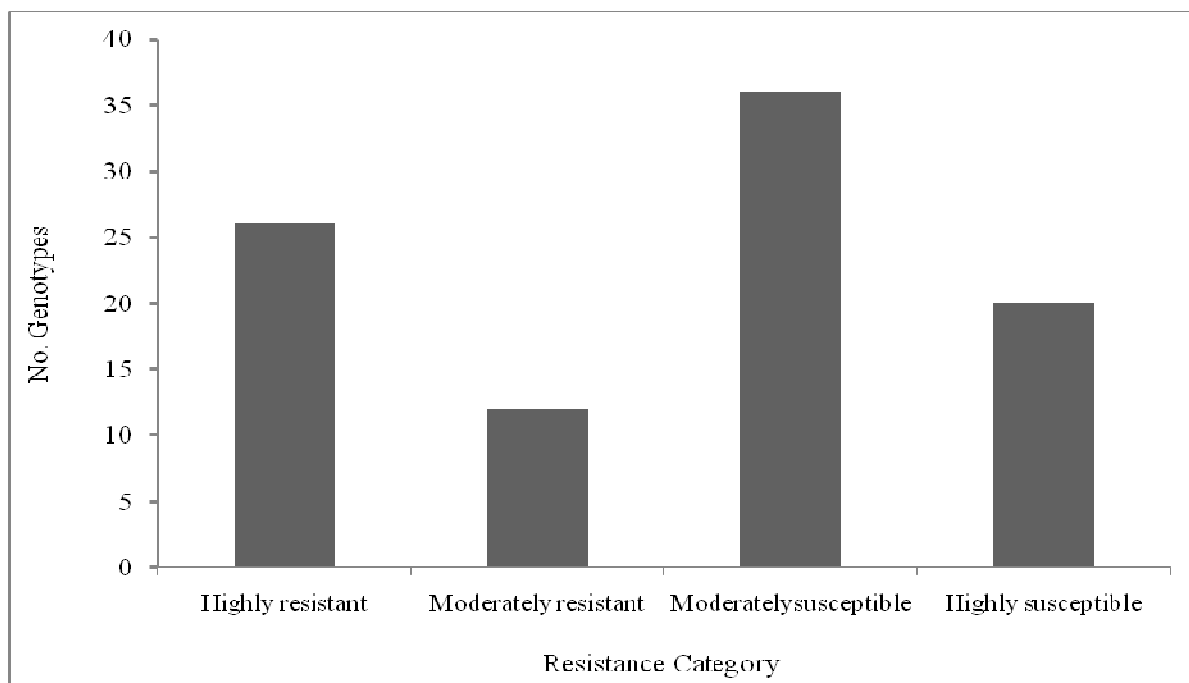


Figure 1. Frequency of maize genotypes found from a screen of 87 maize hybrids and 13 OPVs for weevil resistance

3.3.3 Reaction of landraces to maize weevil infestation

There were significant differences ($P \leq 0.05$) among the landrace accessions for grain damage, weight loss, flour weight, number of adult insects, and weight of damaged and undamaged grains (Table 3). The genotypes were categorised into resistant and susceptible based on the susceptibility indices adapted from the method of Tefera *et al.* (2011b). This showed great variation for resistance against the maize weevil among the landrace accessions. The top four most resistant genotypes were ranked above one of the resistant checks (CKPH09002) which had a susceptibility index of 0.64 and the rest were categorized as moderately resistant. The most susceptible landraces were BRAZ 4 and RIGS GP10 with susceptibility index of 2.34 and 2.22, respectively. Their susceptibility indices were five times higher than that of the most resistant (BRAZ2451) which had a susceptibility index of 0.42. The material was classified into four categories based on susceptibility index. Among the genotypes evaluated, 23% were highly resistant to moderately resistant, 60% were moderately susceptible and 17% were highly susceptible (Figure 2).



Plate 2. Damage of maize weevil on maize grain: tunneling and flour production

Table 3. Weevil damage indices for 19 most resistant and 19 most susceptible among the 75 landraces evaluated

Resistant genotypes			Damage indices				
Entry	Genotype	No. of Insects	Weight-damaged grains (%)	Weight-undamaged grains (%)	Flour weight (%)	Weight loss (%)	Susceptibility index
59	BRAZ 2451	93.67	14.02	72.68	0.59	7.91	0.42
28	SINA 21	99.25	21.33	70.02	0.70	8.65	0.48
54	GUAN 84	111.75	19.59	69.14	0.99	7.41	0.51
11	GUAT 1162	131.92	22.98	67.94	1.01	9.08	0.57
2	GUAT 1008	131.50	27.31	62.70	1.15	9.99	0.64
53	GUAN 36	133.50	24.29	65.94	1.23	9.76	0.65
5	GUAT 1100	128.00	22.49	67.02	1.21	10.50	0.67
44	GUAT 79	145.70	21.43	67.73	1.21	10.80	0.68
42	VENE 897	122.58	21.74	67.44	1.29	10.82	0.70
27	BRAZ 1486	105.83	30.17	57.37	1.20	12.47	0.73
58	CHIS 94	112.92	19.80	70.12	1.53	10.08	0.73
4	GUAT 1014	144.17	23.77	64.46	1.36	11.76	0.75
3	GUAT 1010	128.58	32.07	56.22	1.38	11.71	0.75
52	GUAN 20	185.25	33.79	53.19	1.35	13.02	0.79
17	BRAZ 2454	152.67	24.02	63.44	1.42	12.53	0.79
66	BRAZ 1736	188.83	31.35	58.30	1.75	10.35	0.80
57	VALL 380	136.58	24.10	64.91	1.66	10.99	0.80
12	GUAT 1168	156.33	25.43	61.71	1.50	12.86	0.82
35	BRAZ 1832	166.25	30.24	56.68	1.51	13.08	0.83
Resistant check		CKPH09002	111.33	20.43	69.14	1.11	10.43
Mean		135.90	24.88	63.62	1.27	10.88	0.70
Susceptible genotypes							
20	SONO 72	129.75	34.73	51.97	2.46	13.30	1.08
33	VENE 352	172.96	30.59	53.23	2.15	16.18	1.10
19	BRAZ 2375	203.17	31.01	52.73	2.31	16.26	1.15
65	BRAZ 1495	188.33	33.97	49.83	2.33	16.20	1.15
7	GUAT1034	172.42	26.88	57.83	2.62	15.29	1.19
6	GUAT1030	218.50	36.96	45.73	2.41	17.31	1.21
43	VERA 197	164.88	35.12	46.59	2.46	18.30	1.26
32	BRAZ 2179	179.33	42.34	38.48	2.49	19.18	1.29
34	BRAZ 1364	215.00	39.42	42.96	2.74	17.62	1.30
61	NAYA 129	195.50	38.94	41.99	2.71	19.03	1.34
64	BRAZ 222	163.25	32.76	48.89	2.86	18.35	1.36
56	GUAN 34	195.67	27.95	50.11	2.44	21.94	1.38
36	NAYA 130	212.42	37.36	45.17	3.17	17.47	1.40
21	BRAZ 1470	224.08	33.70	44.84	3.81	21.47	1.71
41	BRAZ 1384	243.92	39.18	40.11	4.28	20.71	1.80
45	PARA GP3	323.02	44.74	31.85	4.62	23.44	1.98
55	PERU 397	253.83	42.60	33.40	4.74	24.00	2.03
23	RIGS GP10	294.83	41.49	32.93	5.29	25.58	2.22
26	BRAZ 4	265.25	44.39	33.83	6.27	21.79	2.34
Susceptible check		H513	190.08	46.26	36.73	2.20	17.01
Mean		211.76	36.43	44.77	3.16	18.80	1.45
CV		17.87	31.97	30.30	32.50	33.80	
P Value		P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	

*Key: CV-coefficient of variation; P-probability level at 5%

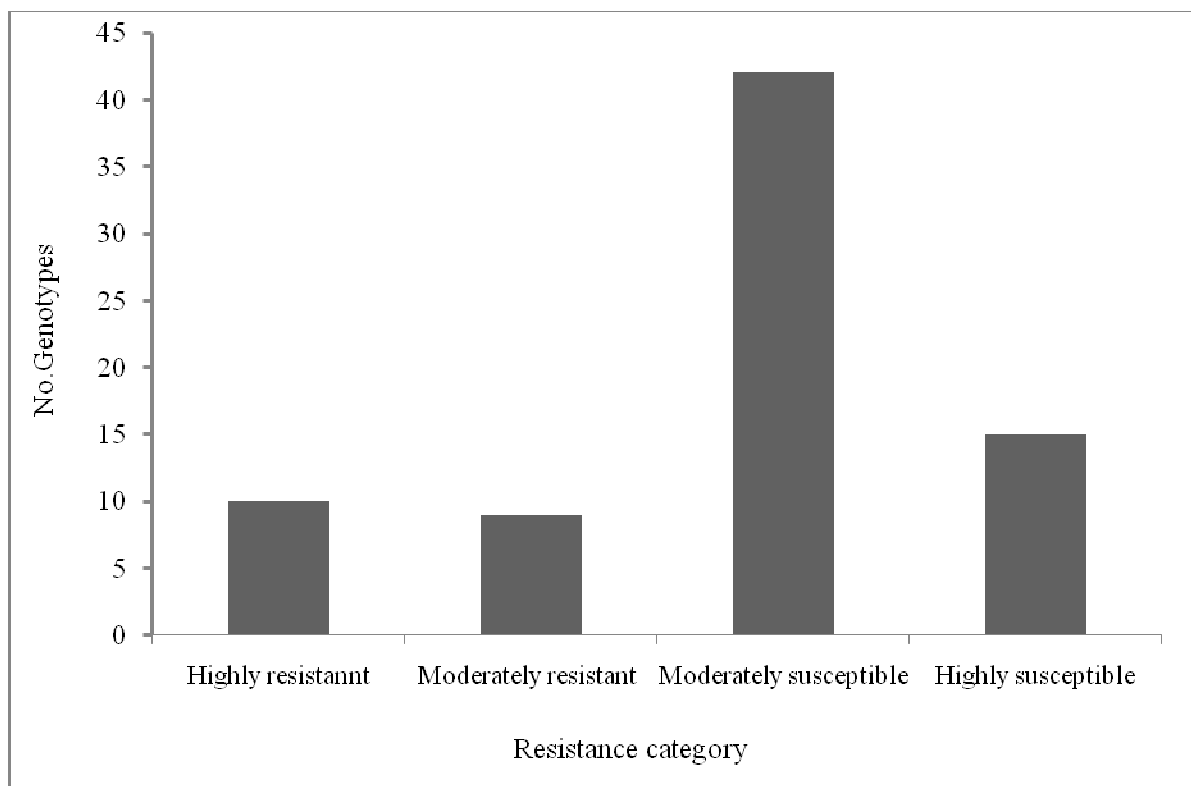


Figure 2. Frequencies of maize genotypes found from a screen of 75 landraces accessions.

3.3.4 Reaction of inbred lines to maize weevil infestation

Analysis of variance showed significant differences ($P \leq 0.05$) among the inbred lines to weevil infestation in terms of weevil progeny emergence, grain weight loss and the flour weight. The mean weight loss for the most resistant and susceptible lines were 3.26 and 24.23%, respectively (Table 4). The genotypes were categorised as highly resistant (1-5 %), moderately resistant (5.1-8 %), moderately susceptible (8.1-13 %) and highly susceptible (> 13.1 %) based on the percentage weight loss. The inbred lines were classified into the four categories based on susceptibility index; and were found to be moderately resistant (47 %), resistant (10 %) and moderately to highly susceptible (43 %) (Figure 3).

Table 4. Maize weevil damage indices of 19 most resistant and 19 most susceptible inbred lines evaluated for resistance to the maize weevil

Entry	Name	No.of insects	Flour weight (%)	Weight loss (%)	Grain damage (%)	Susceptibility index	Reaction category
70	CKSPL10028	27.92	0.11	3.26	5.42	0.2	Resistant
71	CKSPL10035	30.42	0.21	3.3	9.89	0.3	Resistant
66	CKSPL10309	28.92	0.72	3.9	9.94	0.3	Resistant
69	CKSPL10021	20.58	0.2	3.94	5.57	0.3	Resistant
73	CKSPL10042	26.77	0.19	4.13	8.17	0.3	Resistant
86	CKSPL10112	28.46	0.24	4.53	9.34	0.3	Resistant
27	CML204	24.33	0.19	4.56	6.41	0.3	Resistant
67	CKSPL10295	49.17	0.44	4.64	14.91	0.4	Resistant
97	CKSPL10218	41.75	0.29	4.67	12.24	0.4	Resistant
93	CKSPL10186	33.26	0.24	4.8	6.5	0.4	Resistant
78	CKSPL10081	24.75	0.24	4.86	10.31	0.4	Resistant
82	CKSPL10088	34	0.3	4.92	14.82	0.4	Resistant
87	CKSPL10113	82.08	0.75	5.1	23.77	0.5	Resistant
83	CKSPL10089	31.61	0.3	5.11	12.24	0.5	Resistant
75	CKSPL10074	35.75	0.25	5.24	11.03	0.5	Resistant
92	CKSPL10170	35.67	0.2	5.33	10.86	0.6	Resistant
2	CKSPL10177	49.17	0.43	5.45	14.44	0.6	Resistant
4	CKSBL10028	58.75	0.57	5.46	20.67	0.6	Resistant
14	CKSPL10035	66.67	0.44	5.59	15.36	0.5	Resistant
Resistant check	CKSPL10229	58.33	0.22	3.9	7.6	0.3	Resistant
Mean		38.42	0.33	4.67	11.68		
16	CML264	121.83	0.9	12.92	41.94	1.1	Susceptible
105	CKSBL10028	49.75	0.36	13.15	20.2	1.1	Susceptible
61	CML489	133.33	0.91	13.35	38	1.1	Susceptible
28	CKSBL10034	160	1.1	13.44	33.14	1.2	Susceptible
33	CKSBL10025	122.67	0.78	13.72	28.58	1.2	Susceptible
60	CML441	157.42	1.34	13.77	40.78	1.4	Susceptible
11	CKSBL10015	110.42	1.3	13.88	32.17	1.4	Susceptible
6	CKSBL10045	179.42	1.5	14.62	52.91	1.5	Susceptible
59	CZL03007	145.58	1.18	14.84	39.94	1.5	Susceptible
120	CKSBL10045	177.42	1.33	16.51	44.39	1.5	Susceptible
62	CZL01005	183.08	1.7	16.67	45.21	1.7	Susceptible
24	CML334	167.83	1.72	16.69	49.19	1.7	Susceptible
58	CZL00003	191.83	1.78	16.92	55.24	1.8	Susceptible
41	CML159	224.83	3.26	17.46	41.74	2.2	Susceptible
42	CML197	223.17	3.33	18.84	47.08	2.2	Susceptible
56	CML443	168.08	2.09	19.76	48.69	2.3	Susceptible
46	CZL03014	229.08	1.86	20.69	50.66	2.3	Susceptible
10	CML312	153.17	1.4	22.26	48.36	2.3	Susceptible
7	CKSPL10344	186	1.91	23.88	46.05	2.3	Susceptible
Susceptible check	CML395	247.33	2.71	24.23	62.9	2.8	Susceptible
Mean		160.01	1.53	16.33	41.96		
CV		23.65	34.51	32.9	33.2		
P Value		P < 0.001	P < 0.001	P < 0.001	P < 0.001		

The spread of the genotypes across the four categories followed a fairly normal distribution. The number of emerged adult insects was highest among the susceptible inbred lines. Most of the resistant lines originated from “CubaGuard” a Caribbean accession at CIMMYT which showed significant levels of resistance to postharvest insect pests though with poor agronomic traits. Inbred lines which had been developed for stem borer resistance were categorised as moderately resistant to susceptible, hence there was insufficient evidence to conclude that there was an association between resistance to the stem borer and resistance to postharvest insect pests.

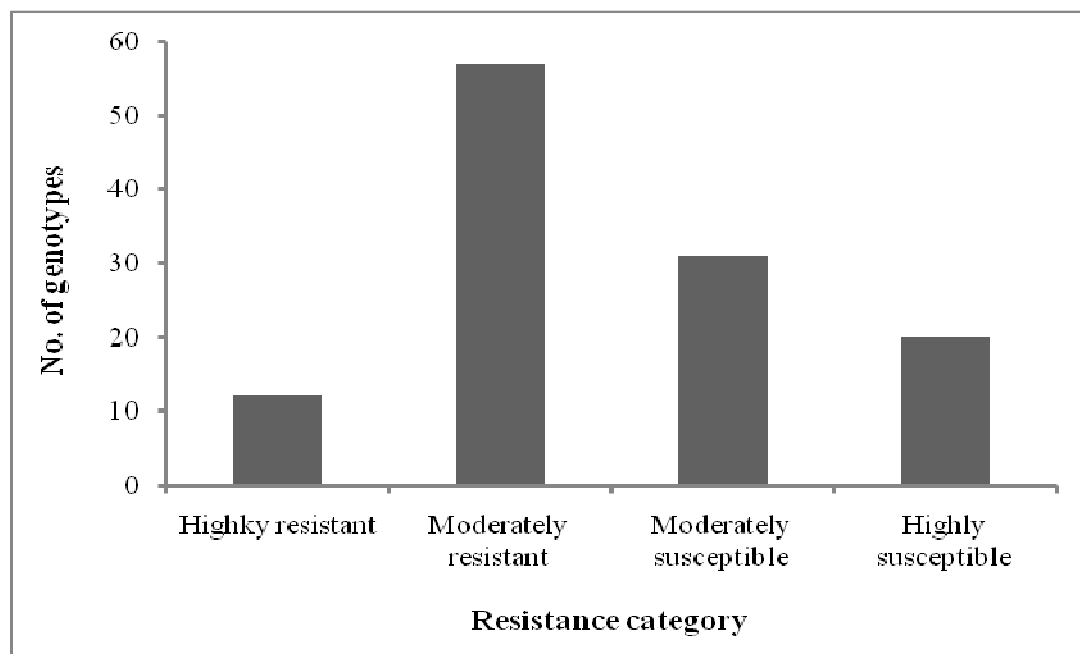


Figure 3. Frequencies of maize genotypes found from a screen of 120 inbred lines for resistance to weevils.

3.3.5 Canonical correlations among insect damage variables

The probability level for the null hypothesis that all the canonical correlations are zero among the variables was highly significant ($P \leq 0.0001$), and, therefore, the two sets of variables were highly correlated. There were great variations in the correlation coefficients between the two sets of variables (Table 5). All the set one variables (number of insects, weight of damaged grains and undamaged grains) accounted for a substantial amount of shared variance between the two sets of variables.

Table 5 Canonical correlation between set 1 (Number of insects, weight of damaged and undamaged grains) and set 2 (weight loss, flour weight and grain damage) variables among 175 genotypes evaluated for maize weevil resistance

Can	Can		Squared	Eigen	Varianc	Cum			No.of	Weight	Weight	Weight	Grain	Flour
Can	corr	SE	can corr	value	e	variance	LR	Pr > F	insects	DG (%)	UDG (%)	loss (%)	damage (%)	weight (%)
1	0.9730	0.005	0.95	17.79	0.98	0.98	0.04	<.0001	0.9433	0.9040	-0.9580	0.962	0.949	0.958
2	0.4330	0.082	0.19	0.23	0.01	0.99	0.73	<.0001	0.0064	0.1318	0.0527	-0.225	0.242	-0.051
3	0.3256	0.090	0.11	0.12	0.01	1.00	0.89	0.0049	0.0793	-0.0567	0.0389	-0.153	-0.201	0.281

Test of H_0 : The canonical correlations in the current row and all that follow are zero.

Key: Can corr = Canonical correlation; SE=Standard error; Cum=Cumulative; LR=Likelihood ratio; DG=damaged grain; UDG=undamaged grain

*Can: Canonical variable coefficient

The squared multiple correlation indicate that the first canonical variable of set one variables have predictive power for number of insects ($r = 0.94$), weight of damaged grains (0.90) and undamaged grains (-0.95), weight loss (0.96), grain damage (0.95) and flour weight (0.94) (Table 5). The first canonical correlation which is the highest possible multiple correlation with the classes that can be achieved using a linear combination of the quantitative variables was 0.973. The likelihood ratio test for the hypothesis that the current canonical correlation and all the smaller ones are zero show that none of them was zero ($P \leq 0.005$) and as expected the first line was equivalent to Wilk's Lambda multivariate test (0.0386). The R^2 between the first canonical variable (CAN1) and the class variable, 0.95, was much larger than the corresponding R^2 for the other two (Table 5). The number of insects and weight of damaged grains were highly and positively correlated to the percentage weight loss, flour weight and grain damage whereas the percentage weight of the undamaged grains was negatively correlated to these.

3.4 Discussion

The results of this study showed that there was great variation in resistance to the maize weevil in the maize germplasm evaluated. The percentage weight loss was the most important variable for discriminating among germplasm for resistance to the maize weevil. The flour weight, number of emerged insects at the end of screening period, and percentage grain damage in that order, were highly correlated as indicated by the canonical correlation analysis. Consequently, these four variables can be integrated to calculate a susceptibility index (Tefera *et al.*, 2011b). Resistant genotypes had low percentage of weight loss, grain damage and reduced number of insects. The low weight loss observed among the resistant genotypes show that resistance against the maize weevil existed in this germplasm. The resistance could be due to physical factors such as grain hardness or antibiosis (García-Lara *et al.*, 2004; Siwale *et al.*, 2009). The extent of damage during storage depend on the number of emerging adults, and the grains permitting faster and higher levels of adult emergence (Abraham *et al.*, 1991). The resistant hybrids can be stored for relatively longer periods of time. The parental lines of the resistant hybrids (CKPH08003, CKPH09004, CKPH08009, CKPH08010, CKPH08028, CKPH08026, CKPH08035) can be used as a source of resistance in breeding programmes. Resistant varieties, therefore, can be adopted as a cheap, effective and ecologically safe method to reduce damage by *S. zeamais* under traditional storage conditions facilities in the tropics.

Commercial varieties were the most susceptible to maize weevil attack among the hybrids evaluated. This indicates that integration of resistance to storage-pests has not been routinely used by many breeding programmes, leading to high postharvest losses. Breeding programs have been addressing yield increase without due focus to postharvest insect pests (Derera *et al.*,

1999). Consequently, it is critical that breeding for resistance to postharvest insect pests be prioritized as equally important to safeguard farmers from huge losses due to maize weevil attack. Superior hybrids resistant to maize weevil were identified among the genotypes that performed better than the susceptible hybrid check. These can be improved further to incorporate the farmer preferred traits such as high yields and resistance to foliar diseases and other pests followed by release to the farmers. For instance, the hybrids CKPH08028 and CKPH08043 have been improved and released as MTPEH 0701 and EMB 0701 for the lowland and mid-altitude of Kenya respectively. These hybrids should be promoted for more adoption by farmers in those ecological zones.

Open pollinated varieties were categorised as moderately resistant to highly susceptible. The great variation observed in the germplasm evaluated could be exploited to raise the levels of resistance to postharvest insect pests while conserving the farmer preferred traits. The classification of the landraces into resistant to highly susceptible followed a normal distribution, an indication of their variability. The high genetic variability observed among the landraces can be attributed to diverse genetic background resulting from polycrosses, since these materials have been subject to pedigree selection (Yamasaki *et al.*, 2007). Inheritance for resistance to the maize weevil is mostly additive and non-additive to a lesser extent (Dhliwayo and Pixley, 2003). Using gene pyramiding approaches, resistance in the landraces, therefore, can be introgressed to modern susceptible varieties. The highly resistant landraces identified: (BRAZ 2451, SINA 21, GUAN 84, and GUAT 1162) can be used in developing inbred lines and hybrid varieties which are resistant to the maize weevil.

There were significant differences among the inbred lines in weevil progeny emergence, grain weight loss and the flour weight. Based on the results, genetic diversity for weevil resistance exists among tropical maize inbred lines thus offering opportunity to exploit the variability towards reducing post-harvest insect pest losses. Genetic diversity is normally exploited for incorporation of important traits lacking in improved crop varieties using conventional and genetic engineering approaches (Dhliwayo and Pixley, 2003). The use of resistant maize varieties leading to low grain weight loss and or total avoidance would reduce the negative impact of the maize weevil. Host plant resistance can, therefore, be used as a vital component of IPM against maize weevil postharvest pests. The development and adoption of weevil resistant varieties will reduce use of storage pesticides, saving costs as well as avoiding negative environmental impacts associated with pesticides, since the technology is packaged in the seed.

3.5 Conclusion and recommendations

The magnitude of damage was high among the hybrids, landraces and inbred lines in that order. Resistant maize hybrids and landraces were identified from the large number of germplasm screened. This indicated that breeding for resistance to the maize weevil is not only feasible, but has already resulted in resistant hybrids being released in Kenya. These could be grown to reduce damage by the maize weevil in storage. The resistant landraces could be used as new sources of resistance. The commercial hybrids were susceptible and, therefore, special attention should be paid to their improvement to produce maize hybrids which are resistant to storage insect pests. It is recommended that proper links be established between entomologists and plant breeders in understanding the mechanisms of resistance and the heritability of the traits, to enhance synergism in breeding varieties with improved resistance.

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Chapter Four

Resistance in Tropical Maize Germplasm to the Larger Grain Borer

4.1 Introduction

The larger grain borer (LGB) (*Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)) is among the major storage pests responsible for losses of maize grain worldwide. This pest is exotic to Africa and was first sighted in East Africa in the 1970s and in West Africa in the early 1980s (Markham *et al.*, 1991). It has now spread to more than 18 African countries by 2009 (Omondi *et al.*, 2009; Tefera *et al.*, 2010a). The LGB causes qualitative and quantitative grain losses by feeding on the kernels and burrowing into them for oviposition. The damaged kernels subsequently provide easy entry for secondary infection by mycotoxin-producing fungi. Losses due to the LGB range from 9%-45%, depending on the duration of storage (Gueye *et al.*, 2008; Kumar, 2002).

Host plant resistance is the most economically and environmentally viable option for the management of the LGB, especially among resource-constrained farmers. Host plant resistance can easily be combined with other mechanisms of pest control in an IPM strategy. Because the LGB is exotic to Africa, sources of resistance to this pest are limited. However, preliminary studies indicate that there is genetic variability in resistance to LGB and other coleopterans among tropical maize germplasm accessions (Mwololo *et al.*, 2010; Tefera *et al.*, 2011).

