Combining *Chilo partellus* Swinhoe and *Sitophilus zeamais* Motschulsky insect pest resistance in early maturing maize hybrids

By

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy (Plant Breeding)

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Pietermaritzburg

Republic of South Africa

August 2016

Abstract

The spotted stem, borer *Chilo partellus* Swinhoe (Lepidopteran, Pyralidae), is an important field pest of maize (*Zea mays* L), widely distributed in the lowland topics and mid-altitude maize growing zones, and causing annual yield loss of 13.5% in Kenya. Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), an equally damaging post-harvest insect pest, causes estimated annual grain losses of 15%. Host plant resistance for both pests has been employed to develop some varieties resistant to either of these pests. However, the traits are in separate cultivars, and most of the released varieties are not early maturing. The specific objectives of the study were to: i) investigate genetic diversity in stem borer and maize weevil resistant S4 maize families for use in breeding for insect resistance in maize hybrids, ii) investigate whether resistance to, *Chilo partellus* and *Sitophilus zeamais*, can be achieved in hybrid combinations using insect resistant maize inbred lines, iii) determine the genome dosage effect on resistance to *C. partellus* and *S. zeamais* by using maize lines with contrasting levels of resistance to the respective pests in designing maize hybrids, iv) determine stability of the new *C. partellus* and *S. zeamais* resistant early and medium maturing hybrids across environments.

Diversity studies results revealed that the markers were polymorphic with 0.46-0.48 polymorphism for both SBR and SPR populations. The cluster analysis revealed three major clusters of germplasm in both SBR and SPR populations. This indicated existence of diversity in both populations which can be exploited in breeding insect resistant varieties.

Diallel analysis using 12 inbred lines revealed highly significant ($p \leq 0.001$) general combining ability (GCA) effects for leaf damage scores and undamaged kernels and contributed 71-77% of the genetic variation. Similarly, the GCA for undamaged grain under *S. zeamais* was favorable ($p \leq 0.001$, 72.5-77.1%). This suggests that these traits are controlled by additive gene action. The analysis further revealed that 34.8% of the genotypes had high levels of stem borer resistance with leaf damage scores of 2-2.5, while 42.4 % had scores of 2.51-3.0, 21.2% had moderate levels of resistance, while 1.5% were susceptible. Grain yield analysis revealed that 3.0-4.5% of genotypes were high yielder (9-11.0 t/ha) from both protected and infested, suggesting that stem borer resistance did not confer yield penalties to the hybrids. Parents; 2, 3, 6, 7, and 10 contributed to higher *C. partellus* resistance genes in hybrids ($p \leq 2.3$), while parents, 2,3,8,9, and 10 contributed to higher *S. zeamais* resistance genes. Hybrids: 3X10, 4X8, 5X10, 3X9, 2X9, 5X8, and 4X9; showed combined resistance to both pests, with grain yield of 5-8 t/ha. The parents can be used as sources of

resistance genes in developing hybrids with combined resistance to field and storage pests in maize production.

Evaluation of hybrids generated through North Carolina II from inbred line parents with contrast resistance levels revealed highly significant ($p \leq 0.001$) mean squares for grain yield, leaf damage scores, grain weight loss and undamaged kernels. The mean grain yield of eight sets was 5.0t/ha, when protected, and 4.7t/ha when infested. The highest grain yield (5.5t/ha, and, 5.2t/ha, protected and infested respectively), were observed in set 3. Sets 1, 7 and 8 had relatively high resistance to maize weevil, and registered the least weight loss of 14.9-16.7%.

Female parent 3 had positive and favourable GCA effects for grain yield (0.95 and 0.70) when protected and infested treatments, as well as for undamaged kernels (1.66). The same parent 3 had favourable negative effects for both leaf damage scores (-0.46) due to *C. partellus* infestation, and, weight loss due to *S. zeamais*. Favourable GCA effects were also observed in set 5, for grain yield (0.69 and 0.57); leaf damage scores, -0.28; weight loss in maize weevil infestation, -3.77; and, undamaged grain, 1.94 under maize weevil infestation for the same parent. Calculated 8.9% of hybrids were highly resistant to stem borer with a leaf damage score of 1.5-2.5; 42% of the test hybrids had a score of 3.0, and, 49% were susceptible with leaf damage scores > 3. Heterosis for grain yield ranged from 26-41% for the best five hybrids. Heterosis for resistant parameters, leaf damage scores was -22% to -17, and, for weight loss was -45 to -32%. Both additive and non-additive gene action were responsible for combined resistance to both *C. partellus* and *S. zeamais* insect pests. Resistance to both *C. partellus* and *S. zeamais* was observed in hybrids from set 3, 7, and 8. Sets 3 and 5 had grain yield above mean of checks, and high levels of resistance for both insect pests.

The overall results and findings of this study demonstrate that it is possible for breeders to develop maize hybrids with dual resistance for *C. partellus* and *S. zeamais* for use by smallholder farmers in Africa. The identified inbred lines parents' with good combining ability for yield and insect pest resistance can also be used in breeding programs to enhance the existing germplasm. The favourable alleles of combined resistance to *C. partellus* and *S. zeamais* can be fixed by further selection. Further, the findings of this research can be used as baseline studies for future research when breeding for combined insect pests' resistance in maize.

Declaration

I, **Regina Mumbua Tende**, declare that:

1. This thesis has not been presented for a degree award in any other university.
2. The research findings reported in this thesis, except where otherwise indicated are my original work.
3. This thesis does not contain other people's writings, pictures, graphs, data or information, unless otherwise, specifically acknowledged.
4. This thesis does not contain tables, text or graphics copied and pasted from the internet, unless specifically acknowledged, and the sources being detailed in the thesis, and then referenced.
5. Where other peoples work, findings or ideas have been used, the same have been duly acknowledged in form of citations, quoted, referenced or otherwise stated.

Signed:

.....

Regina Mumbua Tende (Candidate)

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

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Prof. John Derera (Supervisor)

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Dr. Stephen Mugo (Co-Supervisor)

Acknowledgements

I am grateful to God for giving me a chance to purse a carrier against all expectations; His grace was sufficient to take me through the entire period of this study. To Him be all the glory!

I am indebted to my children, Harry Jeconiah and Hadassah Jan Wavinya, who stood by me even when I spent many days away from them, while either attending classes or conducting field work. Your love, prayers, moral support and understanding kept me going even when my strength threatened to fail me. I am also grateful to my other relations for their continued prayer and moral support. May God richly bless you!

The great supervision from Prof. John Derera of University of KwaZulu-Natal (UKZN) and Dr. Stephen Mugo of International Maize and Wheat Improvement Center (CIMMYT) is gratefully acknowledged. You both gave me wise guidance, encouragement, patience and understanding during this study; for this I salute you!

My appreciation also goes to: My employer, the Kenya Agricultural and Livestock Research Organization (KALRO) especially the Director General, for allowing me time to go and get equipped for better service to the small-holders farmers in Kenya; The University of KwaZulu-Natal through the African Centre for Crop Improvement (ACCI), especially the staff and lecturers, for offering me admission to this PhD program, and impacting knowledge to me; The Alliance for a Green Revolution in Africa (AGRA), for the financial support; CIMMYT, for technical support, germplasm and supervision during field research phase. Technical support from Dr. Edmore Gasure and Dr. Amelework Beyene is gratefully acknowledged. I also do acknowledge my classmates: Hirut, Chauque, Ermias, Stephan, Batiseba and Placide; for both, technical and moral support.

My sincere thanks also go to Mr. Joel Mbithi, Mr. Patrick Chomba and the CIMMYT Kiboko team; Mr. Gabriel Ambani and the KALRO Kakamega team; Eric Munene and Daniel Kanyi, and the Kirinyaga University College (KYUC) team; and Mr. David Karuri and the entire KALRO Embu team. You all ensured that I obtained good quality data from my field trials grown at each of your respective sites. I am equally grateful to Mr. Donald Musembi for availing good quality stem borer neonates, Mr. Charles Marangu for collecting good quality post-harvest screening data and, Mr. Andrew Chavangi for quality data management and guidance on computing issues. Many more people assisted in one way or the other, I do appreciate and may God bless you all.

Dedication

This research work is dedicated to:

God:

You are never far from me when I seek you!

In YOU I live and move and have my being! Acts 17:27-28

My children:

Harry Jeconiah: You were always there to assist whenever I needed help. Many thanks.

Hadassah Jan Wavinya: You made me know, and taught me that, I was first, a full time mother, then second, a part-time student. Many thanks too.

You both are real champions for your Love, prayers, patience and perseverance, even when I made constant travels and spend many days and long hours away from you during the course of this study. My love and blessings are with you and…

May God richly bless you both!

My Mom:

Esther Kailu Tende: You taught me to trust in God, rise above the negative circumstances around me, and forge on. May God richly bless you!

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Chapter 1: Introduction

1.1 Importance of maize

Maize (*Zea mays* L.) is classified as a grass; family, *Poaceae*, subfamily *Panicoideae*, tribe *Andropogoneae*, and genus *Zea*. It is a monoecious plant with both sexes in the same individual, but the inflorescences are different. It is also diploid with chromosome number of $2n = 4x = 20$ (Acquaah, 2007).

Maize is the main staple diet in sub-Saharan Africa for the majority of smallholder farmers and the urban poor population (Oerke, 2006). Current statistics indicate that maize is leading in total tonnage produced in the world with average of 800 million tonnes per year. The mean yield of maize for the year 2004-2013 is estimated at 5.1 t/ha (Figure 1-1) (FAOSTAT, 2014).

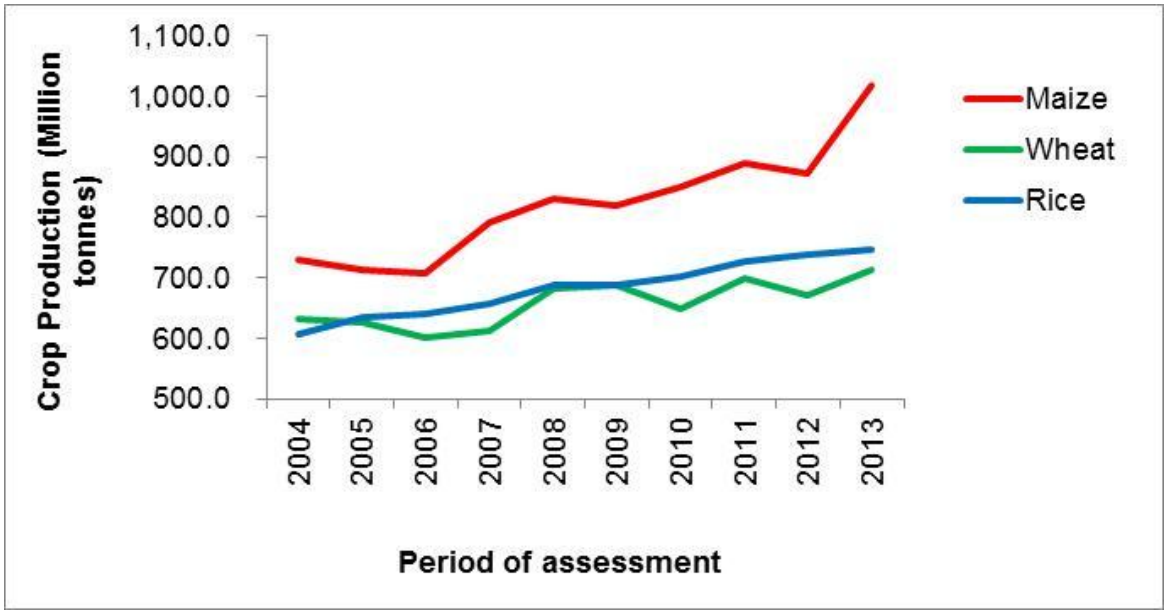

Figure 1-1: Global production tonnage for maize, wheat and rice

Source: FAOSTAT, 2014

It is grown on an estimated global area of over 142 million hectares, with a total production of over 637 million metric tons (MT). The United States of America (USA) is the leading producer of maize with an average yield of 9.5 t/ha for the period 2004-2013, as compared to average yield of 1.9t/ha obtained in Africa for the same period (Figure 1-2) (FAOSTAT, 2014). This low yield being realised in Africa versus the high yield potential justify investments in maize breeding research in sub-Saharan Africa.

1.2 Maize production in Kenya

In Kenya, maize is by far the most important food crop, being grown as both a subsistence and commercial crop for income generation by smallholder farmers (DeGroote, 2002). It is planted on 1.69 million ha, which is more than 30% of the arable land in Kenya (Table 1-1), and is widely distributed throughout the six major agro-ecological zones. The average annual production for the period 2004-2013 is estimated at 3.03 million tonnes (FAOSTAT, 2014). This is far below the actual demand for the crop averaging at 3.75 Million tonnes per annum, which makes Kenya a net importer of maize, with an estimated 140 364 metric tonnes of cereals imported between 2004 and 2006 (FAOSTAT, 2013). Even with the current deficit, the demand for maize is estimated to increase by 45-50% by the year 2020 because of population growth and the diversification of its uses, which include possibilities of producing biofuel and use for animal feed (Anami et al., 2009; Hutňan et al., 2010).

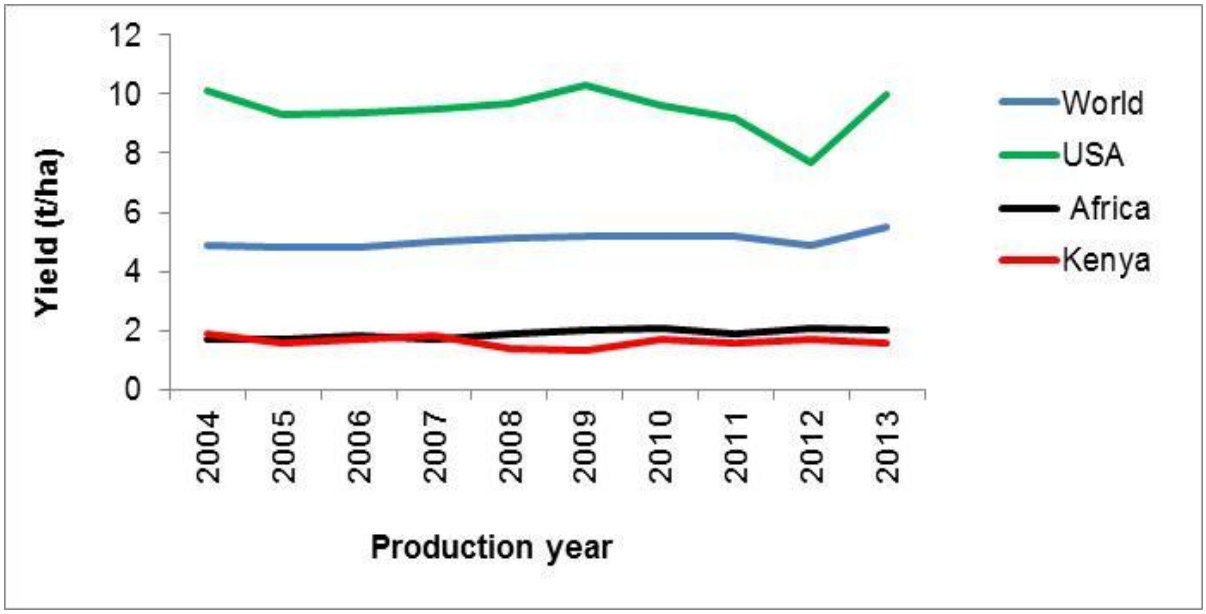

Figure 1-2: Maize yields in Kenya as compared with global yields
Source: FAOSTAT, 2014

Further, Kenya is still producing an average maize yield of 1.6 t/ha maize yields, compared to the recorded average yields of maize in Africa estimated at 1.9 t/ha (Figure 1-2) (FAOSTAT, 2014), yet a large area is used for maize cultivation in Kenya (Table 1-1) (GOK, 2009). Statistics also indicate that maize ranks third to rice and wheat in Kenya in terms of yields per unit area (Figure 1-3) (FAOSTAT, 2014), yet it forms the staple food for the majority of households in Kenya. The low yields can be attributed to various constraints experienced in maize production.

Table 1-1: Area (Ha) under maize cultivation in Kenya

Province	2006	2007	2008	2009
Central	151,596	138,888	146,383	157,063
Coast	88,475	86,786	92,139	129,379
Eastern	523,931	435,773	508,135	462,401
Nairobi	2,137	1,365	1,682	1,053
North Eastern	6,470	4,843	3,606	2,525
Nyanza	273,055	83,333	254,355	262,453
Rift Valley	627,362	664,098	549,448	644,895
Western	223,139	212,049	201,569	225,302
Total	1,896,165	1,627,135	1,757,317	1,885,071
Mean	**237,021**	**203,392**	**219,665**	**235,634**

Source: FAOSTAT 2014. countrystat.kenya@go.ke

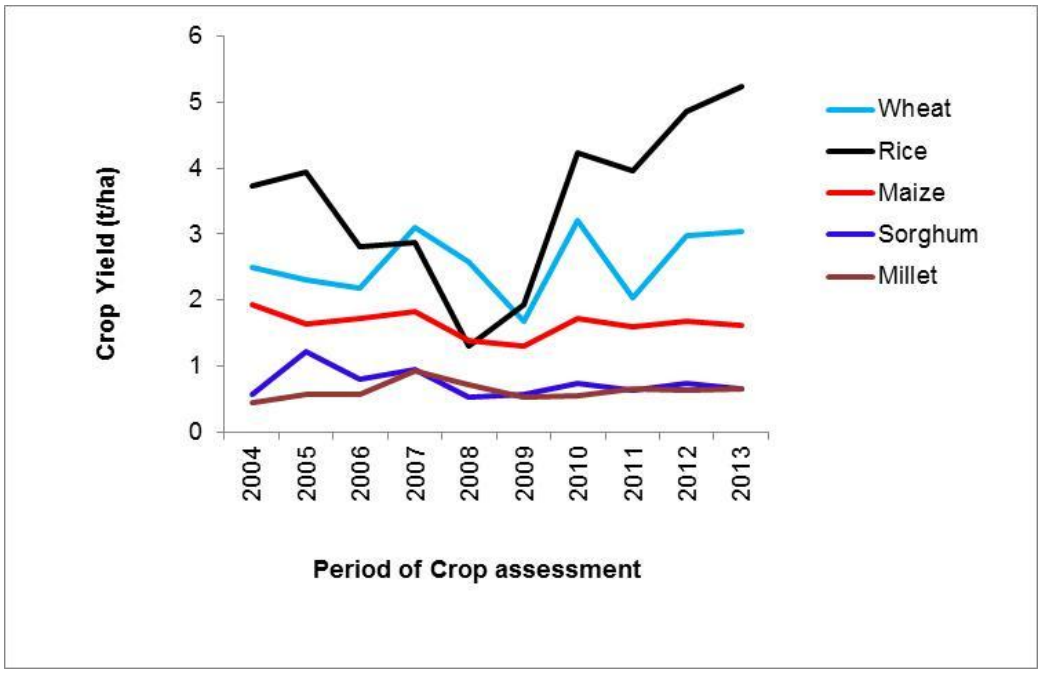

Figure 1-3: Trend of yield for major cereals in Kenya for the period 2004-2013
Source: FAO STATS, 2014

1.3 Constraints to maize production

While the demand for maize is very high and, it is predicted to increase by 45-50% by the year 2020 (Anami et al., 2009), its production is greatly affected by stress factors which can be broadly grouped into three categories, socio-economic, abiotic, and biotic stresses (Oerke, 2006).

The major socio-economic constraints to maize production include lack of credit facilities which makes it difficult to purchase farm inputs especially fertilizer, improved seeds, and chemical pesticides (Wekesa et al., 2003). This is compounded by lack of awareness on the part of the smallholder. Other times certified seed is not available on the market, which leads to use of landraces preserved from previous harvests, which are lower yielding and sometimes prone to other abiotic and biotic stresses (Pixley et al., 2006; Fato et al., 2012).

Abiotic stresses include drought, unreliable rainfall, declining soil nutrients and fertility, salinity and changes in rainfall patterns caused by climate change and global warming (Setimela et al., 2007).

The major biotic stresses to maize production include viruses, fungi, bacteria, parasites, weeds, and harmful insect pests. The insect pests cause direct harm to the crop, and others, act as vectors of disease causing micro-organism. Pathogens and weeds account for 13% each, and insect pests account for 15% (Oerke et al., 1994). Arthropod pests are most common and of great economic importance in maize production (DeVries and Toenniessen, 2001).

1.4 Insects pests

Insect pests are the most damaging to maize crop right from seedling all the way to storage. They can be grouped into two; field and storage pests. The major field pests of maize are the stem borer complex, causing estimated losses of over 50 million MT in Kenya (James, 2003). The most common stem borer species are *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) and *Busseola fusca* Fuller (Lepidoptera: Noctuidae). Maize yield losses due to stem borers in Kenya range between 12-40%, with an average of 13.5% annually, and stem borers have been a major focus for research in an attempt to reduce these losses (DeGroote, 2002).

The major storage insect pests of maize in Kenya are the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the larger grain borer, *Prostephanus truncatus*

Horn, (Coleoptera, Bostrichidae) (Ayertey et al., 1999; Akob and Ewete, 2010). Postharvest losses due to these pests range from 14-40%, with compounding quality loss due to other pest infestation, for example, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) and, create avenues or entry points for secondary infections by mycotoxins in already damaged grains (Bervinson and Garcia-Lara, 2004; Tefera, 2012).

1.5 Rationale of insect resistance breeding

Various methods have been employed in efforts to reduce losses due to field and storage pests. These methods are biological, biotechnological, chemical, cultural, integrated pest management and host plant resistance (Mugo et al., 2001).

Chemical control is effective and commonly used, however, it is expensive for the smallholder farmer, and it has social, environmental and health concerns. These include indiscriminate destruction of target and non-target insects, including beneficial insects like pollinators, decomposers, and biological control agents (Polaszek, 2001). Chemical control also leads to health complications, pollution to the environment, and, pest resurgence due to resistance development on active ingredients by the pest (Meissle et al., 2010) . Cultural control methods, on the other hand, are laborious, and include use of botanicals, field hygiene which encompasses removing of alternate hosts of these pests, burning of leftover stovers and uprooting stems which act as hibernating grounds for the diapausing stem borers (Kfir et al., 2002). Cultural control to some extend also involves hand picking of the individual pests, or use of wood ash (Akob and Ewete, 2010). It is not possible to hand pick stem borers from the maize plant because they enter the stem of the plant and cause stem tunnelling. It is also practically impossible to control weevils on large scale basis using cultural methods because they burrow and are hidden inside the maize grain. Their use is therefore limited. Biological control is effective but slow and it requires thorough knowledge of the pest and its natural enemies which can either be parasitoids, pathogens or predators of the pests (Othira et al., 2009).

Breeding for host plant resistance (HPR) is not easy, since the breeder works with two organisms; the pest and the host. The trait is polygenic and therefore influenced by the environment. However, in terms of benefits, it is the best option for the farmer because the technology is inbuilt within the seed; it is cheaper, easy to use and safe. In addition to HPR, early maturing maize cultivars can escape drought and also avoid pests' early infestation due to lack of synchrony between the critical stages in the plant growth with the critical larval stage of the pest (Mugo et al., 2001).

Despite this awareness, combining different insect resistance traits in breeding has not been done. There are also no maize varieties developed with both stem borer and storage pest resistance. Most of the developed technologies have each resistance trait in separate hybrids, but not a combination of the two into single genotypes. This study aims at developing maize varieties that are early maturing and resistant to both *C. partellus* in the field, and *S. zeamais* in storage in order to reduce both yield and postharvest losses due to these pests. Ultimately the findings of this research will act as baseline studies for breeding for combined stem borer and storage insect pest resistance in maize hybrids, and thereby contribute towards increased food security and productivity by reduction of losses due to these field and storage insect pests.

1.6 Importance of diversity studies and use of marker assisted breeding

Genetic diversity can be defined as "the variety of alleles and genotypes present in a population that is reflected in morphological, physiological and behavioral differences between individuals and populations" (Frankham et al., 2002), as cited by Khoza (2012).

Genetic diversity studies are of great importance in any given breeding program, because they provide baseline information on the genetic resources available for that particular breeding program. Genetic diversity studies also help the breeder make good selection of parents to ensure genetic variability and heterosis. Genetic diversity is also needed for conservation, estimation of alleles in the gene pool of the breeding program, and future follow up of the genetic resources in the population. Genetic diversity studies can also be used for enriching and enhancing the germplasm in the particular breeding program (Prasanna, 2012). Genetic diversity is therefore a critical component for a given breeding program (Jarvis and Hodgkin, 2005).

1.7 Broad objective

The broad objective of this study was to develop early maturing maize hybrids with combined resistance to the important field and storage insect pests in Kenya.

1.7.1 Specific objectives

Specific objectives of the project were to:

1. Investigate the genetic diversity among 130 S_4 maize families with potential for use in breeding for insect resistance in maize hybrids.

2. Determine whether resistance to stem borer, *Chilo partellus*, and storage pest, *Sitophilus zeamais*, can be achieved in hybrid combinations using insect resistant maize inbred lines.

3. Determine the genome dosage effect on resistance to *C. partellus* and *S. zeamais* by using maize lines with contrasting levels of resistance to the respective pests in designing maize hybrids.

4. Determine stability of the new *C. partellus* and *S. zeamais* resistant early and medium maturing hybrids across environments.

1.7.2 Research questions

The following research questions were answered.

1. Does the potential parental insect pest resistance maize germplasm have broad genetic base to act as a good source of genes?

2. Can breeding for insect pest resistance to both stem borers and storage pests be achieved in the same genotype without compromising grain yield?

3. What gene action favours combined stem borer and storage pest resistance in maize hybrids?

4. Is the combined insect pest resistance in the maize hybrids stable across environment?

1.7.3 Hypotheses

There exists genetic diversity in local maize germplasm which can be exploited to provide wide genetic base and insect resistance genes in maize breeding.

Resistance to *Chilo partellus* and *Sitophilus zeamais* can be obtained by crossing inbred lines with the respective resistance to each pest in maize hybrids.

Maize hybrids with combined resistance to *Chilo partellus* and *Sitophilus zeamais* can substantially reduce losses associated with these pests without yield penalty.

There are inbred line parents with resistance to *Chilo partellus* and *Sitophilus zeamais* which have good combining ablity for both grain yield and insect pest resistance which can be used in breeding programs.

Both additive and non-additive gene effects are responsible for controlling resistance to *Chilo partellus* and Sitophilus *zeamais* in maize germplasm, which can be exploited when selecting for host plant resistance in maize.

1.8 Thesis outline

This thesis is divided into six chapters. Each chapter addresses a particular objective and the Chapters 3-5 are designed for publication as standalone potential manuscripts for publication. For this reason there may be unavoidable repetition of some information, contents, and references. They are organized as follows:

Chapter 1: Introduction to thesis. This chapter provides an overview of the importance of maize, its production constraints, and, the objectives for the current study as a way of addressing insect pests constraint in maize production.

Chapter 2: Literature review. The literature on genetic diversity, insect resistance both pre- and post-harvest is discussed. This also includes the gene action and combining ability for insect resistance in maize, and application of the diallel and North Carolina design II in combining ability study.