Kernel resistance to the maize weevil is conditioned by biochemical and physical characteristics (Arnason *et al.*, 1994). High concentrations of simple phenolics, namely hydroxycinnamic acids, have been found in the pericarp of resistant genotypes (Arnason *et al.*, 1997). These phenolics act against the maize weevil by increasing mechanical resistance and antibiosis in the pericarp

and aleurone layer of the kernels, respectively. In other resistant genotypes, high concentrations of dehydrodiferulic acids (DiFAs) and the cross-linking of polysaccharides by DiFAs fortify the pericarp cell wall, thereby increasing resistance to attack by weevils (Arnason *et al.*, 1997; Bergvinson and Garcia-Lara, 2004; García-Lara *et al.*, 2004). In addition, the cross-linking of plant cell wall polymers by DiFAs contributes to the defense mechanisms of the maize against pests and diseases. These cross linkages are involved in the defense mechanisms against insects such as the European corn borer (*Ostrinia nubilalis*), and the Mediterranean corn borer (*Sesamia nonagrioides*), the southwestern corn borer (*Diatraea grandiosella*), the sugarcane borer (*Diatraea saccharalis* (Fabricius), and the maize weevil (*Sitophilus zeamais*) (Santiago and Malvar, 2010). These biochemical mechanisms presumably confer resistance to other coleopteran pests such as the larger grain borer.

Different types of proteins, such as the hydroxyproline-rich glycoproteins (HRGPs) or extensins, protease and trypsin inhibitors, storage proteins and ribosomal inhibitors, are associated with resistance to fungal diseases and insect resistance (Bergvinson and Garcia-Lara, 2004). Extensins are involved in cell wall organization, wound healing, and plant defense mechanisms; and can also be cross-linked within the cell wall, and their presence has been associated with its tensile strength (Cassab, 1998; García-Lara *et al.*, 2004). These cross-linkages control mechanical properties of the cell wall, and it is likely that this complex contributes to insect resistance. This is by fortification of the pericarp cell wall, thereby increasing its physical strength and the overall kernel hardness (García-Lara *et al.*, 2004). Biochemical components with specific and localized activity against insect pests, but not toxic to humans, are ways of enhancing resistance while maintaining the desired nutritional and processing qualities of grain (Arnason *et al.*, 1997). The objective of this study was to characterize tropical maize germplasm for resistance to the larger

grain borer to get an insight on the contribution of protein content and oil content to resistance as well as grain hardness and texture.

4.2 Materials and methods

4.2.1 Materials used in the study

Two hundred and ninety five (295) genotypes were chosen for the study as reported in chapter three. The experimental sites and design were as discussed in the previous chapter three. Evaluation of maize germplasm for resistance to the LGB was done as discussed in chapter three except that LGB insects were used instead of maize weevil.

4.2.2 Biochemical analysis

The protein, oil and starch content percentages were determined from whole grains of each genotype using a grain analyzer machine (Foss Infratec² 1241, FOSS NIRSystems Inc, Slangerupgade 69, Postbox 260, DK-3400 Hillerød, Denmark). Five hundred gram (500 g) grain samples of each genotype were obtained from each of the three replications per genotype and used to assess the traits. The machine was set to divide the sample into five sub-samples to spread the analysis, and then run to give the readings of the different biochemical components in the grain.

4.2.3 Grain hardness and texture

Mechanical hardness of each grain sample was determined using a force displacement meter (model 921A, Tricor Systems Inc, 1650 Todd Farm Drive, Elgin, Illinois, USA) equipped with an electronic force transducer of 20 kg and a 0.8-mm probe Orthogonal Operation for Windows, version 4.25, Tricor Systems Inc., Elgin, IL). Twenty kernels were tested for peak force (Newtons, N) for each genotype $\times$ 3 replication combination to obtain the grain hardness. The

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)

4.2.4 Statistical analyses

Data transformation, analysis of variance, multivariate analysis of variance within a canonical variate analysis and canonical correlations were performed as described in chapter three.

4.3 Results

4.3.1 Canonical discriminant analysis

The R^2 values of the variables ranged from 0.12 for the number of insects to 0.29 for percentage weight loss and all the variables except the number of insects ($P = 0.165$) were significant ($P \leq 0.0001$). The multivariate test for differences between the classes was also significant ($P \leq 0.0001$), thus the multivariate tests of the hypothesis that the class mean vectors are equal indicate that not all mean vectors were equal ($P \leq 0.001$). The Wilks Lambda, Pillais trace, Hotelling-Lawley Trace and Roys Greatest root values were 0.375, 0.828, 1.186 and 0.679, respectively. The first canonical correlation which is the greatest possible multiple correlation with the classes that can be achieved using a linear combination of the quantitative variables was 0.6358 (Table 6). The likelihood ratio test for the hypothesis that the current canonical correlation and all the smaller ones are zero show that none of them was zero and as expected the first line was equivalent to Wilk's Lambda multivariate test. The R^2 between the first canonical variable (CAN1) and the class variable, 0.4043, was much larger than the corresponding R^2 for the rest three (Table 6).

The canonical variable coefficient 1 (CAN1) showed that the linear combination of the centered variables CAN1 $0.7308 \times \text{flour weight} - 0.681978 \times \text{Number of insects} - 0.6442 \text{ Grain damage} + 1.04668 \text{ weight loss}$ separated the entries most effectively (Table 6). In the first canonical variate, weight loss was undoubtedly the most important variable for discriminating among genotypes by having the largest coefficient (loading value). The first canonical variable was the linear combination of the variables flour weight (%), number of insects, grain damage (%) and weight loss (%) that provided the greatest difference (in terms of a univariate F-test) between the class means. This variable also accounted for most of the variation in the data set (65.23) (Table 6). The second canonical variable provided the largest difference between class means while being uncorrelated with the first canonical variable. The third canonical variate was least important in classifying individuals explaining 10 % of the total variation.

Table 6. The canonical loadings of larger grain borer resistance variables and their standardized coefficients from the canonical discriminate analysis

Canonical loading structure											Canonical coefficients		
Variable	Can corr	SE	Squared Can corr	Eigen value	Cum Variance	LR	F Value	Num DF	Den DF	Pr >F	Can 1	Can 2	Can 3
Flour weight (%)	0.6358	0.016	0.4043	0.68	0.57	0.38	2.23	648	5193.80	<.0001	0.731	0.110	1.540
No. Insects	0.4633	0.021	0.2146	0.27	0.83	0.63	1.35	483	3897.60	<.0001	-0.682	0.051	0.429
Grain damage (%)	0.3391	0.023	0.1150	0.13	0.91	0.80	0.95	320	2600.00	0.7280	-0.644	0.928	0.199
Weight loss (%)	0.3067	0.024	0.0940	0.10	1.00	0.91	0.85	159	1301.00	0.9050	1.047	0.166	1.264
Latent roots											0.68	0.27	0.13
Percentage variation											65.23	16.05	10.96

Test of H0: The canonical correlations in the current row and all that follow are zero.

Key: Can corr = Canonical correlation; SE=Standard error; Cum=Cumulative; LR=Likelihood ratio; Num=Numerator; Den=Denominator; DF=Degrees of freedom;

*Can: Canonical variable coefficient

4.4.2 Reaction of maize hybrids and landraces to the larger grain borer infestation

There were significant differences ($P \leq 0.001$) among the hybrids and landraces for all the traits measured except for the number of insects. The genotypes were classified as resistant (weight loss $< 26\%$), moderately resistant (weight loss = 26.1-30 %) and susceptible (weight loss = $>30\%$) based on the weight loss which was shown as the best variable for grouping the genotype (Table 7). The resistant genotypes had low percentages of weight loss, flour weight, and grain damage, and fewer insects. The most resistant genotype among the hybrids was CKPH08024 with a percentage weight loss of 16.7 %, which was a third of the most susceptible hybrid, H628 which had a percentage weight loss of 48.6 %. The resistant check (CKPH9001) had a percentage weight loss of 25.9 %, which was higher than that of the most resistant hybrids and landraces. Four among the resistant and moderately resistant hybrids (CKIR06009, CKIR06007, CKIR07018 and CKIR04003) were CIMMYT hybrids developed for stem borer resistance. The most susceptible genotypes had a percentage weight loss of 50.6 % which was 1.4 times that of PH4, the susceptible check. The LGB reproduction was adversely affected on resistant grains, as indicated by low numbers of emerged insects post incubation (164 among the resistant genotypes compared to 247 among the susceptible ones) (Table 7). The five most resistant hybrids were CKPH08024, CKPH08012, CKPH08009, CKPH08003 and CKPH08014, and the most susceptible were commercial hybrids, H628, H6213, 16210 and H626. The most resistant landraces were BRAZ 2451, GUAT 1162, BRAZ 2100 and GUAN 36 and the most susceptible were RIGSGP10, BRAZ 1470, BRAZ 1384, BRAZ 4 and NAYA 129.

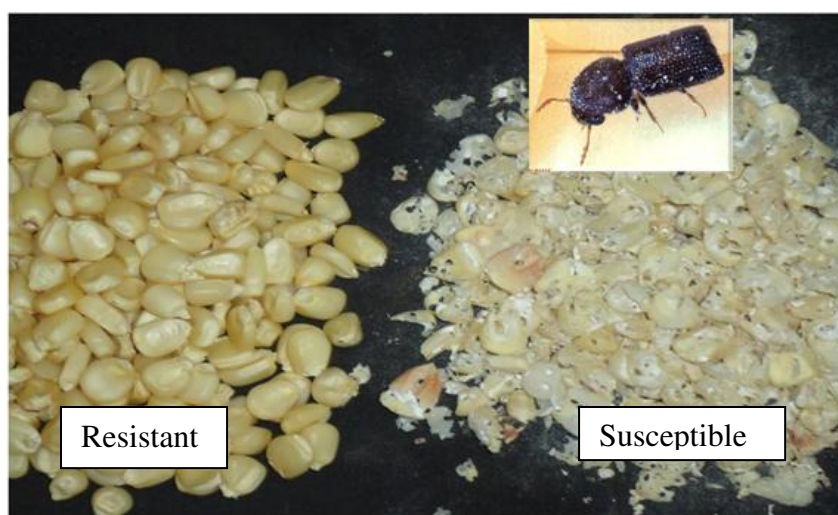


Plate 3. Damage caused by LGB on maize grain

Table 7. Larger grain borer damage indices of most resistant and most susceptible maize genotypes evaluated

Resistant genotypes		Damage indices			
Genotype	Description	Weight loss (%)	Flour weight (%)	Grain damage (%)	No. of insects
CKPH08024	Hybrid	16.66	13.14	27.48	125.0
CKPH08009	Hybrid	20.84	16.78	31.47	178.9
CKPH08012	Hybrid	21.20	14.20	33.01	159.6
CKPH08003	Hybrid	21.87	15.32	33.55	139.6
CKPH08014	Hybrid	22.07	15.40	39.03	160.6
CKPH08033	Hybrid	22.17	14.98	37.50	155.8
BRAZ 2451	Landrace	22.53	13.71	24.59	132.9
BRAZ 2100	Landrace	23.30	16.52	37.77	173.6
ECA-QPOPE	OPV	25.05	21.02	34.59	194.0
CKPH08028	Hybrid	25.40	18.52	36.03	193.7
GUAN 36	Landrace	25.55	21.01	35.73	159.6
ECA-STRIGOFF-VL-125-#	OPV	26.15	15.77	37.81	156.1
EEQPM-8-EA-#-#-#	OPV	26.25	20.01	40.50	174.8
BRAZ 1797	Landrace	26.79	17.75	32.24	154.1
KDV1 - 2-#	Landrace	27.19	17.89	43.21	146.8
VE NE 897	Landrace	27.22	17.69	28.03	136.3
CKPH09001-Resistant check	Hybrid	25.94	23.11	33.96	246.0
Mean		23.85	17.07	34.72	161.0
Susceptible genotypes					
PH 3253	Hybrid	42.32	27.69	50.75	236.7
BRAZ 1371	Landrace	44.23	32.29	39.35	203.4
H6210	Hybrid	44.77	25.32	59.08	284.3
BRAZ 222	Landrace	44.83	34.30	34.90	218.2
BRAZ 1486	Landrace	45.64	44.32	40.90	241.4
H626	Hybrid	45.98	28.61	52.12	229.6
NAYA 129	Hybrid	46.27	31.74	33.09	221.6
H6213	Hybrid	46.85	31.62	55.50	280.6
H628	OPV	48.62	32.06	54.05	257.8
BRAZ 1470	Landrace	50.57	34.65	45.31	230.7
BRAZ 4	Landrace	50.51	33.89	34.13	220.4
PH4-Susceptible check	Hybrid	35.82	23.59	43.84	229.3
Mean		46.42	32.41	45.38	238.60
F Value		3.32	2.75	2.28	5.78
P Value		<.001	<.001	<.001	0.19

*Statistics at df: Rep=2; df-genotypes=162, Residual=1299; Total 14663

*The most resistant and the most susceptible genotypes were presented to reduce the size of the table and for clarity.

4.4.3 Reaction of inbred lines to larger grain borer infestation

There were significant differences ($P \leq 0.05$) among the inbred lines in larger grain borer progeny emergence, percentage grain weight loss, flour weight and grain damage (Table 8). The inbred lines were categorized as highly resistant ($\leq 15\%$); moderately resistant (15.1-20%), moderately susceptible (20.1-25%) and highly susceptible ($> 25.1\%$) based on the percentage weight loss. The mean weight loss for the most resistant and susceptible lines was 13 and 50% respectively. Among the 120 inbred lines evaluated for LGB resistance, 40% were highly resistant to moderately resistant, 40% were moderately susceptible and 20% were susceptible to highly susceptible (Figure 4). The distribution of the lines in the different classes in response to the larger grain borer is evidence of inherent genetic variability among them with respect to resistance. This would form a basis for breeding for resistance as part of an IPM strategy.

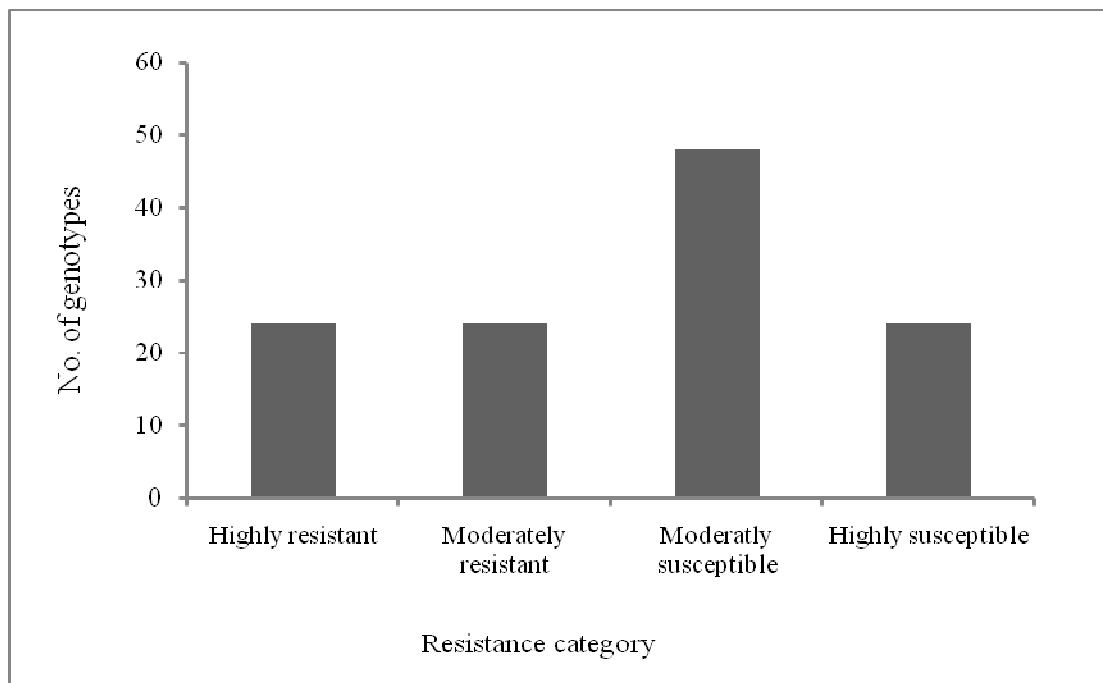


Figure 4. Frequencies of maize found from a screen of 120 maize inbred lines for resistance to LGB.

*The maize inbred lines were classified into 4 categories based on the kernel weight loss classification method of Tefera *et al.* (2010b).

Table 8. Larger grain borer damage indices of 20 most resistant and 20 most susceptible inbred lines from the screen of 120 for resistance to larger grain borer

Entry	Name	No. of insects	Flour weight (%)	Grain damage (%)	Weight loss (%)	S.Index	Reaction category
70	CKSPL10028	92.1	8.9	20.7	13.2	0.5	Resistant
14	CKSPL10035	90.2	8.5	28.4	15.3	0.5	Resistant
84	CKSPL10090	108.3	10	24.9	16.1	0.5	Resistant
98	CKSPL10224	106.7	8.5	23.5	16.3	0.6	Resistant
69	CKSPL10021	122.9	10.3	31.3	16.5	0.6	Resistant
71	CKSPL10035	124.6	11.4	29.2	16.5	0.6	Resistant
82	CKSPL10088	112.5	10.7	27.4	16.8	0.6	Resistant
110	CKSBL10041	108	11.6	32.1	16.9	0.6	Resistant
96	CKSPL10212	136.4	10.5	30.7	16.9	0.6	Resistant
74	CKSPL10070	111.4	10.7	30.3	17.1	0.6	Resistant
83	CKSPL10089	126.5	9.1	24.9	17.4	0.6	Resistant
73	CKSPL10042	119	11.7	30.1	17.5	0.7	Resistant
86	CKSPL10112	127.6	10.9	28.3	17.8	0.7	Resistant
67	CKSPL10295	118.6	12.4	32.4	18.2	0.7	Resistant
78	CKSPL10081	121.3	10.6	28.3	18.4	0.7	Resistant
40	CML144	133.5	12.2	23.6	18.6	0.7	Resistant
75	CKSPL10074	142.5	11.6	36.5	18.8	0.7	Resistant
79	CKSPL10085	119.7	11.5	29.4	19.3	0.7	Resistant
81	CKSPL10087	131	12.7	28.5	19.6	0.7	Resistant
91	CKSPL10164	127.1	12.8	38.5	19.6	0.7	Resistant
R. check	CKSPL10229	127.4	11	29.9	19.7	0.7	Resistant
Mean		119	10.8	28.9	17.3		
20	CKSBL10014	224.2	20.6	41.9	32.8	1.2	Susceptible
113	CKSBL10025	230.8	19.6	38.3	33.2	1.3	Susceptible
109	CKSBL10023	232.5	21.3	44.8	33.3	1.3	Susceptible
10	CML312	174.4	20.1	58.3	33.6	1.3	Susceptible
111	CKSBL10039	218.5	19.9	47.9	34.4	1.3	Susceptible
6	CKSBL10045	206.5	22.4	46	34.6	1.3	Susceptible
42	CML197	89.7	6.8	24.4	34.6	1.3	Susceptible
60	CML441	208.9	22.8	42.1	34.7	1.3	Susceptible
104	CKSBL10013	199.9	20.5	50.5	34.8	1.3	Susceptible
54	P100C6-200-1	198.8	22	40.7	35	1.4	Susceptible
53	DTPWC9-F1	239.6	23.8	43.4	35.5	1.4	Susceptible
34	CML442	215	22.2	54.9	35.7	1.4	Susceptible
46	CZL03014	256.2	22.6	58.6	35.8	1.4	Susceptible
118	CKSBL10046	240.2	25.6	45.4	36.9	1.4	Susceptible
62	CZL01005	213.2	24.6	46.3	37	1.4	Susceptible
120	CKSBL10045	241	24	49.2	37	1.4	Susceptible
11	CKSBL10015	262.4	25.8	42.9	38.5	1.5	Susceptible
58	CZL00003	257.3	27.7	53.7	40.5	1.6	Susceptible
56	CML443	249.7	25.5	50.8	42.8	1.6	Susceptible
S.check	CML395	309.8	32.3	58.6	50	1.9	Susceptible
Mean		218.9	22	46.3	35.8		
	CV	9.7	34.7	33.8	37.2		
	P Value	< 0.001	< 0.001	< 0.001	< 0.001		

4.4.4 Biochemical traits associated with resistance

There were significant differences ($P \leq 0.001$) among the hybrids and landraces for protein and oil content percentages (Table 9). Based on the canonical correlation, the protein content was the best variable which could be used to group the genotypes in relation to their resistance to LGB.

Table 9. Biochemical contents of 15 most resistant and 15 most susceptible among the 175 maize genotypes evaluated for larger grain borer resistance

Susceptible genotypes	Biochemical components		
Genotype	Protein content (%)	Oil content (%)	Starch Content (%)
PH 3253	8.50	5.37	70.55
DH04	8.80	5.60	69.60
PH4-Suceptible check	8.85	5.25	69.50
611D	8.90	5.45	69.10
H6213	8.95	4.85	70.20
H628	9.00	4.70	70.30
SC Duma 43	9.00	4.75	71.10
SUSUMA	9.00	5.32	69.45
DKC8053	9.15	5.55	69.15
KH 600-15A	9.15	5.10	70.30
CKIR06001	9.20	5.03	70.4
CKIR06006	9.20	5.05	70.65
CKIR09007	9.25	4.85	70.25
CML395/CML444	9.25	5.15	70.25
PH1	9.30	5.90	69.50
Mean	9.03	5.20	70.0
Resistant genotypes			
GUAN 34	11.5	6.60	66.90
CKPH08009	11.65	6.50	67.75
CKPH09002	11.67	6.90	67.20
CKPH09001-Reistant Check	12.00	6.55	67.60
CKPH08004	12.03	6.20	68.20
CKPH08020	12.03	6.20	69.30
CKPH09003	12.35	6.05	69.70
CKPH08035	12.65	5.65	67.40
PARA GP3	12.65	6.60	66.05
VERA 197	12.65	5.60	67.55
CKPH08028	12.70	6.40	68.55
Mean	12.20	6.30	67.84
F Value	24.45	11.3	3.44
P Value	< 0.01	< 0.01	< 0.01

*Statistics at df: Rep=2; df-Genotypes 162; residual=1299; Total 1463

*The genotypes were ranked based on the protein content

*The most resistant and the most susceptible genotypes were presented to reduce the size of the table and for clarity.

The most resistant genotypes had high levels of protein and oil. The protein content of the most resistant genotypes was above 11%, whereas that of most of the susceptible ones ranged from 8.5% to 10%. The oil content of the most resistant hybrids was above 5.6%, while that of the most susceptible ones was below 5.5%. The starch content was of little importance in relation to resistance, probably because it does not have antibiosis properties which affect insect pests.

4.4.5 Grain hardness and texture

There were significant differences ($P \leq 0.05$) among the hybrids and landrace accessions for grain hardness. Grain hardness was high (170.71) and (173.34) in the resistant hybrids and landraces respectively (Table 10, Table 11). Likewise, it was lower (155.58) and (159.93) among the susceptible hybrids and susceptible landraces respectively. Analysis of variance showed significant difference ($P \leq 0.05$) among the hybrids and landraces for grain texture (Table 10 and Table 11). The resistant hybrids were semi flint to flint and the susceptible were semi dent to dent with few exceptions. Genotypes CKPH09004 and 500Q were semi-dent and resistant; whereas CKIR09001, CKIR06001, CKIR07008 and H628 were semi-flint, but susceptible. Out of the 15 most resistant landrace accessions, eleven were semi-dent to dent and only four were semi flint to flint. However, the susceptible landraces were semi-dent to dent.

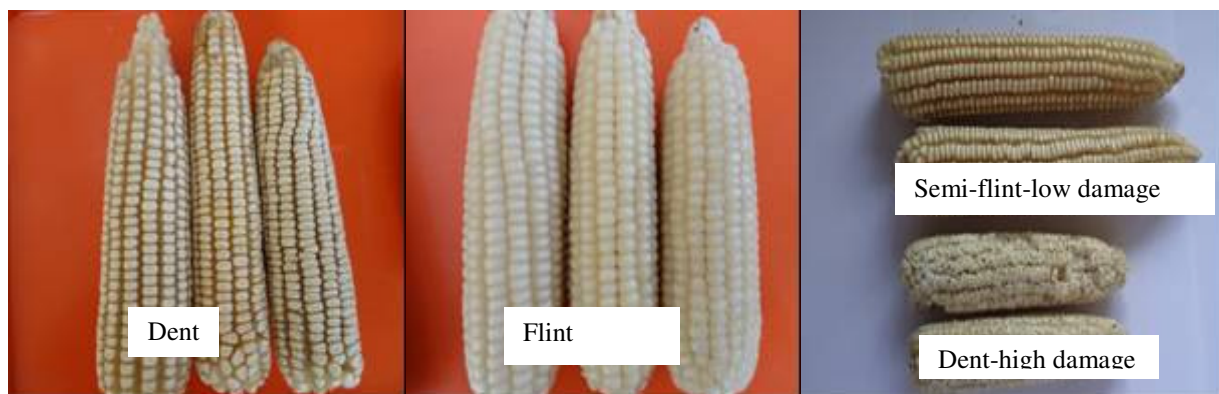


Plate 4. Maize cobs of dent and flint grain texture and levels of damage on flint and dent grain

Table 10. Biophysical traits of 17 most resistant and 17 most susceptible maize hybrids evaluated for resistance to larger grain borer

Entry	Genotype	Grain hardness (N)	Grain texture		Reaction category
			score	Grain texture	
67	CKPH08003	182.67	2.300	Semi-flint	Resistant
69	CKPH09004	176.24	3.000	Semi-dent	Resistant
70	CKPH08009	184.05	2.300	Semi-flint	Resistant
71	CKPH08010	162.78	2.000	Semi-flint	Resistant
78	CKPH08028	182.48	2.000	Semi-flint	Resistant
77	CKPH08026	166.48	2.000	Semi-flint	Resistant
57	CKPH08035	174.83	2.000	Semi-flint	Resistant
73	CKPH08014	172.80	2.000	Semi-flint	Resistant
59	CKPH08037	166.37	2.000	Semi flint	Resistant
72	CKPH08012	163.69	1.000	Flint	Resistant
75	CKPH08024	171.27	2.000	Semi-flint	Resistant
76	CKPH08025	171.55	2.000	Semi-flint	Resistant
52	CKPH09001-Resistant check	169.37	1.000	Flint	Resistant
62	CKPH08040	178.72	2.000	Semi -lint	Resistant
23	500Q	163.86	3.000	Semi-dent	Resistant
54	CKPH09002	177.70	2.000	Semi-flint	Resistant
68	CKPH08004	164.48	2.000	Semi-flint	Resistant
64	CKPH08043	163.52	2.000	Semi-flint	Resistant
Mean		170.71	2.03		
7	H513-Susceptible check	164.09	3.00	Semi-dent	Susceptible
38	CKIR09001	164.12	2.00	Semi-flint	Susceptible
46	CKIR06001	162.12	2.00	Semi-flint	Susceptible
21	531A	137.23	3.00	Semi-dent	Susceptible
14	SC Duma 43	157.00	4.00	Dent	Susceptible
44	CKIR09007	144.58	3.00	Semi dent	Susceptible
36	CKIR07008	169.85	2.00	Semi flint	Susceptible
18	KH 600-15A	155.43	3.00	Semi dent	Susceptible
6	H626	174.55	3.00	Semi dent	Susceptible
33	CKIR07003	175.07	3.00	Semi dent	Susceptible
4	H629	152.39	3.00	Semi dent	Susceptible
3	H6210	154.06	4.00	Dent	Susceptible
24	DK8031	127.24	4.00	Dent	Susceptible
5	H628	151.34	2.00	Semi-flint	Susceptible
13	SC Duma 41	171.14	3.00	Semi-dent	Susceptible
17	611D	136.39	3.00	Semi-dent	Susceptible
1	H6213	156.84	3.00	Semi-dent	Susceptible
16	PH 3253	153.60	4.00	Dent	Susceptible
Mean		155.58	3.06		
CV		6.0	25.0		
P Value		P <0.001	P <0.001		

Table 11. Biophysical traits of 15 most resistant and 15 most susceptible landraces evaluated for resistance to larger grain borer

Entry	Genotype	Grain hardness (N)	Grain texture	Weight loss (%)	Grain texture	Reaction category
54	GUAN 84	181.03	4.00	7.41	Dent	Resistant
59	BRAZ 2451	176.63	3.00	7.91	Semi-dent	Resistant
28	SINA 21	170.61	3.00	8.65	Semi-dent	Resistant
11	GUAT 1162	161.67	2.00	9.08	Semi-flint	Resistant
53	GUAN 36	177.17	2.00	9.76	Semi-flint	Resistant
2	GUAT 1008	179.45	3.00	9.99	Semi-dent	Resistant
58	CHIS 94	171.18	2.00	10.08	Semi-flint	Resistant
71	CKPH09002	178.48	3.00	10.43	Semi-dent	Resistant
5	GUAT 1100	176.60	3.00	10.50	Semi-dent	Resistant
44	GUAT 79	182.18	3.00	10.80	Semi-dent	Resistant
42	VE NE 897	173.68	4.00	10.82	Dent	Resistant
3	GUAT 1010	163.87	2.00	11.71	Semi-flint	Resistant
4	GUAT 1014	169.52	3.00	11.76	Semi-dent	Resistant
27	BRAZ 1486	164.63	3.00	12.47	Semi-dent	Resistant
Mean		173.34	2.79	10.10		
22	CHIS 114	165.30	3.00	14.14	Semi-dent	Susceptible
7	GUAT1034	161.93	4.00	15.29	Dent	Susceptible
33	VE NE 352	159.80	3.00	16.18	Semi-dent	Susceptible
6	GUAT 1030	165.47	3.00	17.31	Semi-dent	Susceptible
36	NAYA 130	139.02	3.00	17.47	Semi-dent	Susceptible
64	BRAZ 222	147.17	2.00	18.35	Semi-flint	Susceptible
61	NAYA 129	156.45	3.00	19.03	Semi-dent	Susceptible
41	BRAZ 1384	154.82	3.00	20.71	Semi-dent	Susceptible
21	BRAZ 1470	177.37	3.00	21.47	Semi-dent	Susceptible
26	BRAZ 4	164.63	4.00	21.79	Dent	Susceptible
56	GUAN 34	162.70	3.00	21.94	Semi-dent	Susceptible
45	PARA GP3	155.90	3.00	23.44	Semi-dent	Susceptible
55	PERU 397	163.39	2.00	24.00	Semi-flint	Susceptible
23	RIGS GP10	165.12	4.00	25.58	Dent	Susceptible
	Mean	159.93	3.07	19.76		
	CV	7.61	7.61	31.80		
	P Value	0.017	0.017	P <0.01		

*CV-coefficient of variation; P Value-probability value at 5 % level

* The 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=1 extremely dent).

4.4.5 Canonical correlations among insect damage variables

The probability level for the null hypothesis that all the canonical correlations are zero (0) among the variables was highly significant ($P \leq 0.0001$) as indicated by the multivariate statistics, so firm conclusions can be drawn from these results. The Wilks Lambda, Pillais trace, Hotelling-Lawley Trace and Roys Greatest root values were 0.629, 0.403, 0.539 and 0.418, respectively. There were great variations in the correlation between grain damage traits and biochemical components (Table 12). From the canonical correlation it was clear that protein content and grain damage accounted for a substantial amount of shared variance between the two sets of variables. The first canonical correlation was 0.543, which was substantially larger than any of the between-set correlations (Table 12). The eigen value corresponding to the first canonical correlations was also high, indicating that it accounted for the most of the variation among the respective canonical variates. The canonical redundancy analysis showed that the first pair of canonical variables was a good overall predictor of the opposite set of variables, the proportions of variance explained being 77.61% and the cumulative proportions for all the three canonical variables being 1.00 (Table 12). Because the variables were measured in the same units, the standardized coefficients rather than the raw coefficients were presented and interpreted. The squared multiple correlation showed that the first canonical variable of the resistance traits had some predictive power for protein content ($r = -0.90$) and very little for oil content and starch content (Table 12). The first canonical variable of the biochemical content is a good predictor of percentage grain damage ($r = 0.97$) and weight loss, a fair predictor of total number of insects ($r = 0.29$) and poor one for the percentage flour weight ($r = 0.15$).

The first canonical variable for the resistance variables had positive signs, an indication that among them there was no suppressor variable, with the most weight on grain damage. The general interpretation of the first canonical correlation was that protein and oil content act as suppressor variables in relation to the resistance traits (grain damage, weight loss and number of insects) whereas starch content is an activator variable. In other words, the starch content stimulates susceptibility to the LGB. The canonical correlation was strong enough to be of practical interest since the sample size was large enough to draw definite conclusions.

Table 12. Canonical correlation between the larger grain borer resistance traits and the grain biochemical content traits of the maize genotypes evaluated for resistance

Canonical correlation between resistance traits and the biochemical content										Correlation coefficients for the variables						
Can	Can corr	Squared can corr	Eigen Value	Prop	Cum	F Value	Num DF	Den DF	Pr > F	Protein	Oil	Starch	Weight loss	Flour weight	Grain damage	No. of insects
Can 1	0.5431	0.2949	0.42	0.78	0.78	6.39	12	399.8	<.0001	-0.90	-0.13	0.12	0.51	0.15	0.97	0.20
Can 2	0.3226	0.1041	0.12	0.22	0.99	2.98	6	304	0.0076	-0.62	1.07	-0.14	-0.87	-0.47	-0.29	0.72
Can 3	0.0674	0.0045	0.00	0.01	1.00	0.35	2	153	0.7062	-0.28	-0.21	-1.02	-0.83	0.64	-0.35	1.08

Key: Can corr=Canonical correlation; Prop=Proportion of variance; Cum=Cumulative variance; Num=Numerator; Den=Denominator; DF=Degrees of freedom;

*Can: Canonical variable coefficient

4.5 Discussion

This study showed that there were differences in resistance levels among maize hybrids, open pollinated varieties and landrace accessions as indicated by grain weight loss, flour weight, grain damage and number of insects. The percentage weight loss was the most important variable for grouping the genotypes into resistant and susceptible. The low percentage of grain weight loss among the resistant genotypes indicated that they have genes that confer resistance to the LGB. Grain resistance to postharvest insect pests has been attributed to physical factors such as grain hardness, and to biochemical traits such as phenolic compounds (García-Lara *et al.*, 2004). The presence of resistant genotypes among the germplasm screened suggests that sources of resistance exist among the landrace accessions and OPVs, and that it is possible to develop improved maize hybrids that are resistant to the LGB. The results show that genetic diversity for LGB resistance exists among tropical maize inbred lines thus offering opportunity to exploit the variability towards reducing post-harvest insect pest losses through genetic improvement. The canonical discriminate analysis and correlation coefficients gives invaluable information that maize resistance against the LGB can be evaluated based on the percentage weight loss followed by grain damage. The level of damage and weight loss during storage is strongly correlated with the number of adult insects (Tefera *et al.*, 2011).

Protein and oil content were high in resistant genotypes compared to susceptible genotype. Protein content was also the most important variable based on the canonical loading values/coefficients. This suggest that these two are among other biochemical components associated with resistance, either directly or indirectly, within the resistant genotypes. However, some genotypes were susceptible to the LGB but had high protein content (> 11%). This could be attributed to the protein composition

within the maize grain and its interaction with starch. Storage proteins in food grain are responsible for the association between starch grains and endosperm matrix proteins, thus influencing the grain hardness (Hoseney, 1987; Dombrink-Kurtzman and Bietz, 1993). The composition and arrangement of storage proteins alter the texture and, consequently, the physical structure of the endosperm (Dombrink-Kurtzman and Bietz, 1993). Siwale *et al.* (2009) reported that weevil-resistant improved maize hybrids and open pollinated varieties (OPV) of genotypes had a high protein content compared to susceptible ones. Arnason *et al.* (1997) indicated that protein content is negatively correlated with the susceptibility of maize to the maize weevil. This is also consistent with other studies, which found that the hydroxyproline-rich proteins (extensins), that are known to accumulate in the pericarp, play a role in resistance to storage pests (Dhliwayo and Pixley, 2003; García-Lara *et al.*, 2004).

These results based on the diverse germplasm evaluated and the canonical correlation indicated that protein content in combination with other factors in the maize grain contributes to LGB resistance. The mechanisms could either be antibiosis by a single, or combination of several biochemical components for possible synergistic effect. For instance, the protein content could be contributing to kernel hardness, which is a putative trait of grain resistance to storage pests (García-Lara *et al.*, 2004). There are key genes responsible for the synthesis of key proteins associated with LGB resistance, but there is a need to investigate further the amino acid composition in the resistant and susceptible genotypes which have high protein content. There is a window for further exploration at the molecular level of the genes which are involved in conferring a high content of specific proteins correlated with resistance to the LGB, through molecular characterization of the germplasm used.

The oil content in most of the resistant genotypes was higher than in susceptible genotypes, despite the low canonical correlation coefficients among the traits measured. The lipids might be playing a role in resistance to the LGB as part of the antibiosis component. This is in agreement with earlier studies that reported a low proliferation of khapra beetles (*Trogoderma granarium*) on wheat, maize and sorghum which had high lipid content (Jood *et al.*, 1996). The basis of resistance due to the presence of lipids in maize kernels could be that oxidization over time leads to the production of volatiles that influence movement of storage pest insects away from the grain (Nawrot *et al.*, 1985).

Grain hardness levels were high in most of the resistant compared to the susceptible genotypes. Soft endosperms have been associated with high infestation by maize weevil due to easy tunneling and adequate flour for feeding (Siwale *et al.*, 2009). Harder grains are difficult to penetrate and yields low flour content. As a result, hard grains are not able to support large number of the damaging insects. In pear millet, soft endosperm was associated with increased susceptibility to the storage weevil *Sitophilus oryzae* (Leuschner *et al.*, 2000). Similar relationship between maize and weevil was reported elsewhere (García-Lara *et al.*, 2004). Grain hardness is highly correlated with extensions and DIFA linkages within the pericarp cell wall of maize grains and this cross-linkage has been reported to provide a biochemical mechanism of controlling the mechanical properties of the cell wall (Waldron *et al.*, 1996). This increases the physical strength, overall grain hardness; and possibly contributing to resistance to postharvest insect pest attack through fortification of the pericarp cell wall.

The resistant hybrids and inbred lines had flint texture, whereas the susceptible genotypes had dent texture. This was contrary to the landrace accessions, whereby the resistant and susceptible were dent. Dent maize genotypes have been reported to be highly susceptible to maize weevil attack and

the results of this study confirms this to a large extent in case of LGB (Kim *et al.*, 1988). Maize genotypes with flint grain texture are resistant to weevil (Kossou *et al.*, 1993). The results corroborate to this view, as evident from the resistant genotypes among the hybrids and inbred lines in response to the weevil and LGB attack. The results further show that grain texture was not the only factor responsible for grain resistance to the postharvest insect pest attack. This was evident among the landraces that the mechanisms of resistance probably had little to do with the grain texture, since both resistant and susceptible cultivars were dent. The grain texture was positively correlated ($r = 0.7$) with the grain hardness among the hybrids, whereby the flints were harder compared to the dents. Flint grains have vitreous endosperm which is hard and crystalline, and possesses a continuous and abundant protein matrix, which has larger and compact, polygonal starch granules (Pratt *et al.*, 1995). Dent grains have a farinaceous endosperm, which is opaque and soft-textured, a discontinuous protein matrix and starch granules which are less aggregated and surrounded by air spaces (Pratt *et al.*, 1995). This study shows that grain texture and hardness are interlinked and worth for consideration in breeding for resistance to postharvest insect pests in maize.

The first canonical correlation showed that protein and oil content act as suppressor variables in relation to the insect grain damage traits (grain damage, weight loss, flour weight and number of insects) indicating that they were associated with resistance to the LGB. Moreover, the correlation coefficients further indicated that the protein content had on average a higher contribution to variation (-0.90) in resistance than the oil content (-0.13). As a result, protein content would be more important as a trait of resistance, subject to further studies.

4.6 Conclusions

The use of resistant genotypes is key in developing a sustainable IPM program against the LGB. Percentage weight loss was observed as the most important trait for discriminating genotypes for resistance against LGB. The resistant hybrids were CKPH08024, CKPH08009, CKPH08012, and CKPH 08014. The most resistant landrace accessions (BRAZ 2451, GUAT 1162, BRAZ 2100, GUAN 36, and GUAT 1162) can be used as sources of resistance in breeding programmes to address the problem of post-harvest losses due to the LGB by developing effective management strategy for LGB. The resistant inbred lines (CKSPL10028, CKSPL10035, CKSPL10088, CKSPL10021, CKSPL10042, CKSPL10295 and CKSPL10112) identified can be used as donor lines to introgress insect pest resistance genes into adapted but susceptible genotypes. The most resistant and susceptible inbred lines can be deployed in association mapping projects for identification of molecular markers for use in marker assisted selection. Protein content and grain hardness are important trait conferring resistance to maize grain against the LGB subject to further studies.

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Chapter Five

Genetic Relationship in Tropical Maize Germplasm in Response to Postharvest Insect Pests

5.1 Introduction

Genetic distance is a measure of genetic divergence among species or among populations within a species. Smaller genetic distances indicate a close genetic relationship whereas large genetic distances indicate a more distant genetic relationship. Analysis of genetic distance is an important tool that underpins successful breeding programmes (Mohammadi and Prasanna, 2003; Mukhtar *et al.*, 2002). In a breeding program, genetic gain achieved through artificial selection is a function of genetic variation and efficiency of selection. Thus, the correct choice of parents employed during development of basic population can influence selection (Bohn *et al.*, 1999). Depending on the objectives of a breeding programme, breeders use different methods in selecting the best parents, including (a) pedigree relationships, (b) morphological and agronomic traits, (c) adaptability and yield stability, and (d) genetic distances estimated from morphological and molecular markers (Bohn *et al.*, 1999; Maric *et al.*, 2004; Bertan *et al.*, 2007). Morphological and agronomic traits were the earliest genetic markers used in germplasm characterization and quantifying genetic distance in crops. Morphological markers have a number of limitations. They generally have low polymorphism, few in number, low heritability, late expression and influenced by environmental (Smith and Smith, 1989).

In contrast, molecular markers, a specialized genetic phenotype are more effective than morphological and agronomic traits for germplasm characterization (Almanza-Pinzón *et al.*, 2003; Maric *et al.*, 2004; Roy *et al.*, 2004). The class of molecular markers classified as selectively neutral are independent of tissue, developmental stages and environmental effects and selection. They are

abundant and considered superior to morphological and agronomic traits for germplasm characterization (Maric *et al.*, 2004). Genetic distance can be estimated from various types of selectively neutral genetic markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random polymorphic DNA (RAPD), simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs). Advances in molecular technology, however, have produced a shift towards SNP markers (Hamblin *et al.*, 2007; Jones *et al.*, 2007). The SSRs perform better at clustering germplasm into populations and providing more resolution in measuring genetic distance than SNPs markers (Hamblin *et al.*, 2007). The SSRs are multiallelic, highly polymorphic, co-dominant and highly reproducible, providing rich genetic information (Balestre *et al.*, 2008; Dandolini *et al.*, 2008). The main problem of using microsatellites is that, high development costs are involved if adequate primer sequences for the species of interest are unavailable, making them difficult to apply to unstudied crop species. Mutations in the primer annealing sites may sometimes arise and result in the occurrence of null alleles leading to failure in PCR amplification, which may lead to errors in genotype scoring.

Genetic diversity for resistance to postharvest insect pests using phenotypic data have been reported (Tefera, 2012). The international maize and wheat improvement center (CIMMYT) in Kenya initiated a breeding programme that has generated germplasm for quality protein maize (QPM), drought tolerance, resistance to stem borers, and resistance to post-harvest insect pests. Genetic diversity among this germplasm and its response to weevil and LGB resistance has not been well studied. Effectiveness of simultaneous use of SSR and biophysical traits to measure relationships among genotypes in response to postharvest insect pests is not yet documented. Better understanding of the genetic basis of genetic variability will improve the efficiency of maize improvement for postharvest insect pest resistance. The objective of the study was to characterize tropical maize

germplasm for resistance to post-harvest insect pests in terms of genetic distance and relationship using biophysical traits and SSR markers.

5.2 Materials and methods

5.2.1 Germplasm and phenotypic evaluation

Two hundred and eighty two (282) maize genotypes comprising of 83 hybrids, 115 inbred lines, 69 landraces and 15 open pollinated varieties selected from CIMMYT Kenya maize breeding programme were used in the study (Appendices1-3). Phenotypic performance of the 282 genotypes was evaluated at the Kiboko and Embu Kenya Agricultural Research Centers (KARI) in October 2010 and April 2011. The experimental design was as described in chapter three. All the 282 genotypes plus resistant and susceptible checks were evaluated for maize weevil and larger grain borer resistance at the postharvest screening laboratory in Kiboko as described in chapter three and four. The parameters measured in the insect bioassay were percentage grain weight loss, percentage grain damage, number of adult progenies and the weight of the flour produced. Resistance among genotypes was determined based on the percentage weight loss (Tefera *et al.*, 2011). Grain hardness and protein content were evaluated after harvest as described in chapter four.

5.2.2 Molecular analysis

Leaf samples were harvested from 10 healthy plants per genotype and bulked about 3 weeks after sowing at the Kiboko station. They were stored in perforated polythene self sealing bags and kept cooled on dry ice in a Styrofoam box. The leaf samples were transported to the Biosciences eastern and central Africa (BecA) hub in Nairobi, cut into pieces, and transferred into 1.2 ml strip tubes that contained two 4-mm stainless steel grinding balls (Spex CetriPrep, USA). The leaf samples were freeze-dried for 4 days using a Labconco freeze dryer (Labconco corporation, Kansas City) (<http://www.labconco.com>) as described in the user's manual. The lyophilized leaf samples were

ground into fine powder at 1500 strokes per minute for 2 minutes using GenoGrinder-2000. Genomic DNA was extracted using a modified version of the high throughput mini-prep Cetyl Trimethyl Ammonium Bromide (CTAB) method (Mace et al., 2003). The quality of the isolated DNA was assessed after running aliquots of DNA samples on a 0.8% agarose gel that contained 0.3 µg/mL Gel-Red-(Biotium). DNA concentration was measured using NanoDrop-ND-1000 Spectrophotometer, (Thermo Scientific, Wilmington, DE 19810, USA).

The samples were genotyped with 56 SSRs (Appendix 4), that were selected from the list of markers used for the genetic characterization of CIMMYT maize inbred lines and OPVs (Warburton *et al.*, 2002). Polymerase Chain Reaction (PCR) was performed in 384-well plates in a total reaction volume of 10 µL that consisted of 30 ng DNA, 1× magnesium-free PCR buffer, 2 mM MgCl₂, 0.20 µM of the forward primer labeled with 6-FAM, PET, VIC or NED fluorescent dyes, 0.20 µM of the reverse primers, 0.20 mM each deoxynucleotide triphosphates (dATP, dTTP, dCTP, dGTP), and 0.25 unit Ampli Taq Gold DNA polymerase. All PCR components were purchased from PE-Applied Biosystems. The PCR reactions were performed for each primer separately using Gene-Amp PCR System 9700 (PE-Applied Biosystems, Foster City, CA 94404, USA) as follows: 10 minutes at 94 °C followed by 35 cycles of denaturation for 30 seconds at 94 °C, annealing for 1 minute at a temperature that ranged between 55 and 60 °C depending on the primer, an extension for 2 minutes at 72 °C, and a final extension of 10 minutes at 72 °C. After the PCR, 3 µl of the PCR amplicon from few randomly selected samples per marker was mixed with bromophenol blue loading dye and assayed for proper amplification and product intensity by running them on 2 % agarose gel. The PCR products were separated by co-loading post-PCR products of four sets of primers based on fluorescent dye and/or fragment size onto ABI 3730 Capillary DNA Sequencer (PE-Applied Biosystems, Foster City, CA 94404, USA) as described in the user's manual. Depending on band

intensity on agarose gel, 1.5 to 2.5 µl of PCR products from each of the 6-FAM (blue), VIC (green), PET (red) and NED (yellow)-labeled markers were pooled together (Schuelke, 2000). The final volume adjusted to 15 µL by adding the required volume from a mix of an injection solution (HiDi) and GS-500LIZ size standard (850 uL HiDi and 12 µl GS500 LIZ for a 96-well plate). DNA fragments were denatured and size-fractionated using ABI 3730 Capillary DNA Sequencer (PE-Applied Biosystems, Foster City, CA 94404, USA). The peaks were sized and the alleles called using GeneMapper software and the internal size standard GS500LIZ-3730. Both DNA extraction and SSR genotyping were done at the BecA hub.

5.2.3 Data analyses

Because of the differences in the nature of germplasm used in the present study, data analyses were done by dividing the 282 genotypes into 3 groups: inbred lines (115), hybrids (83) and landraces and open pollinated varieties (84). Each group had three types of data; molecular data set, grain biophysical/bioassay data set and a combination of the molecular and the biophysical/bioassay data. The percentage weight loss, flour weight and grain damage data was angular transformed ($\arcsin\sqrt{\text{proportion}}$) to normalize its distribution before subjecting them to ANOVA. A univariate analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS statistical package (SAS Institute, 2003) for the individual grain biophysical and insect bioassay traits was done.

The different datasets were standardized before analysis. Stuttering and plus-A amplification often cause ambiguity in automated SSR allele binning (Idury and Cardon, 1997). AlleloBin (<http://www.icrisat.org/bt-software-downloads.htm><http://www.icrisat.org/bt-software-dallelobin.htm>) was used for adjusting inconsistencies in allele calls obtained from GeneMapper software. The number of adjusted alleles per SSR marker, per bulked genotype varied from 2 to 11. Thus, the adjusted allele sizes were converted into binary format (present =1 and absent = 0) using ALS-Binary software

(<http://www.icrisat.org/bt-software-downloads.htm>). Euclidean distance was calculated for all data sets as a measure of genetic distance between the genotypes. Mantel matrix correspondence test (Mantel, 1967) was used to estimate the correlation between the distance matrices resulting from the phenotypic, SSR and combined dataset. The distance matrices were used to generate phenograms using the unweighted pair group method based on arithmetic average (UPGMA) method in MEGA5.1 (5.05 beta). Cophenetic matrices were computed for each phenogram and compared with the distance matrices using the Mantel matrix-comparison test (Mantel, 1967). Euclidean distance matrices, Mantel tests and cophenetic matrices were performed using NTSYS-pc for Windows, version 2.0, Exeter Software (Rohlf, 1998).

5.3 Results

5.3.1 Genetic relationship among maize hybrids

There were significant differences ($P \leq 0.001$) among the maize hybrids for all the biophysical and bioassay traits measured in the study. This indicates that there is a high degree of genetic divergence in relation to storage pest resistance. The euclidean distance among the hybrids ranged between 3 and 9.45 for SSRs, from 0.01 to 0.4 for the biophysical/bioassay, and from 0.39 to 6.0 for the combined dataset. The euclidean distance matrix based on the SSR data indicated that the commercial hybrids (H6 series), SCDUMA41 and CML 312-CML 442 as the most distant genotypes compared to those bred for storage pest (CKPH series) and stem borer (CKIR series) resistance. Among the CKIR series, CKIR07013 and CKIR04003 had the largest genetic distance from the CKPH series; whereas CKIR07009 was the closest to both the CKPH and CKIR series. Three separate dendrograms were generated from the SSR data, biophysical/resistance data and a combination of both data sets. The genotypes were separated into three major clusters based on the molecular data, the phenotypic and a combination of the two data sets (Figure 5, Figure 6, Figure 7).