Chapter 3: Genetic diversity of two S_4 populations; stem borers resistant (SBR), and storage pests resistant (SPR). This chapter provides genetic analysis of S_4 maize families from two maize populations using 30 SSR markers. These populations form part of genetic resources used for host plant breeding for field and storage pest resistance.

Chapter 4: Combining ability for SBR and SPR into hybrids using diallel mating design. This chapter summarizes the findings of obtained after crossing stem borer resistance inbred lines with storage pest resistance one in a diallel design.

Chapter 5: Dosage effect of resistance to both SBR and SPR using North Carolina II mating design. This chapter summarizes the results

obtained when inbred lines with both contrasting and different dosage levels of resistance, to stem borer and storage pests were crossed in a North Carolina II design.

Chapter 6: Overview, general discussions, conclusions and recommendations. This chapter gives a summary of the major findings of this study, and, recommendations for future follow up research on combining stem borer and storage pest resistance in maize hybrids.

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Chapter 2: Literature review

2.1 Introduction

This chapter provides information and literature relating to importance of maize in Kenya, major constraints to maize production, some of the research done to address these constraints. Literature review on earliness and its relevance to breeding for insect resistance was also done. Literature review on economic importance of field pests' stem borer, *Chilo partellus*, and storage pest, *Sitophilus zeamais*; their life cycle, ecology and distribution. It also provides a review of host plant breeding work done addressing insect resistance worldwide, in sub-Saharan Africa and Kenya; identifies some of the gaps in the previous research, which are addressed in the current study. This chapter also identifies the importance and application of other researchers effort and work and how this relates to the current study.

2.2 Maize production in Kenya

Maize in Kenya is grown on 1.6 million ha of land, which is more than 30% of the arable land (FAOSTAT, 2014). Its production is distributed throughout the six agro-ecological zones. These zones are defined by elevation, the amount of rainfall received, length of the growing season and the maturity period of the maize cultivars (Hassan et al., 1998).. Moving from east to west, these zones include; (a) the humid coastal lowland tropics (HCLT) zone, which is found at the coast, (b) the dry mid-altitude (DMA) zone, (c) the dry transitional (DT) zone, (d) the mid-altitude transitional (MAT) zone, (d) the moist mid-altitude (MAM) zone, and (e) the highland tropics (HT) zone (Table 2-1) (Hassan et al., 1998)..

Maize yields from HCLT, DMA, MAM and MAT, are usually low averaging 1.5 ton^{-1}, or even lower, yet they occupy 29% of the area under maize cultivation. The total yield from these zones is estimated to be 11% of the total annual maize production. The HT is sandwiched between the MAT on the eastern side and MAM to the west (Figure 2-1). This zone occupies 30% of the acreage under maize production with average yields of 2.5 t ha^{-1}, which is equivalent to 80% of the total annual maize production (Hassan et al., 1998). The distribution of insect pests of maize follows the maize growing ecologies (Figure 2-1).

Table 2-1: Characteristics of maize growing regions in Kenya

Characteristic	HT	MAT	MAM	DMA	HCLT
Elevation (’00 m)	>18	14-18	14-18	9-18	<9
Annual rainfall (’00 mm)	<18	10-18	8-12	4-8	4-14
National maize area (‘000ha)	307	461	118	118	33
National area (%)	30	46	10	10	4
National Production (%)	35	25	25	10	5
Potential yield (t ha^{-1})	6.7	5.2	3.7	2.7	3.3
Farmer yield (t ha^{-1})	2.0	0.7	1.1	0.5	1
Yield gap (t ha^{-1})	4.7	4.5	2.6	2.2	2.3

Source: Government of Kenya (2009). ‡Data: HT, Highland tropics; MAT, mid-altitude transitional; MAM, md-altitude moist; MAD, mid-altitude dry; HCLT, lowland tropics.

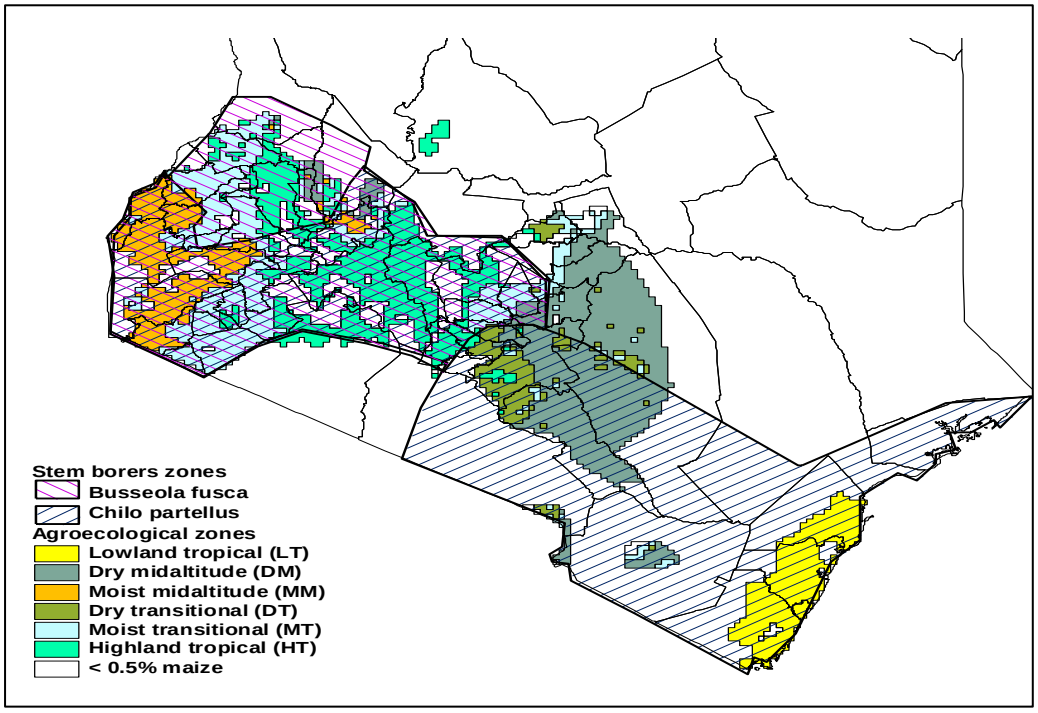

Figure 2-1: Maize growing agro-ecologies in Kenya and the distribution of major stem borers

Source: Modified from Hassan et al., 1998

2.3 General constraints to maize production

Throughout the world, maize cultivation has been limited by various constraints which range from socio-economic, biotic and abiotic stresses. According to Munns and Tester (2008), “stress is an adverse circumstance that disturbs, or is likely to disturb, the normal physiological functioning of an individual”. While the socio-economic stresses mostly affect the smallholder farmer, together with biotic and abiotic stresses, they ultimately contribute to reduced yields as opposed to the potential yields, which could be realised in any one given environment, if they were addressed (Abate et al., 2000). Plant breeders throughout the world are carrying out research on ways of developing maize cultivars that address the needs of the famers, are suited to the various environments, and can withstand the biotic and abiotic stresses in these environments.

Abiotic stress factors are those that occur naturally and are not influenced by humans. These include drought and unreliable rainfall, declining soil nutrients and fertility, salinity, and changes in rainfall patterns caused by climatic changes and global warming (DeVries and Toenniessen, 2001). Plant breeders worldwide have embarked on research to address some of the abiotic stresses through both conventional breeding and biotechnology tools (Anami et al., 2009).

Conversely, biotic stresses, are usually caused by living organisms, including insects and micro-organisms, such as fungi and bacteria (Morais and Pinheiro, 2012). Initially, maize breeding research placed more emphasis on increased yields at the expense of biotic stress resistance (Mwololo, 2010). However, in the recent past, biotic stresses are targeted by breeders worldwide in efforts to reduce crop losses both in the field and post-harvest. These biotic stresses include diseases, weeds and insect pests. The current study emphasises development of hybrids with insect pest resistance both in the field and post-harvest in early maturing hybrids.

2.4 Why emphasis on early maturing maize

Among other factors, challenges of climate change and global warming calls for early maturing maize hybrids. Studies on climate change indicate that there is recorded increase in global temperatures estimated at +0.6^{o}C and projected to increase by between 1^{o}C to 6^{o}C

by the year 2100 (Stige et al., 2006; Mann, 2009). This increase in surface temperatures is predicted to increase evapotranspiration, which favour increased water holding capacity in the atmosphere. However, if the available surface water on land is not sufficient, then this enhanced evapotranspiration is expected to exacerbate naturally occurring droughts (Trenberth, 1998; Trenberth, 2011). These changes have affected low altitude environments in Africa where the growing seasons are increasingly becoming shorter with a higher frequency of drought and higher temperatures due to insufficient surface water (Trenberth, 1998; Stige et al., 2006).

Early maturing maize cultivars have been associated with both drought escaping mechanisms of withstanding water stress (Mugo et al., 1998). Other studies showed that early maturing maize has less aflatoxins and fumonisins contamination as compared to medium and late maturing maize (Löffler et al., 2010). The earliness trait is more so preferred by farmers in semi-arid regions of Kenya and also in other lowland tropics because it is a food security crop, pending maturity of the medium and late maturing maize cultivar (Mugo et al., 1998; Pingali et al., 2001).

On the other hand, drought stressed crops have enhanced damage from insect pest attack. This is because the stressed plants have been found to have high concentrations of nutrients within the leaf tissues (Mattson and Haack, 1987). This favours the growth and development of leaf feeding insects, of which *C. partellus* is among them. Similar studies showed that the altered biochemical composition of drought stressed plants enables insect pests to detoxicate any chemical compounds targeting their control (Mattson and Haack, 1987). These chemicals may be in form of inbuilt mechanisms via biochemical composition of the crop plant or chemical pesticides. As a result, reduced efficacy has been noted in chemicals used on drought stressed crop plants. In order to enhance host plant resistance in maize germplasm targeting drought prone regions, consideration of incorporating the insect resistance trait in early maturing maize germplasm has been factored. Early maturing crops are preferred because they can take advantage of the rains within the first few months before onset of dry spell and escape heavy infestation by insect pests associated with onset of drought conditions (Mattson and Haack, 1987).

It has been noted that early maturing maize varieties experience, not only heavy stem borer infestation levels, but also *S. zeamais* infestations compared to the later maturing cultivars. This can be compounded by late harvesting which allows for infestation to commence in the

field (Ajala et al., 2010). Further, since they mature early they are stored earlier than the late maturing cultivars. The remains of the previous season's stored grains act as sources of infestation by base colony of maize weevil. While these varieties are food security crops, they experience the bulk of insect pest damage both in the field and at post-harvest (Melchinger et al., 1998). Insect resistance trait, for both *C. partellus* and *S. zeamais*, is therefore important in early maturing maize cultivar for management of these pests. Irrespective of these challenges, maize remains the major staple food and the priority crop for the smallholder farmer, and 'earliness' has therefore become a preferred trait by the majority of these farmers.

2.5 Economic importance of *Chilo partellus* and *Sitophilus zeamais*

The devastating effects of *C. partellus* and *S. zeamais* in African agriculture are reflected in the amount of resources spent by farmers in their control. In the year, 1995, Kenyan farmers spent approximately USD 4.5 million on insecticides, USD 10.5 million on fungicides (Ndiritu, 1999). Despite these huge resources spent in efforts to reduce insect pest losses, imported maize is still estimated to increase from the current estimate of around 600 million MT to about 850 million MT in the year 2020 (Meissle et al., 2010). The cost of chemical pesticides in Kenya, Malawi and Tanzania is estimated at USD150–300M every year. This is an emphasis of the importance of developing insect pest resistant maize cultivar, as part of suitable solutions in addressing food security, which is affordable to the smallholder farmer (Oerke et al., 1994).

2.5.1 Biology, ecology and economic importance of *Chilo partellus*

The adult moths emerge and settle on plant debris during the day. The females release pheromones which attract males for mating soon after they emerge (Nesbitt et al., 1979; Lwande et al., 1993). A female moth can lay 200-600 scale-like eggs, within two to three days after emergence. The eggs are laid in batches of 10-80 eggs, on the underside of leaves mostly near mid-ribs (Nwanze, 1988). Neonates hatch 4-8 days later and start feeding on the leaf whorl before tunnelling into the stem and eat out extensive galleries. The larval period lasts between 2-4 weeks before pupating. Just before pupation, the larvae excavate a hole on the stem which is used by adult moths for exit after emergence. Adult moths emerge 5-12 days later after completing a 25-50 days life cycle when environmental

conditions are favourable. Depending on the maize crops maturity period, up to five generations may develop within one growing season, however, when conditions are not favourable, larval diapauses sets in (Nesbitt et al., 1979; Nwanze, 1988).

Chilo partellus is found throughout main land Africa south of the Sahara, especially West, East and Southern Africa. It is prevalent in low altitudes below 1200 metre above sea level (Polaszek, 2001; Kfir et al., 2002). In Kenya, *C. partellus* is distributed in all the maize growing environments, but it is more prevalent in the HCLT, DMA, and MAT (Khan et al., 2008), and therefore, it is the major stem borer species for the mandate region where early maturing maize if grown.

2.5.2 Biology, ecology and distribution of *Sitophilus zeamais*

Sitophilus zeamais Motschulsky (Coleoptera: Curculionadae) is a 2.5 mm long beetle found in the tropics throughout the world. It is a major pest of maize but attacks other cereals like sorghum, rice, wheat, rice, peas and even dried cassava (Danho et al., 2002). In Kenya, the maize weevil is prevalent in all the maize growing ecologies (Mwololo et al., 2012). The adult female makes a hole on the seed coat and deposits one oval white egg in it. The egg is then covered with a waxy secretion which acts as a seal to plug the hole, before adult female moves on to another grain to lay eggs on it (Sallam, 2010). Only one egg is laid on each grain, and a total of 300-400 can be laid by a single adult. The legless larva (grub) feeds inside the grain until pupae stage. A circular hole made during larval stage is used by the adult beetle for exit. The life cycle of an adult maize weevil can be between five to eight months depending on environmental conditions (Maceljski and Korunic, 1973). *Sitophilus zeamais* is distributed throughout the world in warm humid regions where maize is grown, and thrives well in temperature range of 15-34^{o}C and 40% relative humidity (Tefera et al., 2010). In Kenya, it is prevalent mostly in the HCLT, DMA, and MAT, but can be found in other maize growing zones but at a lower frequency (Tefera et al., 2013).

2.5.3 Management of *Chilo partellus* and *Sitophilus zeamais*

Several control options have been employed in order to reduce losses due to stem borers (Kfir, et al., 2002). These measures include chemical, biological, cultural, “push-pull” method (Khan et al., 2008), use of trap crops (Chabi-Olaye et al., 2005), integrated pest

management (IPM) (Nwilene et al., 2008), host plant resistance (HPR) (Arabjafari and Jalali, 2007), and more recent biotechnology through genetic engineering of resistance genes to the host plant (Cerda and Paoletti, 2004).

Chemical control methods are expensive, and they also bring about unfavourable environmental effects in addition to being indiscriminate to the target and non-target insects, including beneficial insects like pollinators, decomposers, and biological control agents (Polaszek, 2001; Romeis et al., 2008). Efforts by all concerned to limit the extensive use of chemicals have been on-going, for example the ban of DDT®, Atrazine® and Furadan® (Dalvie et al., 2009; Meissle et al., 2010), which have had adverse effects on human health and environment.

2.6 Host plant resistance as a method of insects pest control

Host plant resistance (HPR) can be defined as the relative amount of heritable qualities possessed by a plant, which influence the ultimate degree of damage done by the insect in the field (Painter, 1951). This method of control has many advantages to its use as compared to the others because it is compatible with all of the other methods, for example, use of resistant cultivars reduces quantities of chemical use to bare minimum, has no harmful effects on biological control agents and makes a good component of IPM (Dent, 2000). Host plant resistance is not weather-dependent, and does not depend on density of pest, and it affects only the target pests (Russell, 1978). Host plant resistance can be improved through breeding by combining multiple resistance genes which confer different types of resistance within the same cultivar (Kumar, 1997). Development of stem borer and maize weevil resistant maize cultivars will be of great advantage to the smallholder farmer.

2.7 Breeding for host plant resistance to insect pest

Plant breeding has been defined as “the art, science and business of improving plants for human benefits” (Poehlman and Sleper, 1995; Bernado, 2002). An insect resistant plant on the other hand can be defined as the result of heritable plant qualities or traits that make the plant less damaged upon infestation than damage observed in that plant without the traits (Dent, 2000).

In order to initiate a breeding program, it is advisable to first decide on where to source for parental germplasm. There are other factors which are important for insect resistant breeding; adequate source of insects' pests in question, the methodology for field screening, and, protocol for laboratory assays (Tefera et al., 2010).

Breeding for insect resistance has lagged behind research addressing other maize production stresses (Bergvinson and Garcìa-Lara 2004). This is because the breeder has to deal with the dynamics of host–pest interaction, as well as address production of each of the two organisms' enmass. This is costly, time consuming, and requires skilled labour and specialized laboratories (Mugo et al., 2001).

Use of HPR is a better option for *C. partellus* and *S. zeamais* control, since it is easy and less costly for the smallholder farmer compared to chemical control which is expensive and has health implications or cultural control which is laborious and ineffective (Kfir 2002). This is also because the technology is built within the seed, and it does not require sophisticated procedures to administer. However, while various research activities to incorporate HPR option for stem borer control have been carried out in the past decade (CIMMYT, 1989), most of the resistant cultivars are late maturing and therefore not suited for semi-arid regions. There are also no maize varieties developed with both *C. partellus* and *S. zeamais* pest resistance. The current study was geared towards developing maize varieties that have resistance to both stem borers in the field, and maize weevil during post-harvest in order to reduce the yield losses due to these pests. This makes host plant resistance affordable to the smallholder farmer since the technology is built within the seed (Dent, 2000; vanEmden, 2007).

2.8 Genetic diversity and its role in insect pest resistance breeding

In insect pest resistance breeding, genetic diversity studies are important for good genetic base (Jarvis and Hodgkin, 2005). This is because host-plant resistance (HPR) is a quantitative trait, which is polygenic and controlled by multiple genes, and therefore highly influenced by environmental conditions (Bernardo, 2002). Genetic diversity studies can be exploited in charactering insect pest resistance mechanisms (Dhliwayo and Pixley, 2001), as well as making selection of improved superior genotypes for future breeding (Liu et al., 2003; Xia et al., 2004).

Locally adapted maize varieties are known to have good genetic diversity, with alleles that are adapted to the constraints within a given region (Warburton et al., 2008). However, few breeders are willing to use locally grown maize as a source of good alleles and exploit the genetic diversity resource for developing germplasm with desired traits in their breeding programs. This is because the selection process takes a long time before inbred lines with the desired traits are developed. In order to achieve these objectives, markers come in handy for determining the genetic variation in a given population (Jones et al., 1997; Stevens, 2008).

2.9 Types of markers and their importance in breeding

Markers are tools used to identify a trait in a living organism (Lander and Botstein, 1989; Collard et al., 2005). There are two types of markers that have been used for diversity studies; morphological and molecular markers. Morphological markers use phenotypic traits and are biased due to continued selection of preferences in crops morphology (Moose and Mumm, 2008). Molecular markers use variation in macro-molecules in the genome of an organism. Molecular markers can further be classified in two types; Biochemical markers and genetic markers. Biochemical markers are proteins which are produced when a certain gene is expressed. They use dyes and electrophoresis for separation of enzymes, and are less polymorphic (Jones et al., 1997). Genetic markers use fragments of DNA with a specified sequence that detects a desired sequence in an organisms' genome. The discovery of molecular markers enables genetic diversity analysis of plants genomes (Collard et al., 2005).

2.10 Molecular markers and their role in diversity studies

Molecular markers can be defined as specific fragments of DNA that can be readily detected and identified within the whole genome, and whose inheritance can be easily monitored (Ribaut and Hoisington, 1998). Molecular markers detect differences in nucleotide sequence in the DNA of an organism, which are as a result of mutations (Babu et al., 2012).

There are several types of molecular markers that are commonly used, which include random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), DNA amplification finger printing (DAF), restriction fragment length polymorphisms

(RFLPs), and simple sequence repeats (SSR) also known as microsatellites (Semagn et al., 2012).

In the recent past, more markers have been used for different purposes ranging from measuring diversity, to, marker assisted breeding. These recently developed markers include; Inter-simple sequence repeats (ISSR), Cleaved amplified polymorphic sequences (CAPS), Diversity arrays technology (DArT), Expressed sequence tag (EST), Sequence tagged sites (STS), and Sequence characterized amplified regions (SCAR) (Weising et al., 1998). Others include Single-strand conformation polymorphism (SSCP), Sequence characterized amplified region (SCAR), and Single nucleotide polymorphism (SNP). Still others are retrotransposon-based markers, including sequence-specific amplified polymorphism (S-SAP), Inter-retrotransposon amplified polymorphism (IRAP), Retrotransposon-Microsatellite amplified polymorphism (REMAP), Retrotransposon-Based insertional polymorphism (RBIP) (Weising et al., 1998; Semagn et al., 2006).

Before the advent of SNPs and the more recent markers, SSRs, were more preferred since they are codominant, have a simple repetitive DNA sequences consisting of two, three or four nucleotides (di-, tri-, and tetra-nucleotide) repeats. They are also multi-allelic, are easy to use and require low amount of DNA; are reproducible, highly polymorphic, and, affordable. They are also easily transferable among populations, and can be automated for high throughput screening (Enoki et al., 2002). Their use in other maize genetic diversity studies has been documented (Khoza, 2012; Matewele, 2014). They have also been used in genetic diversity studies involving other crops, for example; *Sorghum bicolor* (L.) Moench. Their use involves characterization of germplasm, fingerprinting and identification of germplasm, and calculating genetic distances in populations (Perumal et al., 2007; Beyene et al., 2014). These markers are highly polymorphic and provide quality information on multi-allelic loci, and, they are therefore still valid and were chosen for use in the diversity studies in the current research.

Although genetic diversity has been studied in some maize germplasm in Kenya (Mwololo et al., 2012), there has not been one which focuses on germplasm which is used in breeding for combined insect resistance to both field pests stem borers and storage insect pests. It is therefore, not known whether there is diversity which breeders should exploit in breeding for both stem and postharvest resistance in hybrids. The current study has factored in the need

for genetic diversity studies of maize germplasm which has potential for breeding combined host plant resistance to stem borers and storage pests.

While transgenic technology is a handy tool in breeding, it is important to note that biosafety issues in sub-Saharan Africa are not yet well established. Though Kenya has a biosafety law, no transgenic product has been commercialized to date. While this approach has potential to address pest menace, it remains a tool for complementing conventional approaches to host plant resistance breeding, but cannot replace conventional breeding approach (Araus et al., 2008). Markers have been widely used, not only for diversity studies, but also for other studies relating to insect resistance breeding (Ribaut and Hoisington, 1998).

2.12 Genomic regions for insect resistance in maize

Several studies on insect resistance genetics in maize have been carried out for both leaf feeding insect pests as well as storage pests. Quantitative trait loci (QTL) for resistance to these insect pests have also been documented by several authors: Willcox et al., (2002) reported three QTL regions associated with resistance to southwestern corn borer (SWCB) *Diatraea grandiosella* Dyar, (Lepidoptera, Crambidae) on chromosome 7 (c7), 9 (c9) and 10 (c10). On the other hand, Castro-Alvarez et al., (2015) found six chromosomal regions; 2, 3, 4, 8 and 10; associated with maize weevil resistance. Similar results were obtained by Garcia-Lara et al., (2010) while working on tropical maize. The study found that QTL for grain components; simple phenolic acids, diferulates and hydroxyproline-rich glycoproteins (HGRPs), associated with maize weevil resistance are located in chromosome, 3, 6, 8 and 10.

2.13 Conventional breeding for insect resistance

There are various methods for conventional breeding, but the most common is recurrent selection with screening for resistance at each advanced generation. Different parameters associated with susceptibility and resistance to insects pests in question are first identified, and then measured (KARI and CIMMYT, 2003). The frequency of the resistance genes in the resulting progeny population are increased over time (Pixley et al., 2006).

2.13.1 Sources of bioassay insects

In order to carry out conventional breeding for *C. partellus* resistance in maize, a continuous supply of good quality insects is required. The insects should also be at the required stage and in large enough quantities. For this reason, mass rearing facilities are required before breeding for insect resistance can be initiated (Tefera et al., 2010). The bioassay *C. partellus* neonates used in this study were obtained from KALRO-Katumani mass rearing insectary, while the post–harvest insects were obtained from the mass rearing laboratory located at Kiboko. Having ensured good source of pest supply, a suitable method for breeding is then used for resistance development (Tefera et al., 2010).

2.13.2 Estimation of gene action in conventional breeding

Gene action is defined as the expression of genes within an individual (Chahal and Gosal, 2002). This expression can be identified through genetic studies and classified into additive or dominance and epistasis. Additive gene action can be estimated through narrow sense heritability and acts as guidelines for selection of traits in breeding. Narrow sense heritability can be defined as the degree to which a trait in a parent is passed onto the offspring (Falconer and Mackay, 1996). Dominance can be estimated from the progeny relative to the performance of mid-parent value (Robinson, 1987; Sharma et al., 2007). It can range from over-dominance, when the performance of the progeny is outside the estimated range of parents, to partial-dominance, when the performance of the progeny is inclined towards the performance of one parent (Hallauer et al., 2010). Epistasis is observed when genes controlling a certain trait interact with others to express that trait, which, would otherwise not be observed in presence of other gene combination (Eta-Ndu and Openshow, 1999). It can be expressed as additive by additive, additive by dominance or dominance by dominance effects (Falconer and Mackay, 1996).

Estimation of gene action can be done by making crosses between parents using different types of mating design (Hallauer et al., 2010). The choice of method to use largely rests on the breeder, and the objective of the breeding program and the sources of resistance. After developing the resistant maize cultivar, efficient screening and evaluation methods are important. These methods will identify the genotypes that are expressing the insect

resistance in the progeny. The frequency of the genes can then be assessed (Bernado, 2002).

Studies by Butrón et al. (1999) and Karaya et al. (2009) when investigating combining ability of stem borer resistant lines, reported preponderance of additive gene action for stem borer resistance in maize hybrids. Caution needs to be applied though before we can conclusively say that stem borer resistance is conditioned by additive genes because some other studies have also reported dominance as having a part in stem borer resistance (Kumar, 1997; Sharma et al., 2007). On the other hand, maize weevil resistance is associated with the grain properties since it is the structure of the maize that is attacked by the pest (Arnason et al., 1994; Abebe et al., 2009). Breeding for weevil resistance in maize therefore aims at improving the grain and increasing the characteristics associated with resistance (Bergvinson and García-Lara, 2004). Studies on gene action conditioning maize weevil resistance have been documented (Kim and Kossou, 2003; Derera et al., 2014), with both additive and non-additive genes responsible for resistance to maize weevil, and reported cases of possible maternal effects (Dhliwayo et al., 2005).

Other studies in Kenya by Mwololo et al. (2012) and Tefera et al. (2011) identified maize hybrids with resistance to maize weevil. However, there are no reported studies on gene action conditioning combined stem borer and storage pest resistance, which makes the current study a baseline study for future research on combining resistance to stem borers and maize weevil in maize hybrids.

2.14 Choice of method to use

In the current study, two methods of estimating gene action were used; Griffings' design IV, and North Carolina II (Comstock and Robinson, 1952; Griffing, 1956). There are assumptions based on the method used for sampling the parental inbred lines. When the parents are randomly chosen from a population, the assumptions are that; (1) the genes in the parents are independently distributed, (2) there is no epistasis gene action, (3) there is normal mendelian diploid inheritance, (4) there are no maternal effects, (5) there is no linkage disequilibrium, (6) there are no correlation of environmental effects. However, for this research, the parents were not randomly chosen from the population because the research is inclined towards developing combined insect resistance in maize genotypes. In this case

the experimental material is the population, and, therefore, inferences made in this research are specific to this population.

2.14.1 Diallel mating design and its advantages

The diallel mating design is defined as making all possible crosses among a group of inbred lines (Sprague and Tatum, 1942). Diallel design has been modified over time to factor in evaluation for the parental effects, reciprocals effects, and, the crosses effects, resulting in four methods; method I or complete diallel, method II or half diallel, method III and method IV (Table 2-2).

Complete diallel, also known as method I provides variance due to the parents, the crosses and the reciprocals. Method II, or half diallel provides variance due to parents and crosses, method III evaluates both the crosses and reciprocals while method four provides the variances due to crosses (Griffing, 1956; Hallauer et al., 2010). Diallel mating design is useful when dealing with few parents, and therefore it has limitations on the number of parents that can be crossed. This necessitates the use of North Carolina design II.

Table 2-2: Four methods of diallel mating design

Method	Components
1	Parent + F_1s + reciprocals
2	Parents + F_1s only
3	F_1s + reciprocals
4	F_1s only

The variances estimates obtained from analysis of diallel are translated into genetic variance components, σ^2, additive or dominance. It is from these variance components that fixed effects and random effects are analysed using either analysis of variance or combining ability estimates. In this research, since the parents were carefully selected for insect resistance traits, a fixed model is used for estimating both general and specific combining ability of the parents, and it was used for estimation of unbiased of combining abilities and gene action (Shattuck et al., 1993).

Diallel mating design is a powerful tool, which has been widely used by breeders to estimate general and specific combining ability effects. Favourable GCA effects enable researchers to select suitable inbred lines for use in breeding programs, favourable SCA effects are useful for selecting suitable crosses. Williams and Windharm (2015) used diallel for investigating aflatoxin accumulation in maize. The study reported significant and positive GCA effects on susceptible maize inbred lines. A similar study was carried out by Betrán et al. (2002) to investigate accumulation of aflatoxin in maize in order to identify potential sources of resistance for breeding. The study revealed that yellow maize was more prone to aflatoxin infection as compared to white maize, with a strong influence on the environment. The authors further reported that both GCA and SCA effects were important depending on the method used for screening aflatoxin accumulation.

Diallel mating design has also been used for insect resistance studies. One such study was done by Butrón et al. (1999) when investigating maize ear resistance to pink stem borer. The study revealed that additive, non-additive and cytoplasmic effects were important for ear resistance to the pink stem borer, although additive effects were more important. Further, (Alvarez and Miranda (2002) used diallel method to investigate resistance of maize to fall armyworm (*Spodoptera frugiperda*, Smith. Lepidoptera: Noctuidae). The results also indicated a confounding effect of additive and non-additive effects, with significant non-additive genes for control of *S. frugiperda*. Other studies involving storage pests' resistance have been carried out using diallel mating design. This is evidenced by Dhliwayo et al., (2005) when investigating the combining ability for resistance to maize weevil. The current study showed that both GCA and SCA effects were important for maize weevil resistance. Other studies done using diallel for maize weevil resistance include Kang et al. (1995) to study maize weevil preference to maize grain. The study revealed important and significant GCA and SCA effects for non-preference to grain by maize weevil.

2.14.2 North Carolina II mating design

The North Carolina II mating design, also known as a factorial design, was proposed by (Comstock and Robinson, 1952), as a modification of North Carolina design I. It classifies or groups the female and male parents into separate groups of individuals or families, which are crossed in a factorial scheme, generating half-sib groups (Table 2-3).

North Carolina II design has two advantages over diallel; (1) large number of parents can be crossed, generating few crosses for evaluation, and, (2) two independent estimates of GCA effects are obtained (Sprague and Tatum, 1942; Comstock and Robinson, 1952).

Table 2-3: Schematic representation of North Carolina II design

	Males				
Females	6	7	8	9	10
1	X_{16}	X_{17}	X_{18}	X_{19}	X_{110}
2	X_{26}	X_{27}	X_{28}	X_{29}	X_{210}
3	X_{36}	X_{37}	X_{38}	X_{39}	X_{310}
4	X_{46}	X_{47}	X_{48}	X_{49}	X_{410}
5	X_{56}	X_{57}	X_{58}	X_{59}	X_{510}

Half-sib group of females

Half-sib group of males

Previous studies involving insect resistance have demonstrated the effectiveness of this design for estimation of genetic variances. Derera et al., (2014) demonstrated that North Carolina design II is effective for insect resistance studies when he used this design to study the implications of grain weight loss for breeding resistance of maize to the maize weevil. Another researcher, Matewele (2014), used the North Carolina design II to assess the resistance of maize to larger grain borer and maize weevil in Malawi. Meseka et al. (2013), also used North Carolina design II while studying genetic analysis of maize inbred lines for tolerance to drought and low nitrogen in Nigeria, where he reported favorable SCA effects for grain yield under Low N conditions.

The current study used both diallel and North Carolina II, each with independent parental inbred lines with specified insect resistance levels. In the diallel, 12 parents were used generating 66 test hybrids. A different set of 20 parents were organized in groups of five, and crossed into eight sets using North Carolina design II.

2.15 General and specific combining ability

Combining ability has been defined as the ability of an individual, when crossed, to produce progeny with strong expression of a particular trait (Poehlman and Sleper, 1995). Acquaah (2007), defined combining ability as the performance of a line with others in a cross. Combining ability can be grouped into two, general and specific combing ability.

General combining ability is “the ability of an individual to produce progeny with high genetic quality, when crossed to many other individuals in the population (Maynard, 1996). Falconer and Mackay (1996) also defined GCA as, “the mean performance of a line in all its crosses when expressed as a deviation from all the crosses”.

Specific combining ability is defined as “the performance of a line in a cross with a specific parent (Sprague and Tatum, 1942). It is also defined as, “the performance of a line in a specific cross which is over and above that expected on the basis of general combining ability” (Chahal and Gosal, 2002). Further, Poehlman and Sleper (1995), defined it as “the performance of specific combinations of genetic strains in crosses in relation to the average performance of all combinations. In the current study, GCA and SCA effects were assessed using the diallel method IV, and, North Carolina design II, each method having its own separate set of parents.

A lot of studies have been done on combining ability of maize germplasm for various stress factors. Significant GCA effects were reported by Derera et al. (2008) for grain yield under drought stress when investigating associations between grain yield potential, stress tolerance and yield stability in southern African maize. Menkir and Ayodele (2005) also reported significant GCA effects for grey leaf spot (GLS) disease. Research done to address insect pest resistance to maize include work by Kim and Kossou (2003). The authors reported highly significant GCA and SCA effects for resistance to maize weevil from maize hybrids. Further García-Lara et al. (2009) reported that resistance to maize weevil in maize grain is attributed to varying gene actions. Research findings by Dari et al. (2010) indicated that both GCA and SCA effects were significant, and therefore both additive and non-additive genes action were important for maize weevil resistance in early generation maize inbred lines. In Kenya, Karaya (2009) reported highly significant GCA effects for grain yield and stem borer resistance parameters, with significant SCA effects from few crosses

for leaf damage scores and tunnel length. This was indicative that additive gene action was more important and therefore responsible for stem borer resistance in maize. Similar results were obtained by Beyene et al. (2011), when investigating the combining ability of maize inbred lines resistance to stem borers. The findings revealed that GCA effects were five times greater when compared to SCA effects. However, with all the reported research findings, there is still a gap as on combining resistance to stem borers and storage pests in maize, and there are no publishlications on combined stem borer and maize weevil resistance. This study therefore will act as the baseline findings for future combined field pests and storage pest resistance.

2.16 Summary of literature review

This review showed that maize remains the staple food for the majority in sub-Saharan Africa. This justifies enhanced research in maize improvement in breeding programs. The review further showed that insect pests, both in the field and in storage, still remain a major challenge to maize production, with breeding work addressing individual pests in separate genotypes. This leads to a gap in maize breeding for combined insect resistance, in order to have the two resistance traits in the same genotype. Therefore breeding for combined resistance can lead to securing the losses associated with field and storage pests of maize and thus contribute to food security in sub-Saharan Africa. Again, this review highlighted the importance of having a good source of quality bioassay insect source when breeding for insect resistance traits. The insects should be in the right stage, quality and quantities.

It is evident from the review that no literature has been published on breeding for combined stem borer and post-harvest insect pests' resistance in maize hybrids. The literature indicates that research has been employed separately on stem borer resistance and postharvest insects. As a result there is no evidence of hybrids which carry combined resistance to the two groups of insects. The review also highlighted the importance of having a wide genetic base for a breeding program addressing quantitative traits. Further, the review brought out the importance of engaging modern tools of molecular markers for enhancing breeding. It established that documented work reports additive and non–additive gene action are responsible for stem borer and storage pests resistance in maize, but no literature is documented on combined resistance to field and storage pests resistance in the same maize hybrid.

Again from this review, it is established that secondary traits can be used for selection in breeding for other traits with low heritability, of which insect resistance is one of them. Emphasis of importance of multi-environment testing for genotypes was highlighted. This identifies the main effects due to genotypes and environments, as well as the interaction. It also contributes to selection of genotypes which are stable and those that are specifically adapted for certain environments.

In efforts to address the challenge of combined resistance to maize insect pests, it is important to quantify the level of genetic diversity in germplasm to be used in breeding for insect resistance. The following chapters present research which was conducted to close some of the knowledge gaps which were identified in the literature review.

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Chapter 3: Genetic diversity of maize germplasm for developing insect pest resistant maize hybrids

Abstract

Genetic diversity is important to ensure viability of a breeding programme. The objective of this study was to determine the genetic diversity of 130 S_4 families from two populations; sixty five (65) lines from stem borers' resistant population (SBR) and 65 lines from the storage pests' resistant population (SPR) using 30 SSR markers. Results revealed that the markers were polymorphic with 0.46-0.48 polymorphism for both SBR and SPR populations, except umc1367, which was monomorphic for SPR population. A total of 109 alleles were recorded from SBR population. Allele's scores ranged from 2 to 6 alleles per loci, with a mean of 3.63, and product length ranging 47-362bp. The SPR had total of 103 alleles, with scores of 1 to 6 per loci, and a mean of 3.43 alleles, and product length ranging 47-320bp. Observed gene diversity was 0.27, with expected gene diversity of 0.45-0.48 for SBR and SPR respectively. Mean PIC values for SBR and SBR were 0.48 and 0.46 respectively, while uHe values were 0.4 and 0.45 respectively. Cluster analysis revealed three major clusters in each population; with cluster 1 comprising 33.8-40% of the genotypes in SPR and SBR populations respectively. Cluster two had 55.4% of SBR genotypes, and 53.4% of SPR genotypes. The study indicates that there is ample genetic diversity in the two populations which can be exploited in extracting new inbred lines for use in breeding insect resistant maize hybrids.

Key words: Genetic diversity, stem borer resistant, storage insect pest resistant, SSR markers

3.1 Introduction

Genetic diversity studies have been known to aid breeding programs benchmark their genetic resources (Hartl and Clark, 1997; Dagne, 2008).). These studies also provide useful information on alleles within a given gene pool (Prasanna, 2012).

Identification of breeding gerplasm with good genetic base is of great importance in insect resistance breeding because of its a polygenic trait. Diversity studies therefore ensure that the germplasm being used for breeding has a wide genetic base (Dhliwayo and Pixley, 2003; Hari et al., 2004). This can be achieved at three levels; phenotypic, biochemical or molecular level (Beyene et al., 2014). The most reliable of these is use of molecular markers (Cholastova et al., 2011). There are several types of molecular markers, which include restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), single nucleotide polymorphisms (SNP), and, diversity array technology markers (DATm) (Yuan et al., 2000; Perumal et al., 2007; Bouchet et al., 2012). Genetic diversity studies are therefore of great importance for any given breeding program (Jarvis and Hodgkin, 2005).

Locally adapted maize (*Zea mays* L.) varieties are known to have good genetic diversity, with alleles that are adapted to the constraints within a given region (Warburton et al., 2008). However, few breeders are willing to use locally grown maize as a source of good alleles and exploit the genetic diversity resource for developing germplasm with desired traits in their breeding programs. This could be due to the amount of time and other resources required for identifying the right germplasm for the trait of interest, a challenge that can be addressed by use of molecular breeding approaches which saves time (Semagn et al., 2006) . In Kenya, Katumani composite, early maturing well adapted maize composite, has been grown for many years in the drought prone eastern region of Kenya. It is however, susceptible to insects' pests both in the field and at storage. Unfortunately, its use as a source of good alleles in breeding has been down-played.

The Kenya Agricultural and Livestock Research Organization (KALRO), in collaboration with the International Maize and Wheat Improvement Center (CIMMYT), have developed maize varieties which have insect pest resistance traits (Mugo et al., 2001). Unfortunately, the resistance to field pests is in separate germplasm from the resistance to storage insect pests. To compound the problem, most of the already developed insects' resistant maize hybrids are late maturing yet earliness and insect resistance are among farmer preferences in maize hybrids (Odendo). This makes them unsuitable for production in the dry mid-altitude ecologies of eastern Kenya which experience random drought and high insect pest infestation rates in the field and in storage. Moreover, the warm temperatures prevalence in these ecologies are conducive environment for insect pest multiplication.

A breeding program was started to develop early maturing stem borer and storage pest resistant maize inbred lines. This was done with the aim of developing early maturing maize germplasm with combined resistance to both *Chilo partellus* and *Sitophilus zeamais* insect pests of maize. This required use of divergent germplasm in order to generate superior progeny from which pedigree breeding could be initiated. Therefore, in order to avoid use of parentage stock with narrow genetic base, the potential parental germplasm was subjected to molecular analysis using microsatellite markers to determine the divergence of the germplasm. Use of Katumani composite as a parent for earliness is also important in exploiting the genetic base as a good source of desired alleles in breeding.

The objectives of this study were to:

1. Determine the genetic diversity of the stem borer resistant S_4 maize lines.
2. Determine the genetic diversity of storage pest resistant S_4 maize lines.

3.2 Materials and methods

3.2.1 Maize germplasm and sampling procedure

Seeds of 130 S_4 lines; sixty five from stem borer resistance population, and 65 S_4 lines from a storage insect pest resistant population were selected at random. The populations were developed by crossing elite CIMMYT inbred lines with insects' resistance to the target pests with a locally adapted maize variety, Katumani composite.

The seeds were sent to Biosciences eastern and central Africa (BecA) laboratories in Nairobi, Kenya for genotyping. They were then planted in pots at a greenhouse at BecA during 2012 short rains experienced in the months of November to January (2012A) season. Leaf tissues were harvested three weeks after germination, and placed in tubes of a 96-well tube containing one stainless steel ball in each tube. The 96-well tube box was then placed in a bucket containing liquid nitrogen at a temperature of -210°C (-346°F) to chill the tubes. The chilled samples were placed in a genogrinder machine set at 500 strokes per minute and ground for two minutes.

3.2.2 DNA extraction and genotyping

The plates were weighed and placed in a centrifuge machine for three minutes, which was set at 3500rpm. Using a multichannel pipette, 450ul of 65^0C pre-heated extraction buffer was added to each tube and capped. The samples were then placed in a water bath set at 65^0C, and incubated for 40 minutes. Deoxyribonucleic acid (DNA) was extracted by solvent modified method as described by Dellaporta et al. (1983), and, Semagn et al. (2012). A set of 30 microsatellites (SSR) markers, were used for genotyping the samples. The PCR products were run and detected on capillary system ABI-3730 using the LIZ500 as internal size standard.

3.2.3 Data collection and analysis

The data were then captured and done using the Genscan® software (Applied Biosytems) with reference dyes used as; Ned(Y) Pet R) 6-FAM (B) and Vic (G). The resulting fragments were analyzed and the alleles scored using the Genemapper® software ver4.1 (Applied Biosystems, 2009), and then compiled into a spreadsheet as a standard Genemapper output file.

3.2.4 Genetic diversity analysis

The genotypic data was subjected to analysis using DarWin version 6.0.10, and GENALEX version 6.5 (Peakall and Smouse, 2007). Data analysis was done using the protocol of Nei and Li (Nei and Li, 1979), to determine differences in allelic frequencies among the SSR markers. Information on polymorphic information content (PIC), the number of effective alleles per locus, total number of alleles per locus (Na), allelic richness observed heterozygosity (Ho), the average gene diversity (He), and, total gene diversity (Ht), were generated (Botstein et al., 1980). This information was calculated using the following formulae;

(PIC) was calculated using the formula $PIC=1- i=1-n(\sum fi^2)– (fi^2)^2$ ……..(1)

Na = No. of Different Alleles;

Ne = No. of Effective Alleles = 1 / (Sum pi^2);

I = Shannon's Information Index = -1 Sum (pi * Ln (pi));*

Ho = Observed Heterozygosity = No. of Hets / N;

He = Expected Heterozygosity = 1 - Sum pi^2;

Ht = Ho + He

*uHe = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He;*

F = Fixation Index = (He - Ho) / He = 1 - (Ho / He);

Where pi is the frequency of the ith allele for the population & Sum pi^2 is the sum of the squared population allele frequencies.

Cluster analysis was done for both SBR and SPR populations with neighbour-joining algorithm. The unweighted pair group method of DARwin 6.0 software was used, with Bootsrap values set at 10,000 iterations. Genetic dissimilarity dendograms were generated for each population, and graphical representation of populations done (Perrier and Jacquemoud-Collet, 2006).

3.3 Results

3.3.1 Microsatellite markers characterization

A total of 3859 data points were achieved out of the expected 3900 data points giving an overall success rate of 98.95%, from the analysed marker data. Summary of the motif and coloading information of 30 SSR markers, which were used for genotyping SBR and SPR populations, is recorded in Table 3-1. Microsatellite analysis revealed that 6.7% of the SSR motifs represented are dinucleotide, 40% are trinucleotide, and 53.3% are compound nucleotide (Table 3-1).

The shortest product size in both SBR and SPR population was observed in locus umc2250 with 47 base pairs (bp), while the longest for SBR was observed in locus phi062 with 362 bp, and, the longest for SPR was observed in locusPhi227562 with 320 bp. The highest difference in variation from the same locus was 212 bp, observed in phi072 (Table 3-1).

Table 3-1: Background information on 30 microsatellite markers, their motif and coloading information

Marker Entry	Name	Repeat Sequence	Repeat number	Bin Number	Coloading set
1	nc130	AGC	Tri	5	6
2	nc133	GTGTC	Penta	2.05	19
3	Phi227562	ACC	Tri	1.11	*
4	phi029	AG/AGCG	Compound	3.04	10
5	phi031	GTAC	Tetra	6.04	2
6	phi041	AGCC	Tetra	10	3
7	phi046	ACGC	Tetra	3.08	*
8	phi056	CCG	Tri	1	3
9	phi062	ACG	Tri	10.04	23
10	phi065	CACTT	Penta	9.03	17
11	phi072	AAAC	Tetra	4	3
12	phi075	CT	Di	6	4
13	phi076	AGCGGG	Hexa	4.11	7
14	phi079	AGATG	Penta	4.05	1
15	phi084	GAA	Tri	10.05	6
16	phi102228	AAGC	Tetra	3.06	2
17	Phi112	AG	Di	7.01	1
18	Phi114	GCCT	Tetra	7.03	23
19	phi123	AAAG	Tetra	6.07	21
20	phi299852	AGC	Tri	6.07	23
21	Phi308707	AGC	Tri	1.1	1
22	Phi331888	AAG	Tri	5.04	4
23	Phi374118	ACC	Tri	3.02	5
24	Phi96100	ACCT	Tetra	2.01	5
25	umc1161	(GCTGGG)5	Hexa	8.06	22
26	umc1304	(TCGA)4	Tetra	8.02	10
27	umc1367	(CGA)6	Tri	10.03	5
28	umc1545	(AAGA)4	Tetra	7	18
29	umc1971		Tri		*
30	umc2250	(ACG)4	Tri	2.04	7

* Coloading information undefined

3.3.2 Allelic content of stem borer resistant population

Analysis using 30 SSR markers identified total of 109 alleles from SBR population. The number of polymorphic alleles scored ranged 2 - 6 per loci, with a mean of 3.63 alleles. A graphical summary of the markers and the respective alleles amplified for SBR population is presented in Figure 3-1.

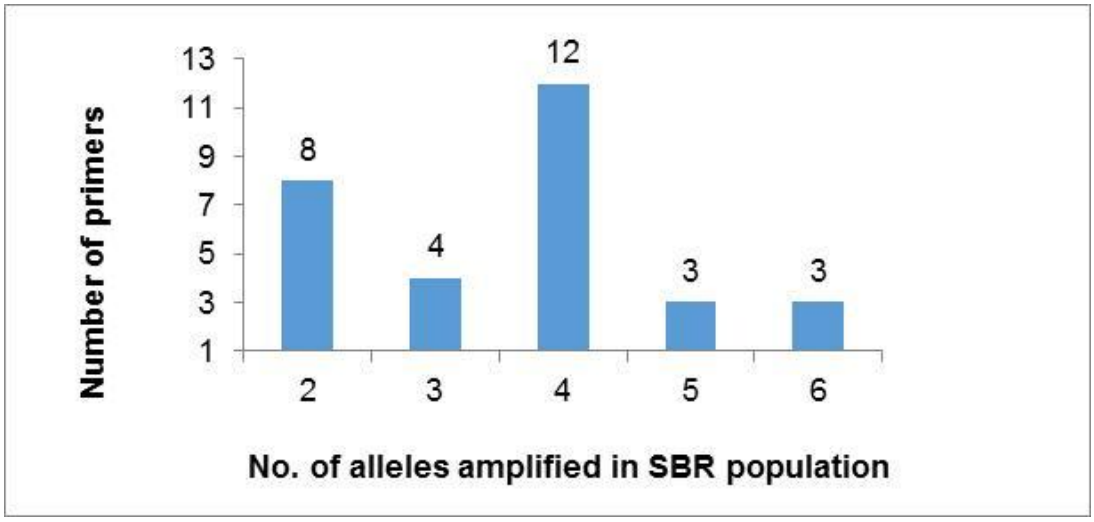

Figure 3-1: Alleles amplified from SBR population using 30 SSR markers

Out of the 30 markers used, eight markers, (nc133, phi065, phi084, phi102228, Phi112, phi123, Phi227562, umc2250), amplified two alleles each; four markers (phi079, umc1304, umc1367, umc1971), amplified 3 alleles each; 12 markers (nc130, phi029, phi062, phi075, phi076, Phi114, Phi308707, Phi331888, Phi374118, Phi96100, umc1161, umc1545) amplified 4 alleles each, and, three markers each (phi031, phi041, phi056, and, phi046, phi072, phi299852) amplified 5 and 6 alleles, respectively. The PIC values for SBR population ranged from 0.06 observed in locus umc1367 to 0.79 observed in phi299852, with mean of 0.48. Observed uHe indicated gene diversity of 0.06-0.80 in SBR population, and a mean of 0.48 (Table 3-2). Conversely, 43% of the loci have Ne value less than 2, 36% have Ne value of 2-3, and 20% have Ne value greater than 3.

Table 3-2: Genetic diversity information generated from analysis of 65 S4 stem borer resistant population using 30 microsatellite markers

Locus	Na	Ne	I	Ht	Ho	He	uHe	F	PIC
nc130	4	1.54	0.73	0.51	0.16	0.35	0.35	0.55	0.35
nc133	2	1.35	0.43	0.48	0.22	0.26	0.26	0.17	0.26
phi029	4	2.37	1.00	0.94	0.37	0.58	0.58	0.37	0.58
phi031	5	2.81	1.21	1.45	0.81	0.64	0.65	-0.25	0.64
phi041	5	3.23	1.37	0.84	0.15	0.69	0.70	0.79	0.69
phi046	6	2.12	1.00	0.67	0.14	0.53	0.53	0.74	0.53
phi056	5	3.63	1.44	1.00	0.28	0.72	0.73	0.62	0.72
phi062	4	1.98	0.79	0.71	0.22	0.49	0.50	0.56	0.49
phi065	2	1.30	0.40	0.34	0.11	0.23	0.24	0.52	0.23
phi072	6	2.40	1.09	0.81	0.23	0.58	0.59	0.60	0.58
phi075	4	2.61	1.08	0.88	0.26	0.62	0.62	0.58	0.62
phi076	4	3.26	1.26	1.28	0.59	0.69	0.70	0.15	0.69
phi079	3	2.39	0.96	0.98	0.40	0.58	0.59	0.32	0.58
phi084	2	1.76	0.62	0.54	0.11	0.43	0.44	0.75	0.43
phi102228	2	1.10	0.19	0.15	0.06	0.09	0.09	0.30	0.09
Phi112	2	1.13	0.23	0.15	0.03	0.12	0.12	0.73	0.12
Phi114	4	2.65	1.16	1.00	0.38	0.62	0.63	0.40	0.62
phi123	2	1.43	0.48	0.45	0.15	0.30	0.30	0.49	0.30
Phi227562	2	1.45	0.49	0.34	0.03	0.31	0.31	0.90	0.31
phi299852	6	4.74	1.66	1.19	0.40	0.79	0.80	0.49	0.79
Phi308707	4	3.97	1.38	1.05	0.30	0.75	0.75	0.60	0.75
Phi331888	4	2.56	1.04	1.01	0.40	0.61	0.61	0.34	0.61
Phi374118	4	2.94	1.17	1.16	0.50	0.66	0.66	0.24	0.66
Phi96100	4	1.88	0.85	0.62	0.15	0.47	0.47	0.67	0.47
umc1161	4	2.40	1.08	0.75	0.17	0.58	0.59	0.71	0.58
umc1304	3	1.08	0.18	0.12	0.05	0.07	0.08	0.38	0.07
umc1367	3	1.06	0.15	0.12	0.06	0.06	0.06	-0.03	0.06
umc1545	4	3.12	1.23	1.01	0.33	0.68	0.68	0.52	0.68
umc1971	3	1.42	0.55	0.47	0.17	0.30	0.30	0.42	0.30
umc2250	2	2.00	0.69	1.48	0.98	0.50	0.50	-0.97	0.50
Mean	3.63	2.26	0.86	0.75	0.27	0.48	0.48	0.42	0.48
SE	0.23	0.17	0.08	0.07	0.04	0.04	0.04	0.07	0.04

‡Data: N_a = total number of alleles per locus; N_e = number of effective alleles per locus; I = Shannon's Information Index; H_t = total gene diversity; H_o = observed gene diversity within genotypes; H_e = average gene diversity within genotypes; uH_e = Unbiased Expected Heterozygosity; F_{IS} = inbreeding coefficient; PIC = polymorphic information content.