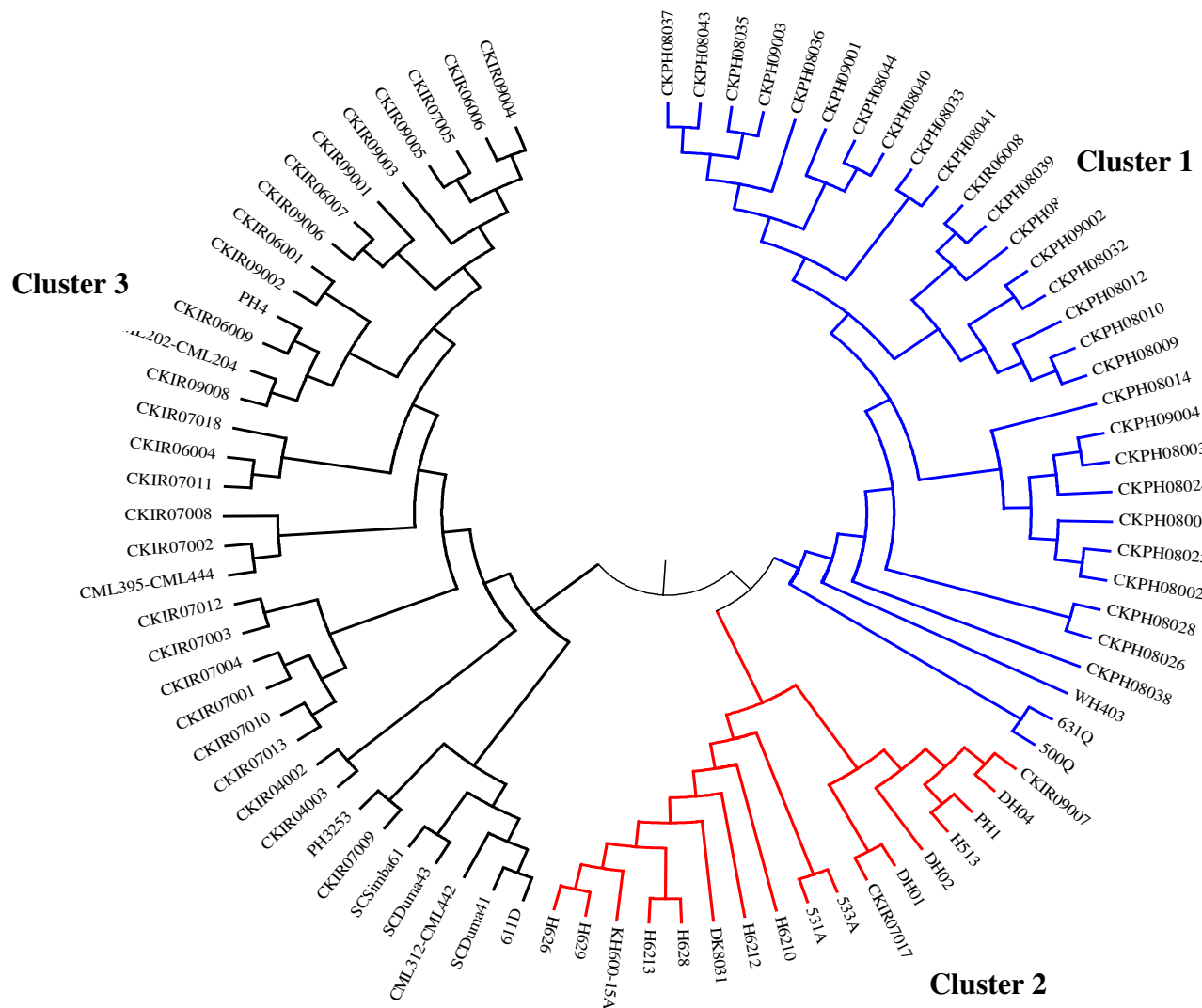


Figure 5. Dendrogram of 83 maize hybrids derived by UPGMA from the euclidian genetic distance matrix of the SSR marker data.

Most of the commercial and CIMMYT bred stem borer resistant (CKIR series) hybrids were grouped together (cluster 3), an indication of their close relationship (Figure 5). Hybrids which had been bred for storage pest resistance (CKPH series) with few commercial and stem borer resistant hybrids were also grouped together (cluster 1). The third cluster was largely made up of the commercial hybrids. The resistant check (CKPH09001) was grouped together with the CKPH series whereas the susceptible checks (PH4) was grouped together with the CKIR series.

The cluster analysis based on the biophysical/bioassay data set and a combination of both molecular and latter data sets separated the maize hybrids according to their response to postharvest storage insect pests (Figure 6, Figure 7). These include the resistant in clusters 3 (CPKH series), moderately resistant in cluster 2 (CKPH series, few CKIR series and commercial); moderately susceptible in clusters 1 (CKIR series and few commercial) and susceptible in cluster 4 which was composed of the commercial hybrids and few genotypes of the CKIR series. The separation among the genotypes based on the molecular data alone was comparable to that based on the biophysical/bioassay data alone and or a combination of both.

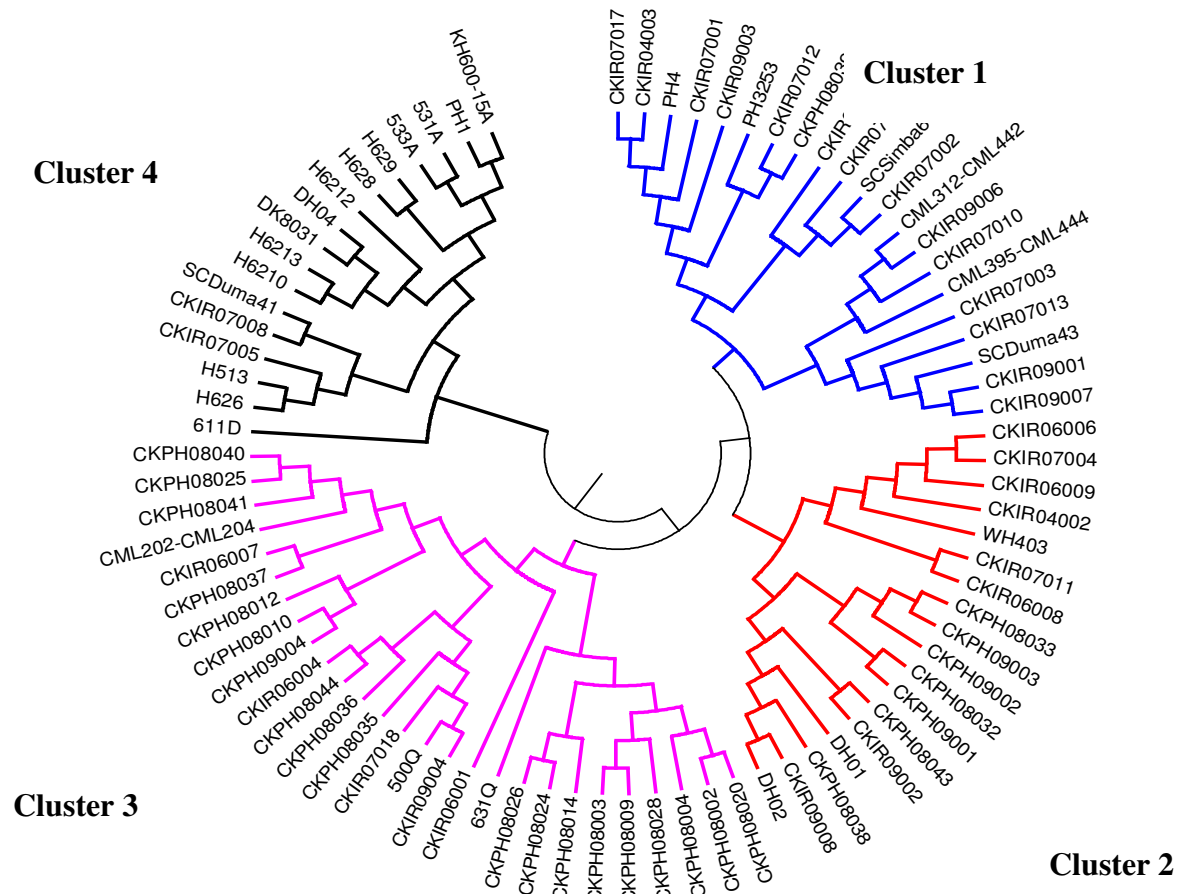


Figure 6. Dendrogram of 83 maize hybrids derived by UPGMA from the euclidian genetic distance matrix of the biophysical/bioassay data.

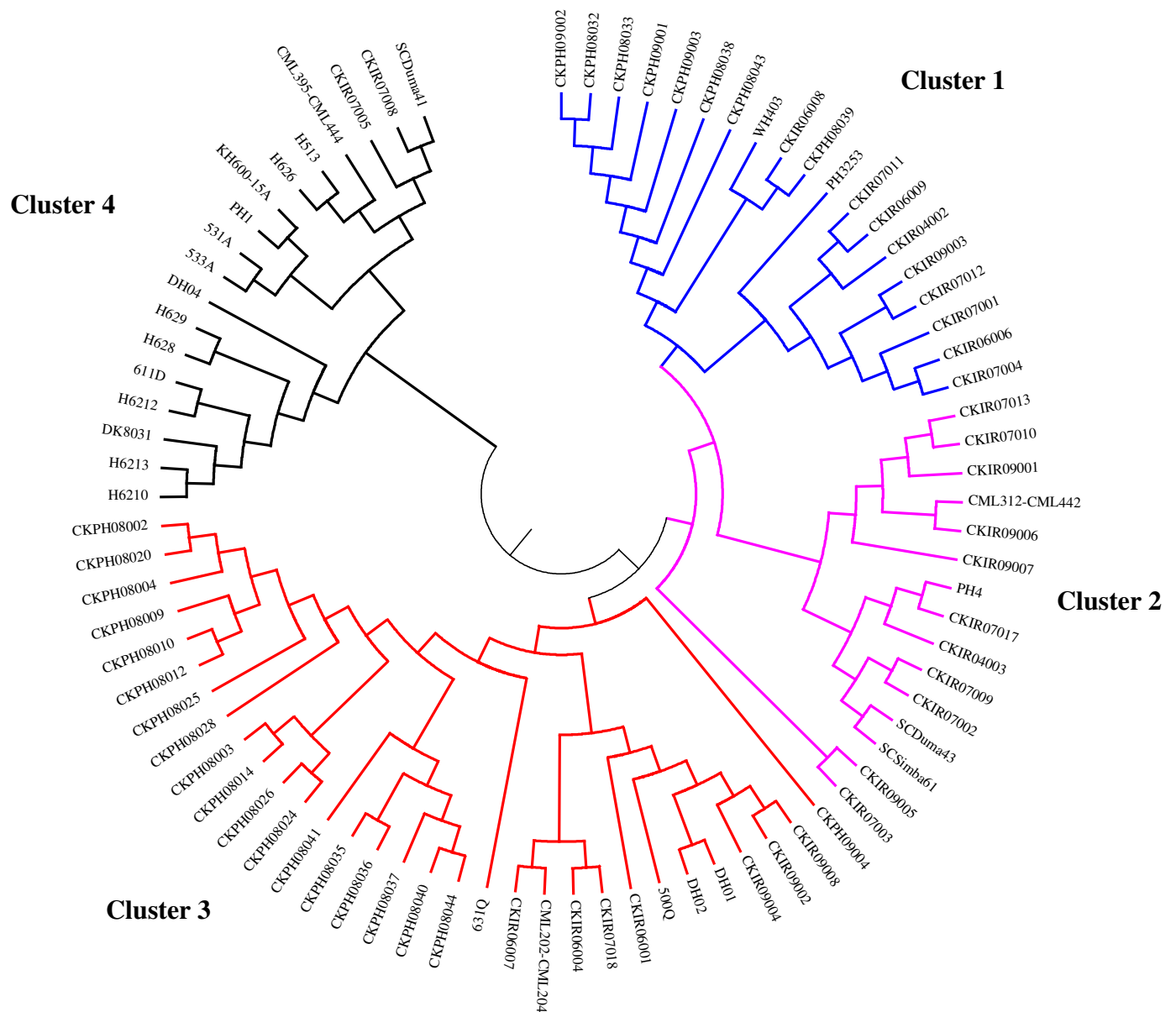


Figure 7. Dendrogram of 83 maize hybrids derived by UPGMA from the euclidean genetic distance matrix of the SSR and biophysical/bioassay data.

The Mantel (1967) matrix correspondence test showed that there was low correspondence between the distance matrices generated from SSR and agronomic traits ($r = 0.27$). It was only high (0.96) between the molecular data and the combined (molecular and agronomic) data sets. The dendrograms derived from the distance matrices of the different data sets showed similarities to a large extent (Figure 5, Figure 6). The cophenetic correlations, which are measures of goodness of fit

for the cluster analysis among the three data sets were 0.84, 0.80 and 0.83 for the molecular, phenotypic and combined (molecular + phenotypic data sets), respectively. The degree of fitness is interpreted subjectively as follows: $r > 0.9$ very good fit; $0.8 < r < 0.9$ good fit, $0.7 < r < 0.8$ poor fit, and $r < 0.7$ very poor fit.

5.3.2 Genetic relationship among maize inbred lines

There were significant differences ($P \leq 0.001$) for all the agronomic traits among the inbred lines evaluated. The euclidean distance among the inbred lines ranged between 2.83 and 10 for SSRs, from 0.01 to 2.20 for the biophysical/bioassay, and from 2.05 to 12.50 for the combined dataset. Dendrograms were generated from the genetic distances of the SSR data, biophysical, bioassay and a combination of both data sets, separating genotypes into 5 clusters with distinct sub-clusters (Figure 8-10). Based on the SSR marker data, CML197, CML312, CML440, CZL03007, CKSBL10034 and CKSBL1008 had the greatest genetic distance among all the inbred lines. The inbred lines, CKSBL10034 and CKSBL1008 were even more distinct from the CKBL series which had been bred for multi-stem borer resistance. The CKPS series which had been bred for storage pest resistance were genetically distinct from CML 442, CML 441, CMK 334, CML 511, CML 395, CML 312, CKSBL0039 and CKSBL10030. The inbred line, CML 395, a susceptible check, was closely related to CKSBL1003, CKSBL10026-35, CKSBL10043, CKSBL10029-12 and CKSBL1005-119 though not close to CML 312, another susceptible check with a genetic of 3.6. The SSR marker data estimated successfully the close relationship among most of the CKPS series and the CKSBL series on the other hand. The 5 major clusters found in this study included inbred lines which had been bred for postharvest insect pest resistance, CKPH series in cluster 1; multi-stem borer resistant inbred lines (cluster 3 and 4); distinct CIMMYT breeding program lines in clusters 2 and 5. The grouping in cluster 5 was not robust and the lines had the largest distance from the rest. The insect

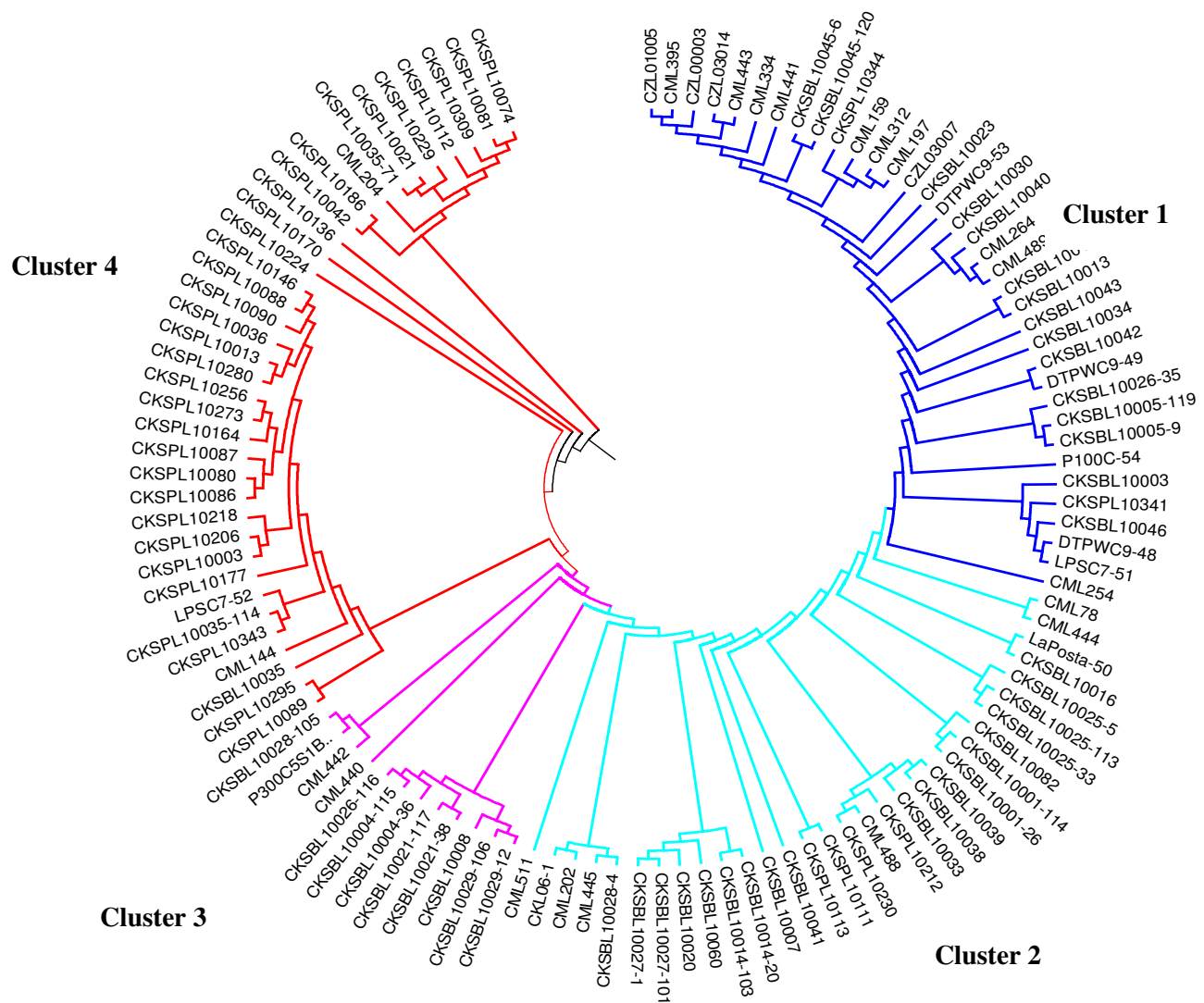


Figure 10. Dendrogram of 115 maize inbred lines derived by UPGMA from the euclidean genetic distance matrix of the SSR marker and biophysical/bioassay data.

The Mantel (1967) matrix correspondence test demonstrated that there was low correspondence between the distance matrices generated from SSR and biophysical/bioassay data ($r = 0.24$) and high (0.95) between the molecular data set and the combined (molecular and biophysical/bioassay) data sets. However, similarities were evident among the groupings derived from the different data sets. The cophenetic correlations, which are measures of goodness of fit for the cluster analysis among the three data sets were 0.71, 0.78 and 0.71 for the molecular, biophysical/bioassay and combined

(molecular + biophysical/bioassay data sets), respectively. The degree of fitness was interpreted subjectively as follows: $r > 0.9$ very good fit; $0.8 > r < 0.9$ good fit, $0.7 > r < 0.8$ poor fit, and $r < 0.7$ very poor fit.

5.3.3 Genetic relationship among landraces and open pollinated varieties

The euclidean distance among the landraces and open pollinated varieties ranged between 6.00 and 10 for SSRs, from 0.01 to 2.0 for the biophysical/bioassay, and from 0.5 to 13.50 for the combined data set. The distance matrix based on the SSR molecular data showed that RIGSGP10, the most susceptible landrace, had the largest distance from BRAZ 2451, SINA 30, BRAZ 2017, NAYA 129, JALIA, BRAZ 403. On the other hand, it was closest to PARAGP3, GUAN 36, BRAZ 1470, VENE 352, GUAN 84, BRAZ 222, GUAT 1008, GUAT 1010, BRAZ 2179, BRAZ 1919, BRAZ 1384 and VENE 897, which were all susceptible to moderately susceptible except GUAN 36, GUAT 1008 and GUAT 1010, GUAN84 and VENE 897 which were moderately resistant to resistant. Similarly those with largest distance from RIGSGP10 included both susceptible and resistant genotypes. The most distinct genotypes in respect to all the germplasm were SINA 30 and NAYA 129; whereas the closest were VENE 897 and BRZ 2454. Cluster analysis using the genetic distances of the SSR data, biophysical/bioassay and a combination of both data sets separated the germplasm into 5 major clusters with distinct sub-clusters based on the molecular data, biophysical/bioassay and a combination of the two data sets (Figure 11-13).

Cluster analysis based on the SSR data gave mixed results in relation to resistance to the postharvest insect pests. Some of the resistant germplasm was grouped together with the susceptible. For instance GUAT 1100 which was resistant was closely associated with GUAN 34 and BRAZ 4 which are susceptible and GUAN 36 which was resistant with RIGSGP10, the most susceptible (Figure

11). The major clusters had many subgroups compared to the grouping among the inbred lines and hybrids, an indication of the great diversity and or divergence among the landrace accessions and the pen pollinated varieties (OPVs).

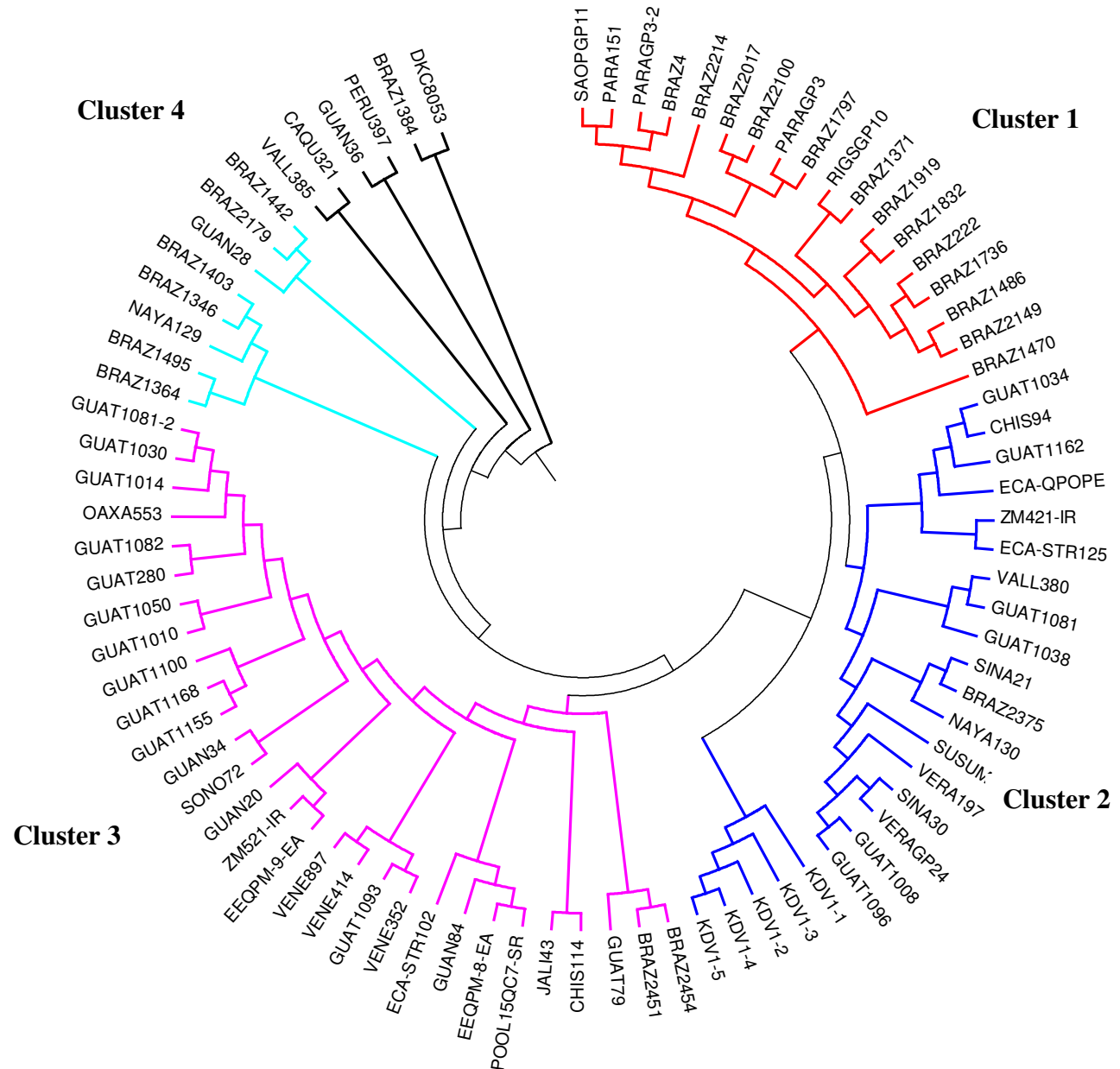


Figure 11. Dendrogram of 84 maize landraces accessions and open pollinated varieties derived by UPGMA from the euclidian genetic distance matrix of the SSR data.

The major clusters based on the biophysical data included landrace accessions that were resistant, moderately resistant, moderately susceptible and highly susceptible. The genotypes BRAZ2451,

GUAN 84, SINA21, GUAT 1162, GUAT1008, GUAN 36 and CHIS 94 were the most resistant and grouped together. Similarly, GUAN 34, RIGSGP10, PERU 397, BRAZ 1384, BRAZ 1470, NAYA 130 and BRAZ 4, the most susceptible were grouped together (Figure 12).