Fifty seven (57%) of the markers had a PIC value greater than 0.5, and Ht values ranged from 0.12 observed in locus umc1304, to 1.48 observed in locus umc2250, with a mean of 0.75 (Table 3-2). The locus differentiation, F_{IS}, for SBR population was extreme from -0.97 (umc2250) to 0.9 (Phi227562), having a mean of 0.42 (Table 3-2). A summary of genetic analysis for the SBR population is presented in Figure 3-2.

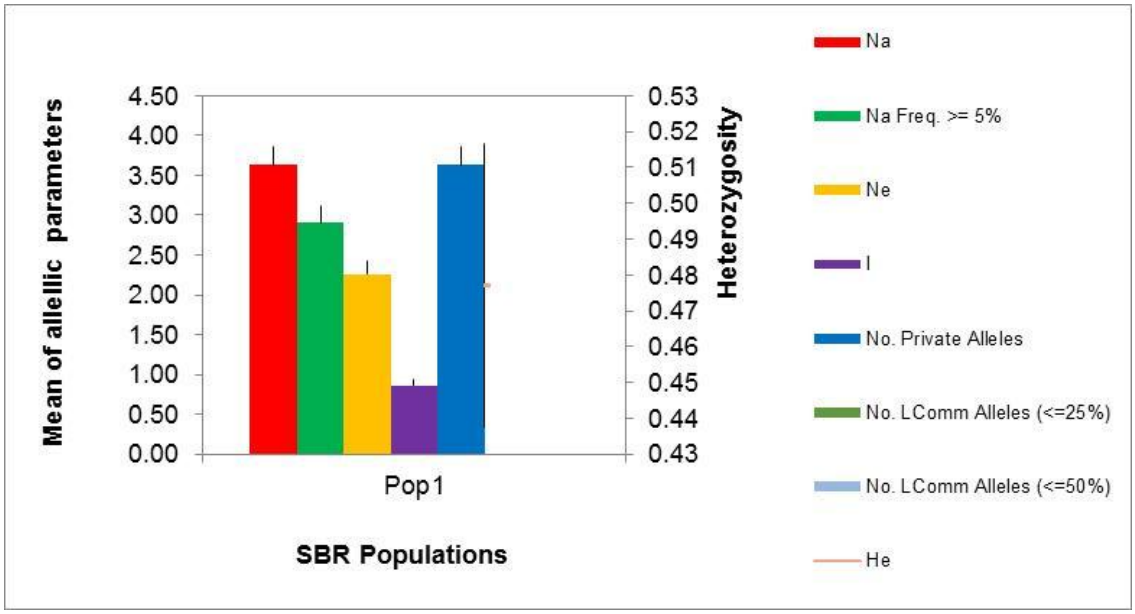

Figure 3-2: Mean Allelic Patterns across SBR Population using 30 SSR markers

3.3.3 Cluster analysis of stem borer resistant population

Three major cluster groups (C1, C2, and, C3), were observed in the SBR population from cluster analysis of the SBR population using SSR markers. Cluster C1, comprised 40% of the genotypes, and was further classified into sub-clusters. The second cluster, C2, was by far the largest comprising of 55.4% of genotypes. It had two major sub-clusters. The third grouping, C3, was the smallest of the clusters comprising 3 genotypes (4, 16, and 64), and constituting only 4.6% of genotypes in SBR population. The pairwise dissimilarity values for SBR generated ranged from a minimum of 0.18 to a maximum of of 0.71, with a mean value of 0.445 (Figure 3-3).

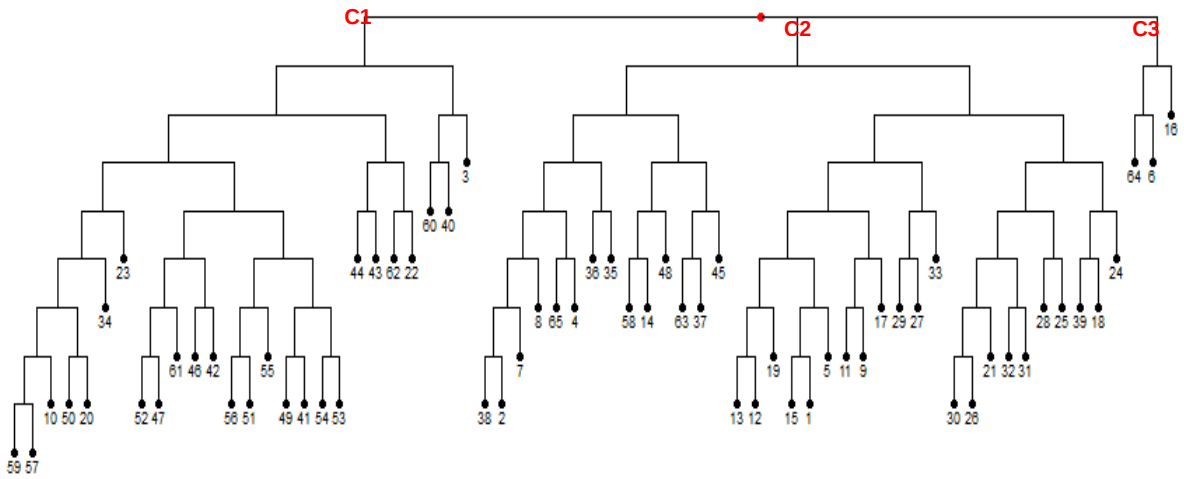

Figure 3-3: Dissimilarity matrices dendogram of SBR population using neighbour joining algorithm indicating genetic relationships of genotypes

3.3.4 Allelic content of storage pests' resistant population

Similar analysis with the same 30 SSR markers on SPR population identified a total of 103 alleles. The allele's scores ranged from 1 to 6 alleles per loci, with a mean of 3.43 alleles. Only one marker (umc1367) was monomorphic out of the thirty SSRs markers used. Five markers, (phi046, phi062, phi084, phi123, phi102228), amplified two alleles each; 11 markers (nc133, phi065, phi072, phi079, phi112, phi308707, phi331888, phi374118, umc1304, umc1971, umc2250), amplified 3 alleles each; eight markers (nc130, phi029, phi041, phi075, phi076, phi96100, phi227562, umc1545) amplified 4 alleles each, and, three markers (phi056, phi114, umc1161), amplified 5 alleles each, and, two markers (phi031, phi299852) amplified 6 alleles (Table 3-3).

Ne values for the SPR population ranged between 2-5.3, with 50% of loci having Ne values less than 2, 33% of loci had between 2-3, and, 17% had Ne values greater than 3. Apart from umc1367, which had a PIC value of 0, the PIC values for SPR population ranged from 0.07 (umc1304) to 0.85 (phi079), with a mean of 0.46. Fifty three (53%) of the loci had pic values greater than 0.5.

Similar to SBR population, the locus differentiation, F_{IS}, for SPR population was also extreme ranging from -0.91 (umc2250) to 0.82 (Phi041), having a mean of 0.41. Observed uHe indicated gene diversity of 0.08-0.82 in SPR population, with a mean of 0.45 (Table 3-3).

Table 3-3: Genetic diversity information generated from analysis of 65 S4 storage insect pests resistant population using 30 microsatellite markers

Locus	N_a	N_e	I	H_t	H_o	H_e	uH_e	F_{is}	PIC
nc130	4	2.07	0.91	1.02	0.50	0.52	0.52	0.03	0.52
nc133	3	1.73	0.76	0.70	0.28	0.42	0.42	0.34	0.42
phi029	4	2.22	0.90	0.81	0.26	0.55	0.55	0.52	0.55
phi031	6	5.34	1.73	1.78	0.97	0.81	0.82	-0.19	0.81
phi041	4	3.20	1.26	0.81	0.13	0.69	0.69	0.82	0.69
phi046	2	1.69	0.60	0.61	0.20	0.41	0.41	0.51	0.41
phi056	5	3.00	1.28	0.96	0.30	0.67	0.67	0.55	0.67
phi062	2	1.20	0.31	0.23	0.06	0.17	0.17	0.63	0.17
phi065	3	1.27	0.43	0.38	0.17	0.21	0.21	0.19	0.21
phi072	3	1.64	0.63	0.59	0.20	0.39	0.39	0.48	0.39
phi075	4	2.70	1.12	0.83	0.20	0.63	0.63	0.68	0.63
phi076	4	2.69	1.10	1.14	0.51	0.63	0.63	0.19	0.63
phi079	3	2.52	1.01	0.87	0.27	0.60	0.61	0.56	0.85
phi084	2	2.00	0.69	0.70	0.20	0.50	0.50	0.60	0.50
phi112	3	1.37	0.54	0.36	0.09	0.27	0.27	0.66	0.27
phi114	5	2.46	1.03	0.93	0.34	0.59	0.60	0.43	0.59
phi123	2	1.47	0.50	0.50	0.18	0.32	0.32	0.42	0.32
phi96100	4	3.25	1.27	1.06	0.37	0.69	0.70	0.47	0.69
phi102228	2	1.10	0.19	0.15	0.06	0.09	0.09	0.30	0.09
phi227562	4	1.75	0.80	0.53	0.10	0.43	0.43	0.78	0.43
phi299852	6	4.64	1.64	1.22	0.43	0.78	0.79	0.45	0.78
phi308707	3	2.15	0.91	0.75	0.22	0.53	0.54	0.60	0.53
phi331888	3	2.18	0.85	0.74	0.20	0.54	0.55	0.63	0.54
phi374118	3	1.97	0.72	0.80	0.31	0.49	0.50	0.37	0.49
umc1161	5	2.17	1.07	0.71	0.17	0.54	0.54	0.69	0.54
umc1304	3	1.08	0.18	0.12	0.05	0.07	0.08	0.38	0.07
umc1367	1	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
umc1545	4	1.56	0.68	0.57	0.22	0.36	0.36	0.40	0.36
umc1971	3	1.08	0.18	0.12	0.05	0.08	0.08	0.38	0.08
umc2250	3	2.03	0.73	1.48	0.97	0.51	0.51	-0.91	0.51
Mean	3.43	2.15	0.80	0.72	0.27	0.45	0.45	0.41	0.46
SE	0.22	0.18	0.08	0.08	0.04	0.04	0.04	0.06	0.04

‡Data: N_a = total number of alleles per locus; N_e = number of effective alleles per locus; I = Shannon's Information Index; H_t = total gene diversity; H_o = observed gene diversity within genotypes; H_e = average gene diversity within genotypes; uH_e = Unbiased Expected Heterozygosity; F_{IS} = inbreeding coefficient; PIC = polymorphic information content.

A graphical summary of the markers and the respective alleles amplified for SPR population is presented in Figure 3-4.

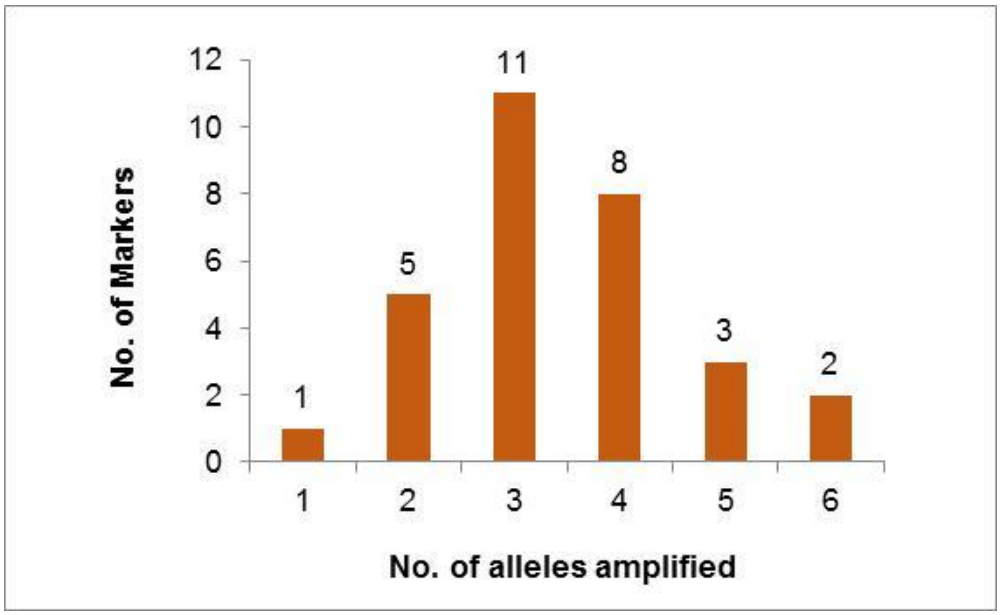

Figure 3-4: Alleles amplified from SPR population using 30 SSR markers

The mean allelic performance observations for the SPR population are summarized in Figure 3-5.

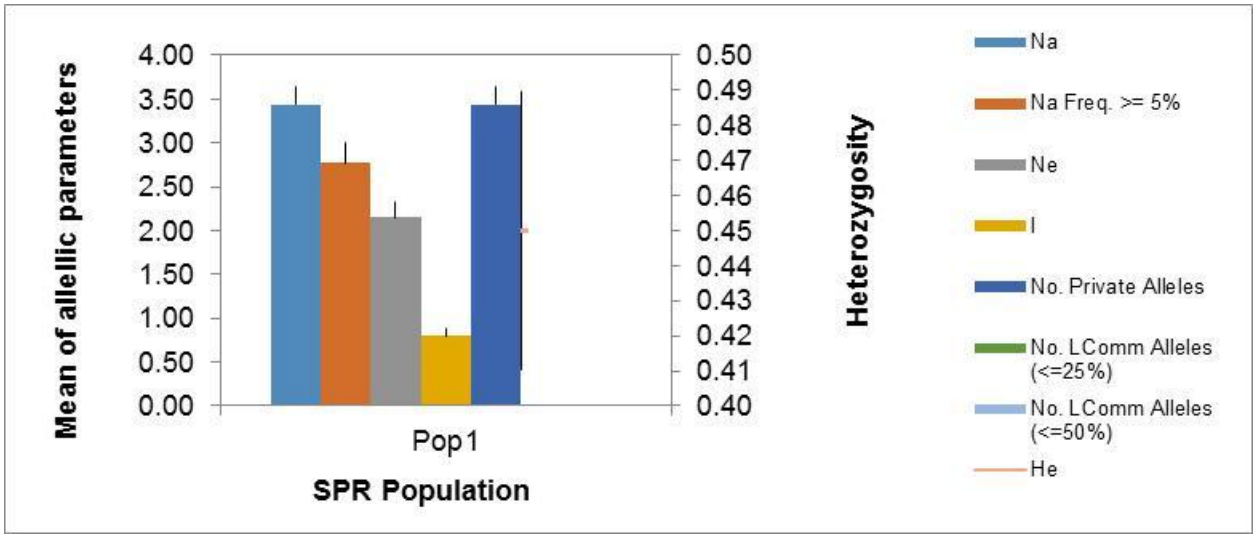

Figure 3-5: Mean allelic patterns across SPR population using 30 SSR markers

3.3.5 Cluster analysis of storage insect pest resistant population

The SPR population revealed three major cluster groupings (C1, C2, and, C3) after analysis using SSR markers. Cluster C1, comprised 33.8% of the genotypes, and was further classified into two major sub-clusters. The second cluster, C2, comprised of 53.8% of the genotypes, and was further sub-divided into two major clusters, one of which is composed of only one genotype, entry 38. The other sub-cluster had two major sub-clusters with different number and list of genotypes each.

The third grouping, C3, was the smallest of the clusters comprising 8 genotypes, constituting only 12.3% of genotypes in SPR population (Figure 3-6).

The pairwise dissimilarity values for SPR generated ranged from a minimum of 0.17 to a maximum of of 0.64, with a mean value of 0.4.

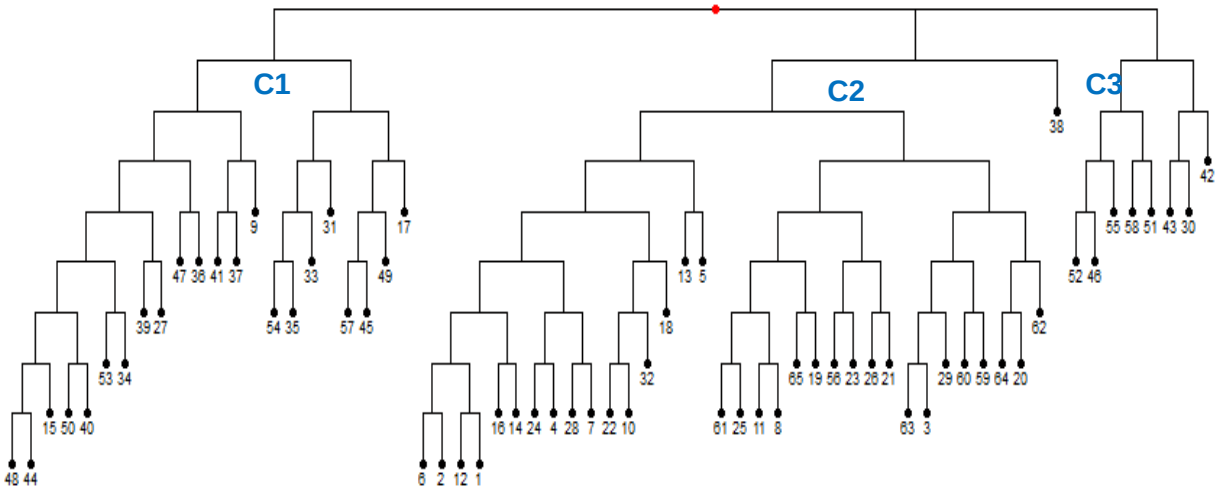

Figure 3-6: Dissimilarity matrices dendogram of SPR population using neighbour joining algorithm indicating genetic relationships of genotypes

3.4 Discussion

Information obtained from the SSR markers used in the current study indicates that these markers were polymorphic. It is only one of the markers that was monomorphic for the SPR population. High success rate was achieved when these markers were used and therefore, it can be deduced that they had the ability to differenciate genotypes within the populations.

Legesse et al. (2007) and Wende et al. (2013), reported that dinucleotide SSR loci amplified the largest number of alleles as well as high PIC values. However, there was no observed correlatedness between marker nucleotide repeats and number of alleles amplified for the current study. Similar results with no correlatedness between allele numbers, PIC and nucleotide repeats were reported in the literature (Matewele, 2014).

The avearge alleles obtained from the current study of 3.43 and 3.63 for SPR and SBR populations respectively, are comparable to those reported by (Choukan et al., 2006). However, Xia et al. (2004) reported that the total number of alleles in diversity studies is proportional to sample size. Given that the sample size for the current study was 65 genotypes, this may be linked to the number of alleles observed. There were also differences in allele number and effective alleles which can be attributed to the variation of major allele frequencies in the genotypes (Beyene et al., 2014). These results also agree with other studies done by Kostova et al. (2006), who reported low mean value of 1.9 alleles per locus. However, Zhi-zhai et al. (2010) observed mean allele value of 9.57 in 143 maize genotypes. The variation of fragment sizes within locus in the current study may be attributed to a phenomenon called slip-strand mispairing which occurs during DNA replication and can lead to great variation in allele size, as observed in loci phi062 with 362 bp and Phi227562 with 320 bp (Levinson and Gutman, 1987; Beyene et al., 2014). The same phenomenon can be due to potential mutations occuring on the binding site of primers leading to low primer binding (Dillon et al., 2005).

The levels of diversity obtained using these 30 SSR microsatellite markers in the two populations was 0.45 – 0.48. This agrees with previous studies (Musundire, 2013) where average diversity of 0.53 was observed. Similar studies by Matewele (2014), while working on diversity of maize germplasm from Malawi, reported diversity values of 0.51. Other authors have reported both lower values of diversity (Akinwale et al., 2014) as well as high values (Kong et al., 2000).

The discriminatory power of loci was further estimated using PIC values having put into consideration the number of alleles and their relative frequencies (Smith et al., 2000). In the current study, PIC values were high, with 57% of loci having pic values greater than 0.5, and two of the loci (Phi308707, phi299852), giving values above 0.75 in SBR population, and two (phi031, phi079) giving values greater than 0.8. This is evidence that these markers were able to effectively discriminate among all test genotypes and the results demonstrate

their informative nature when detecting differences among genotypes. These findings agree with studies (Ganapathy et al., 2012) done elsewhere with reported high PIC values. Conversely, 43% of the loci in the current study yielded PIC values of less than 0.5 for two populations. This is not to indicate that their discriminatory power is questionable, given that other authors have reported low pic value of 0.33 (Legesse et al., 2007). It is therefore important to note that these findings agree with studies by Smith et al. (2000) and Geleta et al. (2006), who reported that even moderate PIC values can be useful for classification of lines.

Presence of high values for uH_e confirm that these lines were not yet pure inbred lines since the lines were at S_4 and still in the process of being selfed after selection based on the breeding goals. The results further indicate that there are high levels of polymorphism in the test populations. This is in agreement with findings by Smith et al., (1997) while evaluating the utility of SSR markers in maize. Other authors (Senior et al., 1998) have also reported similar findings as reported in this study.

Genetic distances revealed the relatedness of the S4 lines used for this study, with clarity of markers being able to distinguish closely related lines with minimum genetic distances (Smith et al., 1997).

Cluster analysis of the two populations, SBR and SPR, showed a good fit to the data with the dendrograms showing clear distinction of the different clusters. The three major clusters observed in the two populations could be an indication of pedigree relatedness of the S4 lines. This agrees with previous finding (Reif et al., 2006), when he used SSR markers for heterotic groupings of maize. Similar findings were reported by (Senior et al., 1998), when investigating the genetic similarity and relatedness in maize. Furthermore, the clustering could be due to insect resistance levels in the test genotypes. This can be confirmed through other studies incorporating phenotypic data after screening the genotypes for resistance to *C. partellus* or *S. zeamais* insects. It is also important to note that clustering could be due to maturity grouping of the S_4 lines, since most of lines used were of early to medium maturity regime. This data was, however, not reported in the current study. Of great interest was entry 38 genotype, which fell in a sub-cluster of its own. This could be due to either genetic mix-up or incomplete pedigree records. Such observations have been reported by other researchers as occurrences due to effects of selection, some mutations, or genetic drift, as well as human error (Warburton et al., 2008). Hartl and Clark (1997) argued that the

differentiation of genotypes provides reason for breeders to select their preferred germplasm and fix desired alleles in each population.

3.5 Summary conclusions

The findings of this study have demonstrated that:

1. There is genetic variability in the genotypes from both the stem borer resistant as well as the storage pest resistant S_4 population.
2. The chosen SSR markers differentiated the S_4 lines, indicating that they were robust for the current diversity studies.

These findings can therefore be used for the following recommendation:

1. The genetic variability could be exploited for further breeding.
2. These populations can act as a valuable source of alleles especially in insect resistance studies.

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Chapter 4: Estimating combined insect pest resistance for *Chilo partellus* and *Sitophilus zeamais* in maize hybrids

Abstract

The spotted stem borer, *Chilo partellus* Swinhoe, a major field pest of maize, and the storage pest, *Sitophilus zeamais* Motschulsky, are widely distributed in all major maize growing ecologies in Kenya. The two make destructive loss force of estimated at 15% in the field and 17-40% in storage. Host plant resistance has been achieved for each of these pests in separate inbred lines and hybrids, however, no combined field and post-harvest insects' pests resistance studies have been studied in Kenya. A study was carried out to determine whether resistance to the two pests can be combined in hybrids using 12 parents diallel. The seedlings were screened for stem borer resistance by artificially infesting with 10 *C. partellus* neonates, and at harvest, 100g of grain was infested with 45 unsexed adults of *S. zeamais* and *P. truncatus*, for the post-harvest resistance screening. There were highly significant ($p \leq 0.001$) mean squares for grain yield, both under infestation and protected plots. The stem borers' and postharvest insects' resistance traits; leaf damage scores, exit holes, cumulative tunnel length, dust weight, live insect and weight loss; were also highly significant ($p \leq 0.001$) in six environments. Parents 2, 3, 6, 7, and 10 contributed to higher *Chilo partellus* resistance genes in hybrids (≤ 2.3), while parents, 2,3,8,9, and 10 contributed to higher *Sitophilus zeamais* resistance genes. General combining ability effects for lead damage scores and undamaged kernels for *Sitophilus zeamais* were highly significant ($p \leq 0.001$, R^2 =71-77%). Hybrids 3X10, 4X8, 5X10, 3X9, 2X9, 5X8, and 4X9; showed combined resistance to both pests, with grain yield ranging between 5 and 8 t/ha respectively. These inbred lines can be used as sources of resistance genes in developing hybrids with combined resistance to field and storage pests.

Key words: Insect Pests, Maize, Resistance, Combining Ability

4.1 Introduction

Maize, (*Zea mays* L), remains the major food crop for the majority of households in sun-Saharan Africa (FAOSTAT, 2014). Production is, however, still below the demand of the crop because of yield losses caused by both field and storage pests (DeGroote, 2002). Stem borers form the major pest complex of maize in the field especially in the tropics where temperatures are high and the environmental conditions are conducive for the pests' multiplication. The maize weevil and the larger grain borer form the major pests of maize in storage.

The spotted stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) is of great economic importance, not only in lowland tropics, but also in mid-altitude and highland tropics, where it is progressively advancing due to global warming which has led to climate change. It is therefore distributed in all major maize growing ecologies in Kenya up to altitude of 1200masl. It attacks maize from seedling stage through to maturity (Appert and Deuse, 1982). In highly humid and hot environments, up to 100% infestation levels have been recorded in maize fields (Mohamed et al., 2004). Losses due to stem borers are two-fold: Quantity (lower yields), and also quality, (faecal waste and secondary microbial infections). De Groote (2002) reported average grain yield losses of 13.5% in Kenya, while more than 50 million metric tons (MT) of global maize production are estimated lost due to stem borers annually (James, 2003).

On the other hand, the storage pest, maize weevil, (*Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a major pest in Kenya distributed also in all maize growing ecologies. It is known to thrive well in temperatures ranging between 15-34^{o}C, and causes both quantity and quality loss (Giga et al., 1991; Derera et al., 2014). Infestation starts in the field especially in the early maturing maize genotypes, especially, in the open-tipped genotypes (Dari et al., 2010). This can lead to ear rot and secondary pathogen infections, for example, mycotoxins, resulting in production of aflatoxins and fumonisins, which are harmful to human health (Kankolongo et al., 2009). The result of such infection is low market value for the grain, and also endangers the lives of the consumers.

Chemical pesticides, are not affordable to smallholder farmers, they are indiscriminative in nature, and have side-effects both directly to the health of the farmer, and then to the

environment, whereas biological control methods are generally not effective (Dalvie et al., 2009; Meissle et al., 2010). Therefore host plant resistance, which is a noble method for pest control and stands to be promising because the protection is in-built within the seed. This is affordable to the farmer and has no side effects both to the health of the farmer and consumer, and the environment (Mugo et al., 2001). Various stem borer and storage pest resistant maize germplasm has been developed, and released to the farmers (Dhliwayo et al., 2005; Tefera et al., 2011). Unfortunately, the hybrids already released confer resistance to only one type of these pests; either stem borer resistant or maize weevil resistant maize hybrids. The challenge here is that each of the insect resistant traits is in separate maize germplasm. This necessitates the smallholder farmer to use chemicals at some point; either in the field to control stem borers when growing storage pests' resistant maize, or in store, after harvesting the stem borer resistant maize. This is because the two insect resistance traits are in different genotypes; either way, the cost of production and storage of maize is high and not feasible to the resource-constrained smallholder farmer.

In efforts to address this challenge, studies were carried out to determine whether resistance to both stem borers and storage pests can be achieved in hybrid combination without compromising the yield. This study therefore aimed at finding out whether stem borer resistant inbred lines can be used in hybrid formation along with elite maize weevil resistant inbred lines to develop hybrids which have resistance to each of these pests. In addition, the study aimed at identifying the best combiners of lines in general combining ability (GCA) and specific combining ability (SCA) to confer resistance to the maize crop when in the field and in storage. The findings can be used for devising the strategy for breeding multiple resistance to maize pests and therefore contribute towards reduced yield losses in the field, increase length of storing maize, increased food security and improved livelihoods for the small-holder farmers in Africa.

The objectives of this study were to:

1. Identify inbred lines with stem borer resistance that combine well with the selected post-harvest insect resistance lines.
2. Determine the general and specific combining ability of elite inbred lines with resistance to the individual pests; *Chilo partellus* and *Sitophilus zeamais*.
3. Identify the best combiners of inbred lines which give hybrids with combined *C. partellus* and *S. zeamais* resistance for use in future breeding.

4.2 Materials and methods

4.2.1 Choice of parents and making crosses

Ten test inbred lines, and two CIMMYT maize lines (CML), developed by the International Center for Maize and Wheat Improvement (CIMMYT) through the Insect Resistant Maize for Africa (IRMA) project (Table 4-1), were crossed in a half diallel combination.

Table 4-1: List of parents used in the diallel crosses, their codes and pedigrees

Parent	CODE	Pedigree
1	CKSBL10039	P590 C7 Blancos F156-1-2-1-B-B-B-B -B-B
2	CKSBL10025	MBR C5 Bc F60-2-1-2-B-B-BxCML 384-B-1-2-B-B-B-B -B-B
3	CKSBL10026	MBR C5 Bc F8-1-1-1-B-2-2-B -B-B
4	CKSBL10014	CML311/MBR C3 Bc F43-2-1-1-B-B-B-B -B-B
5	CKSBL10034	MBR-Et(W)/P590C3 F6-1-1-B-2x761B A1 Bco x 751B-B-3-B-1-2-B-B-B-B -B-B
6	CKDHL120030	5K_CUBA/GUAD//KILIMA_ST94A/MSV/3/CML395:@.@%3052.-B
7	CKDHL120493	5K_CML442/3/CUBA/GUAD//KILIMA_ST94A/MSV:@.@%3007.-B
8	CKSPL10090	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-277-1-B-3-B-B-B-B
9	CKDHL120517	5K_CML442/3/CUBA/GUAD//KILIMA_ST94A/MSV:@.@%3031.-B
10	CKDHL120731	5K_CML444/3/CUBA/GUAD//KILIMA_ST94A/MSV:@.@%3042.-B
11	CML442	CML442
12	CML444	CML444

The diallel mating design was identified and chosen as suitable for making the crosses of the parents from the SBR and SPR groups (Griffing, 1956). This method is useful for estimation of both the general combining ability (GCA) and the specific combining ability (SCA). The GCA will generate information for estimating additive gene action, while the SCA information generated will be used for estimation of the non-additive (dominance and epistasis) gene effects. It has been used widely in genetic studies and for parental selection in maize breeding and also gives good estimates of GCA and SCA (Machida et al., 2010; Makumbi et al., 2011).

4.2.2 Sites for nursery and evaluation of F1 hybrids

The nursery was planted at CIMMYT's new site at Kenya Agricultural and Livestock Research Organization (KALRO)-Kiboko (2°12' 50.24" S, 37°43' 30.11" E, 945 masl) on the 27th December 2012, during the 2012B season. The females' plots were four rows of 5 m

length planted at 75 cm between rows and 25 cm within rows. Two seeds were sown per hill and later thinned down to one plant per hill. The male plots were sown on one row per planting, preceding each set of female rows at three intervals (-5, 0 and +5 days) after planting the females to synchronize flowering for continued pollen supply at the time of pollination (CIMMYT, 1985). The nursery was harvested on the 30th April 2013 and, clean seed from each female row per plot was bulked, dried and shelled, and the grain weighed.

Table 4-2: Climatic description of the sites used for evaluation of the F1 maize hybrids

Location	Co-ordinates	Altitude (masl)	Soil type	Zone	Temp (°C)		RF (mm)
					Minimum	Maximum	
Kiboko	02° 15' S 037° 75' E	975	Sandy-loam	LT	23	29	530
Kirinyaga	00° 34'08.37' S 037° 19'21.31' E	1286	Andisols	MAM	14	26	1250
Embu	00° 30'18.76' S 037° 27'18.22' E	1533	Humid-Nitisols	MT	14	28	1100
Kakamega	00° 02'20.02' N 034° 48'57.42' E	1589	Clay-Loam	MT	17	28	1900

‡ LT, lowland tropics; MAM, moist mid-altitude; MT, Moist transitional; RF, Rainfall; mm, millimetres; masl, meters above sea level; Temp, average annual temperature in °C (degrees centigrade).

Three sites, Kirinyaga, Embu and Kakamega, were selected for trial evaluation of the resulting F1 hybrids (Table 4-2). All routine agronomic practices and proper nursery management procedures were followed up to pollen shedding (Badu-Apraku et al., 2012). Pollen from four male plants was harvested, bulked and used to hand pollinate as many designated females as possible. Data was collected on flowering dates, ear height, and plant height.

The choice of these sites was done based on the special constraints each site experiences: In Kirinyaga, there is natural infestation occasioned by *C. partellus*, while in Embu, there is both *C. partellus* and *Busseola fusca* Fuller (Lepidoptera: Noctuidae) stem borer species. In addition to these, Embu is a hot spot of maize streak virus (MSV) infection. On the other hand, Kakamega is known for high disease incidences, and this makes it a good site for evaluation of hybrids under disease pressure.

Season by site combination constituted an environment, therefore from three sites and two seasons (2013B and 2014A), this gave a total of six environments. Season A are the short rains experienced during the months of November to January, while season B are the long rains experienced during the months of March to June. These environments were Kirinyaga in 2013B as Environment 1, and in 2014A Environment 2; Embu by 2013B was environment 3, and 2014A environment 4, and, Kakamega 2013B was Environment 5, and 2014A Environment 6. Sixty six (66) F1 hybrids, and four commercial checks, were evaluated under rain fed conditions, with supplementary irrigation when needed, and, artificial infestation with *C. partellus*.

4.2.3 Design used for evaluating F1 maize hybrids obtained after making crosses

The trial consisted of 66 test hybrids and three commercial available hybrids (checks) and one internal check (within the breeding program check) with known stem borer resistance trait. The trial design was a 7 x 10 α-lattice, with two rows per plot and three replications. The rows were five meter-long, with 21 hills per row, and row to row spacing of 0.75 m and hill to hill spacing of 0.25 m. Two seeds were planted per hill, and later thinned to one plant per hill two weeks after germination, except the border hills which maintained two plants per hill. All routine agronomic practices of maize production; weeding and fertilizer application; were followed, except the use of pest control sprays. The plots were partitioned into two parts using a nylon rope two weeks after germination at the time of thinning, to create two, 10-plant portions, excluding the first hill, to allow for artificial infestation.

4.2.4 Artificial infestation with *Chilo partellus* neonates

The front half of the mapped field was infested with ten first instar larvae of *C. partellus* neonates, into the maize seedlings whorl using a camel hair brush, two weeks after germination. The neonates were obtained from a mass rearing laboratory situated at the Kenya Agricultural and Livestock Research Organization (KALRO) (37^{o} 37' 36.75" E, 1^{o} 38' 1.54" S, 1571masl), Katumani (Tefera et al., 2010). The second half and the back side was protected using Bulldock® 0.05 GR granule, a synthetic pyrethroid with Beta-cyfluthrin 0.5g/kg as the active ingredient, which is systemic. The granules were placed in the whorl of the developing seedlings one shake in the funnel of each plant amounting to 3 kg/ ha.

After infestation, the neonates were allowed two weeks to feed and burrow into stem of the developing maize seedlings. A four week interval between infestation and second scoring was allowed to give the neonates a chance to develop within the maize plant while inflicting damage on the plant up to sixth instar stage, prior to pupae stage.

4.2.5 Stem borer resistance screening data collection

The first data scores on leaf damage scores were taken after the expiry of two weeks, and, a second scoring was done four weeks after infestation, then the plants were allowed to develop to maturity. The leaf damage scores were recorded using a scale of 1-9 (Table 4-3) (Tefera et al., 2010). Other data collected include, days to flowering, plant heights and ear heights. At harvest, yield data and other stem borer resistance parameters, which include exit holes and cumulative tunnel length, were taken and recorded. The harvested cobs were weighed and taken to the post-harvest resistance screening laboratory, which is situated at KALRO-Kiboko (037^{o} 42' 50.98" E, 02^{o} 13' 56.56" S; 947 masl). Other post-harvest parameters were collected and they include oils, starch and protein content, before setting up the storage pests' resistance screening experiments.

Table 4-3: Leaf damage scores rating for insect resistance assessment

Score	Leaf damage scores	Resistance rating
1	Few pin holes to indicate feeding activity (<10%)	Highly resistant
2	Few shot holes on several leaves (<20%)	Resistant
3	Several holes on leaves (<30% perforation on leaf)	Resistant
4	Several small holes on leaves (40-50%)	Moderately resistant
5	More severe feeding activity (>50%) on a few leaves	Moderately resistant
6	Elongated lesions on several leaves	Susceptible
7	Heavy perforation on leaves leading to leaf tattering	Susceptible
8	More severe leaves perforation (>80%)	Highly susceptible
9	Dead hearts (when growing point is completely damaged)	Extremely susceptible

Source: Tefera et al., 2010

4.2.6 Post-harvest insect resistance screening and data collection

At harvest, clean ears from each plot were selected and shelled, and then post-harvest data were taken on moisture, oil, starch, and protein content of the grain. The grain was then dried to relative uniform moisture content and fumigated using Aluminum Phosphide 55%, marketed as Phostoxin®, for two weeks, in order to eliminate any pests from the field. Equal weights of 100g samples were taken from each plot and placed in 0.5 litre glass jars in the post-harvest laboratory with enough room to allow for proper aeration within the jar for the period of incubation. Forty five unsexed, three week-old adults insects of both *S. zeamais* and *P. truncatus* were placed in each sample. This age allowed for reduction of time from infestation to emergence of the next progeny. The jars were covered with a lid perforated in small enough holes not to allow the insects escape from the jar. They were then incubated for 90 days, under controlled conditions in the laboratory with temperatures of 28 ± 2^{o}C, relative humidity (RH) of 65 ± 5% and a 12:12 hour light:dark regime, using the field randomization (Haines, 1991; Tefera et al., 2010).

The jars were opened after the 90-day screening period, and the products sieved through appropriate screens in order to separate the various components; grain, insects and flour. This incubation period was to ensure that there was enough time for the insects to infest and feed on the grain so that any clean grains observed was not due to escapes. The large screen mesh size 4.7mm was to separate grains from insects and flour, and a smaller screen mesh size 1.0mm for separating the insects from the flour. Data on number of live and dead insects was recorded to give progeny count. The weights of both the damaged and undamaged kernels were also recorded. Both damaged and undamaged kernels were counted and recorded, and, finally, weight loss of grain calculated. All the data was captured in excel before analysis.

4.3 Data analysis

The data on leaf damage scores, powder produced (flour), and weight loss of grain was transformed to the square root prior to statistical analyses. Grain yield was adjusted to 12.5% moisture content, and plot yield calculated to tonnes per hectare using the formula:

GY = (Fwt/1000)((100-Moisture) / (100-12.5)*(10000/Plot area))*shelling %* .....(2)

Where;

GY = Grain yield;

FWT = field weight.

Analysis of variance was done for the traits of interest; grain yield, leaf damage scores, weight grain loss, and tunnel length; using PROC GLM of SAS, for all the traits. A fixed model, for Griffings method 4 (Griffing, 1956) for a partial diallel, was used (Hallauer et al., 2010) which excluded the parents and the reciprocal crosses. The following statistical model was used:

$x_{ij} = \mu + r_k + g_i + g_j + s_{ij} + e_{ijk}$ …….(3)

Where:

xij = the effect of the ijth genotype ($i,j = 1,2$ ----------p, $i < j$)

μ = grand mean

rk = kth replication effect

gi and gj = GCA effects of parents *i* and *j*

sij = SCA effects between parent *i* and *j*

e*ijk* = experimental error for the observation *x*ijk ($k = 1,2$, ---------r, $i = j = 1,2$,---------n).

The environments were considered as random effects while the genotypes were fixed. A combined analysis across the environments was also performed taking into consideration that environments are random effects and genotypes are fixed effects (SAS, Institute, 2014).

Percentages of GCA and SCA were calculated using the formulae:

%GCA = 100*(GCA ss)/ Entry ss, %SCA = 100*(SCA ss)/ Entry ss ……...…….(4)

4.4 Results

4.4.1 Grain yield and stem borer resistance parameters

The results revealed that out of 66 genotypes which were evaluated, 23 of these hybrids had high levels of stem borer resistance with leaf damage scores of 2-2.5. This accounts for

34.8% of the genotypes. Twenty eight (28) of the hybrids scored 2.51-3.0 leaf damage which is equivalent to 42.4 %. Moderate levels of resistance with scores of 3.1-3.5, were observed in 14 of the test hybrids which is equivalent to 21.2% of the genotypes. Only one hybrid was observed to be susceptible with leaf damage scores of greater than 3.5, accounting for 1.5% of the test hybrids (Figure 4-1).

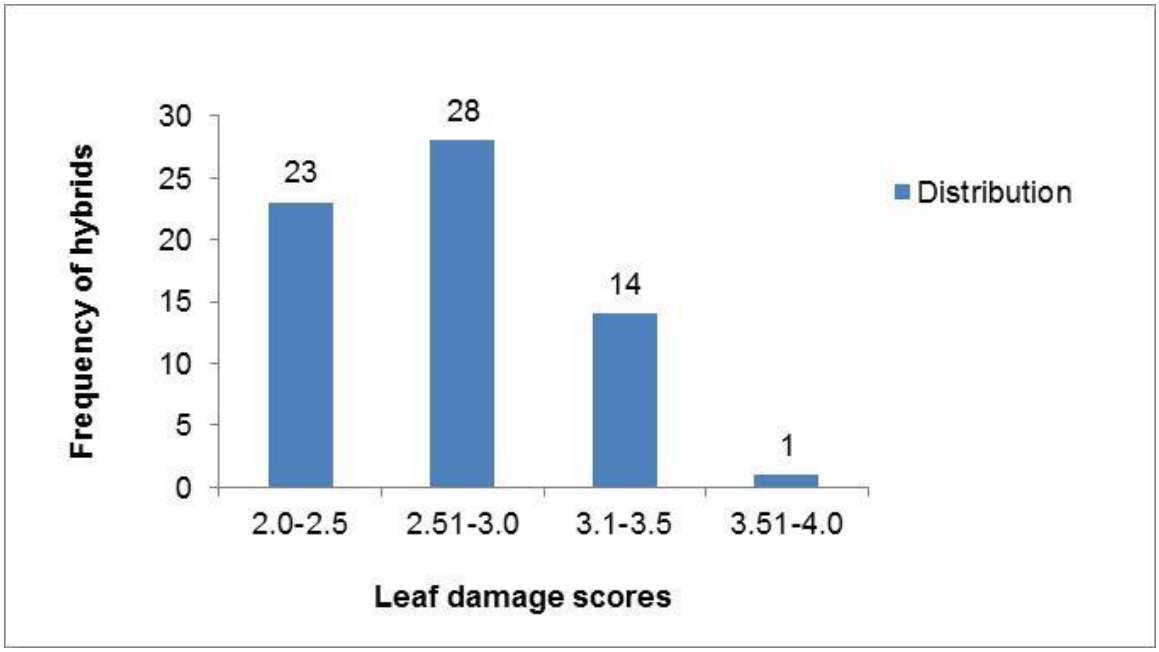

Figure 4-1: Frequency distribution of leaf damage scores among test hybrids

Analysis of variance revealed highly significant (p≤0.001) mean squares for yield, both under infestation and protected plots, and stem borers' resistance traits, leaf damage scores, exit holes and cumulative tunnel length across different environments, for genotypes (Table 4-4).

Similarly, the oil, protein and starch content showed highly significant (p≤0.001) mean squares across different environments, for genotypes. General combining ability (GCA) effects for leaf damage scores and undamaged kernels for *S. zeamais* were highly significant (p≤0.001, R^2= 71-77%). Specific combining ability was not significant for cumulative tunnel length, and, there were no significant interactions for specific combining ability by environment, for grain yield under infestation, exit holes, leaf damage scores and protein content. It was observed that Entry 1 had the highest starch content of 70.5%, yet the same entry had the lowest oil content of 4.9%. Similar results were observed for entry 26 which had high protein content of 12.0%, yet with low starch content of 68.2% (Table 4-4).

Table 4-4: Analysis of variance for grain yield, stem borer resistance parameters and biochemical traits

		Mean squares							
		Grain Yield		*Chilo partellus*			Biochemical traits		
Source	**DF**	**GYP**	**GYI**	**CTL**	**ExHls**	**LDS**	**Oil**	**Protein**	**Starch**
ENV	5	771.6***	1100.5***	4552.0***	340.7***	53.4***	34.2***	78.8***	162.8***
REP(ENV)	12	30.3	45.3	214.2	25.4	5.68	0.442	7.6	5.9
ENTRY	65	36.1***	37.3***	25.6***	5.0***	2.33***	1.14***	4.2***	3.6***
ENV*ENTRY	325	8.2***	9.3***	15.5**	2.2***	0.48*	0.215***	0.92***	0.93***
GCA	11	105.9***	92.8***	82.1***	17.4***	10.6***	4.2***	20.0***	11.5***
SCA	54	21.9***	26.0***	14.1	2.5***	0.66***	0.52***	0.97***	1.96***
GCA*ENV	55	20.5***	20.1***	24.9***	3.8***	0.70***	0.61***	2.2***	1.83***
SCA*ENV	270	5.7*	7.04	13.5	1.9	0.44	0.13***	0.67	0.75*
Error	780	4.6	7.1	12.7	1.6	0.394	0.093	0.62	0.63
R^2		0.72	0.67	0.76	0.71	0.68	0.82	0.69	0.74
Coeff Var		31.6	40.4	67.7	76.5	23.4	6.1	7.3	1.14
% GCA (ss)		0.5	0.42	0.54	0.58	0.77	0.62	0.81	0.54
% SCA (ss)		0.5	0.58	0.46	0.42	0.23	0.38	0.19	0.46
Mean		6.8	6.6	5.3	1.7	2.7	5.03	10.8	69.6
Max		10.5 (38)	10.1 (5)	3.7 (12)	3.5 (49)	3.9 (65)	5.6 (66)	12.0 (26)	70.5 (1)
Min		3.0 (53)	3.3 (53)	8.5 (65)	0.96 (2)	2.1 (2)	4.9 (1)	9.9 (9)	68.2 (26)

‡Data significance: *** Highly significant at ≤ 0.001; **significant at ≤ 0.01; * significant at ≤ 0.05. †GYP, grain yield protected (t/ha), GYI, grain yield infested, CTL, cumulative tunnel length, ExHls, exit holes, LDS, leaf damage scores. Numbers in parentheses indicate the entries with the adjacent means.

Grain yield from five test hybrids were high (9-11.0 t/ha), which constitute 3.0-4.5% of genotypes. Grain yields from 36.4 - 43.9% of the test hybrids ranged 7.0 - 9.0 t/ha, while 43.9 - 45.5% of the test hybrids yielded 5.0 – 7.0 t/ha. Only 9.1-13.6% of the test hybrids gave low yields of 3.0 - 5.0 t/ha (Figure 4-2).

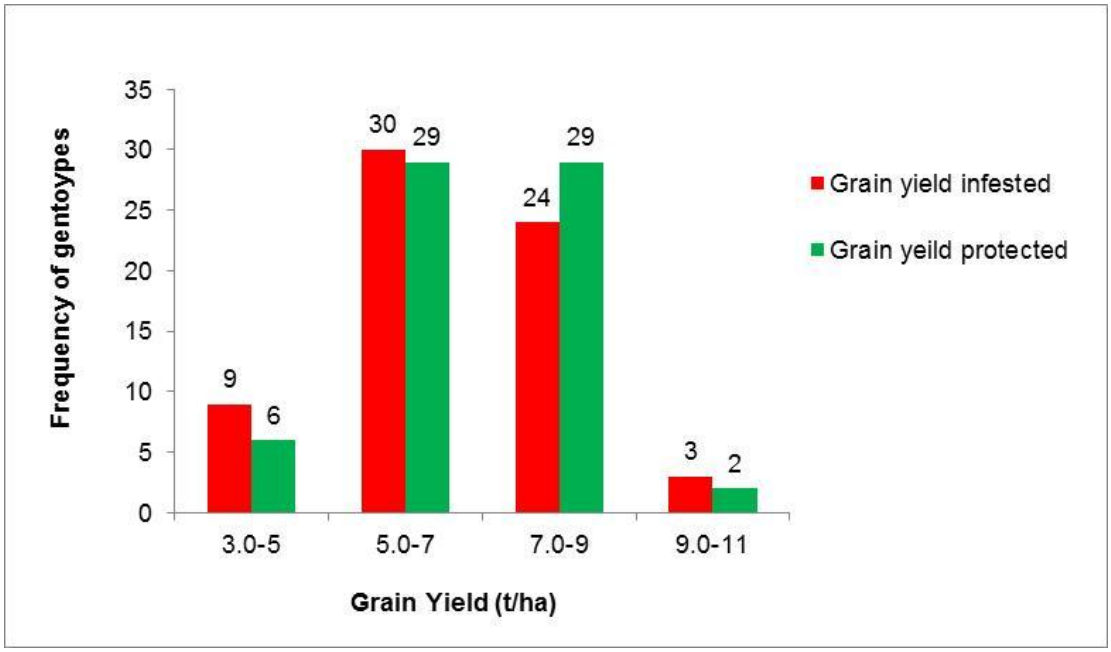

Figure 4-2: Distribution of grain yield when infested and protected among test hybrids

4.4.1 Mean performance for yield and insect resistant parameters

The highest yielding entry for grain yield when protected was 10.5 t/ha (entry 38, single cross 4×12), and mean of 6.8t/ha, while grain yield when infested was 10.1 t/ha (entry 5, 1×6), and mean of 6.6 t/ha for all the entries. Entry 35 (4×9) gave the high yields both when protected (8.4t/ha) and when infested (8.3 t/ha). It had a leaf damage score of 2.2, cumulative tunnel length of 4.1 and exit holes count of 1 (Table 4-5).

The lowest score for leaf damage was 2.1 (entry 2, 1×3), and high of 3.9 (entry 65, 10×12), the mean for all the entries was 2.7. Cumulative tunnel length had mean of 5.3 cm, with minimum measurements of 3.7 cm for entry 12 (2×3), and maximum of 8.8 cm from entry 49 (6×10). Exit holes count yielded mean of 1.7, with minimum of 1 from entries 2, 12, 22, 35 and 7 (1×3, 2×3, 3×4, 4x9 and 1x8), while the highest count was 3.5 from entry 49 (6×10). These hybrids ranked among the top four for leaf damage score except entry 7 which ranked eight for leaf damage (Table 4-5). Entry 53 was observed to give the lowest yields both when protected and also infested with *C. partellus*.

Table 4-5: Means of stem borer resistance traits, cumulative tunnel length, exit holes and leaf damage scores of best and worst ten hybrids

Rank	Entry	SC	GYP	Entry	SC	GYI	Entry	SC	CTL	Entry	SC	ExHls	Entry	SC	LDS
1	38	4X12	10.5	5	1x6	10.1	12	2x3	3.1	2	1x3	1	2	1x3	2.1
2	66	11X12	10	38	4x12	9.2	4	1x5	3.5	12	2x3	1	12	2x3	2.2
3	11	1X12	8.8	48	6x9	9.1	27	3x9	3.6	22	3x4	1	22	3x4	2.2
4	9	1X10	8.7	9	11x12	8.8	1	1x2	3.7	35	4x9	1	35	4x9	2.2
5	21	2x12	8.7	66	1x10	8.8	29	3x4	3.8	7	1x8	1	27	3x9	2.2
6	45	5x12	8.7	37	4x11	8.6	22	3x11	3.8	27	3x9	1.1	18	2x9	2.2
7	33	4x7	8.6	56	7x12	8.4	2	1x3	3.9	1	1x2	1.1	8	1x9	2.2
8	50	6x11	8.5	11	1x12	8.3	21	2x12	4	3	1x4	1.1	7	1x8	2.3
9	35	4x9	8.4	35	5x12	8.3	9	4x9	4.1	4	1x5	1.2	1	1x2	2.3
10	56	7x12	8.4	45	4x9	8.3	35	5x9	4.1	19	2x10	1.2	3	1x4	2.3
57	19	2x10	5.5	22	3x4	5	54	7x10	6.5	59	8x11	2.1	53	7x9	3.1
58	1	1x2	5.4	49	6x10	5	66	8x12	6.6	6	1x7	2.2	59	8x11	3.1
59	61	9x10	5.4	26	3x8	4.9	60	11x12	6.6	20	2x11	2.2	64	10x11	3.1
60	65	10x12	5.1	23	3x5	4.8	55	7x11	6.7	48	6x9	2.3	51	6x12	3.2
61	23	3x5	4.9	61	9x10	4.6	59	8x11	6.9	56	7x12	2.4	55	7x11	3.2
62	26	3x8	4.6	57	8x9	4.4	64	10x11	7	54	7x10	2.5	60	8x12	3.2
63	57	8x9	4.6	52	7x11	4	56	7x12	7.1	64	10x11	2.5	56	7x12	3.3
64	52	7x8	3.7	55	7x8	4	53	7x9	7.6	66	11x12	2.9	66	11x12	3.3
65	58	8x10	3.7	58	8x10	3.6	65	10x12	8.5	65	10x12	3.4	49	6x10	3.4
66	53	7x9	3	53	7x9	3.3	49	6x10	8.8	49	6x10	3.5	65	10x12	3.9
	Mean		6.8			6.6			5.3			1.7			2.7
	Max		10.5 (38)			10.1 (5)			3.7 (12)			3.5 (49)			3.9 (65)
	Min		3.0 (53)			3.3 (53)			8.8 (49)			0.96 (2)			2.1 (2)
	SE		0.65			0.77			1.28			0.4			0.18

†GYP, Grain yield (t/ha) protected; GYI, grain yield (t/ha) infested; CTL, cumulative tunnel length (cm); ExHls, exit holes (counts); LDS, leaf damage scores (scores 1 = clean , 9 = most susceptible); SC, single cross.

4.4.2 Post-harvest insects' resistance parameters

Highly significant (p≤ 0.001) differences were observed from the analysis of variance for percent live insects recovered, both from larger grain borer and maize weevil infestation across different environments, for genotypes, entry, entry-by-environment interactions, and for GCA (Table 4-6). Both GCA and SCA were highly significant for undamaged kernels, weight loss, and live insects.