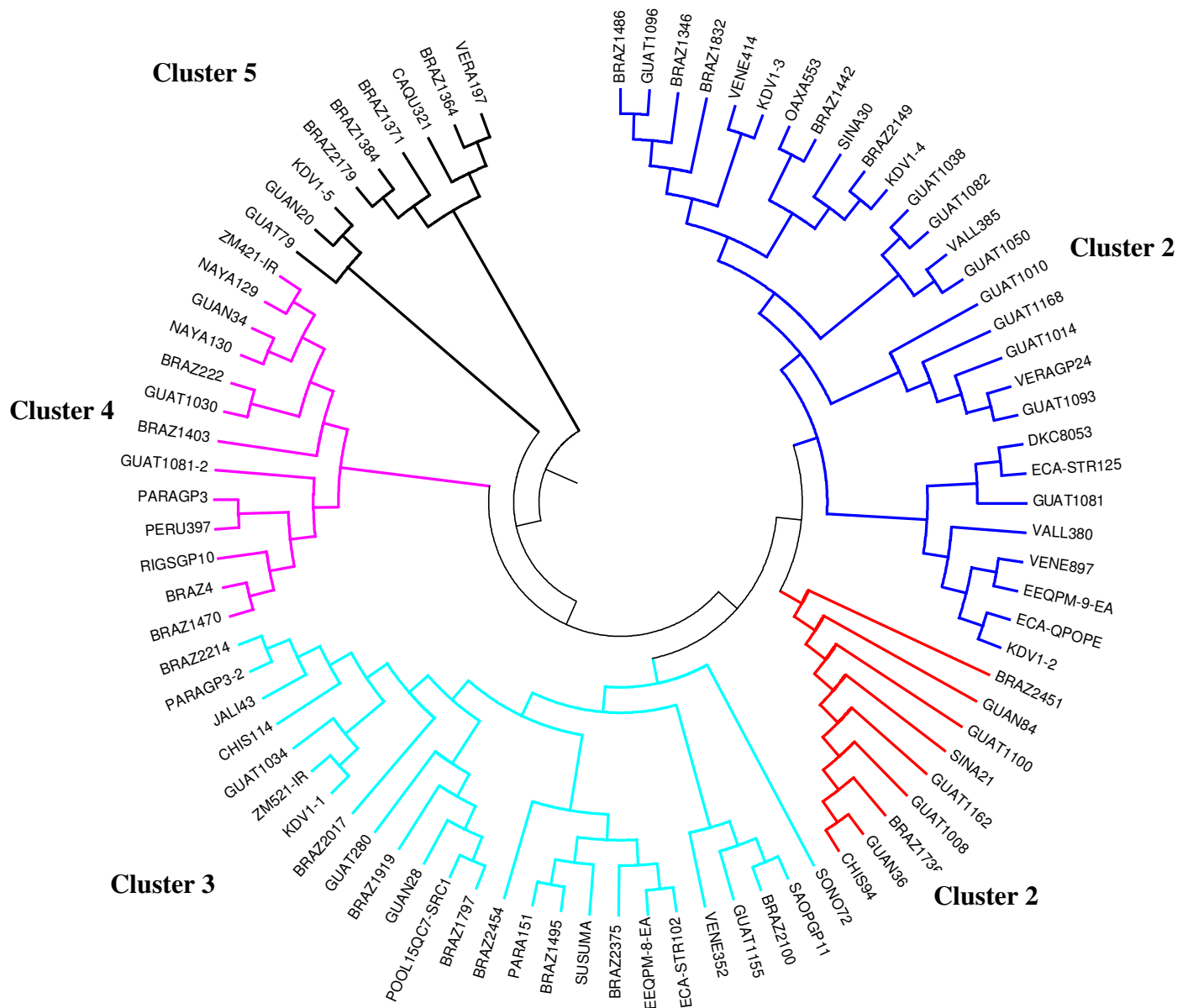


Figure 12. Dendrogram of 84 maize landraces accessions and open pollinated varieties derived by UPGMA from the euclidian genetic distance matrix of the phenotypic data

Phenetic analyses based on SSR and biophysical/bioassay data sets generated dendrograms that had similarity to that based on biophysical/bioassay data set alone. The germplasm was grouped into four

clusters. The most susceptible genotypes GUAN 34, PARAGP3, RIGSGP10, BRAZ 4, BRAZ 1384, BRAZ 1470, PERU 397, NAYA 130 and BRAZ 222 clustered together in cluster 4, whereas the resistant genotypes BRAZ 2451, SINA 21, GUAN 84, GUAT 1162, GUAT 1008, GUAN 36) and moderately resistant genotypes DKC8053, KDV1-3, GUAT 1081, VENE 897, VALL 380 were grouped together in cluster 3 (Figure 13).

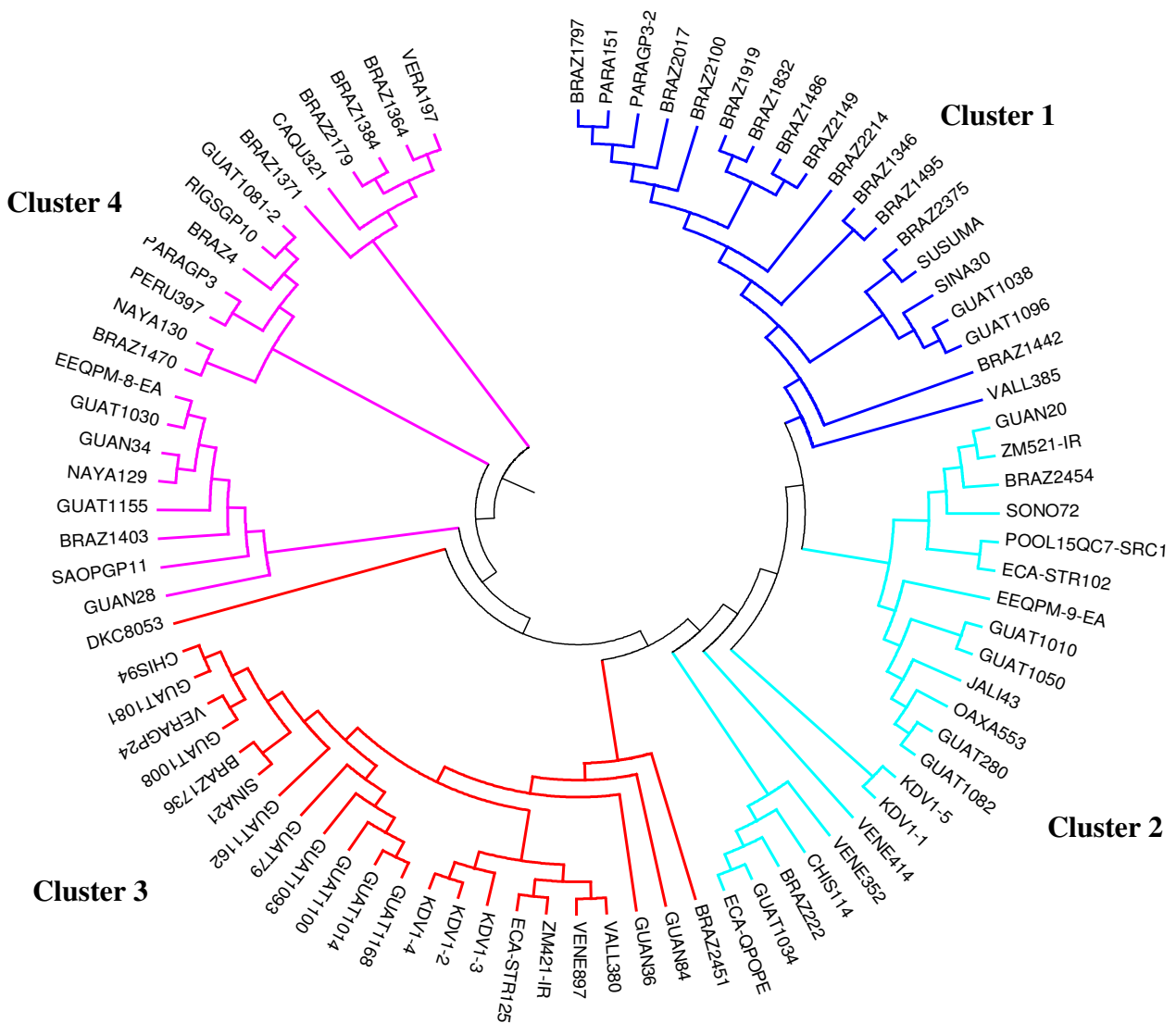


Figure 13. Dendrogram of 84 maize landraces accessions and open pollinated varieties derived by UPGMA from the euclidian genetic distance matrix of the SSR and biophysical data

In the three data sets, the grouping into many subgroups from the major clusters is an indication of the greater diversity among the landrace accessions and OPVs compared to the hybrids and inbred line. The Mantel (1967) matrix correspondence test showed that there was low correspondence between the distance matrices generated from SSR and biophysical/bioassay data ($r = 0.07$) and high (0.12) between the molecular data set and the combined (molecular and biophysical/bioassay) data sets. The cophenetic correlations, which are measures of goodness of fit for the cluster analysis among the three data sets were 0.84, 0.81 and 0.83 for the molecular, biophysical/bioassay and combined (molecular + biophysical/bioassay data sets), respectively.

3.4 Discussion

The significant differences and wide range in the means of the biophysical/bioassay traits related to resistance among the germplasm shows that there is great potential for the development of improved maize genotypes that are resistant to the postharvest insect pests. The biophysical/bioassay and molecular data confirm the existence of genetic divergence in tropical maize germplasm in response to the maize weevil and larger grain borer. This is in agreement with earlier studies that reported the existence of genetic variability of resistance to the maize weevil, larger grain borer and other coleopterans among tropical maize germplasm (Arnason *et al.*, 1994; Mwololo *et al.*, 2010; Tefera *et al.*, 2011). This genetic diversity can be exploited in breeding programmes to introgress resistance to postharvest insect pests into improved varieties using conventional and genetic engineering approaches (Dhliwayo and Pixley, 2003).

Clustering of the individual candidates among the large germplasm evaluated in relation to resistance to the postharvest insect pests was evident. There was no clear cut pattern observed in genetic distances among some of the germplasm evaluated in relation to resistance, particularly among the landrace accessions. For instance some of the susceptible genotypes were closely related with the resistant. This can be explained by the fact that, selectively neutral markers used were not subject to selection and thus resistance, an adaptive trait had low correlation with SSR data. Clear estimates of the genetic distances would be closer when there is association between the loci controlling the phenotypic trait of interest (QTL) and the markers used and when a larger number of traits of interest to a particular situation are evaluated (Roy *et al.* 2004; Lefebvre *et al.*, 2001).

There were more clusters among the landraces compared to the inbred line and the hybrids, an indication of the great variability among them. Landraces have diverse genetic background resulting

from polycrosses, since these materials were not necessarily subject to pedigree selection over a long period of time. These landraces in general are well adapted to different agro-ecological niches and can act as important genetic resources, for useful traits (Yamasaki *et al.*, 2007). The clustering among the hybrids and inbred lines based on the molecular and agronomic data separately and combined had similarities. This implies that the hybrids and inbred lines are more related, and therefore, share common ancestry. In addition, maize hybrids and inbred lines have been subjected to artificial pedigree selection. The hybrids and inbred lines as well were clustered based on their ancestry using the molecular data. Genetic distance within the same series might be large owing to the parents used to constitute them, since only one parent among them might have been common. On the other hand, the differences in the clustering among the landraces based on the molecular and biophysical/bioassay data separately is an indication that the phenotypic variation had little association with the genetic markers used in the study; and a wide genetic variability among them.

There was low correlation (0.26) between the distance matrices generated using SSR and biophysical/bioassay data for all the three data sets in the maize germplasm. This is in accordance with other studies involving cereals such as wheat and barley whereby correlation as low as 0.006 were reported (Roy *et al.*, 2004; Roldan-Ruiz *et al.*, 2001). The lack of correlation between molecular and biophysical/bioassay traits were due to the fact that, molecular markers measure neutral DNA variation and are not subject to selection (Koeber *et al.*, 2002). The molecular analysis provides a wider genome sampling than the phenotypic analysis, therefore, able to give a clear picture of genetic distance. The variation detected by the molecular markers is non-adaptive, hence not affected by natural or artificial selection. Most desirable phenotypic traits in plant breeding are as a result of interaction among expressed genes, but agronomic studies are still vital in germplasm description, and determination of molecular genetic distance is a complement (Donini *et al.*, 2000).

Earlier studies have reported that it is necessary to consider the molecular and phenotypic data separately in genotype divergence studies (Warburton *et al.*, 2002). When the matrices for SSR and biophysical data were merged the correlation was high and this simply due to the auto correlation between same SSR data.

Using the biophysical/bioassay traits, it was possible to discriminate the resistant from the susceptible. However, some of the closely related genotypes, for instance the resistant maize hybrids had been bred using a common parent but were grouped separately if they fell in either of the categories regardless of their origin. The use of phenotypic traits, is therefore, relatively less efficient in discrimination of closely related genotypes and analysis of their genetic relationships compared to the use of molecular markers. Nevertheless, the use of phenotypic traits serves as a general approach in germplasm classification within a collection in relation to a particular trait. However, both methods showed some consistency in clustering of the genotypes regardless of the ability to provide similar description of relationships. The cophenetic correlation computed from the cophenetic value and the original distance matrices is a measure of goodness of fit for a cluster analysis (Rohlf, 1998). The phenograms derived from the microsatellite and agronomic traits data showed good agreement ($r > 0.8$) except for inbred lines between the graphical display of distances and the original matrix, hence being in support of the visual inferences.

5.5 Conclusion

Genetic divergence for resistance to postharvest insect pests exists in tropical maize germplasm. Using the biophysical/bioassay traits which are adaptive, it was possible to discriminate the resistant from the susceptible but not according to their pedigree. The maize hybrids and inbred lines have common ancestry based on the clustering derived from the SSR and biophysical/bioassay data. The landraces were more diverse and had many sub-groups compared to the maize hybrids and inbred lines. The lack of correlations between the divergence measurements emphasizes the need for using more than one method in classifying germplasm in respect to the traits of interest. The most resistant landraces, BRAZ 2451, GUAN 84, GUAT 1162, GUAT 1008, had Latin American origin and the most susceptible landraces had both Latin American and African origins. The highly diverse individuals identified in the large germplasm in relation to insect pest resistance in maize form a basis for future advancements in breeding for resistance as a strategy of IPM.

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Chapter Six

Quantitative Trait Loci Mapping in Maize for Resistance to Maize weevil and Larger Grain Borer

6.1 Introduction

Diseases and insect pests are key biotic factors leading to low yields in maize production. Globally, insect pests destroy approximately 14% of all potential food production, including maize, despite the yearly application of more than 3,000 million kilograms of pesticides (Pimentel, 2007). Farmers in African countries experience grain weight losses as high as 80% during storage (Tapondjou *et al.*, 2000). Host plant resistance through crop breeding remains a cornerstone of an integrated pest management (IPM) strategy to minimize storage losses and impact on grain quality. Genetic variation for resistance to postharvest insect pests has been reported (Mwololo *et al.*, 2010). Studies that have used germplasm with more diversity confirmed the heritability of insect pest resistance (Derera *et al.*, 2001). Antibiosis and non-preference were found to be important mechanisms of resistance to postharvest insect pests in maize grain (Derera *et al.*, 2001). Biochemical and physical characteristics have been associated with resistance to the maize weevil (Arnason *et al.*, 1994). The biochemical components of grain that have been reported to confer resistance to insects or diseases through antibiosis include phenolic acids, hydroxyproline-rich glycoproteins, sugar content, soluble peroxidase and protein inhibitors. Grain hardness is a physical factor which has been closely associated with resistance to grain storage pests by conditioning non-preference (García-Lara *et al.*, 2004). Most of these traits are quantitative in nature which are polygenic, hence under the influence of more than one gene each contributing smaller effects towards expression of that trait.

A quantitative trait locus (QTL) is a region associated with a particular phenotypic trait, and these QTL are often found on different chromosomes (Edwards *et al.*, 1987). The main goals of QTL

mapping in plants are to (a) increase our biological knowledge of the inheritance and genetic architecture of quantitative traits, both within a species and across related species, and (b) identify markers that can be used as indirect selection tools in breeding (Bernardo, 2008). Linkage analysis and association mapping are the two most commonly used methods for QTL mapping. Linkage analysis requires biparental population whereas association mapping requires collections of germplasm to find genes underlying quantitative traits (Yu *et al.*, 2006). Linkage analysis is critical for preliminary location of QTLs and association mapping for precise location (Mackay, 2001).

The availability of a wide range of molecular markers and powerful statistical methods has significantly facilitated QTL mapping. The ability to transfer target genomic regions using molecular markers has resulted in QTL mapping experiments in economically important crops, to identify molecular markers for MAS (Xu, 1998, Semagn *et al.*, 2006). The most common molecular markers used earlier were restriction fragment length polymorphisms, random amplified polymorphic DNA, amplified fragment length polymorphisms and microsatellites (Pinto *et al.*, 2003). Recently, the single nucleotide polymorphism (SNPs) markers have gained preference in the scientific community as the markers of choice in molecular genetics (Slate *et al.*, 2009). They are the most abundant type of genetic polymorphism in most genome and genotyping cost is low. Molecular markers enable breeders to exercise selection on genotypic or DNA-based differences rather than phenotypic differences (Mohan *et al.*, 1997). They therefore, have the potential to increase selection efficiency in breeding through marker-assisted selection by tagging of agriculturally important genes.

Molecular markers have been used to map genes for insect resistance in most major crop species such as maize, rice, wheat, potato and beans. These studies examined specific plant crosses to determine QTLs affecting aspects of plant chemistry, such as maysin in corn and acyl sugars in

tomato, or aspects of plant morphology (trichomes), traits already known to affect resistance to insects (Yencho *et al.*, 1996; Byrne *et al.*, 1996). The Br gene from mung bean, effective against several species of bruchids and the bean bug, *Riptortus clavatus* for Coleopteran resistance have been tagged (Kaga and Ishimoto, 1998). In maize, most of the QTL mapping and identification of candidate genes for resistance has been done for Lepidopterans, particularly the corn ear worm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis* (Hübner) (Byrne *et al.*, 1997). The use of robust DNA markers that map QTL's associated with postharvest insect pests in maize is an important approach in fine mapping and marker assisted recurrent selection (MARS). The first attempt on QTL mapping for Coleopterans was on the maize weevil (García-Lara *et al.*, 2009). There are limited reports on QTL for resistance to the larger grain borer in tropical maize. The objective of this study was to identify QTL for maize weevil and LGB resistance.

6.2 Materials and methods

6.2.1 Germplasm and experimental design

A mapping population of 203 $F_{2:3}$ derived progenies were developed from a cross between susceptible and resistant inbred parents identified from earlier studies, namely CML444 and CKSPL10116, respectively. The $F_{2:3}$ progenies were further crossed to a tester (CML 312/CML 442) to generate F1s which were evaluated in three replicates across six environments at Kenya Agricultural Research Centers (KARI-Kiboko, KARI-Mtwapa, Kirinyiga Technical Institute (KTI), KARI-Kakamega, KARI-Embu and Bukura Agricultural Center) (Table 13, Appendix 5).

Table 13. Characteristics of the 6 trial sites used in the evaluation of the mapping population

Site	Name	Longitude	Latitude	Elevation (masl)	Rainfall (mm)	Temp max (°C)	Temp (min)	Soil texture
1	Bukura	34° 36' E	0° 15' N	1397	1800	20	22	Orthic ferralsol
2	Kakamega	34° 44' E	0° 16' N	1530	1916	29	13	Sandy loam
3	Kiboko	37° 75' E	2° 15' S	975	530	35	14	Sandy clay
4	Embu	37° 42' E	0° 49' S	1540	1200	24	18	Clay loam
5	KTI	37° 19' E	0° 34' S	1282	1500	24	18	Clay loam
6	Mtwapa	39° 219 E	4° 347 S	30	965	29	22	Sandy soils

masl=meters above sea level; Temp=temperature; max=maximum; min=minimum; KTI=Kirinyaga Technical Institute farm

The experimental design for evaluation of the test cross F1s was an Alpha lattice design (35 x 6) of two rows replicated 3 times. Data was collected on husk cover tip length. Harvested grains from the six locations were evaluated in the laboratory for phenotypic insect pest resistance traits for quantitative trait loci analysis namely: grain damage (GD), grain weight loss (WL), flour weight (FW) and number of insects (AP). The Grain hardness (GH) and protein content (PC) were determined as putative traits of resistance as described in chapter four.

Leaf samples were picked from 10 plants of each of the 203 $F_{2:3}$ families before crossing with the tester on 3 weeks old seedlings for genotyping. DNA was extracted from the leaves using the 96-

well format high throughput protocol (Mace *et al.*, 2003). Six hundred (600) single nucleotide polymorphic (SNPs) markers were screened for polymorphism between the parental lines, 340 SNPs were polymorphic, and used to genotype the F_{2:3} derived progeny. Genotyping was done using the K-Biosciences Competitive Allele-Specific PCR genotyping system (KASP)-(KBiosciences Ltd Unit 7 Maple Park Essex Road Hoddesdon EN11 0EX UK).

6.2.2 Bioassay and biophysical data analyses

All traits were subject to analysis of variance within and across environments by the proc mixed procedure of SAS statistical package (SAS Institute, 2003). Normality test was done using Anderson-Darling test of MINITAB 14 software. Data on flour produced (%) was arcsine transformed before statistical analyses for normalization. Complete and incomplete blocks and locations were considered as random effects and entries as fixed effects when calculating the means for the mean environment. The means were used for the QTL analysis. Estimates of variance components, including genotypic variance (σ^2_g), genotype $\times$ environment interaction ($\sigma^2_{g \times e}$), phenotypic variance (σ^2_p) and residual (σ^2) were calculated by equating the mean squares to their expected values (Searle, 1971). Broad-sense heritability (H) on entry mean basis was estimated according to Hallauer and Miranda, (1981). Phenotypic correlation coefficients were calculated among resistance and biophysical traits from adjusted entry means across environments for each parameter.

6.2.3 Genetic mapping and QTL analyses

Genetic linkage maps for the population were constructed using Joinmap 4.0 (VanOoijen and Voorrips, 2006). Segregation at each marker locus was tested against the expected Mendelian segregation ratio (1:2:1) using a Chi-square test. Grouping of the markers (loci) was done with a

minimum logarithm of odds (LOD) score of 2.0. Minimum distance between loci of 5 cM and a goodness of fit jump threshold of 5 for removal of loci and 1 as the number of added loci after which to perform a ripple for verification of locus orders was adapted (VanOoijen and Voorrips, 2006). Recombination threshold was set at 0.4 and the recombination frequencies between marker loci was estimated by multi-point analyses and transformed into centimorgans (map distance) using the Kosambi mapping function.

Quantitative trait loci mapping was performed for protein content, grain hardness, husk cover tip length, grain damage, weight loss, adult progenies and flour weight in 203 test cross progenies. Composite interval mapping with a LOD-score of 3.0 as implemented in PlabQTL was used in QTL mapping (Zeng, 1994; Utz and Melchinger, 1996). The QTL analyses were based on adjusted entry means of the 203 test cross progenies across six environments for the maize weevil and LGB grain damage traits and the additional traits associated with resistance. Previous studies suggest a LOD threshold value between 2 and 3 (Lander and Botstein, 1989). A threshold value of 2.5 has been used in other studies of QTL in maize and this minimizes the risk of type 11 error (Cardinal *et al.*, 2003). The QTL mapping was initially carried out using simple interval mapping to identify the major QTL, a genetic model without epistasis based on the average effect of an allele substitution (Bernardo, 2002). Subsequently, composite interval mapping was employed. The cov/+select option was used to detect closely linked QTL of opposite effects.

Estimates of QTL positions were obtained at the point where the LOD score reached its maximum value in the region under consideration. Gene action was defined in relation to direction of resistance for each trait based on the additive effect value. The parameters associated with resistance after insect pest infestation (grain damage, weight loss, flour weight and adult progeny) indicate resistance if the values are smaller, therefore QTL with additive effect negative values ranging to

+0.20 indicate additive gene action. In relation to protein content, husk cover tip length and grain hardness, greater resistance is associated with positive trait values, hence the larger the value in the positive direction the better.

6.3 Results

6.3.1 Analyses of biophysical and resistance traits

There were highly significant differences ($P \leq 0.001$) among the test crosses for all the traits evaluated (Table 14). The environment variance was also significant among all the traits. However, the genotype $\times$ environment interaction was not significant for all the traits. The components of variance, σ^2_g and $\sigma^2_g \times e$ of the test cross progenies were highly significant for all the agronomic and resistance traits evaluated, and the heritability ranged from 0.23 to 0.54 (Table 14). They were low for the grain hardness and number of insects (< 0.3) and intermediate (0.4-0.58) for the protein content, husk cover tip length, grain damage and flour weight.

Table 14. The mean estimates of variance components and heritability for postharvest insect pest resistance parameters for test cross of F2:3 families from the cross CML44 $\times$ CKSPL10116

Parameter	Protein Content (%)	Husk cover tip length (cm)	Grain hardness (N)	Weight loss (%)	Grain damage (%)	Adult progeny	Flour Weight (%)
Means	10.91	5.72	160.61	14.37	43.90	207.04	2.01
Range	10.20-11.50	3.90-7.80	146.20-175.60	10.90-21.40	30.90-57.00	140.5-290.05	1.10-3.30
σ^2_l	0.26**	7.04**	4.92	47.82**	218.28**	3940.04**	0.95**
σ^2_g	0.04**	0.30**	9.78	2.19**	10.98**	228.48**	0.10**
$\sigma^2_{g \times l}$	0.04	0.14	0.033	0.36	9.24	364.16	0.05
σ^2	0.42	1.58	233.76	45.93	286.66	8852.68	1.70
H	0.54	0.58	0.23	0.45	0.41	0.29	0.51
h^2	0.21	0.22	0.15	0.20	0.14	0.16	0.19

σ^2_l —environmental variance; σ^2_g —genotypic variance; $\sigma^2_{g \times l}$ —genotype $\times$ location variance; H—heritability; h^2 —narrow sense heritability

The phenotypic correlations among the resistance traits were significant ($P < 0.001$) except for the grain hardness (Table 15). The flour weight, grain damage, weight loss and the number of insects

were positively correlated, but negatively correlated with the protein content and grain hardness. The protein content and grain hardness were positively correlated.