The storage pests' resistance traits of weight loss for *S. zeamais* had a mean score of 17.4%, with minimum of 8.8% for entry 57 (8×9). Percentage undamaged kernels recorded were mean of 42.7%, with the best genotype recording 75.1% undamaged kernels (entry 57, 8×9), and the lowest being 15.6 (entry 61, 9×10) for *S. zeamais*, The percentage of live *S. zeamais* insects recovered were a maximum of 77.8% from entry 44, and a minimum of 40.5% form entry 57 (Table 4-6).

Entry 57 was observed to have the best combination for low percentages of weight loss, live *S. zeamais* insects and the highest percentage of undamaged grain after infestation. Conversely, entry 4 had both high percentage of grain weight loss of 26.7% and the lowest percentage of undamaged grains of 15.6% (Table 4-6).

Table 4-6: Analysis of variance for grain yield and post-harvest insect pest resistance parameters due to infestation by the *Sitophilus zeamais*

		Grain Yield		Maize weevil		
Source	**DF**	**GYP**	**GYI**	**MLI**	**MuDkn**	**Mwtls**
ENV	5	771.6***	1100.5***	17741.2***	44994.7***	9068.8***
REP(ENV)	12	30.3	45.3	1328.3	1019	339.1
ENTRY	65	36.1***	37.3***	997.5***	2768.5***	297.8***
ENV*ENTRY	325	8.2***	9.3***	330.8***	413.4***	65.3***
GCA	11	105.9***	92.8***	3903.0***	11853.1***	1250.6***
SCA	54	21.9***	26.0***	405.6***	917.9***	103.8***
GCA*ENV	55	20.5***	20.1***	664.8***	537.9***	86.6***
SCA*ENV	270	5.7*	7.04	262.8***	388.0***	60.9***
Error	780	4.6	7.1	186.8	293.6	47.2
R^2		0.72	0.67	0.66	0.71	0.71
Coeff Var		31.6	40.4	21.8	40.2	39.4
% GCA (ss)		49.7	42.1	66.2	72.5	71.1
% SCA (ss)		50.3	57.9	33.8	27.5	28.9
Mean		6.8	6.6	62.7	42.7	17.4
Max		10.5 (38)	10.1 (5)	77.8 (44)	75.1 (57)	26.7 (4)
Min		3.0 (53)	3.3 (53)	40.5 (57)	15.6 (4)	8.8 (57)

‡Data significance: *** Highly significant at p≤ 0.001; **significant at p≤ 0.01; * significant at p≤ 0.05. †GYP/GYI, grain yield (t/ha), protected/infested; MLI, percentage live maize weevil insects, MuDkn, percentage of undamaged kernels/grains under mw infestation, Mwtls, percent weight loss by grains due to mw infestation. Numbers in parentheses indicate the entries with the adjacent means.

4.4.3 General combining ability effects

Parents 12, 11, 4, 6, and 1, had positive and favourable effects (1.47, 0.72, 0.95, 0.38, and 0.19) for grain yield respectively, when protected, with parents 4, 11, and 12 being highly significant ($p \leq 0.001$). The same parents also gave positive and favourable estimates for grain yield when infested. Parents 1,3,2,4 and 5, gave the most favourable and negative effects (-0.93, -0.92, -0.64, -0.62, -0.35), for cumulative tunnel length, but only parents 1 and 3 were significant ($p \leq 0.01$) (Table 4-7).

Table 4-7: General combining ability effects for grain yield, stem borer resistance parameters, biochemical components and storage pest resistance parameters

Parent	GYP	GYI	CTL	ExHls	LDS	OIL	Protein	Starch	MLI	MuDkn	Mwtls
1	**0.19**	0.47	**-0.93***	**-0.32***	**-0.27*****	-0.29***	-0.17*	0.29***	4.8***	-13.03***	4.65***
2	-0.19	-0.17	**-0.64**	-0.25	**-0.23*****	-0.17***	-0.31***	0.5***	3.24**	0.15	-0.03
3	-0.73***	-0.90***	**-0.92***	**-0.37****	**-0.35*****	0.06	0.34***	-1.0***	2	-4.06*	1.13
4	**0.95*****	0.85***	**-0.62**	**-0.36****	**-0.24*****	0.24***	-0.43***	-0.15	-0.56	-0.71	-0.82
5	-0.16	0.03	**-0.35**	-0.2	**-0.13***	-0.06	-0.16*	0.11	2.68*	-6.39***	1.73*
6	**0.38**	0.75**	0.68	0.25	0.25***	0.17***	-0.06	-0.09	1.65	-3.60*	0.29
7	-0.21	-0.58*	0.90*	0.26	0.15**	-0.13***	0.22**	-0.06	0.51	-0.9	0.89
8	-1.12***	-0.95***	0.16	-0.15	0.063	0.05	0.57***	-0.32***	-8.42***	12.72***	-4.33***
9	-0.67**	-0.27	-0.08	-0.02	-0.07	-0.07	0.41***	-0.05	-8.62***	14.71***	-4.55***
10	-0.62**	-0.70**	0.6	0.43**	0.23***	0.12**	0.14	-0.17*	-4.38***	7.93***	-1.57*
11	**0.72*****	0.28	0.65	0.36**	0.27***	0.1**	-0.12	-0.06	4.24***	-6.45***	2.65***
12	**1.47*****	1.20***	0.55	0.37**	0.32***	-0.02	-0.44***	0.31***	2.86*	-0.37	-0.03
Mean	6.78***	6.60***	5.26***	1.66***	2.68***	5.03***	10.77	69.6***	62.65	42.65***	17.43***
StdErr	0.22	0.26	0.43	0.13	0.06	0.04	0.08	0.09	1.27	1.65	0.7

‡Data: GYP/GYI, grain yield protected/infested; CTL, cumulative tunnel length; ExHls, exit holes; LDS, leaf damage scores; MLI, percentage live mw insects; MuDkn, percentage of undamaged kernels; Mwtls, percent grain weight loss; Significance: *** means data is significant at P ≤ 0.001, ** data is significant at p ≤ 0.01, * data is significant at p ≤ 0.05.

Parents 1, 3, and 4, gave significant favourable negative estimate (-0.32, -0.37, and -0.36) of exit holes, respectively. Leaf damage scores favourable effects came from parents 1, 2, 3, 4, and 5 (-0.27, -0.23, -0.35, -0.24, and -0.13), respectively which were highly significant ($p \leq 0.01$), except parent 5 ($p \leq 0.01$) (Table 4-7).

Parents 1 and 2 gave negative estimates for both oil and protein (-0.29, 10.17 and -0.17, 10.31), but gave positive estimates for starch (0.29, 0.50), respectively. Inbred line parent 8 and 9 had the most favourable negative effects on percentage live insects for *S. zeamais* (-8.42, -8.62). The same parents had favourable positive effects for undamaged kernels (12.71, 14.71) and also favourable negative effects for weight loss (-4.33, -4.55) (Table 4-7). The contributions of these two parents to the three traits of interest gave negative and significant effects for oils (-0.29, -0.17), and proteins (-0.17, -0.34). Parents 8 and 9 gave positive effects for proteins (0.57, 0.41) respectively (Table 4-7). Overall, general combining ability accounted for 50.9-66.2% of live insects, 71.1-74.9% of weight loss and 72.5-77.1% of undamaged kernels.

4.4.1 Specific combining ability effects

The single cross (SC) 7×10, had favorable positive specific combining ability (SCA) effects (2.33) for yield when protected, which translated into 39.2% yield gain from expected (predicted) yield of 5.95 to observed yield 8.28 t/ha. Other hybrids with favorable and positive effects for yield were SC 1×10 (2.31), giving yield gain of 36.4% from the expected of 6.3 t/ha to observed 8.65 t/ha. More favorable SCA effects for grain yield were observed for SC 1×10 (2.43), SC 1×6 (2.27), and SC 2×9 (1.89) when infested (Table 4-8).

Further, the analysis revealed negative unfavorable SCA effects as well. These were observed on some specific single crosses for grain yield and insect resistance traits. The single cross 10×12 gave unfavorable SCA effects of -2.54, for yield which resulted in yield reduction of 33.3% from expected 7.63 t/ha to 5.0 t/ha. Similar results were observed for grain yield for SC 6×12 (-2.31), SC 7×9 (-2.89), as well as SC 11×12 (-0.042) for grain yield protected, and infested (-0.12). Although the hybrid SC 11×12 revealed a negative SCA (-0.02), the observed yield of 10.02 t/ha, was far more than expected (predicted) yield of 8.97 t/ha. Other hybrids include SC 7×9 (-2.48), and SC 10×12 (-1.68) with negative and unfavorable effects on grain yield when infested (Table 4-8).

Three single crosses had significant SCA effects for leaf damage scores. These are SC 4×5 (15.1%), SC 10×12 (19.9%), and SC 7×9, (11.1%). It was also observed that SC 6×10 and

SC 10x12, showed significant SCA effects, 51.4% for exit holes and 40.2% respectively, (Table 4-8).

Table 4-8: Specific combining ability effect on grain yield, *Chilo partellus* and *Sitophilus zeamais* insect resistance traits observed in selected crosses

		GYP			GYI			LDS		
Entry	SC	SCA	Obsv	%effect	SCA	Obsv	%Eff	SCA	Obsv	%Eff
5	1X6	-0.3	7.0	-4.4	**2.3**	**10.1**	29	0.1	2.8	4.2
9	1X10	**2.3**	**8.7**	36.4	**2.4**	**8.8**	38.2	-0.1	2.6	-3.2
18	2X9	1.5	7.4	25.6	**1.9**	**8.1**	30.7	-0.1	2.2	-5.9
26	3X8	-0.4	4.6	-7.4	0.2	4.9	3.3	0	2.4	1.2
31	4X5	-0.9	6.6	12.2	-1.0	6.4	-13.8	0.4	2.7	15.1
47	6X8	1.7	7.8	28.7	0.3	6.7	4.4	0	3.0	0.9
49	6X10	-0.8	5.7	-12.2	-1.7	5.0	-24.8	0.2	3.4	7.5
51	6X12	**-2.3**	**6.3**	-26.8	-2.0	6.5	-23.5	0	3.2	-1.0
53	7X9	**-2.9**	**3.0**	-48.9	-2.5	3.3	-43.2	0.3	3.1	11
54	7X10	**2.3**	**8.3**	39.2	1.7	7.0	32.1	-0.3	2.8	-9.3
58	8X10	-1.4	3.7	-26.8	-1.3	3.6	-26.9	0.1	3.0	1.7
65	10X12	**-2.5**	**5.1**	-33.3	-1.7	5.4	-23.7	0.6	3.9	19.9
66	11X12	**-0.02**	**10.0**	-0.5	-0.1	8.0	-1.5	0.1	3.4	2.9
		MLI			**MuDkn**			**Mwtls**		
Entry	**SC**	**SCA**	**Obsv**	**%Eff**	**SCA**	**Obsv**	**%Eff**	**SCA**	**Obsv**	**%Eff**
5	1X6	-1.7	67.4	-2.5	3.1	29.1	11.8	-2.4	20.0	-11
9	1X10	4.2	67.3	6.7	**-15**	**22.5**	**-40**	**4.6**	**25.1**	**22.4**
18	2X9	-6.1	51.2	-10.6	6.5	64.0	11.3	-2.4	10.5	-18
26	3X8	-4.7	51.5	-8.4	2.2	53.5	4.2	-1.3	12.9	-9.2
31	4X5	3.4	68.2	5.3	-0.8	34.8	-2.2	0.4	18.8	2.2
47	6X8	11.9	67.8	21.4	-13	38.9	-24.9	3.6	17.0	26.8
49	6X10	-4.4	55.6	-7.3	0.2	47.2	0.4	-0.3	15.8	-2.0
51	6X12	-4.5	62.7	-6.6	8.1	46.8	20.9	-1.8	15.9	-10.0
53	7X9	-6.3	48.2	-11.6	0.2	56.6	0.3	0.1	13.9	0.9
54	7X10	13.4	72.1	22.7	**-26.0**	**23.6**	**-52.5**	**8.8**	**25.5**	52.3
58	8X10	2.8	52.7	5.7	-0.5	62.9	-0.7	1.8	13.3	15.7
65	10X12	0.4	61.5	0.7	5.7	55.9	11.4	-0.1	15.7	-0.7
66	11X12	-12	64.3	-16.9	5.5	41.0	15.3	-0.9	18.7	-4.3

Data: GYP, grain yield protected; GYI, grain yield infested; LDS, leaf damage scores; CTL, cumulative tunnel length; MLI, live maize weevil insects; MuDkn, undamaged kernels after maize weevil infestation; Mwtls, weight loss due to maize weevil infestation; SC, single cross; SCA, specific combining ability; %Eff, percentage effect contributed to observed mean; Obsv, observed mean of trait.

The single cross 7×10 gave highly significant SCA effects for Mwtls (8.76), MuDGN (-26.11), and MLI (13.35) effects. Other genotypes with significant SCA effects were; 1x10 having MuDGN (15.01), and Mwtls (4.60); cross between 6 and 8 also with, MuDGN (11.95), and Mwtls (12.92) (Table 4-8). Overall, SCA accounted for 22.9-27.5% of undamaged kernels, 25.1- 28.9% weight loss, and 33.8-49.1% live insects (Table 4-8).

4.4.2 Combined resistance for stem borers and maize weevil in hybrids

There was observed resistance to stem borers by crosses derived from stem borer resistant inbred lines, 1 to 5. Inbred lines parents 1, 2, 3 and 4, gave crosses with high resistance levels to stem borers (Table 4-9).

Table 4-9: Means of leaf damage scores for stem borers and undamaged grain for maize weevil

Rank	Ent	SC	LDS	Entry	SC	MuDGn
1	2	1×3	2.1	57	8×9	75.1
2	12	2×3	2.2	61	9×10	64.4
3	22	3×4	2.2	**18**	**2×9**	**64**
4	**35**	**4×9**	**2.2**	58	8×10	62.9
5	27	**3×9**	2.2	28	3×10	59.9
6	**18**	**2×9**	**2.2**	62	9×11	57.1
7	8	1×9	2.2	60	8×12	56.8
8	7	**1×8**	2.3	53	7×9	56.6
9	1	1×2	2.3	41	5×8	56.6
10	3	1×4	2.3	65	10×12	55.9
Mean			2.7			42.7
Max			3.9 (65)			75.1 (57)
Min			2.1 (2)			15.6 (4)
SE			0.18			4.93

†Ent, entry; LDS, leaf damage scores (scores 1 = clean, 9 = most susceptible); MuDgN, undamaged grain infested with maize weevil.

However, among the best 10 crosses with high levels of stem borer resistance, five of them; SC 1×8, SC 1×9, SC 2×9, SC 3×9, and SC 4×9; had parents 8 and 9 which are storage pest resistant inbred lines (Table 4-9). Similarly the best 10 crosses with high levels of storage pest resistance where derived from storage pest resistance inbred lines 6 to 10 and demonstrated high resistance levels to *S. zeamais*. Four of these crosses; SC 7×9, SC 8×9, SC 8×10, and SC 9×10; were purely from storage pests resistant inbred lines 7, 8, 9 and 10. The other six had parents from the stem borer resistant inbred lines 2, 3 and 5. The neutral CIMMYT inbred lines 11 and 12 also combined well with storage pest resistance inbred lines and contributed to grain yield in three of these top ten hybrids with combined insect resistance (Table 4-9).

Some hybrids demonstrated existence of combined stem borer and maize weevil resistance in the same genotypes. These hybrids are summarized in Table 4-10.

Table 4-10: Single cross hybrids which exhibited combined resistance to stem borers, maize weevil and high grain yield

Entry	SC	Gyp	Gyi	LDS	MuDKN
35	4×9	8.4	8.3	2.2	59.7
41	5×8	5.9	8.2	2.4	56.5
18	2×9	7.4	8.1	2.2	62.5
27	3×9	6.5	6.3	2.2	66.4
43	5×10	5.9	6.3	2.6	58.4
34	4×8	6.8	6.2	2.4	60.3
28	3×10	6.0	5.7	2.3	64.3
17	2×8	6.1	5.6	2.7	63.2
	Mean	6.6	6.8	2.4	61.4
Best Check	**PH3253**	**7.1**	**6.0**	**3.2**	**16.5**
Grand mean	**Pop**	**6.7**	**6.5**	**2.7**	**42.9**
	LSD	0.6	0.6	0.1	1.9
	CV	14.2	15.2	11.6	13.4
	Max	10.5 (38)	10.1 (5)	3.9 (65)	69.5 (58)
	Min	3.0 (53)	3.3 (53)	2.1 (2)	49.8 (4)

‡Data: Gyp/i, grain yield (t/ha) protected/infested; LDS, leaf damage score (1=clean, 9=dead heart); MuDKN, undamaged grain (%) under maize weevil infestation. Figure in parentheses are the respective entries.

Entry 35, a cross between inbred lines 4 and 9, gave the highest yields both when protected (8.4 t/ha), and when infested (8.3 t/ha). It had a leaf damage score of 2.2, and 59.7% of undamaged grain after 90 days infestation with maize weevil (Table 4-10). Yields of the best check when protected were high (7.1 t/ha), when compared to the mean of hybrids with combined resistance which stood at 6.6 t/ha, as well as the mean of the population, 6.7 t/ha. However, insect resistance traits, leaf damage scores and undamaged kernels after infestation with *C. partellus* and *S. zeamais* were low at 3.2 and 16.6% respectively (Table 4-10).

4.5 Discussion

4.5.1 Grain yield, stem borer and post-harvest resistance parameters

The significance of environments implies that each of the environments was unique and important for evaluating the test hybrids, which is in agreement with work done by (Beyene et al., 2012). Significance of test genotypes (entry) suggests that these hybrids had variation

and each one was a unique genetic constitution, there existed variability within the test hybrids.

The significant Environment by Entry (GXE), suggests that there was interaction between the genotypes and the environments. It also justifies evaluating the test hybrids across different environments so that unbiased estimates of phenotypic information can be obtained. Again this confirms the importance of multiple environments for evaluating genotypes particularly under infestation because each environment not only affects the genotypes, but also interferes with the bioassay insects in specific ways. This can be explored further to determine which environments can separate the test genotypes based on insect resistance traits of interest. Again this can be explored further to determine which genotypes are suited to which environment, although, data for yield stability across many environments would be more preferred for general adaptation rather than specific adaptability. This agrees with work done by Machida et al. (2010), when investigating the combining ability of quality protein maize, and reported that some of the traits were highly influenced by environment, and to make selection based on few environments is important for release of maize varieties.

Significant general combining ability and specific combining ability for grain yield (protected and infested), exit holes, leaf damage scores, oil, protein and starch all suggest that there were both additive and non-additive genes effects conditioning these traits. This partially agrees with findings by Dhliwayo et al. (2005), Beyene et al. (2011), and Derera et al. (2014) who reported additive effects for insects' resistance traits. However, in this study, non-additive effects are reported to have played a major role in insect pests' resistance as well. Significant GCA for cumulative tunnel length suggests that the trait is mostly conditioned by additive effects, since SCA was not significant for these test hybrids.

The presence of significant effects, for the post-harvest traits of undamaged kernel, live insects, and weight loss of the test genotypes is a confirmation of the variability existing in the genotypes. The significant GCA and SCA for all the traits except live insects of larger grain borer, further suggests that resistance to post-harvest insect pests is due to both additive and non-additive effects. This agrees with Derera et al. (2010), and Matewele (2014), that resistance to maize weevil was due to additive, non-additive effects. Dhliwayo et al. (2005), further reported that there are maternal and non-maternal effects influencing kernel qualities of a maize grain on response to maize weevil attack.

4.5.2 Mean performance for yield and insect resistant parameters

The highest yielding entries for grain when protected and infested suggest that stem borer resistance can be obtained in maize hybrids without yield penalty. This is because the differences between the two treatment means were not significant, and the overall means within the treatments was also not significant. These findings agree with work done by Beyene et al. (2011), where different inbred lines were combined and evaluated for resistance to stem borers.

On the other hand, the most resistant hybrid for maize weevil and larger grain borer was not necessarily among the best yielding hybrids. This suggests that post-harvest insect resistance breeding may have yield penalties on the genotypes. This agrees with work done by Butrón et al. (2002), which reported yield penalties on selection for resistance to European corn borer, however, the present study identifies this penalty only in association with post-harvest insect pests resistance. The different ranking of genotypes per trait is a confirmation of the variability within the test genotypes. The observed resistance to post harvest insects contributed by parent 8 and 9 is suggestive of the high frequency of good alleles which can be exploited for future insect resistance breeding.

4.5.3 General combining ability effects

The observed favourable positive effect for yield from parents 12, 11, 4, 6 and 1, can be deduced to indicate that the allele frequency for yield is high in these parents, irrespective of the treatment. Parent 12 exhibited dominance effects for yield when the observed yield exceeded the predicted. The same can be deduced of parents 1, 2, 3, and 4 which gave favourable negative effects for exit holes, cumulative tunnel length and leaf damage scores. These suggest that they have high frequency of stem borer resistance alleles.

The overall effects due to general combining ability (GCA) and specific combining ability (SCA) were significant for all post-harvest resistance traits; live insects, undamaged kernel, and weight loss. This confirms that both additive and non-additive gene actions were responsible for resistance in the test genotypes. Storage pest resistance parents 8, 9 and 10 had hybrids with high levels of *S. zeamais* resistance which means there contribution to storage pest resistance was favourable. The favourable negative effects contributed by the two inbred lines parents 8 and 9 for live insects and weight loss resistance traits and the favourable positive effects for undamaged kernels under infestation can therefore be deduced that they have high frequency of the favourable alleles for resistance to post-

harvest insect pest. These parents can therefore be used for breeding for post-harvest insect pest resistance in a breeding program.

4.5.4 Specific combining ability effects

Non-additive gene action was observed for grain yield and other traits as noted in yield of cross, 7×10 when the observed yield was far much more than the predicted. This can be deduced to mean dominance or epistatic gene effects. Of great interest was the single cross 11×12, which even when it had negative SCA effect, still gave far greater yield than expected (predicted) yield. Such observations can be attributed to non-additive gene action, and therefore can be exploited for yield gain as well as resistance parameter. Again from the findings of this research, SCA contributed to both yield and resistance traits, and it can be deduced that there are non-additive gene action conditioning these traits. This agrees with findings of Kim and Kossou (2003) who reported non-additive gene action for both grain yield and maize weevil resistance in maize germplasm.

4.5.5 Combined resistance for stem borer and maize weevil in hybrids

High resistance levels observed from SBR × SBR inbred lines 1, 2, 3 and 4, is an indication that there is synergy in terms of resistance with the respective inbred lines contributing favorable alleles for stem borer resistance in the hybrids. This confirms the already reported favorable GCA effects for stem borer resistant traits leaf damage scores, exit holes and cumulative tunnel length, already reported in this study. It is important to therefore note that additive gene action was responsible for stem borer resistance in these hybrids. Similarly hybrids derived from crosses of SPR × SPR inbred lines showed synergy in resistance to maize weevil. This confirms the findings of this study where GCA effects for resistance were favorable. The combination of good grain yield and high levels of resistance to *C. partellus* and *S. zeamais* is an indication that it is possible to develop hybrids with combined insect resistance to these pests.

4.6 Conclusion

The findings of this research provide evidence from which the following deductions can be made:

1. Four *C. partellus* parents; 1, 2, 3 and 4, and three *S. zeamais* resistant inbreds lines parents 8, 9 and 10 were good combiners and can be good sources of resistance genes in breeding for combined resistance to field and storage pests in maize.
2. Stem borer resistant hybrids did not show yield penalties, however, there were yield penalties' in the observed post-harvest resistance hybrids.
3. Some single crosses exhibited high yields, stem borer resistance and maize weevil resistance. These are entry 35, SC 4X9; entry 18, SC 2X9; entry 34, SC 4x8; entry 27, SC 3x9; entry 17, SC 2x8. These hybrids had parents 2, 3, 4, 8 and 9.
4. Resistance was conditioned mostly by both additive and no-additive gene action.
5. Non-additive gene action can be exploited for hybrids that exhibited high performance exceeding the expected in insect resistance traits.

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Chapter 5: Genetic analysis of combined stem borer and storage insect pest resistance in maize hybrids

Abstract

Field pest stem borer, *Chilo partellus* Swinhoe is a major pest, and widely distributed in all maize producing ecologies of sub-Saharan Africa causing 12.9-17%. In storage, maize weevil, *Sitophilus zeamais* Motschulsky, causes estimated 15-30% maize yield losses in developing countries. Hybrids developed through host plant resistance have separate resistance to each of these insect pests and there is no record of hybrids with dual resistance to these pests. A study was carried out to determine the effects of combining resistance to the two pests using parental inbred lines with contrasting and varying levels of resistance to each of these insect pests. Twenty inbred lines were selected and organized into four sets of five (5) inbred lines each, based on resistance levels to *C. partellus* and *S. zeamais*. The lines were crossed in accordance with a North Carolina Design II, in eight sets. The resulting F1s were evaluated for yield in four (4) environments. The young seedlings were screened for resistance to *C. partellus* in the field, and the grain screened for *S. zeamais* resistance after harvest. There were highly significant ($p \leq 0.001$) mean squares for grain yield, leaf damage scores, grain weight loss and undamaged kernels. Both additive and non-additive gene action were responsible for combined resistance to both *C. partellus* and *S. zeamais* insect pests. The female parent 3 diplayed favourable GCA effects for grain yield, when protected and infested treatments, as well as positive and favourable effects for undamaged kernels, and for both leaf damage scores (-0.46) due to *C. partellus* infestation, and, weight loss due to *S. zeamais*. At least 8% of the hybrids were highly resistant to stem borer with a leaf damage score of 1.5-2.5; 42% of the test hybrids had a score of 3.0, and, 49% were susceptible with leaf damage scores > 3. Heterosis for grain yield ranged from 26-41% for the best five hybrids. Heterosis for resistant parameters, leaf damage scores was -22% to -17, and, for weight loss was -45 to -32%. Some hybrids from different sets exhibited resistance to both *C. partellus* and *S. zeamais* and displayed grain yield levels above the mean of checks, and high levels of resistance for both insect pests.

Key word: Combining ability, Heterosis, Insect pests, Maize, North Carolina II.

5.1 Introduction

Maize crop yields in sub-Saharan Africa remain low at 1.9 t/ha as compared to the potential yields of 9.5 t/ha realized in more developed countries (Bänziger et al., 2000; Araus et al., 2008). Unfortunately smallholder farmers bear the brunt of these low yields because they have resource-constrained, and also depend largely on the crop for food (Mugo, 2005). The reasons for the low yields are partly due to poor agronomic practices, lack of use of certified seed (DeVries and Toenniessen, 2001), and losses due to insect pests both in the field and during storage (Appert and Deuse, 1982). Field pest stem borer, *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae), is a major pest in maize production, which is widely distributed in all maize producing ecologies of sub-Saharan Africa (Guofa et al., 2001; Ong'amo et al., 2006). In Kenya, it is found in the lowland tropics and mid-altitude agro-ecologies (Ong'amo et al., 2006). Due to climate change which has contributed to global warming, the spread of this pest is rapidly moving to the highland tropics (Guofa et al., 2001; Le Rü et al., 2006).

Infestation by *C. partellus* starts after crop emergence all the way to the grain filling stage of the developing cob, and up to 100% infestation has been reported (Ebenebe et al., 2000; Mohamed et al., 2004). The feeding action of larvae causes damage to leaves, which reduces the photosynthetic area of the maize plant. The developing larvae tunnels within the maize stem, creating tunnels which affect the translocation of nutrients and water from the roots to the rest of the plant, and also interferes with the translocation of photosynthates from the leaves (Mohamed et al., 2004).

The second generation of *C. partellus* feeds on the developing grains and this lowers the value of the grain, as well as opening entry points for secondary pathogens to infest the developing grain. Secondary effects of *C. partellus* infestation include increased stem lodging of maize plants, weak maize plants which are vulnerable to attack by disease causing pathogens, and increased mycotoxin infections on the young developing cob. DeGroote (2002), estimated yield losses of 13.5%, amounting to estimated $90M in Kenya due to this pest.

On the other hand, the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a major pest in maize storage. Maize grain losses due to this pest are estimated at 15-30% in developing countries (Derera et al., 2014). Infestation starts in the field especially on the open tipped varieties (Sallam, 2010). The complex yield losses from stem borers in the field, to post-harvest losses by maize weevil in storage; is a major threat to food security in Africa.

Breeders, in an effort to address this challenge, have developed maize hybrids with separate resistance to each of these insect pests; stem borer resistant hybrids, and storage pests' resistant hybrids. There is, however, no record of hybrids with dual resistance to these pests.

The objectives of the current research were therefore to:

1. Combine stem borer and storage pests resistance in maize hybrids;
2. Evaluate the performance of these hybrids under *C. partellus* infestation in the field;
3. Screen for postharvest *S. zeamais* resistance of the hybrids after harvest; and
4. Investigate the mode of gene action and heritability of the resistance traits in the hybrids

5.2 Materials and Methods

5.2.1 Germplasm

The parental inbred lines used in this study were obtained from International Maize and Wheat Improvement Centre (CIMMYT), Nairobi (Table 5-1). Beyene et al., (2011a), evaluated and reported the levels of resistance for stem borer field pest, while the maize weevil storage pest resistant inbred lines were evaluated and reported by Mwololo et al., (2012). The procedure used for stem borer and maize weevil resistance is as described in chapter four sections 4.2.4 and 4.2.6.

Table 5-1: Groups of inbred lines used for making sets in North Carolina II design and their insect pests' resistance information

	SBR	SPR Males		SBR	SPR Males
Group	Cp_r/Sz_s	Cp_s/Sz_r	Group	Cp_s/Sz_r	Cp_r/Sz_s
1	CKSBL10039	CKSPL1001	1	CKSBL10041	CKSPL10186
2	CKSBL10014	CKSPL1009	2	CKSBL10029	P100C6-200-1-1-B***-#-#
3	CKSBL10027	CKSPL1008	3	CKSBL10001	CKSPL10036
4	CKSBL10025	CKSPL1011	4	CKSBL10008	LPSC7-F180-3-1-1-1-BB-#-#
5	CKSBL10004	CKSPL1034	5	CKSBL10021	CML440
6	CKSBL10040	CKSPL1008	6	CKSBL10008	DTPWC9-F16-1-1-1-1-BB-#-
7	CKSBL10007	CKSPL1002	7	CKSBL10020	CKSPL10230
8	CKSBL10045	CKSPL1022	8	CKSBL10001	CKSPL10003
9	CKSBL10043	CKSPL1008	9	CKSBL10028	CKSPL10113
10	CKSBL10033	CKSPL1011	10	CKSBL10023	CML159

†Cp_r/Sz_s, *Chilo partellus* resistant but *Sitophilus zeamais* susceptible; Cp_s/Sz_r, *Chilo partellus* susceptible but *Sitophilus zeamais* resistant; SBR, stem borer resistant; SPR, storage pest resistant; CKSBL, code for CIMMYT/KARI stem borer resistant lines; CKSPL, code for CIMMYT/KARI storage pest resistant lines.

A total of forty parents were obtained from two populations; twenty from stem borer resistant (SBR) population, and, twenty from storage pests' resistant (SPR) population. These parents had varying levels of resistance to *C. partellus* and *S. zeamais* insect pests. These inbred lines were divided into groups of five parents based on resistance levels using the SBR lines as the female parents, and SPR lines as the males (Table 5-1).

Two major groups of the lines were formed; group one had 10 SBR line with resistance to *C. partellus* but susceptible to *S. zeamais* used as females and 10 SPR lines susceptible to *C. partellus*, but resistant to *S. zeamais*. The lines had varying levels of resistance among themselves. Group two had 10 lines drawn from SBR population which were susceptible to *C. partellus* but resistant to *S. zeamais*, and, 10 lines drawn from SPR population which were resistant to *C. partellus* but susceptible to *S. zeamais* (Table 5-1).

The resulting crosses had parents with resistance to *C. partellus* but susceptible to *S. zeamais* crossed with parents resistant to *S. zeamais* but susceptible to *C. partellus*, ($Cp_r/Sz_s \times Cp_s/Sz_r$), and the reverse (($Cp_s/Sz_r \times Cp_r/Sz_s$), each with varying levels (dosage) of resistance. A total of 200 single crosses were expected (25 from each set). However, some of the crosses were unsuccessful leading to a total of 190 singles crosses (Appendix 1, list of F1 entries per set). Five check hybrids were used alongside the test hybrids during across sites evaluation.

5.2.2 Experimental sites

The nurseries were planted at Kiboko (ENV 0), while the evaluation sites were at Kirinyaga, Embu and Kakamega sites during the 2013B and 2014A seasons. Season by site interaction was classified as one environment (Table 5-2).

Table 5-2: Experimental sites for nursery and evaluation of hybrids derived from North Carolina II design

ENV	Location	Latutude (N/S)	Longitude (E)	Soils	Altitude (masl)	Season	RF (mm)	Temp (^oC).
0	Kiboko	02^o 15' S	037 ^{o} 75'	Sandy-Loam	975	2013A	530	26
1	Kirinyaga	00^o 33.703'	037 ^{o} 19.346'	Andisols	1308	2013B	1250	25
2	Kirinyaga	00^o 33.678'	037 ^{o} 19.328'	Andisols	1305	2014A	1200	26
3	Kakamega	02^o 12.882'	37 ^{o} 43.534'	Clay-loam	1526	2013B	1900	23
4	Kakamega	00^o 16.872'	34 ^{o} 46.237'	Clay-loam	1521	2014A	1700	24

†masl, meters above seas level; RF, rainfall, N/S, north or South, except where indicated; E, east.

5.2.3 Design of the experiment

5.2.3.1 The nurseries

Females were planted in four rows for every one row of males, but in a block of twenty rows per female parent. The respective males were planted in one row planting staggered three times in intervals of -5, 0, +5 days, corresponding to the female planting. These males were planted adjacent to each of the female twenty-row-block rows. The plots were five meter rows per entry with row to row distance of 75 cm and hill to hill distance of 25 cm. Two seeds were sown and later thinned to one plant per hill (CIMMYT, 1985). Different colour tags were used to label females and males. Chemical stem borer control was applied on seedlings at the 6-leaf, and, 10-leaf stages. Routine agronomic practices for weeding, fertilizer application and rogueing off-types were practiced to ensure clean, uniform and healthy plants. Shoots were covered as they emerged in all plants (CIMMYT, 1985).

Pollen was harvested and bulked from at least four plants in the male rows. As many females as possible were hand pollinated, to ensure large number for seeds for multi-location field-testing. Harvested ears from each female were kept separate. The rotten ears were eliminated, and the seeds from each female were dried, shelled, weighed and protected with a suitable storage pesticide. The commonly used chemical for post-harvest insect pests of maize among the smallholder farmers in Kenya is Actellic Super, with 1.6 % Pirimiphosmethyl and 0.3 % Permethrin as active ingredients (Farrell and Schulten 2002).

5.2.3.2 Evaluation of F1 crosses for resistance to stem borer and storage pests' resistance

The hybrids were evaluated in a 13x15 α-lattice design with three replications at Kirinyaga University College (KYUC), Embu and Kakamega sites in Kenya during the 2013B and 2014A seasons. Planting, spacing, seeding rate, and thinning were carried out as described in section 5.2.3.1. Infestation was carried out as described in chapter four section 4.2.4 (Artificial infestation using *C. partellus* neonates) (Tefera et al., 2011a). Routine agronomic practices of weeding, fertilizer application and rogueing off-types were practiced to ensure clean, uniform and healthy plants.

5.2.4 Data collection and analysis

Data on leaf damage was collected using a scale of 1-9, where 1 is clean and 9 is heavily damaged (dead hearts) (Tefera et al., 2011a). At harvest, grain yield, exit holes, cumulative

tunnel length data was collected. Data on oil, protein and starch content was collected using the grain analyser®. Grain yield was then adjusted to 12.5% moisture (Bänziger et al., 2000), using the formula:

Grain Yield (t/ha) = [Grain Weight (kg/plot) x 10 x (100-MC)/ (100-12.5)/(Plot Area)]……(5)

Where MC = Grain Moisture Content.

The harvested grain was then screened for post-harvest insects pests resistance as described in the previous chapter (Post-harvest insects' resistance screening and data collection) using the protocol described by Tefera et al., (2011a).

Analysis of variance was done using PROC GLM of SAS, for all the traits (SAS, 2000).

$Y_{ijkpq} = \mu + S_p + gi(S_p) + g_j(S_p) + h_{ij}(S_p) + E_q + r_k(SE)_{pq} + (ES)_{pq} + (Eg)_{iq}(S_p) + (Eg)_{jq}(S_p) + (Eh)_{ijq}(S_p) + e_{ijkpq}$…….....(6)

Where i =1, 2, 3, 4, 5; j = 1, 2, 3, 4, 5; k = 1, 2, 3; p = 1, 2, 3, 4, 5, 6, 7, 8; q = 1, 2, 3, 4 and Y_{ijkpq} denotes the value of the hybrid of a mating of the i^{th} female line, the j^{th} male line, in the k^{th} block, within set p and in the q^{th} environment. The terms are defined as follows:

μ = Grand mean,

S_p = the average effect of the p^{th} set,

$g_i(S_p)$ = the GCA effect common to all hybrids of the i^{th} female line nested within p^{th} set,

$g_j(S_p)$ = the GCA effect common to all hybrids of the j^{th} male line nested within p^{th} set,

$h_{ij}(S_p)$ = the SCA effect specific to hybrid of the i^{th} female and j^{th} male line nested within p^{th} set,

E_q = average effect of q^{th} environment,

$r_k(SE)_{pq}$ = the effect of the k^{th} replication nested within the p^{th} set and q^{th} environment

$(ES)_{pq}$ = the interaction between set effects and the environment

$(Eg)_{iq}(S_p)$ and $+(Eg)_{jq}(S_p)$ = the interaction between environment and GCA nested within sets

$(Eh)_{ijq}(S_p)$ = the interaction between environment and SCA nested within sets, and

e_{ijkpq} = the random experimental error.

Using the variance ratios, heritability estimates were calculated in REML (Payne et al., 2011) as suggested by Hallauer et al. (2010) for the fully inbred parents (F = 1)

(Where $\sigma^2_m = \sigma^2_f = ½\sigma 2A$; and $\sigma^2_{mf} = \sigma 2D$) using the formulae:

a) $h^2 = 2\sigma^2_m/(\sigma^2/r + \sigma^2_{mf} + 2\sigma^2_m)$ ……(7)
for one environment, and

b) $h^2 = 2\sigma^2_m/(\sigma^2/re + \sigma^2_{fme}/e + 2\sigma^2_{me}/e + \sigma^2_{mf} + 2\sigma^2_m)$ .....(8)
for across environments,

Where σ^2_m = male (set) variance, σ^2 = random error variance; σ^2_{mf} = male x female (set) variance; σ^2_{fme} = environment x male x female (set) variance; σ^2_{me} = environment x male (set) variance; r = number of replications and e = number of environments. σ^2_f = female (set) variance; σ^2_A = additive variance and σ^2_D = dominance variance.

5.3 Results

5.3.1 Gene Action

Results of analysis of variance for grain yield (protected and infested), leaf damage scores and weight loss and undamaged kernels are presented in Table 5-3.

Table 5-3: Analysis of variance for grain yield, leaf damage scores, percent weight loss and grain damage

Source	DF	Gyp (t/ha)	Gyi (t/ha)	LDS	MWTLS	M-uDgrn
Set	7	25.3***	34.2***	9.5***	1852.3***	536.5***
ENV	3	1216.5***	1534.6***	16.7***	17851.3***	6514.2***
Rep(ENV)	8	25.4	36.2	11.1	269.6	2108.7
Female(Set)	30	21.6***	15.3***	2.2***	177.7***	2108.7***
Male(set)	32	48.3***	42.2***	3.7***	358.7***	484.6***
Female*Male(Set)	115	3.2***	4.0***	1.1***	73.3 ns	155.2***
Set*ENV	21	4.8***	3.2*	4.4***	100.2*	168.4*
Female*ENV(set)	90	6.2***	4.3***	1.0*	97.5***	142.6*
Male*ENV(set)	96	5.5***	4.7***	1.9***	102.0***	154.9***
Female*Male*ENV(set)	345	2.7***	2.5***	0.9 ns	79.3***	107.3 ns

‡Data: Gyp/i, grain yield (t/ha), protected /infested; LDS, leaf damage scores; MWTLS, percent weight loss; M-GD, percent grain damage. ENV, environment; Rep, replication; DF, degrees of freedom.

Significant differences were observed for grain yield, leaf damage scores and undamaged kernels for test hybrids. The female (set) main effects for all traits were highly significant ($p \leq 0.001$), with undamaged kernels accounting for 69% of gross sum of squares. On the other hand, the male (set) main effects for grain yield; infested, protected; and, leaf damage scores accounted for 55%, 58%, and 37% of sum of squares, respectively.

The sets, and, environment were all highly significant ($p \leq 0.001$) for grain yield and insect resistant parameters. The sets x environment interactions, were also highly significant (($p \leq 0.001$), for grain yield (protected) and leaf damage scores; and significant ($p \leq 0.05$) for grain yield (infested), weight loss and undamaged grains (Table 5-3).

The interaction of females and males within sets was highly significant ($p \leq 0.001$), except for weight loss due to maize weevil infestation, which was not significant. Similarly, the interaction of female by environment within sets, was highly significant ($p \leq 0.001$), except for leaf damage scores and undamaged kernels which were significant ($p \leq 0.05$). The male x environment (sets) were highly significant ($p \leq 0.001$), for grain yield (protected and infested) for all traits. Female x male x environments (sets) were highly significant for grain yield (protected and infested), and weight loss, but not significant for leaf damage scores and undamaged kernels.

5.3.2 Mean performance of eight sets

The mean performance of the eight sets was 5.0t/ha, when protected, and 4.7t/ha when infested. Differences were observed in performance of the sets depending on traits in consideration (Table 5-4).

The highest grain yield of 5.5t/ha, and, 5.2t/ha were recorded in set 3, both when protected and infested respectively. Conversely, the lowest yields were recorded in set 1 (4.5 t/ha, and, 4.2t/ha), both when protected and infested respectively. Sets 1, 4 and 6 yielded lower; 4.2-4.5 t/ha, 4.3-4.9t/ha, and 4.4-4.7t/ha; than the mean of all the sets, when protected as well as when infested (Table 5-4).

Set 7 had the least difference in weight between protected and infested grain yield, while set 4 had the highest difference in yield due to treatments (Table 5-4). The performance of best check for yield when protected was high, but this yield was heavily damaged by stem borer (score of 3.8), and also in storage with only 14.4% undamaged grain (Table 5-4).

Table 5-4: Mean performance and ranks of eight sets for grain yield and insect pests resistance traits

Set	Gyp (t/ha)	Gyi (t/ha)	LDS	MWTLS	M-uDgrn
1	4.5(8)	4.2(8)	3.2(7)	16.7(3)	58 (3)
2	5.1(4)	4.8(5)	3.1(5)	18.7(5)	55.0 (8)
3	**5.5(1)**	**5.2(1)**	**2.9(3)**	**18.6(4)**	**57.4 (6)**
4	4.9(6)	4.3(7)	3.2(6)	20.6(7)	57.9 (4)
5	5.2(3)	4.9(3)	3.0(4)	19.0(6)	57.5 (5)
6	4.7(7)	4.4(6)	3.3(8)	22.8(8)	56.6 (7)
7	**5.1(5)**	**4.9(4)**	**2.9(2)**	**14.9(1)**	**58.6 (2)**
8	**5.3(2)**	**5.0(2)**	**2.7(1)**	**15.7(2)**	**59.3 (1)**
Sets Mean	**5.0**	**4.7**	**3.0**	**18.4**	58 (3)
PH3253	**6.6**	**5.1**	**3.8**	**33.3**	**14.4**
Checks	**5.9**	**5.0**	**3.3**	**25.8**	**26.7**
R^2	0.8	0.8	0.49	0.6	0.5
CV	27.9	28.7	29.7	43.3	24.3
MSE	1.4	1.4	0.9	8.0	10.3

‡Data: Gyp/Gyi, grain yield (t/ha), protected /infested; LDS, leaf damage scores; MWTLS, percent weight loss; M-uDgrn, percent undamaged grain under maize weevil infestation. Figures in parentheses are the respective ranks for the trait.

Storage pest resistance trait, weight loss, showed high differences across the eight sets. Sets 1, 7 and 8 had relatively high resistance to maize weevil, and registered the least weight loss of 14.9-16.7%. Resistance to both *C. partellus* and *S. zeamais* was observed in hybrids from set 8, 7, and 3 (Table 5-4).

5.3.3 General combining ability of parents

The general combining ability effects for both the female and female parents used in this study are recorded in Table 5-5.

Female parent 3 had positive and favourable GCA effects for grain yield (0.95 and 0.70) when protected and infested treatments, as well as positive and favourable effects for undamaged kernels (1.66). The same parent 3 had favourable negative effects for leaf damage scores (-0.46) due to *C. partellus* infestation, and, negative and favourable effects for weight loss when infested with *S. zeamais*. Similar results were observed with the same parent when used in set 5, where favourable GCA effects were obtained for grain yield (0.69 and 0.57), -0.28 for leaf damage scores, -3.77 for weight loss in maize weevil infestation, and, 1.94 for undamaged grain under maize weevil infestation (Table 5-5).

Table 5-5: General combining ability of selected traits grain yield, leaf damage scores and weight loss from North Carolina II analysis

Set	Parent	GYP	GYI	LDS	Mwtls	M-uDgrn
1	3	0.95	0.70**	-0.07	-0.46	1.66
5	3	0.69	0.57**	-0.28	-3.77**	1.94
2	22	0.30	0.39**	-0.12	-2.76**	4.14
5	22	-0.33	-0.47	-0.104	-0.3	**0.35**
7	12	1.37	0.85**	0.16	-1.238	-2.41
5	1	-1.46	-1.23	0.27	2.35	-1.15
2	6	-0.25	-0.094	-0.36	2.76	-0.59
6	36	-2.38	-2.4	0.13	1.41	7.6**
4	36	-2.51	-2.29	-0.04	2.2	7.05**

†Data: GYP/I, grain yield protected/infested; LDS, leaf damage scores; Mwtls, weight loss due to maize weevil: Significance: *** means data is significant at p ≤ 0.001, *** data is significant at p ≤ 0.01, * data is significant at p ≤ 0.05.

The male parent 22 exhibited favourable GCA for all the traits in consideration in set 2, however, when used with different females in set 5, the GCA effect for undamaged grains of 0.35 was not favourable. Similar results were observed with parent 36 in sets 4 and 6, where negative GCA effects of -2.38 and -2.51 were observed (Table 5-5).

The highest and positive favourable GCA for grain yield were observed in female parent 12 (1.37 and 0.85) in set 7, while the lowest was in parent 1 (-1.46 and 1.23) for protected and infested respectively. Leaf damage scores highest negative GCA was contributed by parent 6 (-0.36) in set 2, while parent 36 contributed the highest positive GCA effect (7.6 and 7.05) for undamaged kernels in sets 6 and 4 respectively (Table 5-5).

Overall, favourable GCA obtained translated to yield gains of between 37% infested to 45% protected respectively, leaf damage scores (42%), and weight loss (45%) and, undamaged grain (53%) for the female parents. The male parents contributed favourable GCA for grain yield (53% and 55%) protected and infested respectively, 45% for leaf damage scores, 43% weight loss, and, 48% of undamaged kernels (Table 5-6).

Table 5-6: Percentage heterosis for grain yield when protected and infested with *Chilo partellus*

Best five hybrids for grain yield for protected and infested treatments							
Set	**Female**	**Male**	**gyp**	**Set**	**Female**	**Male**	**gyi**
1	**3**	**25**	**40.7**	**4**	**17**	**40**	**37.4**
4	**17**	**40**	**29.2**	6	8	40	31.2
4	**19**	**40**	**28.3**	**1**	**3**	**25**	**28.0**
5	3	34	28.1	1	2	23	27.1
1	2	23	26.5	**4**	**19**	**40**	**25.9**
Best five for leaf damage scores and grain weight loss							
Set	**Female**	**Male**	**LDS**	**Set**	**Female**	**Male**	**Mwtls**
1	1	23	-45.1	4	16	36	-47.7
6	7	36	-48.8	6	7	36	-47.7
4	19	36	-48.8	1	2	24	-50.7
4	20	36	-48.9	4	19	36	-61.1
1	2	24	-49.3	6	6	36	-61.7

‡Data: Gyp/Gyi), LDS, Mwtls; % heterosis of grain yield (protected/infested); LDS, leaf damage scores, and Mwtls, grain weight loss.

5.3.4 Hybrids resistance to insect pests

The mean leaf damage score across eight sets was 3.0. Four sets; 3, 5, 7, and 8 were resistant to stem borer, *C. partellus*, with leaf damage scores of 2.7 (set 8) to 3.0 (set 5). Lowest scores were registered in sets 1, 4, and 6 with means scores of 3.2 and 3.3.

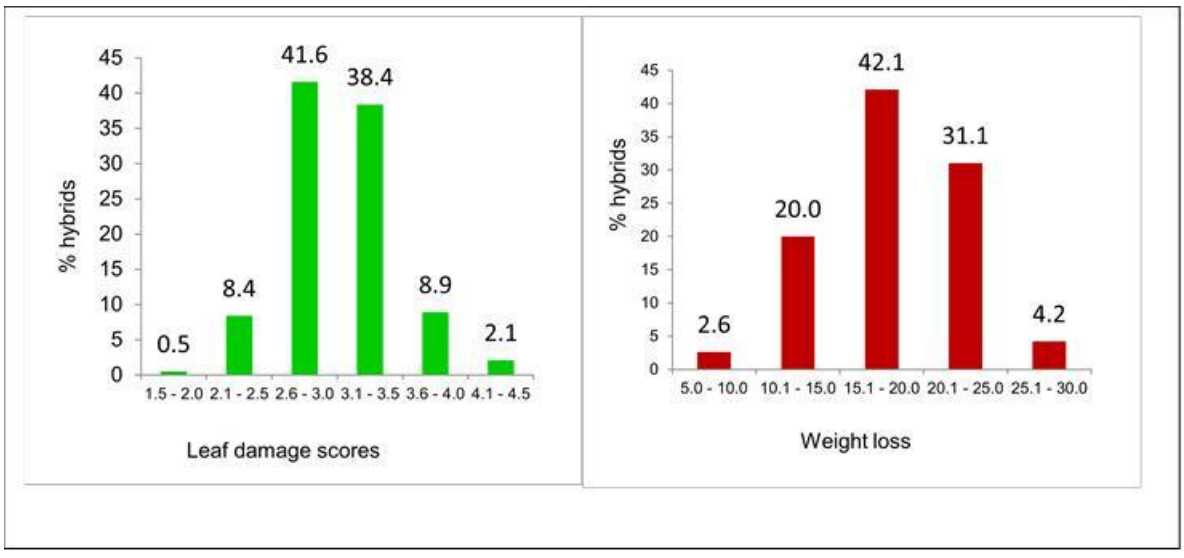

Figure 5-1: Distribution of leaf damage scores due to stem borers and weight loss due to maize weevil infestation

In terms of individual hybrids, calculated 8.9% were highly resistant to stem borer with a leaf damage score of 1.5-2.5. There were 42% of the test hybrids with a score of 3.0, with the remaining 49% being susceptible with leaf damage scores greater 3. The minimum weight loss was 7.3%, while the maximum was 28.4%, with less than 5% of hybrids registering minimum weight loss and, only 4% of hybrids had more than 25% weight loss (Figure 5-1).

5.3.5 Performance of hybrids across different ecologies

The hybrids performed differently across the environments. Environment 1 had the highest grain yield (6.7 t/ha and 7.0 t/ha) for protected and infested treatments. Conversely, environment 3 had the lowest grain yield (3.6 t/ha and 3.1 t/ha).

In terms of insect pests' resistance, environment 2 had the lowest leaf damage scores of 2.0, and environment 4 had the highest scores of 3.5. More exit holes were observed in environment 1, followed by 3. Environment 2 had the least number of exit holes. The percentage of undamaged kernels ranged from 54% (ENV 4) to 61% (ENV 3) (Table 5-7).

Similarly, inbred line male parents 34 and 40, had four out of ten best hybrids in grain yield even when infested, while the female parent inbred line 12 contributed genes for grain yield in two of the top ten hybrids in vigour and value for breeding.

Table 5-7: Mean performance of hybrids in four environments for grain yield and insect pests resistance traits

ENV	Gyp (t/ha)	Gyi (t/ha)	ExH	LDS	MuDKN	Mwtls
1	6.7 A	7.0 A	3.4 D	2.7 B	58.7 B	17.4 B
2	4.0 C	4.1 C	0.2 A	2.0 A	55.4 C	25.5 D
3	3.6 D	3.1 C	2.1 B	2.4 C	61.8 A	11.8 A
4	5.8 B	4.8 B	0.7 C	3.5 D	54.1 D	19.1 C
Mse	1.9	1.8	1.8	0.5	106.5	64.0
LSD	1.12	0.16	0.16	0.58	8.3	0.93

†Data: ENV, environment; Gyp/Gyi, grain yield (t/ha) protected/infested; ExH, exit holes (#); LDS, leaf damage scores (1=clean, 9=dead hearts); MuDKN, undamaged grain under maize weevil infestation; Mwtls, weight loss under maize weevil infestation.
†Means with the same letter are not significantly different.

5.3.6 Heterosis and the value for breeding for insect resistance

There was observed heterosis for grain yield, leaf damage scores, weevil weight loss, and, undamaged grains in the test hybrids (Table 5-7). Heterosis for grain yield when protected

ranged from 26-41% for the best five hybrids, when protected, and 26-37% when infested. The worst affected five hybrids displayed -45% to -61% reductions in grain yield.

Heterosis for leaf damage scores for the best five hybrids was -22% to -17, while weight loss, registered -45 to -32%. Conversely, the worst five hybrids had a reduction of 15-22% increase in leaf damage scores, while weevil weight loss had an increase of 25-33% (Table 5-7).

The best performing hybrid, single cross 17x40, had percent heterosis of 29-37% when both protected and infested. The same hybrid had grain yields of 154% higher above the population mean. Other hybrids that had high heterosis and reasonable value for breeding are single cross 19x40, and single cross 3x25. Conversely there are some hybrids which performed badly with the worst single cross 6x6, yielding 24% grain yield below the population mean (Table 5.8).