Table 15. Phenotypic correlations among resistance parameters to the maize weevil and larger grain borer

	FW	GD	WL	AP	PC	GH
FW		0.70**	0.85**	0.82**	-0.30**	-0.20ns
GD	0.67		0.74**	0.74**	-0.28**	-0.30ns
WL				0.78**	-0.35**	-0.28ns
AP					-0.5**	-0.25ns
PT						0.4**

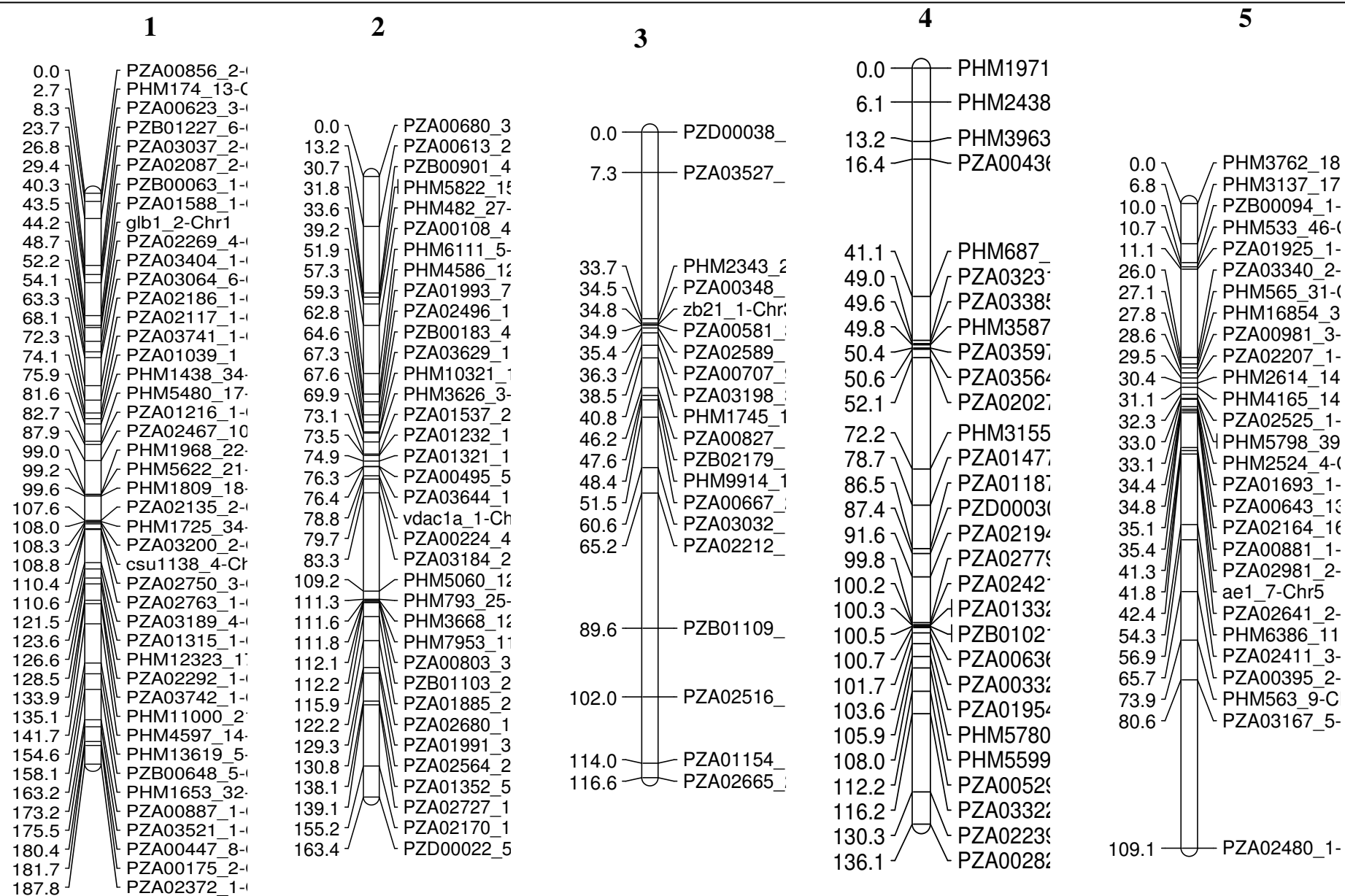
Key: FW-flour weight; GD=grain damage; AP=No. of insects; PC=protein content; GH=grain hardness; WL=weight loss

6.3.2 Linkage map

The final linkage map was drawn from a set of 249 SNPs, following exclusion of those which did not conform to the 1:2:1 Mendelian segregation ratio. The distortion observed was due to surplus of heterozygotes. Overlapping markers were excluded from the map thereby ending up with a total of 244 markers. This led to 10 linkage groups corresponding to the 10 chromosomes of the haploid maize genome, ranging in length from 66.7 to 188 cM. The linkage map was 1102 cM in length with average marker distance of 4.5 cM between loci (Figure 14). The number of markers mapped per chromosome ranged from 13-44. The order and placement of the SNP markers were generally in a good agreement with the maize reference map except in chromosome 7.

Figure 14. Genetic map of CML444 × CKSPL10116 F2:3 maize mapping populations generated using the 249 polymorphic SNP markers

*Numerical numbers (1-10) represent the chromosome numbers of the maize genome



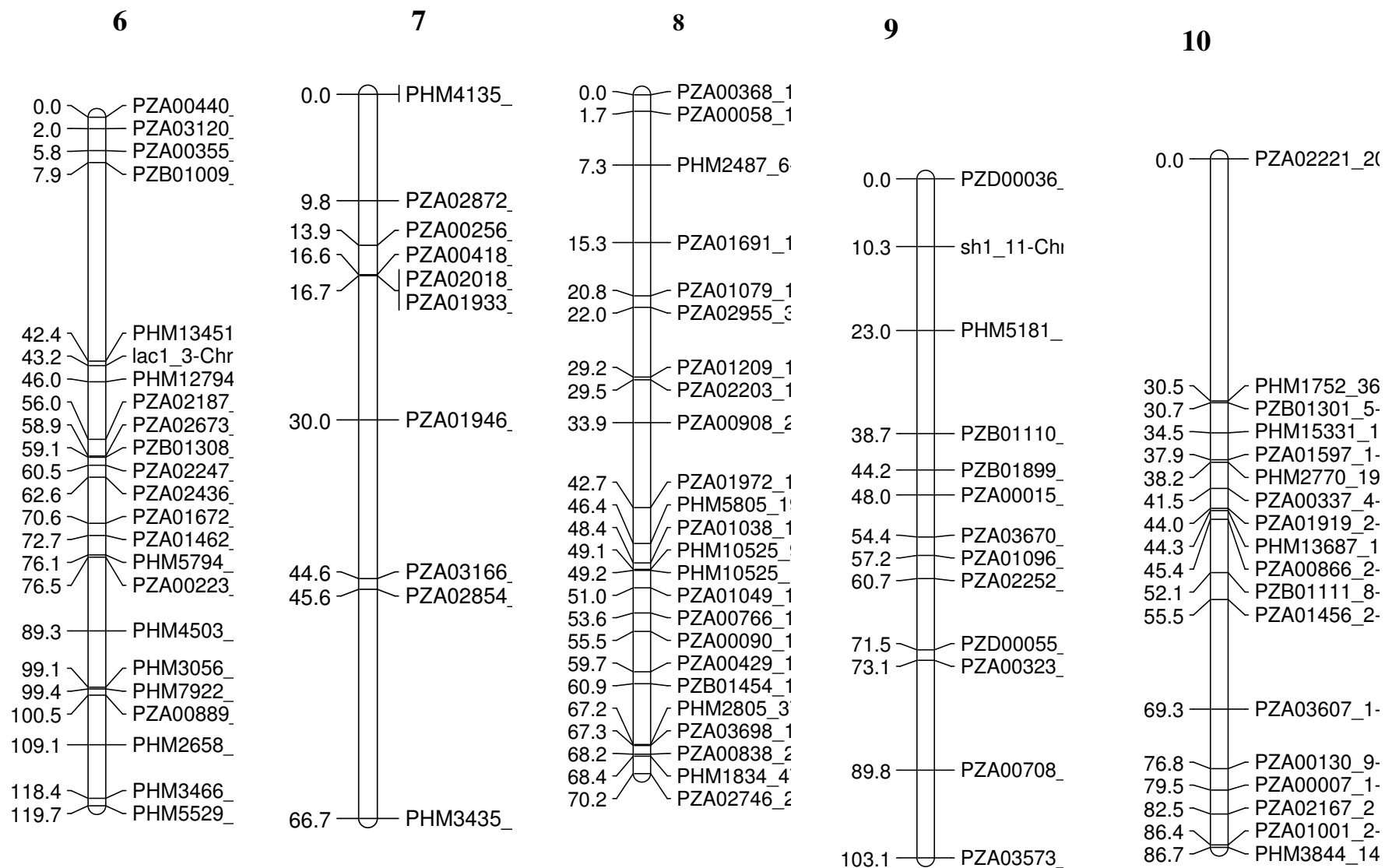


Figure 15. Genetic map of CML444 x CKSPL10116 F2:3 maize mapping populations generated using the 249 polymorphic SNP markers

*Numbers (1-10) represent the chromosome numbers of the maize genome

6.3.3 Quantitative trait loci analysis

Four QTLs for protein content were detected on chromosomes 1, 2, 3 and 8 respectively (Figure15; Figure 16; Table 17) which explained 47.8 % of the phenotypic variation, with individual QTL accounting for 10-15 %. A simultaneous fit with all the four QTL based on the cross validation explained 75.8 % of the adjusted genetic variance. The additive gene action ranged from 0.11 to 0.15. All the QTLs (alleles) which were from the resistant parent contributed to the increased protein content, which is a putative trait of resistance in maize grains to postharvest insect pests.

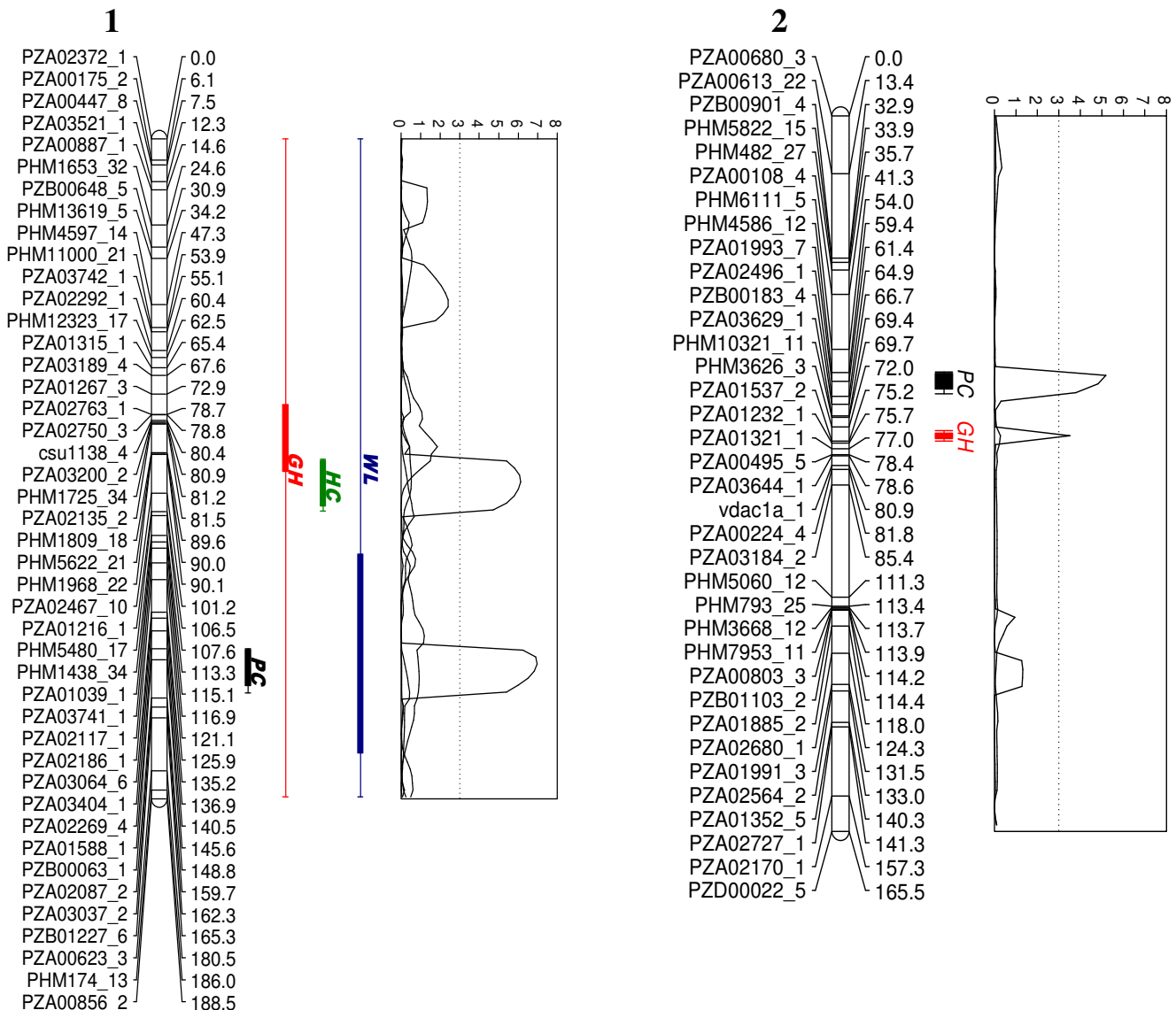


Figure 16.Quantitative trait loci (QTL) for grain hardness (GH), husk cover tip length (HC), weight loss (WL) and protein content (PC) in chromosomes 1 and 2

*Numbers represent the chromosome numbers of the maize genome

Table 16. Summary of QTL positions and additive effects for protein content, grain hardness and husk cover tip length as traits of resistance to postharvest insect pests in maize grain estimated from test crosses of 203 F2:3 families of the cross CML44 × CKSPL10116

Trait	QTL	Chromosome	Position (cM)	Marker and Loci interval		Supportive Interval	LOD	R ² (%)	Additive Effect	SE	Adj σ^2_g (%) explained
Protein content	1	1	138	PZA03064_	106-111	134-142	4.36	11.7	0.112	0.022**	75.8
	2	2	58	PHM4586_1	202-253	54-66	3.34	9.0	0.22	0.021**	
	3	3	98	PZB01109_	286-92	92-110	5.81	15.3	0.15	0.023**	
	4	8	34	PZA00908_	61-251	32-42	5.23	13.8	0.144	0.021**	
Husk cover	1	1	110	PHM5480_1	32-10	106-114	5.65	15.1	-0.405	0.089**	37.3
	2	4	26	PZA00529_	50-34	22-32	3.5	9.6	0.291	0.086**	
	3	7	66	PZA0284_	100-22	62-66	4.17	12.3	-0.385	0.093**	
Grain hardness	1	1	88	PZA02135_	257-15	84-90	3.64	8.3	5.84	1.464**	24.3
	2	1	120	PZA3741_	80-116	116-126	4.01	9.1	-8.36	1.469**	
	3	2	74	PHM3626-3	34-244	72-76	3.51	7.7	5.41	1.30**	
Oil content	1	3	90	PZB01109_	92-286	78-98	3.92	8.5	0.11	0.029**	32.1
	2	4	30	PHM5599_	234-35	28-34	7.66	16.0	0.15	0.03**	
	3	8	3	PZA00440_	108-224	0-4	3.38	8.1	0.10	0.026**	

***Key:** cM=centimorgan; LOD=likelihood-ratio test statistic; R²=phenotypic variation; SE=standard error; Adj σ^2_g =adjusted genetic variance (%)

* The coefficient of determination (R²) gives the phenotypic variation (%) explained by a given QTL

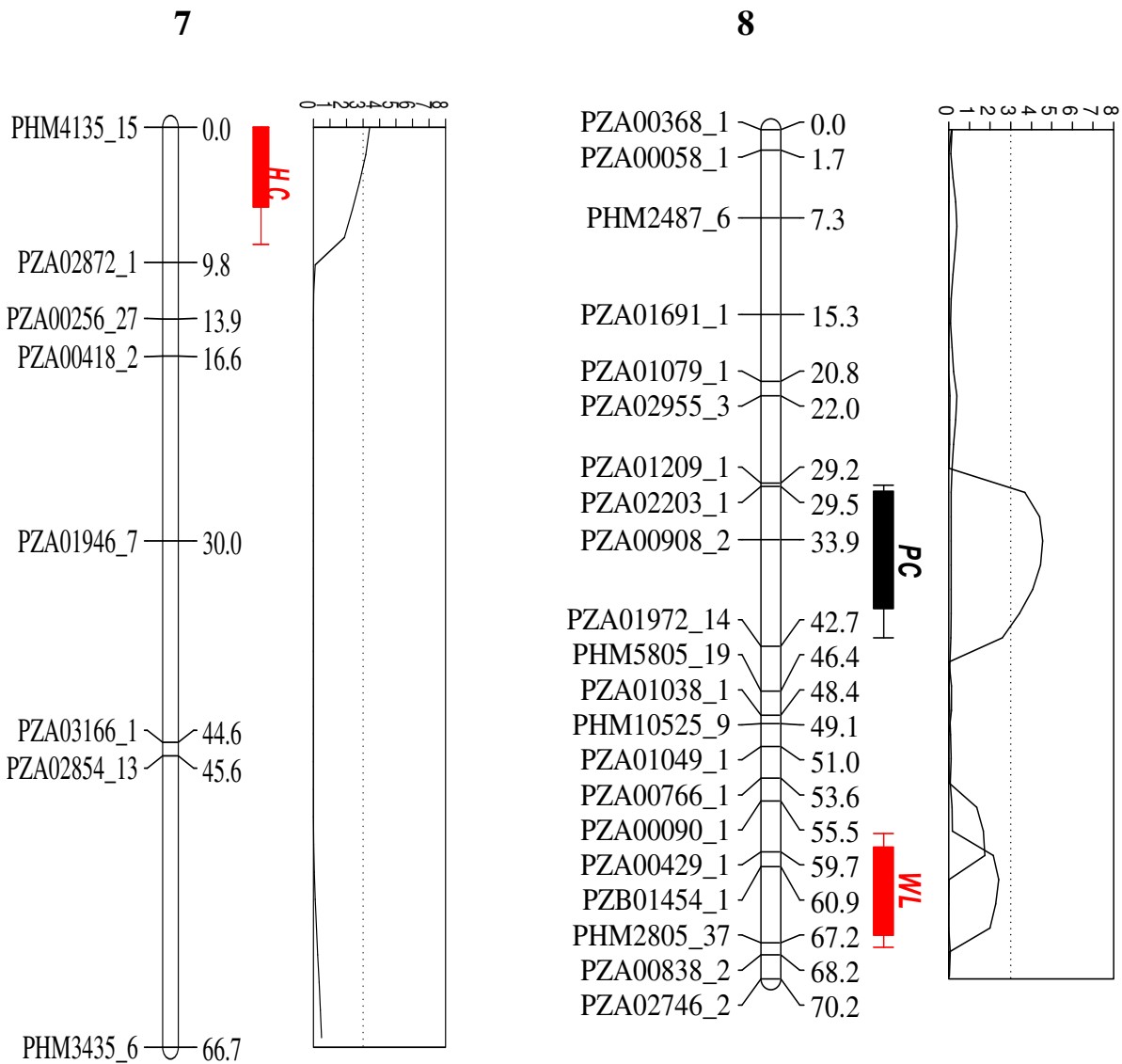


Figure 17. Quantitative trait loci (QTL) for husk cover tip length (HC), protein content (PC) and weight loss (WL) in chromosomes 7 and 8.

*Numerical numbers represent the chromosome numbers of the maize genome

Three QTL were identified in chromosome 1 (two) and 2 (one) for grain hardness, one coming from the susceptible and two from the resistant parent. The phenotypic variation explained by the QTLs in chromosome 1 was 7% and that from chromosome 2 was 5%. The additive effects were 5.84 and -

8.36 for the QTLs in chromosome 1 and 5.41 for that in chromosome 2. Three QTL on chromosomes 3, 4 and 8 and they all came from the resistant parent. The additive effect ranged between 0.1 and 0.15 and they explained 32% of the phenotypic variation. Three QTL on chromosomes 1, 4 and 7, respectively were detected for husk cover tip length (Table 17). These were all stable based on the cross validation statistics. The additive effects were -0.405, 0.291 and -0.385, respectively. This indicates that the first and third QTL were from the susceptible whereas the second was from the resistant parent. As a result, the alleles for increase in the husk cover tip length came from the resistant and the susceptible parent. The simultaneous fit with all the three QTLs explained 21.6% of the phenotypic variation and 37.3% of the genetic variance.

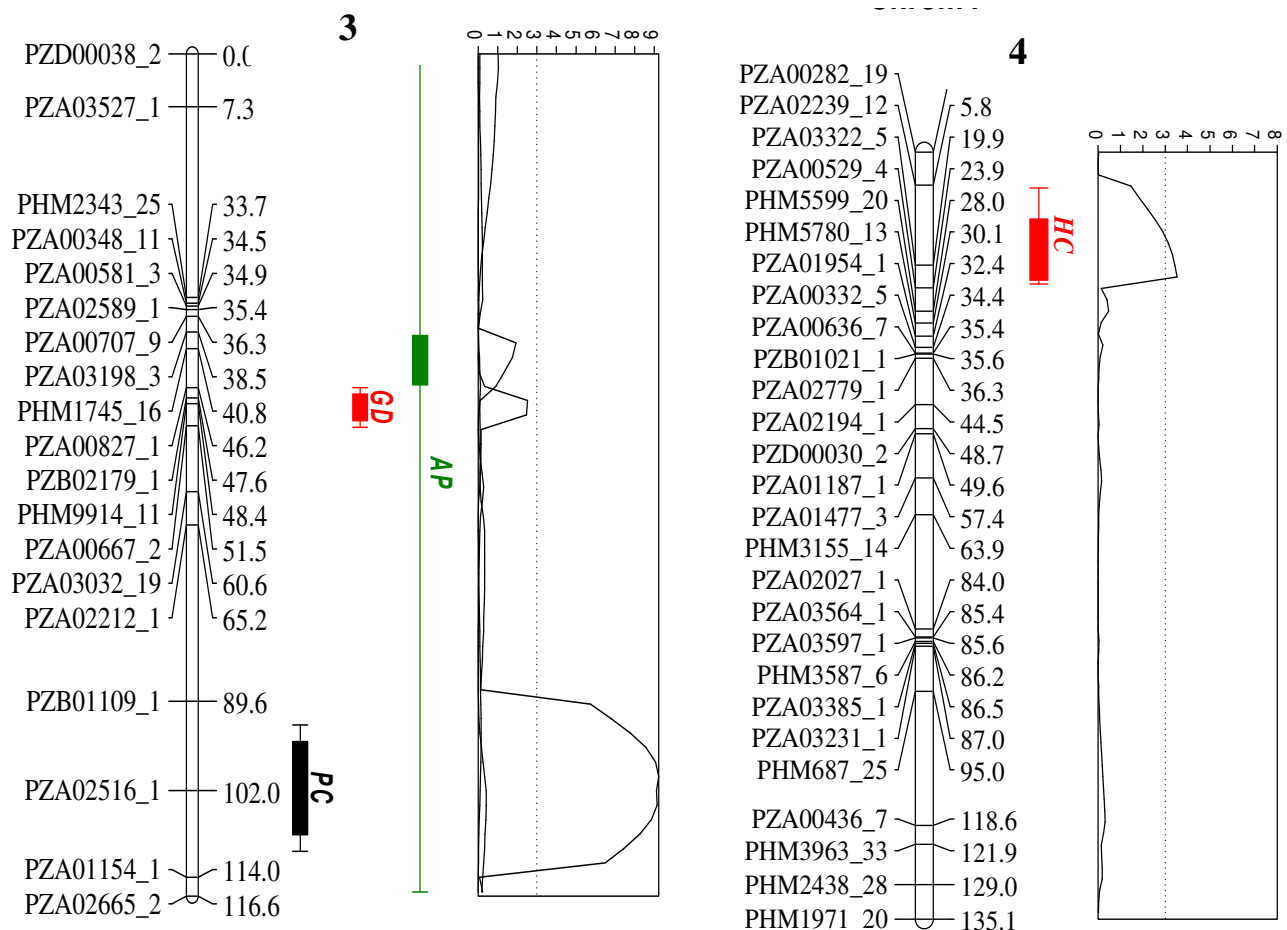


Figure 18. Quantitative trait loci (QTL) positions for grain damage (GD), protein content (PC), number of insects (AP) and husk cover tip length (HC) in chromosomes 3 and 4.

Four chromosomal regions were identified as common putative QTL for grain damage by the maize weevil and LGB (Figure 18, Table 18). The QTLs were on chromosomes 3, 5 and 9. The additive effects ranged from -2.6 to -1.25, therefore, the QTLs were all from the resistant parent. The simultaneous fit with all the three QTLs explained 21.8% of the phenotypic variation and 43.3% of the genetic variance. Four QTLs were detected for weight loss in chromosomes 1, 5, 8 and 9. The additive effects ranged from -1.13 to -0.823. All the four QTLs explained 18.3% and 70.3% of the phenotypic and genetic variation respectively; and all alleles came from the susceptible parent. Two QTLs were detected for the flour weight in chromosomes 5 and 9, with additive effects of -0.20 and -0.19, respectively. The two explained 14 % of the phenotypic variance and 27.4% of the genotypic variance. The alleles came from the resistant parent. Two QTLs were detected for the number of insects in chromosomes 5 and 9, which explained 12.4% and 55.4% of the phenotypic and genetic variance respectively. The additive effects were -12.44 and -12.95, respectively. The alleles came from the resistant parent.

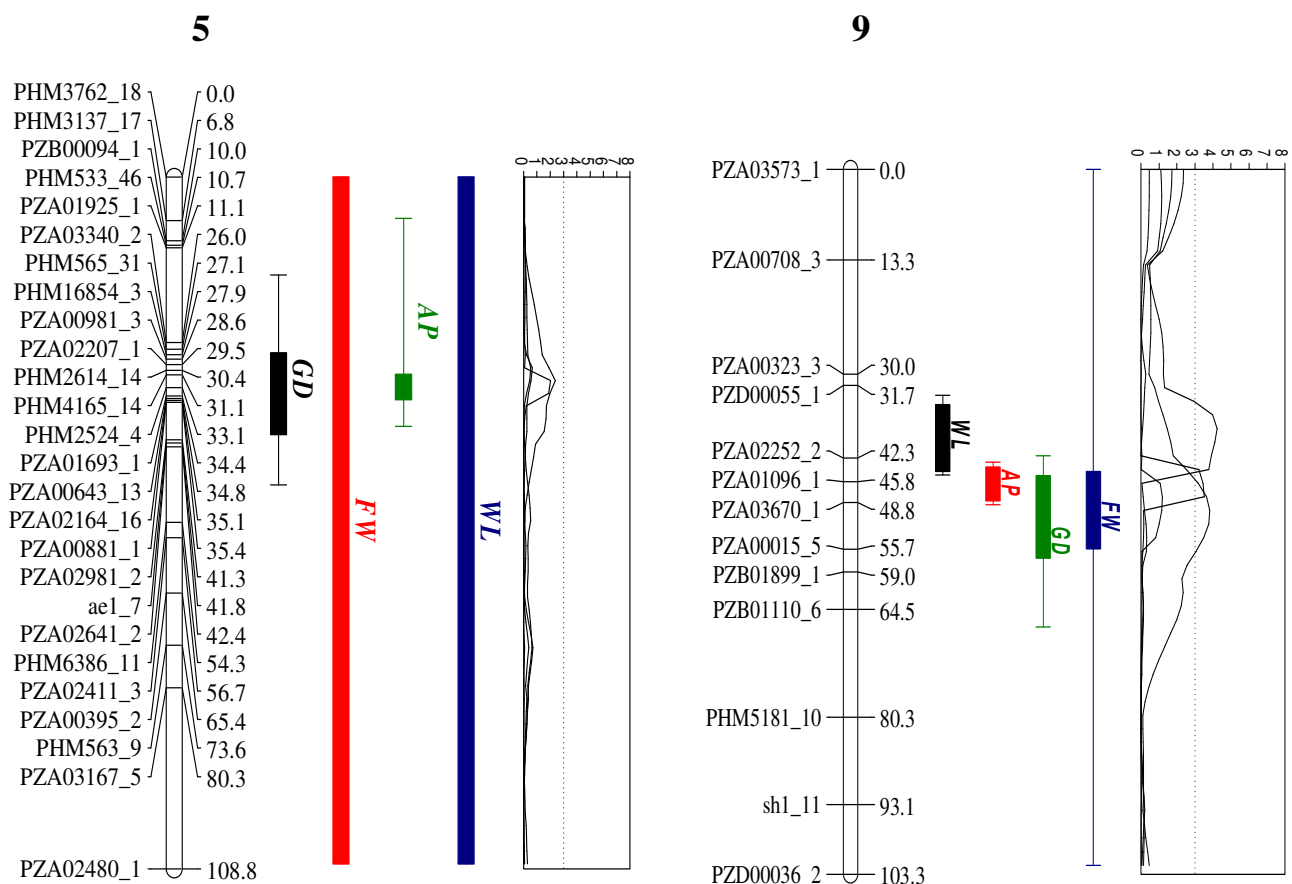


Figure 19. Quantitative trait loci (QTL) for grain damage (GD), flour weight (FW), number of insects (AP) and weight loss (WL) in chromosomes 5 and 9.

Table 17. Summary of QTL positions and additive effects for maize weevil and larger grain borer damage indices on maize grain estimated from test crosses of 203 F2:3 families from the cross CML44 × CKSPL10116

Trait	QTL	Chromosome	Position (cM)	Marker and Loci interval	Supp interval	LOD	R ² (%)	Additive Effect	SE	Adj σ^2_g
Grain damage	1	3	20	PZA02135_ 281-16	0-52	3.51	9.7	-1.42	0.344**	43.4
	2	5	36	PZA00881_ 27-135	26-40	4.23	8.9	-2.5	0.61**	
	3	5	32	PHM4165_1 27-135	28-40	3.31	7.2	-1.24	0.332**	
	4	9	48	PZAOIO96_ 38-114	42-58	4.09	10.5	-2.6	0.61**	
No. of insects	1	3	40	PZA03198_ 277-14	38-48	2.7	7.2	-8.13	2.417**	55.4
	2	5	32	PH4165_1 27-135	30-34	3.77	10.2	-12.44	2.934**	
	3	9	46	PZA01096_ 65-114	44-50	4.36	11.7	-12.94	2.823**	
Flour weight	1	5	30	PZA02207_ 196-261	28-34	3.88	10.4	-0.2	0.49**	27.4
	2	9	48	PZA01096_ 65-114	42-50	3.81	10.2	-0.196	0.47**	
Weight loss	1	1	186	PHM174_13 13-59	182-188	5.25	14.7	-1.033	0.253**	70.5
	2	5	10	PHM3137_1 19-177	6-12	3.72	10.3	-0.935	0.261**	
	3	8	60	PZA00429_ 47-123	58-66	3.14	8.5	-0.823	0.239**	
	4	9	44	PZA02252_ 86-65	42-46	3.62	10	-1.127	0.240**	

Key: cM= centimorgan; Supp=supportive interval; LOD=likelihood-ratio test statistic; R²=phenotypic variation; SE=standard error; Adj σ^2_g =adjusted genetic variance.

*The QTLs presented for the damage indices in the table were common for both maize weevil and LGB.

6.4 Discussion

There were high and significant phenotypic correlation coefficients among the traits for assessing resistance in maize grains, namely grain damage, weight loss, flour weight and number of insects. The clustering of QTL linked to these traits in chromosomes 3, 5, and 9 supports this observation. Most of the QTL for maize weevil and LGB damage indices were common. This confirms that the mechanisms of resistance to the maize weevil and LGB are common and controlled by genes located in the same chromosome. The QTL for some of the resistance traits were located in common genomic regions found on chromosomes 3, 5 and 9. Strong association was observed between grain damage, number of insects, flour weight and weight loss (chromosomes 3, 5, 9). Chromosomes 5 and 9 had QTL associated with all four traits in the latter, whereas chromosome 3 had QTL for grain damage, number of insects and protein content. The results are in agreement with other reports whereby most QTL for resistance were contributed by the resistant parent with few from the susceptible parent (Jampantong, 1999). Additive genetic effects were prevalent for all the detected QTL.

Resistance to insect pests in maize grain is conditioned by biophysical and or biochemical traits. The QTL for protein content accounted for over 75% of the genetic variation, an indication that this is a reliable trait for consideration in breeding for resistance. The mapping of QTL for protein content in the same region with the resistance traits was an indication of their close association. Previous studies have reported the role of proteins in maize grain for weevil and larger grain borer resistance (García-Lara *et al.*, 2004; Mwololo *et al.*, 2012; Siwale *et al.*, 2009). The protein is involved in cell wall structure strengthening together with biochemical components such as simple phenolic acids and diferulates leading to harder grains. The QTL in chromosome 3 are mapped to a genomic region associated with genes linked to cell wall sugar

components in maize grain pericarp, and for whole plant fiber (Holland, 2007; Hazen *et al.*, 2003; Cardinal *et al.*, 2003). Quantitative trait loci for galactase, xylose and arabinose have also been mapped to chromosomes 2 and 3 (Hazen *et al.*, 2003). Genes for peroxidase and simple phenolic compounds which are linked to maize weevil resistance were mapped to chromosome 2 (Brewbaker *et al.*, 1985). Genes of structural proteins like HRGPs using cDNA probes, have been mapped to chromosome 2 (Davis *et al.*, 1999). Similarly, the protein content had QTL in chromosomes 2 and 3, therefore, it is closely linked with the cell wall components associated with grain resistance to insect pest. Two QTL for protein and oil content were mapped to the same genomic region (chromosomes 1 and 8) as coumaric acid (CA) and ferulic acid (FA), found to confer resistance to storage pests (Davis *et al.*, 1999; García-Lara *et al.*, 2010). Extensions, which are proteins, are bound to the cell wall complex through covalent linkages with pectins by cross linking with sugars or oxidation by peroxidase forming stable protein links (Cassab, 1998).

Earlier studies have reported grain hardness as a putative trait of resistance to postharvest insect pests in maize (García-Lara *et al.*, 2004). There were negative correlations between grain hardness and the resistance traits though low, and was positive to protein content. The QTL for grain hardness were located in chromosomes 1, 2 and 3. Earlier studies have indicated that genes linked to the kernel characteristics such as kernel size and endosperm softness are located in chromosome 1 (Gudrups *et al.*, 2001). The three QTL correspond to those for protein content given that out of the four, two were in chromosomes 1, 2 and 3. The results from this study show links between proteins and grain hardness; and insect damage traits, suggesting common genetic basis for traits associated with resistance to postharvest insect pests. Storage proteins in food grain are responsible for the association between starch grains and endosperm matrix proteins, thus influencing the grain hardness (Hoseney, 1987; Dombrink-Kurtzman and Bietz, 1993). The

presence of abundant and organized protein bodies is associated with greater adherence and better distribution of starch granules in the endosperm and, consequently, higher physical resistance of the grain (Gibbon *et al.*, 2003). Husk cover (bare tipped or complete husk cover) has been associated with controlling the level of field infestation of the maize cobs by postharvest insect pests including maize weevil and larger grain borer (Warfield and Davis, 1996). Three QTL were identified for husk cover tip length in chromosomes 1, 4 and 7. These genomic regions have been found to have the genomic regions for both biochemical and biophysical characteristics of maize grains (García-Lara *et al.*, 2010). The husk cover characteristics have been reported to be under control of additive gene action with non additive playing a minor role and this concurs with the results from this study (Brewbaker and Kim, 1979).

The results show that there was clustering of QTL for postharvest insect pest resistance with QTL for disease, lepidopteran insect resistance and structural cell wall biochemical components. Most of the QTL for resistance traits are located in chromosomes 1, 5 and 9. According to Papst *et al.*, (2004), QTL for stem borer resistance are not randomly distributed in the maize genome but occur in chromosomes 1, 5 and 9. In addition, genes for lignin biosynthesis, a biochemical component associated with stem borer resistance are located in the same genomic region as those for stem borer resistance. Furthermore, the study supports the functional relationship between gene and QTL mapping for insect pest resistance, which has been previously established by other authors for insect pests of maize (Cardinal and Lee, 2005; Krakowsky *et al.*, 2007; Meyer *et al.*, 2007). The QTL for disease resistance, which have been mapped before and recently, map in some genomic areas reported in this study (Kelley *et al.*, 2012). The clustering of QTL forms an important basis for breeding for resistance. These should be prioritized in identifying markers for use in stacking multiple resistance into one variety (Bergvinson and García-Lara, 2006).

6.5 Conclusion

Quantitative traits loci (QTL) associated with postharvest insect resistance traits were identified. The QTL for maize weevil and LGB indices were common, therefore, the mechanisms of resistance are similar. The QTL affecting weevil and LGB resistance traits in tropical maize are important in analyzing genetic variation and increasing the efficiency of maize breeding programmes. The chromosomal regions containing genes involved in the synthesis of cell wall components could be associated with resistance to different insect species in maize. Marker assisted recurrent selection would be useful in transferring the QTL alleles into susceptible and promising inbred lines. The identification of QTL associated with weevil and LGB resistance in tropical maize will enable breeders to exploit the genetic variation and increase the efficiency in delivering maize lines resistant to storage pests to increase food security for subsistence farmers. Stable QTL are needed for further validation and adoption in marker assisted selection. The QTL identified in this study explained high genetic variation compared to phenotypic variation, thus implying that they are novel in relation to resistance. Consequently, this forms a basis for further work towards establishment of a breeding program which depends on molecular tools.

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Chapter Seven

General Discussion and Conclusion

7.1 Discussion

Postharvest insect pests cause serious losses to cereals in both quality and quantity. The maize weevil and LGB are insect pests that are among major pests responsible for serious losses of maize in storage worldwide. Their damage results in direct loss of food, and may also reduce future maize production for farmers who save grain as seed (Tefera *et al.*, 2010; Pingali and Pandey, 2001). Protection of food grains against the deleterious effect of postharvest insect pests is important in order to attain food security. Host plant resistance through genetic improvements remains a cornerstone of integrated pest management to minimize losses due to postharvest insect pests. This study highlighted the use of resistant genotypes as key in developing sustainable integrated pest-management (IPM) programmes for the maize weevil and LGB. Effective breeding for resistance requires identification of resistant germplasm within the maize gene pool and understanding the underlying mechanisms of resistance. Moreover, breeding progress in relation to postharvest insect pests would also depend on the magnitude of genetic variability within the maize germplasm. The higher the variability for a given trait, the higher the genetic gain expected during selection processes.

There was great variation in resistance to the maize weevil and LGB among the maize hybrids, OPVs and landraces evaluated. The low percentage of grain weight loss among the resistant genotypes indicates that they have genes that confer resistance to the weevil and LGB. Dual resistance to the weevil and LGB was observed in the maize germplasm evaluated. These results therefore suggest possibility of identification of sources for a breeding programme to develop genotypes with multiple resistance to postharvest insect pests. Grain hardness levels were high in

the resistant compared to susceptible genotypes. Grain resistance to postharvest insect pests have been attributed to physical factors such as grain hardness, and to biochemical traits such as phenolic compounds (Garcia-Lara *et al.*, 2004). Soft endosperms have been associated with high infestation by maize weevil due to easy tunneling and adequate flour for feeding (Siwale *et al.*, 2009). Grains with hard texture are difficult to penetrate and produce low flour content upon attack by storage pests. As a result, hard grains are not able to support large number of the damaging insects, thus this trait can be selected for in breeding for resistance insect pests.

In this thesis, evaluation of a large and diverse set of germplasm showed that mechanisms conferring resistance to maize weevil and LGB were highly correlated. Phenotypic and genotypic correlations between the traits in respect to the two insect pests were high and significant. The high significance and genetic correlation for the weight loss implies that the genetic influence on resistance to the two postharvest insect pests among the genotypes is similar. This suggests that genotypes selected for resistance to one insect would also be resistant to the other insect. The presence of resistant genotypes among the germplasm screened suggests that sources of resistance exist among the landrace accessions and open pollinated varieties that could be used to develop improved maize hybrids resistant to the maize weevil and LGB.

Protein content was high in resistant genotypes compared to susceptible genotypes. The current results based on the large number of and diverse germplasm evaluated confirm that, protein content in combination with other factors in the maize grain contribute to insect pest resistance. Storage proteins in cereal grain are responsible for the association between starch grains and endosperm matrix proteins, thus influencing grain hardness (Hoseney, 1987; Dombrink-Kurtzman and Bietz, 1993). Regions of hard endosperm in the corn grain have a higher alpha- and gamma-zein content (Paiva *et al.*, 1991). In other studies it has been shown that the presence

of organized protein bodies is associated with greater adherence and better distribution of starch granules in the endosperm and, consequently, higher physical resistance of the grain (Gibbon *et al.*, 2003). It is, therefore, clear that there are key genes responsible for the synthesis of key proteins associated with postharvest insect pest resistance. There is thus an opportunity to investigate further the protein composition in the resistant and susceptible genotypes. Other studies have similarly shown that genotypes resistant to the maize weevil have high levels of protein content compared to susceptible genotypes (Arnason *et al.*, 1997; Siwale *et al.*, 2009). This is also consistent with other studies, which found that hydroxyproline-rich proteins (extensins), that are known to accumulate in the pericarp, play a role in resistance to insect pests (Dhliwayo and Pixley, 2003; García-Lara *et al.*, 2004). Other factors which have been associated with resistance of the grain to insect attack include phenolic compounds and soluble peroxidase (García-Lara *et al.*, 2004).

The commercial cultivars were the most susceptible to weevil and LGB attack among the hybrids evaluated. This shows that integration of resistance to postharvest insect pests has not been routinely done by breeding programmes, thereby leading to high postharvest losses. Most breeding programmes focus on increasing yield without due focus to postharvest insect pests (Derera *et al.*, 1999). Breeding for resistance to postharvest insect pests should be prioritized as equally important to safeguard farmers from huge losses due to their attack on food grains. High genetic diversity was observed among the landrace accessions compared to the hybrids and inbred lines. The high genetic variability can be attributed to diverse genetic background resulting from polycrosses, since these materials are not necessarily subject to pedigree selection (Yamasaki *et al.*, 2007).

The inheritance of resistance to the post-harvest insect pests is mostly additive and non-additive to a lesser extent (Dhliwayo and Pixley, 2003). The identification of resistant maize hybrids and landraces showed that breeding for resistance to the maize weevil is not only feasible, but has already resulted in resistant hybrids being released in Kenya. These could be grown to reduce damage by weevil and LGB in storage. The resistant landraces could be used as additional new sources of resistance. Exploiting the additive effect of inheritance for resistance to postharvest insect pests would ensure quick genetic gain, thereby being able to make progress in breeding for resistance.

Most of the resistant hybrids and inbred lines were flints whereas the susceptible were dent; and this was contrary in the landrace accessions, whereby the resistant and susceptible were dent. Dent maize genotypes are highly susceptible to the maize weevil attack (Kim *et al.*, 1988); and the results of this study confirm this to a large extent. Maize genotypes with flint grain texture were reported to be resistant to weevil (Kossou *et al.*, 1993). The results further show that grain texture was not the only factor responsible for grain resistance to the postharvest insect pest attack; as was evident among the landraces that the mechanisms of resistance had little to do with the grain texture. As such, resistance in the landraces can be associated with biochemical components such as phenolic acids and soluble peroxidase. Breeding programs can therefore target tagging of genes which are associated with these biochemical components to identify tightly linked markers for use in loci introgression through marker assisted recurrent selection. The grain texture was positively correlated ($r = 0.7$) with the grain hardness among the hybrids, whereby the flints were harder compared to the dents. Flint grains have vitreous endosperm which is hard and crystalline, and possesses a continuous and abundant protein matrix, which has larger and compact, polygonal starch granules (Pratt *et al.*, (1995). Dent grains have a

farinaceous endosperm, which is opaque and soft-textured, a discontinuous protein matrix and starch granules and are less aggregated and surrounded by air spaces (Pratt *et al.*, 1995). These attributes may account for why kernels with flint texture were more resistant to insect attack and are thus worth for consideration in breeding for postharvest insect pest resistance in maize.

The results obtained from this study based on the biophysical/bioassay and SSR data further confirm existence of genetic variability in tropical maize germplasm for resistance to maize weevil and LGB. Earlier studies have reported the existence of genetic diversity in resistance to the maize weevil, larger grain borer and other coleopterans among tropical maize germplasm (Arnason *et al.*, 1994; Mwololo *et al.*, 2010; Tefera *et al.*, 2011). Such genetic diversity can be exploited in breeding programmes to introgress resistance to postharvest insect pests into improved varieties using conventional and genetic engineering approaches (Dhliwayo and Pixley, 2003). There was no clear distinction in genetic distances among some of the germplasm evaluated in the entire experiment in relation to resistance, particularly among the landrace accessions. Absence of longer genetic distances was due to the fact that neutral genetic markers are not necessarily the same loci conditioning resistance or selection. Neutral genetic markers are not useful for measuring adaptive genetic diversity, where the phenotypic traits are subject to selection (Koeberner *et al.*, 2002). Clear estimates of the genetic distances would be closer when there is association between the loci controlling the phenotypic trait of interest (QTL) and the markers used and when a larger number of the traits of interest in relation to a particular situation are evaluated (Lefebvre *et al.*, 2001; Roy *et al.* 2004;). There were more clusters among the landraces compared to the inbred line and the hybrids, an indication of the variability among them. This is in line with the view that, landraces have diverse genetic background resulting from polycrosses, since they were not necessarily subject to pedigree selection (Yamasaki *et al.*,

2007). The pattern of clustering among hybrids and inbred lines based on molecular and agronomic data separately and when combined were similar suggesting a common ancestry or high genetic relatedness.

The QTL for maize weevil and LGB damage indices were in the same genomic regions suggesting that the mechanisms of resistance in maize to insect and disease could be introgressed via breeding. Indeed, the significant phenotypic correlations among the resistance traits to insect damage such as were also supported by the clustering of QTL linked to these traits in chromosomes 3, 5, and 9. Among the four QTL linked to protein content, one was located in chromosome 3, an indication of association between protein content and postharvest insect pest resistance traits. Previous studies have implicated proteins in resistance metabolism to the maize weevil and LGB through, cell wall structure strengthening and grain hardness (García-Lara, *et al.*, 2004; Siwale *et al.*, 2009, Mwololo *et al.*, 2012). Other QTL for protein content were in this study mapped to chromosomes 1 and 4; and the same region has been mapped for coumaric acid (CA) and ferulic acid (FA) which is a key biochemical component for resistance to storage pests (García-Lara *et al.*, 2010). In addition, phenylalanine ammonio lyase and caffeate *O*-methyltransferase genes have been found in chromosome 4 (Davis *et al.*, 1999).

In maize, chromosomes 2 and 3 are associated with genes linked to cell wall biochemistry such as cellulose synthase, cell wall sugar metabolism, grain pericarp and plant fiber development (Holland *et al.*, 2000; Cardinal *et al.*, 2003; Hazen *et al.*, 2003). Quantitative trait loci for components such as galactase, xylose and arabinose were also reported in chromosomes 2 and 3 (Hazen *et al.*, 2003). Other metabolites implicated in host resistance such as peroxidase and simple phenolic compounds and structural proteins such as HRGPs are conditioned by loci in chromosome 2 of maize (Brewbaker *et al.*, 1985; Davis *et al.*, 1999). The results from this study

show that there are links among protein content and grain hardness; and insect damage traits since there was correspondence between QTL for these traits. This suggested to a large extent, common genetic basis for traits associated with resistance to postharvest insect pests. The presence of abundant and organized protein bodies is associated with greater adherence and better distribution of starch granules in the endosperm and, consequently, higher physical resistance of the grain (Gibbon *et al.*, 2003). Interestingly, QTL identified for husk cover tip length in chromosomes 1, 4 and 7 mapped to genomic regions that have been found to carry the genes for both biochemical and biophysical characteristics of maize grain (García-Lara *et al.*, 2010). This indicates the possibility of incorporating this trait into maize varieties concurrently with other key traits of resistance to postharvest pest attack. Thus, these results are indicative of a functional relationship between resistance genes and QTL for insect resistance. Moreover, the genes and QTL for disease resistance, which have been mapped before and recently, map in most of the genomic areas reported in this study (Kelly *et al.*, 2012).

Overall, these diverse sets of studies undertaken in this thesis aimed at elucidating resistance in maize to LGB and maize weevil in order to underpin design and implementation of breeding experiments clearly show that (i) resistance to the LGB is very similar to resistance to maize weevil (ii) resistant genotypes exist and can be used to improve the many susceptible commercial hybrids were identified, genetic correlations were high among traits in relation to maize weevil and LGB resistance-therefore the mechanisms of resistance are similar, genetic variability was observed and QTL for resistance to post-harvest insect pests were identified.

7.2 Conclusions

Deployment of resistant genotypes is fundamental to the success of an integrated pest-management programme. The main focus of this thesis was to identify and characterize resistance to the maize weevil and LGB. Whereas resistance to the maize weevil had been identified earlier elsewhere, in this thesis, it was used to help characterize resistance to another emergent coleopteran pest the LGB. In line with a hypothesis, that both coleopteran pests attack maize grain in a similar manner and thus could illicit similar reaction, the mechanisms of resistance to weevil and LGB attack were similar. Thus resistant genotypes (landraces and inbred lines) identified could be used as sources of resistance for further breeding to develop resilient varieties whereas hybrids could be released immediately. Development and deployment of such resistant varieties will reduce usage of storage pesticides, saving costs, minimizing pollution and secure harvests against losses strengthening food security. Other major findings of this study that could support breeding include, the demonstration of potential for development of genotypes with dual resistance to the maize weevil and LGB. Tools to support selection such as role of seed proteins, grain resistance traits and QTL for resistance will all further support breeding activities. Starch matrix interacts with storage proteins to influence grain hardness and, therefore, further studies could be done to understand the genetics of compact starch matrix which is desirable. Additionally, the management options for the maize weevil seem to work for the larger grain borer, therefore a single integrated pest management strategy would work for both.

7.3 Future perspective in breeding for resistance to storage insect pests in maize

This study shows that screening for multiple resistance can be challenging and costly. The cost of screening for resistance to the storage insect pests was approximately USD 30.00 per sample for both pests. Thus the entire screening process to identify resistance is expensive with regard to technical and resource requirements. As such, development of cost effective means especially for plant breeders is critical. Development of effective screening methods is, however, influenced by the nature of resistance traits being evaluated. Resistance phenotypes may also vary under different environments. Cheap and high-through put screening methods for quick identification of resistant genotypes are needed. Image analysis and electronic monitoring of grain tunneling would represent potential for automated indexing of insect performance parameters (eggs, larvae, adults) and measuring of multiple factors (host preference, mobility, food intake) involved in host selection, respectively.

Currently the approaches employed to breed for resistance to insects, particularly those aimed at introgressing resistance genes to cultivars, need refinement and optimization. Exploring possibility of using robust molecular markers in marker assisted recurrent selection will be the key towards enhancing selection efficiency. The availability of high density genetic maps together with phenotypic and pedigree data would form the basis for genome wide selection, an approach that supports prediction of genetic values in relation to resistance. Resistance to pests belonging to the same genus or family seems to be conditioned by the same mechanisms as was evident in the current study for both maize weevil and LGB. This implies that genotypes with multiple resistance to insect pests could be found through large scale screening including both Coleopterans and Lepidopterans as the test insects. Introgression of resistance genes through

marker assisted back crossing could also be exploited under such circumstances. Marker assisted selection can also be used in resistance gene pyramiding since it would be easier to determine whether a plant has multiple resistance genes than by using insect bioassays.

Understanding the physiological basis of resistance and developing meaningful bioassays or other quantitative measurements to assess resistance to insects in the laboratory and field is crucial. For many crop plants, the science has moved on beyond genomics to establishing metabolic pathways linked to resistance. These advances in science provide an opportunity for the identification of genes and their related metabolism pathways improving prospects for maximizing resistance levels while minimizing negative consequences. This is an emergent area that will require collaborative studies involving geneticists, physiologists, entomologists, and biochemists, with the ultimate goal to engineer plant metabolic pathways for improved postharvest insect pest resistance.

In this study quantitative trait loci (QTL) for resistance to both maize weevil and LGB were mapped based on biparental crosses. The major limitation with this approach is that mapped QTL are fixed usually to the respective populations developed. However, recombination events that have accumulated over thousands of generations could be exploited via association mapping to dissecting complex traits such as insect pest resistance, finding QTL that may be more usable to other populations of maize. Conducting genome wide association mapping would complement traditional QTL mapping and is thus proposed as next steps beyond this thesis. Given the large number of maize accessions available at CIMMYT and use of single nucleotide polymorphic (SNP) markers, the use of simulation models can be exploited. Further molecular and biochemical analysis should be carried out to determine the function of the target genes, and in this way to reveal new sources of resistance as the last step in map-based cloning.

In addition, the results obtained from this study show that protein content contributes to insect pest resistance. There is therefore an opportunity to explore this further, through transcriptome analysis and re-sequencing using next generation sequencing methods. This approach will help in identifying the genes concerned and could be directly tagged, thus being more reliable. Since protein content and cell wall components (reported in other studies) have significant influence on resistance to postharvest insect pests including maize weevil and larger grain borer (LGB), the components for their synthesis could be candidates for fine mapping for resistance. Further saturation of genetic linkage map with additional informative markers is necessary for tagging of candidate genes involved in regulating insect pest resistance.

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Appendices

Appendix 1 List of maize hybrids used in the evaluation for resistance and genetic relationship studies

Entry	Name	Origin	Entry	Name	Origin
1	H6213	Kenya	43	CKIR09006	CIMMYT-Kenya
2	H6212	Kenya	44	CKIR09007	CIMMYT-Kenya
3	H6210	Kenya	45	CKIR09008	CIMMYT-Kenya
4	H629	Kenya	46	CKIR06001	CIMMYT-Kenya
5	H628	Kenya	47	CKIR06004	CIMMYT-Kenya
6	H626	Kenya	48	CKIR06006	CIMMYT-Kenya
7	H513	Kenya	49	CKIR06007	CIMMYT-Kenya
8	PH4	Kenya	50	CKIR06008	CIMMYT-Kenya
9	PH1	Kenya	51	CKIR06009	CIMMYT-Kenya
10	DH02	Kenya	52	CKPH09001	CIMMYT-Kenya
11	DH04	Kenya	53	CKPH08032	CIMMYT-Kenya
12	WH403	Kenya	54	CKPH09002	CIMMYT-Kenya
13	SC Duma 41	Africa	55	CKPH08033	CIMMYT-Kenya
14	SC Duma 43	Africa	56	CKPH09003	CIMMYT-Kenya
15	SC Simba 61	Kenya	57	CKPH08035	CIMMYT-Kenya
16	PH 3253	Africa	58	CKPH08036	CIMMYT-Kenya
17	611D	Kenya	59	CKPH08037	CIMMYT-Kenya
18	KH 600-15A	Kenya	60	CKPH08038	CIMMYT-Kenya
19	DH01	Kenya	61	CKPH08039	CIMMYT-Kenya
20	533A	Kenya	62	CKPH08040	CIMMYT-Kenya
21	531A	Kenya	63	CKPH08041	CIMMYT-Kenya
22	631Q	Kenya	64	CKPH08043	CIMMYT-Kenya
23	500Q	Kenya	65	CKPH08044	CIMMYT-Kenya
24	DK8031	Kenya	66	CKPH08002	CIMMYT-Kenya
25	CKIR07010	CIMMYT-Kenya	67	CKPH08003	CIMMYT-Kenya
26	CKIR07011	CIMMYT-Kenya	68	CKPH08004	CIMMYT-Kenya
27	CKIR07012	CIMMYT-Kenya	69	CKPH09004	CIMMYT-Kenya
28	CKIR07013	CIMMYT-Kenya	70	CKPH08009	CIMMYT-Kenya
29	CKIR07017	CIMMYT-Kenya	71	CKPH08010	CIMMYT-Kenya
30	CKIR07018	CIMMYT-Kenya	72	CKPH08012	CIMMYT-Kenya
31	CKIR07001	CIMMYT-Kenya	73	CKPH08014	CIMMYT-Kenya
32	CKIR07002	CIMMYT-Kenya	74	CKPH08020	CIMMYT-Kenya
33	CKIR07003	CIMMYT-Kenya	75	CKPH08024	CIMMYT-Kenya
34	CKIR07004	CIMMYT-Kenya	76	CKPH08025	CIMMYT-Kenya
35	CKIR07005	CIMMYT-Kenya	77	CKPH08026	CIMMYT-Kenya
36	CKIR07008	CIMMYT-Kenya	78	CKPH08028	CIMMYT-Kenya
37	CKIR07009	CIMMYT-Kenya	96	CML395/CML444	CIMMYT-Kenya
38	CKIR09001	CIMMYT-Kenya	97	CML312/CML442	CIMMYT-Kenya
39	CKIR09002	CIMMYT-Kenya	98	CKIR04002	CIMMYT-Kenya
40	CKIR09003	CIMMYT-Kenya	99	CKIR04003	CIMMYT-Kenya
41	CKIR09004	CIMMYT-Kenya	100	CML202 /CML204	CIMMYT-Kenya
42	CKIR09005	CIMMYT-Kenya			

Appendix 2. List of Landraces and open pollinated varieties used in evaluation for resistance and genetic relationship studies

Entry	Name	Description	Origin	Entry	Name	Description	Origin
1	GUAT 1081	Landrace	Latin America	41	BRAZ 1384	Landrace	Latin America
2	GUAT 1008	Landrace	Latin America	43	VERA 197	Landrace	Latin America
3	GUAT 1010	Landrace	Latin America	45	PARA GP3	Landrace	Latin America
4	GUAT 1014	Landrace	Latin America	46	VALL 385	Landrace	Latin America
5	GUAT 1100	Landrace	Latin America	48	PARA GP3	Landrace	Latin America
6	GUAT 1030	Landrace	Latin America	49	BRAZ 1442	Landrace	Latin America
7	GUAT 1034	Landrace	Latin America	50	BRAZ 1919	Landrace	Latin America
8	GUAT 1038	Landrace	Latin America	51	BRAZ 1346	Landrace	Latin America
10	GUAT 1155	Landrace	Latin America	53	GUAN 36	Landrace	Latin America
11	GUAT 1162	Landrace	Latin America	54	GUAN 84	Landrace	Latin America
12	GUAT 1168	Landrace	Latin America	57	VALL 380	Landrace	Latin America
13	GUAT 1093	Landrace	Latin America	58	CHIS 94	Landrace	Latin America
14	GUAT 1096	Landrace	Latin America	59	BRAZ 2451	Landrace	Latin America
15	GUAT 1082	Landrace	Latin America	61	NAYA 129	Landrace	Africa
16	OAXA 553	Landrace	Latin America	62	JALI 43	Landrace	Africa
18	SINA 30	Landrace	Africa	63	CAQU 321	Landrace	Latin America
19	BRAZ 2375	Landrace	Latin America	64	BRAZ 222	Landrace	Latin America
21	BRAZ 1470	Landrace	Latin America	65	BRAZ 1495	Landrace	Latin America
22	CHIS 114	Landrace	Latin America	66	BRAZ 1736	Landrace	Latin America
23	RIGS GP10	Landrace	Latin America	67	BRAZ 2214	Landrace	Latin America
24	PARA 151	Landrace	Latin America	68	BRAZ 2100	Landrace	Latin America
25	VERA GP24	Landrace	Latin America	79	DKC8053	OPV	Africa
26	BRAZ 4	Landrace	Latin America	80	ECA-QPOPE	OPV	Latin America
27	BRAZ 1486	Landrace	Latin America	81	SUSUMA	OPV	Africa
28	SINA 21	Landrace	Africa	82	POOL15QC7-SRC1-F	OPV	Africa
29	BRAZ 2017	Landrace	Latin America	83	EEQPM-9-EA-#-#-#	OPV	Africa
30	GUAN 28	Landrace	Latin America	84	EEQPM-8-EA-#-#-#	OPV	Africa
31	BRAZ 2149	Landrace	Latin America	85	KDV1 - 1-#	OPV	Africa
33	VENE 352	Landrace	Latin America	86	KDV1 - 2-#	OPV	Africa
34	BRAZ 1364	Landrace	Latin America	87	KDV1 - 3-#	OPV	Africa
35	BRAZ 1832	Landrace	Latin America	88	KDV1 - 4-#	OPV	Africa
36	NAYA 130	Landrace	Africa	89	KDV1 - 5-#	OPV	Africa
37	VENE 414	Landrace	Latin America	90	ZM521-IR-#-#-#	OPV	Africa
38	GUAT 280	Landrace	Latin America	91	ZM421-IR-#-#-#	OPV	Africa
39	BRAZ 1403	Landrace	Latin America	92	ECA-STRIGOFF-102-#	OPV	Latin America
40	BRAZ 1797	Landrace	Latin America	93	ECA-STRIGOFF-125-#	OPV	Latin America

Appendix 3. List of inbred lines used in the evaluation for resistance and genetic relationship studies

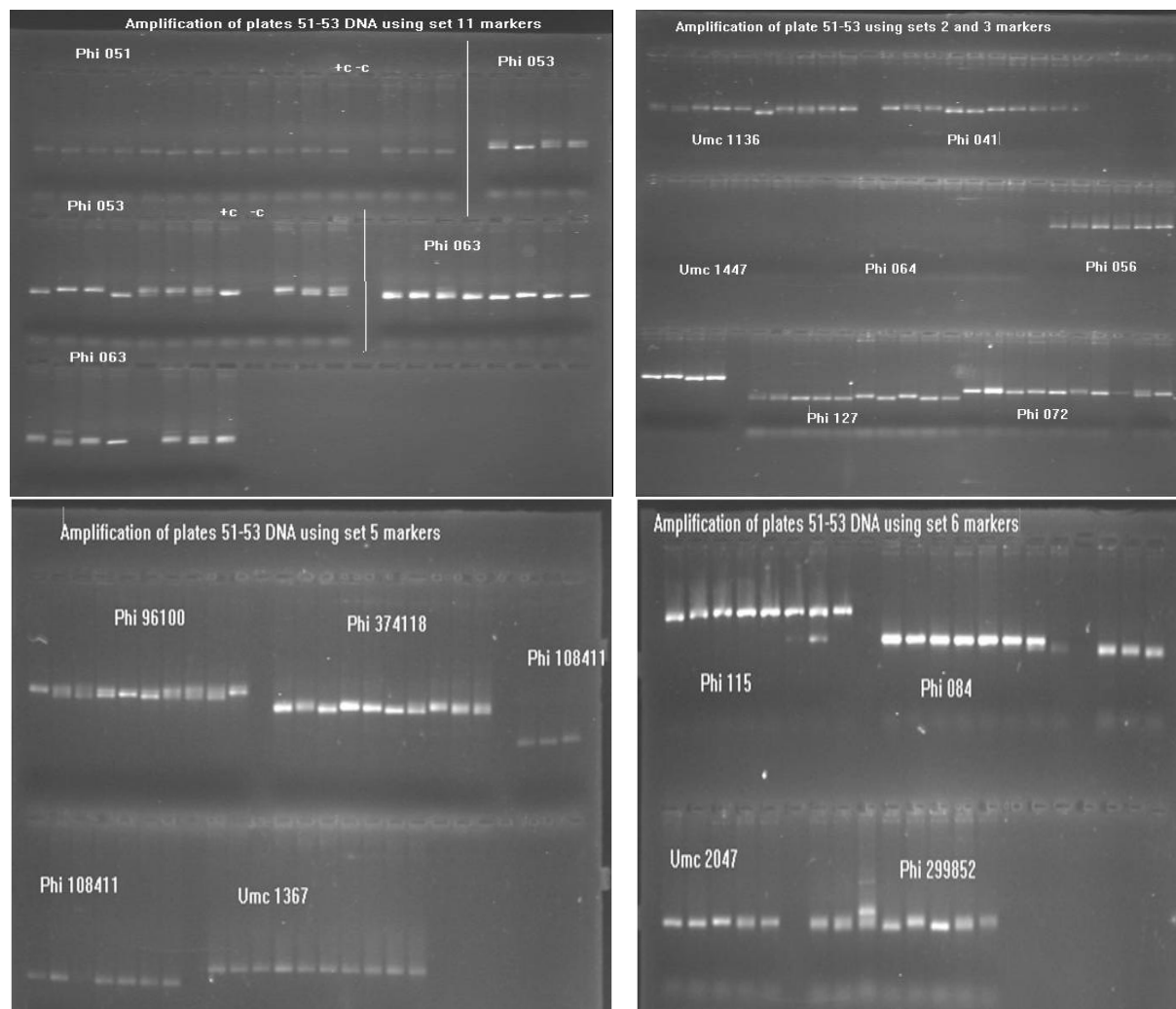
Entry	Name	Origin	Entry	Name	Origin	Entry	Name	Origin
1	CKSBL10027	America	41	CML159	L.America	81	CKSPL10087	L.America/Africa
2	CKSPL10177	L.America/Africa	42	CML197	L.America	82	CKSPL10088	L.America/Africa
3	CML395	L. America	43	CML440	L.America	83	CKSPL10089	L.America/Africa
4	CKSBL10028	America	44	CML78	L.America	84	CKSPL10090	L.America/Africa
5	CKSBL10025	America	45	CML445	L.America	85	CKSPL10111	L.America/Africa
6	CKSBL10045	America	46	CZL03014	L.America	86	CKSPL10112	L.America/Africa
7	CKSPL10344	Latin America	47	CML511	L.America	87	CKSPL10113	L.America/Africa
8	CML254	Latin America	48	DTPWC9-F104-5-4-1-1-B-B-#-#	L.America	88	CKSPL10111	L.America/Africa
9	CKSBL10005	America	49	DTPWC9-F115-1-4-1-1-B-B-#-#	L.America	89	CKSPL10136	L.America/Africa
10	CML312	Latin America	50	La Posta Seq C7-F64-2-6-2-1-B-B-#-#	L.America	90	CKSPL10146	L.America/Africa
11	CKSBL10015	America	51	LPSC7-F86-3-1-1-1-BB-#-#	L.America	91	CKSPL10164	L.America/Africa
12	CKSBL10029	America	52	LPSC7-F180-3-1-1-1-BB-#-#	L.America	92	CKSPL10170	L.America/Africa
13	CKSBL10038	America	53	DTPWC9-F16-1-1-1-1-BB-#-#	L.America	93	CKSPL10186	L.America/Africa
14	CKSPL10035	Latin America	54	P100C6-200-1-1-B-***-#-#	L.America	94	CKSPL10003	L.America/Africa
15	CKSBL10040	America	55	P300C5S1B-2-3-2-#-#-1-2-B-B-#-#	L.America	95	CKSPL10206	L.America/Africa
16	CML264	Latin America	56	CML443	L.America	96	CKSPL10212	L.America/Africa
17	CKSBL10003	America	57	CML488	L.America	97	CKSPL10218	L.America/Africa
18	CKSBL10020	America	58	CZL00003	L.America	98	CKSPL10224	L.America/Africa
19	CKSBL10082	America	59	CZL03007	L.America	99	CKSPL10229	L.America/Africa
20	CKSBL10014	America	60	CML441	L.America	100	CKSPL10230	L.America/Africa
21	CKSBL10016	America	61	CML489	L.America	101	CKSBL10027	Asia/L.America
22	CKSBL10042	America	62	CZL01005	L.America	102	CKSBL10060	America
23	CKSBL10035	America	63	CKSPL10256	L.America/Africa	103	CKSBL10014	America
24	CML334	Latin America	64	CKSPL10273	L.America/Africa	104	CKSBL10013	America
25	CML202	Latin America	65	CKSPL10280	L.America/Africa	105	CKSBL10028	America
26	CKSBL10001	Asia/L.America	66	CKSPL10309	L.America/Africa	106	CKSBL10029	America
27	CML204	L. America	67	CKSPL10295	L.America/Africa	107	CKSBL10033	America
28	CKSBL10034	America	68	CKSPL10013	L.America/Africa	108	CKSPL10343	America
29	CKSBL10007	America	69	CKSPL10021	L.America/Africa	109	CKSBL10023	America
30	CKSBL10030	America	70	CKSPL10028	L.America/Africa	110	CKSBL10041	America
31	CKSBL10043	America	71	CKSPL10035	L.America/Africa	111	CKSBL10039	America
32	CKSBL10008	America	72	CKSPL10036	L.America/Africa	112	CKSPL10341	America
33	CKSBL10025	America	73	CKSPL10042	L.America/Africa	113	CKSBL10025	America
34	CML442	Latin America	74	CKSPL10070	L.America/Africa	114	CKSBL10001	America
35	CKSBL10026	America	75	CKSPL10074	L.America/Africa	115	CKSBL10004	America
36	CKSBL10004	America	76	CKSPL10075	L.America/Africa	116	CKSBL10026	America
37	CML444	L.America	77	CKSPL10080	L.America/Africa	117	CKSBL10021	America
38	CKSBL10021	America	78	CKSPL10081	L.America/Africa	118	CKSBL10046	America
39	CKL06-1	Africa	79	CKSPL10085	L.America/Africa	119	CKSBL10005	America
40	CML144	L.America	80	CKSPL10086	L.America/Africa	120	CKSBL10045	America

Appendix 4. Summary of SSR markers used in the genetic relationship study among tropical maize germplasm

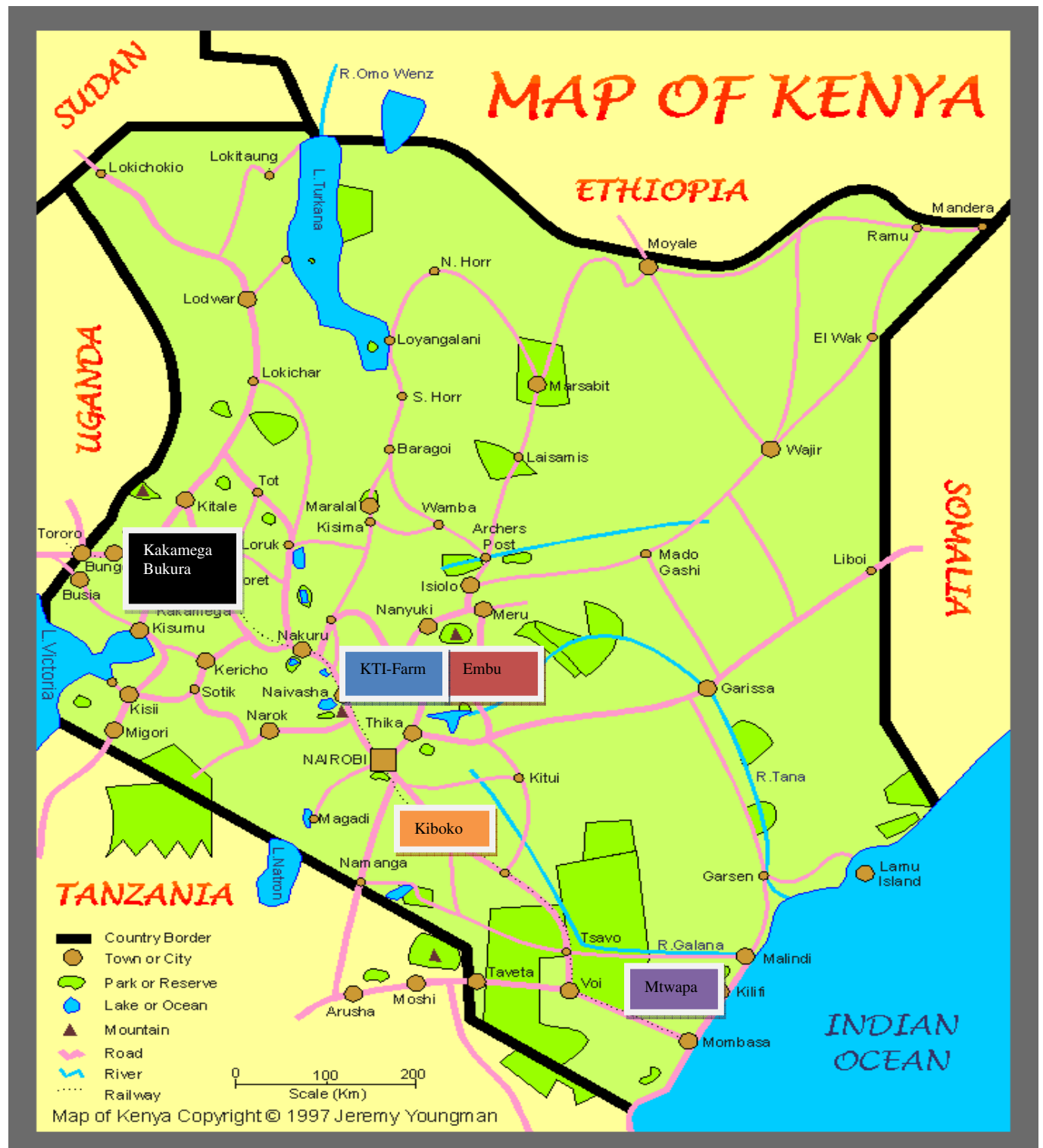
Locus/Marker	Bin position	Repeat length	Allele size range (bp)	No. of alleles for inbred lines (n=115)	No. of alleles for hybrids (n=83)	No. of alleles for OPVs and landraces (n=84)	Total number of alleles
nc133	2.05	5	106-113	2	2	2	2
phi008	5.03	3	55-96	8	5	1	9
phi014	8.04	3	147-162	3	4	4	4
phi015	8.09	4	82-108	5	7	7	7
phi022	9.03	4	134-166	2	2	1	2
phi029	3.04	2 and 4	144-158	3	5	4	7
phi031	6.04	4	185-222	5	5	5	5
phi041	10	4	187-210	5	4	5	6
phi050	10.03	4	73-84	3	1	1	4
phi051	7.05	3	135-144	3	3	2	4
phi053	3.05	4	170-195	3	4	3	4
phi056	1.01	3	234-244	5	5	5	7
phi059	10.02	3	124-158	5	5	6	6
phi062	10.04	3	160-164	2	1	1	2
phi063	10.02	4	150-222	5	4	8	9
phi064	1.11	4	72-107	7	9	9	9
phi072	4.01	4	136-161	4	7	6	7
phi075	6	2	222-239	5	5	4	8
phi076	4.11	6	153-176	3	5	5	5
phi079	4.05	5	179-193	4	3	3	4
phi084	10.04	3	148-160	4	4	4	5
phi085	5.07	5	230-257	4	4	5	6
phi089	6.08	4	86-94	2	2	2	2
phi090	2.08	5	138-146	1	1	1	2
phi093	4.08	4	281-292	3	3	2	4
phi102228	3.04	4	100-132	2	0	2	4
phi104127	3.01	4	152-169	3	3	4	5
phi108411	9.06	4	110-139	3	1	2	7
phi109188	5	4	154-170	4	3	4	5

phi109275	1	4	51-137	7	10	6	11
phi112	7.01	2	133-158	4	3	2	5
phi114	7.02	4	128-169	5	5	5	6
phi115	8.03	2 and 4	280-301	2	2	2	5
phi123	6.07	4	142-146	2	2	2	2
phi127	2.08	4	109-129	3	1	4	6
phi227562	1.12	3	304-327	6	4	7	8
phi308707	1.1	3	109-131	4	5	5	5
phi331888	5.04	3	129-135	3	3	3	3
phi374118	3.02	3	205-233	4	6	6	7
phi453121	3.01	3	206-224	3	4	4	4
phi96100	2	4	266-298	5	7	8	9
umc1061	10.06	3	100-112	2	4	3	5
umc1136	3.1	3	130-157	6	7	5	7
umc1143	6	5	73-85	3	3	3	3
umc1161	8.06	6	136-149	3	2	3	3
umc1196	10.07	6	133-158	4	4	4	5
umc1266	3.06	3	120-144	3	0	1	3
umc1304	8.02	4	124-133	2	1	5	3
umc1332	5.04	3	116-143	5	5	1	6
umc1367	10.03	3	146-159	0	0	3	3
umc1447	5.03	3	113-124	2	3	2	4
umc1545	7	4	69-85	3	2	3	3
umc1917	1.04	3	132-153	2	4	3	6
umc2047	1.09	4	100-134	7	7	7	8
umc2250	2.04	3	153-153	0	0	0	1

Appendix 5. DNA amplification by different sets of polymorphic markers

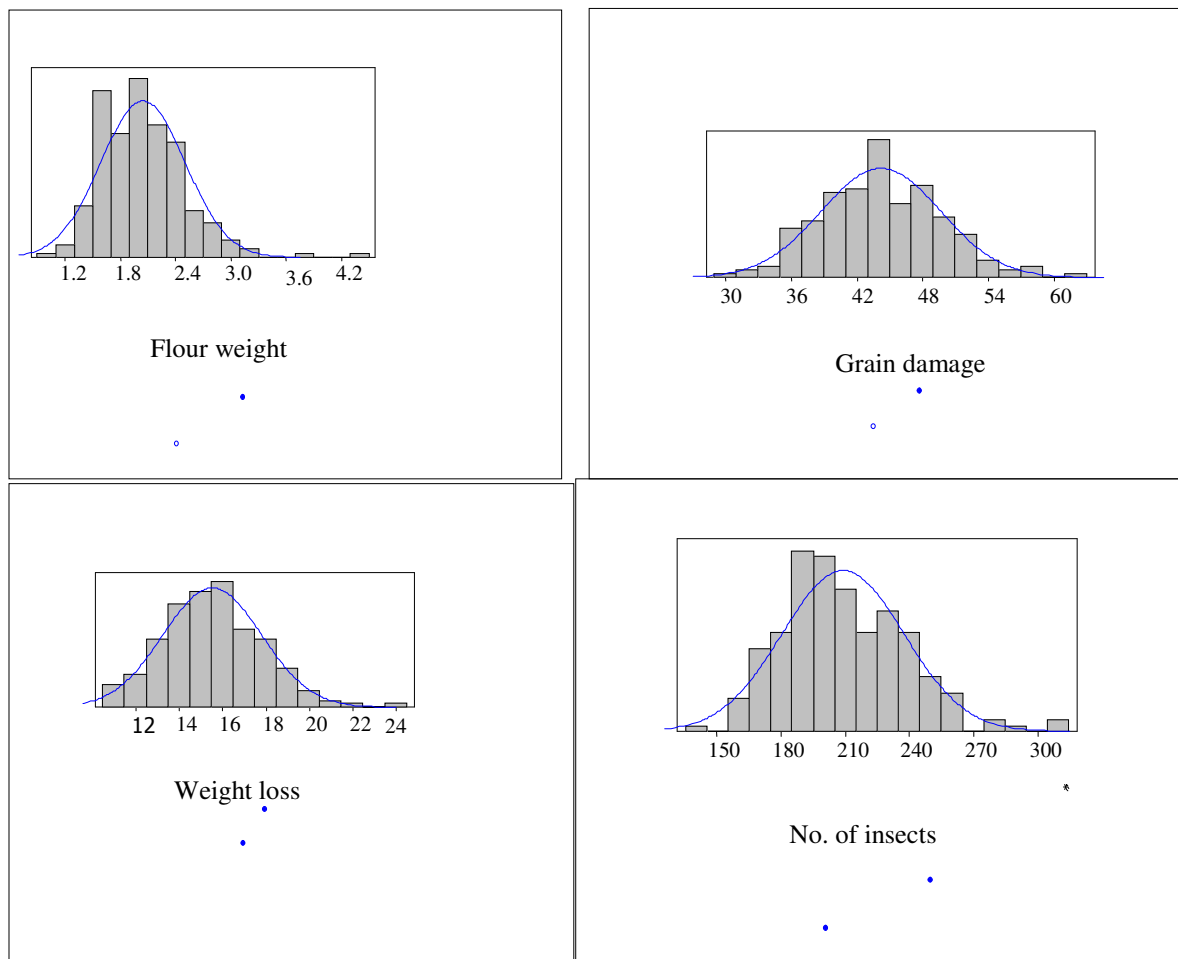


Appendix 6. Map of Kenya with the field trial sites where experiments were conducted shown in text boxes



*The experimental sites for evaluation of test crosses were Kakamega, Bukura, Embu, Kirinyaga Technical Institute (KTI), Kiboko and Mtwapa enclosed in a text box and each shaded with a different color on the map.

Appendix 7. Test for normality for postharvest insect pest resistance traits in mapping populations



*Flour weight ($P \leq 0.005$); Grain damage ($P = 0.883$); weight loss ($P = 0.4$); No. of insects ($P = 0.09$)