Table 5-8: Grain yield advantage of best ten hybrids over and above the mean of population

			Best ten hybrids for grain yield under insect infestation				
SET	**Female**	**Male**	**GYP**	**GYI**	**LDS**	**MWTLS**	**M-Udgrn**
4	**17**	**40**	**153.1**	**154.6**	113.8	123.3	112.3
6	8	40	136.9	152	106.5	137.5	94.3
3	12	34	147.2	145.8	100.8	106.2	92.6
5	2	32	151	143.6	112.6	104.9	93
3	12	32	150	143.3	113.2	134.5	96
6	7	40	135.2	142	123.1	120.3	94.6
4	**19**	**40**	**157.3**	**141.3**	113.2	121.6	101.3
5	4	34	107.4	140.3	94.7	124.8	103.3
3	14	34	135.1	137.4	87.1	124.7	97.2
3	15	34	133.3	135.8	96	101.1	83.1
			Worst five hybrids for gran yield under insect infestation				
6	7	36	37.4	37.9	127.2	130.2	114.7
1	2	24	37.9	36.7	113.5	77.4	92.5
4	16	36	50.8	34.9	73	105.9	137.8
4	19	36	39.9	27.5	91.5	105.9	111
6	**6**	**36**	**53.5**	**24.4**	119.6	145.5	127.8

†Data: Gyp/Gyi, grain yield (t/ha) protected/infested; LDS, leaf damage scores (1=clean, 9=dead hearts); Mwtls, grain loss due to maize weevil infestation; MuDKN, undamaged grain under maize weevil infestation; Mwtls, weight loss under maize weevil infestation.

5.4 Discussion

5.4.1 Gene Action

The presence of significant GCA and SCA for grain yield, leaf damage scores and undamaged kernels indicate that both additive and non-additive gene action are important for conditioning combined resistance to *C. partellus* and *S. zeamais* in maize hybrids.

These findings can be used when making selections for breeding for insect resistance through recurrent selection and pedigree methods. The presence of non-additive gene action places emphasis on the need to consider dominance effects. Similar findings were reported by Butrón et al. (2009), while investigating genetic resistance to pink stem borer in maize.

This research work further observed that up to 69% of gross sum of squares for undamaged kernels under maize weevil infestation were realized; an indication that it is possible to combine insect pest resistance through proper selection of resistant parents with the respective resistance levels. It was recorded that the female and male main effects for grain yield were significant and almost equivalent (55% and 58%). It can be concluded that there was a balanced contribution of both the male and female parent for insect resistance. This makes it complicated to deduce that additive gene action was more predominant over non-additive as earlier studies suggested (Menkir and Ayodele, 2005). The results agree also with work done by Derera (2005) when breeding for yield potential, stress tolerance and yield stability in maize. Similar findings were reported by Dari et al. (2010), when investigating resistance of early generation maize inbred lines and their hybrids, that both additive and non-additive gene actions were responsible for weevil resistance.

A slight drop in male main effects GCA for leaf damage score (37%) may be attributed to the fact that the male parents were selected from the storage insect pest resistance population with predetermined levels of resistance to both *C. partellus* and *S. zeamais* insect pests.

The significance of sets in this study showed the role each combination had in resistance breeding. Sets 3, 7 and 8; which showed good combination of resistance genes as well as grain yield both protected and infested; had parents with contrast; susceptible to *C. partellus*, but resistant to *S. zeamais*. These parents may have had combination of complementary genes for resistance as well as grain yield.

Similarly, Matewele (2014) found that parents obtained for resistance to maize weevil and larger grain borer produced F1 which had varying levels of resistance to one of either pest, and, some with resistance to both pests. This can be further explored through molecular work to identify the right parents with these genes for future breeding. Conclusions can be made that both additive and dominant gene action have a major role to play in insect resistance breeding. Breeding for insect resistance and yield can be achieved in the same hybrid, with the right parents, the correct selection procedure, and, the right model.

5.4.2 Mean performance of eight sets

Set 1 F1 hybrids produced the lowest grain yield when under protected and infested treatment. The female parents from SBR had *C. partellus* resistance but *S. zeamais* Susceptible, while the male parents from SPR population had *C. partellus* susceptible but *S. zeamais* resistance. The challenge of high resistance to the insect pests but low yields observed in set 1 could mean that there is presence of tightly linked genes for insect resistance with undesired low yields.

Parental combination for set 7 gave the most resistant to both *C. partellus* and *S. zeamais* F1 hybrids. Ironically, the parents, though drawn from different populations; females from SBR and males from SPR; had similar attributes in terms of resistance, with both being susceptible to *C. partellus*, but resistant to *S. zeamais*. The F1s from this set showed higher yields and combined resistance to the two pests. This can be explained as having some linkage disequilibrium for *C. partellus* resistance, which breaks when crossed, and the resistance expressed in the F1 progeny. This also explains the importance of screening maize germplasm for resistance to other biotic stresses even when they have not been developed for resistance to the particular stress. The SPR population was screened for *C. partellus* and the SBR population screened for *S. zeamais* resistance. This is useful for sourcing for resistance genes to insect pests. The findings agree with work done by Matewele (2014) and Mwololo et al. (2012) that there are available sources of maize weevil resistance. These resistance sources can be explored for combined resistance. Other researchers; (Butrón et al., 2004; Tefera et al., 2011b; Beyene et al., 2011b); reported resistance to each of the pests in hybrids without compromising yield.

5.4.3 General combining ability

Female parent 3, showed favourable GCA effects for grain yield, undamaged kernels, leaf damage scores, and, weight loss, in sets 1 and 5. Similarly, male parent 22 had favourable

GCA effects for set 2. This suggests that these parents have good alleles for insect pests' resistance and can be used for breeding for combined resistance to these two pests. Male parents 22 and 38 however shared favourable GCA for all traits except for undamaged kernels. The results can be deduced to mean that a good donor of favourable alleles for undamaged kernels can be used in combination with these parents for breeding.

The high GCA for grain yield observed in female parent 12 can be exploited for yield in combination with other lines that can contribute resistance genes in hybrids. Similarly, parents 6, 31, 33, 36, and 38 displayed favourable GCA for other traits; leaf damage scores, weight loss and undamaged kernels, suggesting that they can be exploited as good sources of resistance alleles in breeding. Other lines had combination of favourable alleles for some traits and unfavourable for others; which can be exploited with the right combination in hybrids.

5.4.4 Hybrids resistance to insect pests

Variation among test hybrids existed. This is evidenced by the observed differences in genotypes to infestation by *C. partellus* and *S. zeamais*, an indication that the hybrids were diverse in terms of response to infestation. These differences were registered as levels of leaf damage scores, percentage of weight loss and percentage of undamaged grain after infestation. Use of such parameters as measures of resistance has been documented previously when screening maize germplasm for resistance to insect pests (Beyene et al., 2011b; Mwololo et al., 2012; Derera et al., 2014; Matewele, 2014). Leaf damage scores have been tested and validated as a measure of resistance and susceptibility to stem borers in Kenya (Tefera et al., 2011a).

Tefera et al. (2011b) reported that resistant maize varieties had less leaf damage score as compared to susceptible varieties. Using this parameter as a determinant of *C. partellus* resistance, the test hybrids in this study possessed reasonable resistance as indicated in the results where 8.9% were highly resistant, 41.6% resistant, 38.4% moderately resistant, and, 11% susceptible. The resistance distribution in the population followed the normal distribution curve; an indication that the genetic variation existed among the test hybrids (Kim and Kossou, 2003).

Similarly, weight loss and grain damage have been used in previous studies involving breeding for post-harvest resistance in maize. The varieties with resistance to *S. zeamais* had low weight loss compared to susceptible maize varieties (Abebe et al., 2009; Derera et

al., 2010; Mwololo et al., 2012). Grouping of the test hybrids into resistance groups also revealed that maize weevil resistance was normally distributed.

The parameters have, therefore, been found to discriminate and separate genotypes into resistant and susceptible groupings based on levels of damage and weight loss. A more recent study by Matewele (2014), showed that percentage of grain weight loss was a conservative indicator of resistance in maize varieties. It is this indicator that is preferred for the current study so that it can discretely discriminate hybrids that are truly resistant to *S. zeamais* from those that are susceptible.

In the current study, combination of high yields when infested with low leaf damage scores, low weight loss due to weevil infestation and high percentage of undamaged grains suggests that the hybrids in sets 3, 7 and 8, had combined resistance to *C. partellus* and *S. zeamais* insect pests. The best check performance when protected and the drop in yield when infested emphasises the importance of incorporating insect resistance in breeding programs. Further, at storage the minimum undamaged grain percentage is an indication that yield loss at storage is of great economic importance.

The findings of this study show also that breeders can select for combined resistance from within their breeding program and enhance the resistance of insects in the existing germplasm. It can therefore be concluded that favourable alleles of combined resistance to *C. partellus* and *S. zeamais* can be fixed through selection.

5.4.5 Performance of hybrids across different ecologies

The hybrids' differential performance across the environments for grain yield and insect pest resistance parameters was observed. This is evidenced by analysis of variance which revealed significant differences through Set*ENV, Female*ENV(set), Male*ENV(set), Female*Male*ENV(set). Lack of significance for leaf damage scores and undamaged grain for Female*Male*ENV(set), can be attributed to the consistent and uniform artificial infestation both in the field as well as in controlled laboratory screening. This is important for this study because it shows that any differences in performance in terms of leaf damage scores and undamaged grain were due to genotypic variation rather than environmental influence. However, in terms of grain yield and weight loss, there was observed significant differences in the test hybrids.

Consistency in ranking grain yield both infested and protected, where environment 1 had the highest grain yield and environment 3 had the lowest grain yield could be an indication that

these hybrids are more suited to environment 1. Although environment 2 had the lowest leaf damage scores, and environment 4 had the highest scores, analysis of variance revealed no significance differences for LDS and undamaged kernels. This suggests that resistance parameters can be considered for the whole population rather than per environment basis for this study. Further, field infestation can be done at selected sites to save on costs, and use the other sites to evaluate for genotype stability. This agrees with studies by (Derera, 2005), when working on breeding for resistance to grey leaf spot, where he reported that maize screening for biotic stress can be done in an established nursery with high disease pressure to save on resources.

In other studies, Matewele (2014) reported that correlation between grain yield and resistance to maize weevil was not significant. Hence resistance to maize weevil can be advanced without affecting grain yield. The correlations between yield and resistance parameters for both maize weevil and larger grain borer were not significant. This means that selection for resistance can be done without significantly affecting yield.

5.4.6 Heterosis and value for breeding

High percentage values for increased vigour in test hybrids are an indication of progress in insect pest resistance. The high reduction in leaf damage indicates good resistance genes combination. It is not without a challenge though, because some of the test hybrids demonstrated increase in leaf damage scores as well as reduced grain yield. This is an indication of the variation existing in the test hybrids from which breeders can select what is desirable and discard what is not favourable. This confirms Mwololo et al. (2012) findings that there are good sources of resistance to both maize weevil and stem borers in Kenya. Using these sources, some hybrids, for example, single cross 17x40 and 19x40 had good heterosis and performed well above population mean. It can be deduced that inbred line 40 had favourable genes for grain yield, even when infested. The hybrids can be said to be tolerant because despite the moderate levels of resistance observed, they yielded well. These hybrids can be recommended for release in mid-altitude areas.

5.5 Conclusion

The results obtained demonstrated that there is genetic variability for insect resistance and yield in the test hybrids. Furthermore, the study demonstrated that resistance to *C. partellus* and *S. zeamais* can be combined in a single maize hybrid. The findings of this research therefore lead to the following conclusions:

1. Combined *C. partellus* and *S. zeamais* resistance can be achieved with proper selection of parents which combine well in hybrid combination.
2. The results of field evaluation demonstrated that resistance alleles for *C. partellus* and *S. zeamais* followed the Mendelian law of independent assortment. This is because some the hybrids were susceptible to the insect pest although the parents had known levels of resistance to the pest.
3. Hybrids from sets 3, 7 and 8 demonstrated combined resistance to *C. partellus* and *S. zeamais* with high grain yield both when infested and protected, and good levels of resistance to the two pest. This shows that combined resistance can be achieved.
4. Significant gain in breeding was achieved for selected hybrids for increased grain yield and reduced leaf damage scores as evidenced in high heterosis and percentage gain above mean of population.
5. Both additive and dominant gene action played a major role in conferring *C. partellus* and *S. zeamais* resistance in the current study, and therefore breeding for insect resistance and yield can be achieved in the same hybrid.

Considering the outcome of the results, the following recommendations can be made:

1. Presence of tolerance type of resistance in some hybrids is an indication that there is need to incorporate molecular marker for identifying the correct germplasm for insect resistance development.
2. Use the SPR inbred lines as the females and the SBR inbred lines as the males and screen for insect resistance to determine whether there could be variation in resistance levels and maternal effects.

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Chapter 6: Research Overview

Introduction

The current study focused on “combining stem borer and storage insect pest resistance in early maturing maize germplasm”. This was accomplished through various chapters, of which each was addressing an objective. Summary of the main objectives, the findings, and breeding implications, and, recommendations are outline in this overview.

Four research questions needed to be answered by the findings of this research, which are:

a) Insect resistance is a polygenic trait, which is highly influenced by the environment. Is there wide enough genetic base on the selected maize germplasm for resistance breeding in early maturing maize?
b) Can resistance to the field pests stem borer, *Chilo partellus*, and storage pests maize weevil *Sitophilus zeamais*, and *Prostephanus truncatus*, be combined in the same hybrids through direct hybrids development using inbred lines parents which have separate resistances to each pest without compromising grain yield?
c) Can combined resistance to *Chilo partellus* and *Sitophilus zeamais*, be achieved when the parent inbred lines have contrast and varying “dosage” or levels of resistance to each of these pests?
d) What gene action favours combined stem borer and storage pests’ resistance?

6.1 Findings of the study and their implications

6.1.1 Genetic diversity studies using 30 SSR markers for S_4 stem borer and storage insect pest resistant populations

Genetic diversity analysis revealed that the selected S_4 maize germplasm for stem borers and storage pests were diverse and had a wide genetic base. A total of 3859 data points were achieved out of the expected 3900 data points giving an overall success rate of 98.95%, from the analyzed marker data.

The shortest product size in both SBR and SPR population was observed in locus umc2250 with 47 base pairs (bp), while the longest for SBR was observed in locus phi062 with 362 bp,

and, the longest for SPR was observed in locusPhi227562 with 320 bp. The highest difference in variation from the same locus was 212 bp, observed in phi072.

Diversity analysis revealed a total of 109 and 103 alleles from SBR and SPR populations respectively. The number of polymorphic alleles scored ranged from 2 to 6 per loci, with a mean of 3.63 alleles for SBR and 1 to 6 alleles per loci, with a mean of 3.43 alleles, for SPR. Observed uHe indicated gene diversity of 0.06-0.82 in both SBR and SPR populations, with means of 0.48and 0.45 respectively. Observed mean PIC values were 0.45-0.46, with over 50 of the loci having PIC greater than 0.5.

Cluster analysis revealed three major clusters for each of the population with observed 6 and 8 major sub-clusters for SPR and SBR populations respectively.

6.1.2 Estimating combined insect pest resistance for *Chilo partellus* and *Sitophilus zeamais* in maize hybrids

Diallel analysis of combined SBR and SPR inbred lines revealed that it is possible to combine the two types of insect pests' resistance in hybrids. Grain yields for stem borer resistant hybrids were comparable, or even better than the checks. However, the hybrids which exhibited storage pest resistance were not necessarily high yielding. Some hybrids however had combined resistance to the two pests without compromising yield. This was evidenced by some select entries which were high yielding both when protected and also infested, a good example is the single cross, SC 4X9, entry 35. This entry gave high yields both when protected (8.4t/ha) and when infested (8.3 t/ha). It had a leaf damage score of 2.2, cumulative tunnel length of 4.1, exit holes count of 1, and maize weevil - larger grain borer weight loss, of 15 - 49.1%.

Inbred line parents with favourable alleles for grain yield, resistance were identified. These are 12, 11, 4, 6, and 1, which had positive and favourable effects (1.47, 0.72, 0.95, 0.38, and 0.19) for grain yield respectively, when protected, with parents 4, 11, and 12 being highly significant ($p \leq 0.001$) and also when infested. Other inbred line parents; 1,2,3,4 and 5; were identified with favourable alleles for stem borer resistance, while inbred lines parents 8 and 9 gave favourable allele contributions for storage pests' resistance.

The SBRxSPR hybrids combination showed higher grain yield above mean of checks had modest leaf damage scores as well as modest grain damage. Heterosis was also observed since this combination yielded better than the SPRXSPR, SBRXSR, and internal checks.

Significant general combining ability and specific combining ability for grain yield (protected and infested), exit holes and leaf damage scores, as well as, significant effects, for the post-harvest traits of undamaged kernel, live insects, and weight loss, were observed on the test genotypes. This was suggestive that there were both additive and non-additive genes effects conditioning these traits. While other researcher had placed a lot of emphasis on additive effects, in this study, non-additive effects are reported to have played a major role in insect pests' resistance as well. The findings were in agreement with reported studies by other scientist working on insect pests host plant resistance (Kumar, 1997; Dhliwayo et al., 2005; Dari et al., 2010; Derera et al., 2014).

6.1.3 Genetic analysis of combined stem borer and storage insect pest resistance in maize hybrids

This study involved 20 parental inbred lines organized into groups of five parents each and eight sets to investigate whether resistance to *Chilo partellus* and *Sitophilus zeamais* can be achieved when the parents have contrast resistance to each pest and at different levels.

The results revealed that there were differences in performance of the sets depending with traits in consideration. Set three (3), gave more favorable grain yield and resistance to both stem borers and storage pests respectively, with the highest grain yield of 5.5t/ha, and, 5.2t/ha, both when protected and infested respectively. Grain yield mean performance of the eight sets was 5.0t/ha, when protected, and 4.7t/ha when infested.

There was observed heterosis for grain yield; when protected ranged from 26-41% for the best five hybrids, and 26-37% when infested. Also heterosis for leaf damage scores, for the best five hybrids was -22% to -17%. Post-harvest resistance traits, weevil weight loss, registered -45% to -32%. The best performing hybrid, single cross 17x40, had percent heterosis of 29-37% both when protected and infested. The same hybrid had grain yields of 154% higher above the population mean. Other hybrids that had high heterosis and reasonable value for breeding are single cross 19x40, and single cross 3x25. The presence of significant GCA and SCA for grain yield, leaf damage scores and undamaged kernels indicate that both additive and non-additive gene action are important for conditioning combined resistance to *C. partellus* and *S. zeamais* in maize hybrids. The presence of non-additive gene action places emphasis on the need to consider dominance effects brought about by presence of either epistasis, pleiotropic genes, or, genotype by environment interactions. The validity of these results is confirmed by other researchers who observed similar findings (Butrón et al., 2009), while investigating genetic resistance to pink stem borer in maize.

The findings of this study also show that breeders can select for combined resistance from within their breeding program and enhance the resistance of insects in the existing germplasm. It can therefore be concluded that favourable alleles of combined resistance to *C. partellus* and *S. zeamais* can be fixed through selection.

6.2 General conclusions and recommendations

The findings of this research have demonstrated that:

1. There was gene diversity in the identified maize germplasm for use in insect resistance breeding program jointly developed by Kenya Agricultural and Livestock Research Organization (KALRO), and the International Maize and Wheat Improvement Center (CIMMYT).
2. It is possible to combine stem borer and storage insect pest resistance into hybrids. However, there is need to identify sources of resistance to storage pests which have favourable alleles for high grain yield.
3. Some of the germplasm with susceptible alleles for one pest can contribute favourable alleles for resistance to another pest in hybrid combination.
4. The findings of this research are important because they will act as baseline studies for future research when breeding for combined insect pest resistance in maize.

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Appendix

Appendix 3.1: List of stem borer resistant S_4 lines and their respective pedigrees and origins

Stem Borer Resistant population							
Entry	Stock ID	Pedigree	Origin	Entry	Stock ID	Pedigree	Origin
1	SM-405-1	(CKSBL10001/KCB)-1-B-1	IR-KIB-11B-12-1-1	34	SM-405-400	(CKSBL10001/KCB)-41-B-1	IR-KIB-11B-12-41-1
2	SM-405-13	(CKSBL10001/KCB)-2-B-1	IR-KIB-11B-12-2-1	35	SM-405-411	(CKSBL10001/KCB)-42-B-1	IR-KIB-11B-12-42-1
3	SM-405-22	(CKSBL10001/KCB)-3-B-1	IR-KIB-11B-12-3-1	36	SM-405-421	(CKSBL10001/KCB)-43-B-1	IR-KIB-11B-12-43-1
4	SM-405-27	(CKSBL10001/KCB)-4-B-1	IR-KIB-11B-12-4-1	37	SM-405-437	(CKSBL10001/KCB)-45-B-4	IR-KIB-11B-12-45-4
5	SM-405-36	(CKSBL10001/KCB)-5-B-2	IR-KIB-11B-12-5-2	38	SM-405-443	(CKSBL10001/KCB)-46-B-1	IR-KIB-11B-12-46-1
6	SM-405-56	(CKSBL10001/KCB)-12-B-1	IR-KIB-11B-12-12-1	39	SM-405-457	(CKSBL10001/KCB)-48-B-3	IR-KIB-11B-12-48-3
7	SM-405-63	(CKSBL10001/KCB)-13-B-1	IR-KIB-11B-12-13-1	40	SM-405-469	(CKSBL10001/KCB)-49-B-1	IR-KIB-11B-12-49-1
8	SM-405-80	(CKSBL10001/KCB)-15-B-1	IR-KIB-11B-12-15-1	41	SM-405-478	(CKSBL10001/KCB)-50-B-1	IR-KIB-11B-12-50-1
9	SM-405-93	(CKSBL10001/KCB)-16-B-1	IR-KIB-11B-12-16-1	42	SM-405-489	(CKSBL10001/KCB)-51-B-1	IR-KIB-11B-12-51-1
10	SM-405-111	(CKSBL10001/KCB)-17-B-1	IR-KIB-11B-12-17-1	43	SM-405-501	(CKSBL10001/KCB)-52-B-1	IR-KIB-11B-12-52-1
11	SM-405-127	(CKSBL10001/KCB)-18-B-1	IR-KIB-11B-12-18-1	44	SM-405-518	(CKSBL10002/KCB)-1-B-1	IR-KIB-11B-12-53-1
12	SM-405-136	(CKSBL10001/KCB)-19-B-1	IR-KIB-11B-12-19-1	45	SM-405-532	(CKSBL10002/KCB)-2-B-1	IR-KIB-11B-12-54-1
13	SM-405-149	(CKSBL10001/KCB)-20-B-1	IR-KIB-11B-12-20-1	46	SM-405-547	(CKSBL10002/KCB)-3-B-1	IR-KIB-11B-12-55-1
14	SM-405-162	(CKSBL10001/KCB)-21-B-1	IR-KIB-11B-12-21-1	47	SM-405-552	(CKSBL10002/KCB)-4-B-1	IR-KIB-11B-12-56-1
15	SM-405-170	(CKSBL10001/KCB)-22-B-1	IR-KIB-11B-12-22-1	48	SM-405-558	(CKSBL10002/KCB)-5-B-1	IR-KIB-11B-12-57-1
16	SM-405-181	(CKSBL10001/KCB)-23-B-1	IR-KIB-11B-12-23-1	49	SM-405-563	(CKSBL10002/KCB)-6-B-1	IR-KIB-11B-12-58-1
17	SM-405-189	(CKSBL10001/KCB)-24-B-1	IR-KIB-11B-12-24-1	50	SM-405-564	(CKSBL10002/KCB)-7-B-1	IR-KIB-11B-12-59-1
18	SM-405-202	(CKSBL10001/KCB)-25-B-1	IR-KIB-11B-12-25-1	51	SM-405-568	(CKSBL10002/KCB)-8-B-1	IR-KIB-11B-12-60-1
19	SM-405-209	(CKSBL10001/KCB)-26-B-1	IR-KIB-11B-12-26-1	52	SM-405-577	(CKSBL10002/KCB)-9-B-1	IR-KIB-11B-12-61-1
20	SM-405-219	(CKSBL10001/KCB)-27-B-1	IR-KIB-11B-12-27-1	53	SM-405-592	(CKSBL10002/KCB)-10-B-1	IR-KIB-11B-12-62-1
21	SM-405-227	(CKSBL10001/KCB)-28-B-1	IR-KIB-11B-12-28-1	54	SM-405-598	(CKSBL10002/KCB)-11-B-1	IR-KIB-11B-12-63-1
22	SM-405-245	(CKSBL10001/KCB)-29-B-1	IR-KIB-11B-12-29-1	55	SM-405-608	(CKSBL10002/KCB)-12-B-1	IR-KIB-11B-12-64-1
23	SM-405-262	(CKSBL10001/KCB)-30-B-1	IR-KIB-11B-12-30-1	56	SM-405-614	(CKSBL10002/KCB)-13-B-1	IR-KIB-11B-12-65-1
24	SM-405-269	(CKSBL10001/KCB)-31-B-1	IR-KIB-11B-12-31-1	57	SM-405-618	(CKSBL10002/KCB)-14-B-1	IR-KIB-11B-12-66-1
25	SM-405-275	(CKSBL10001/KCB)-32-B-1	IR-KIB-11B-12-32-1	58	SM-405-621	(CKSBL10002/KCB)-16-B-1	IR-KIB-11B-12-68-1
26	SM-405-288	(CKSBL10001/KCB)-33-B-1	IR-KIB-11B-12-33-1	59	SM-405-623	(CKSBL10002/KCB)-17-B-1	IR-KIB-11B-12-69-1
27	SM-405-305	(CKSBL10001/KCB)-34-B-1	IR-KIB-11B-12-34-1	60	SM-405-629	(CKSBL10003/KCB)-1-B-1	IR-KIB-11B-12-70-1
28	SM-405-323	(CKSBL10001/KCB)-35-B-1	IR-KIB-11B-12-35-1	61	SM-405-641	(CKSBL10003/KCB)-2-B-1	IR-KIB-11B-12-71-1
29	SM-405-334	(CKSBL10001/KCB)-36-B-3	IR-KIB-11B-12-36-3	62	SM-405-649	(CKSBL10003/KCB)-3-B-1	IR-KIB-11B-12-72-1
30	SM-405-341	(CKSBL10001/KCB)-37-B-1	IR-KIB-11B-12-37-1	63	SM-405-655	(CKSBL10003/KCB)-4-B-1	IR-KIB-11B-12-73-1
31	SM-405-351	(CKSBL10001/KCB)-38-B-1	IR-KIB-11B-12-38-1	64	SM-405-663	(CKSBL10003/KCB)-5-B-1	IR-KIB-11B-12-74-1
32	SM-405-367	(CKSBL10001/KCB)-39-B-1	IR-KIB-11B-12-39-1	65	SM-405-671	(CKSBL10003/KCB)-6-B-1	IR-KIB-11B-12-75-1
33	SM-405-386	(CKSBL10001/KCB)-40-B-1	IR-KIB-11B-12-40-1				

Appendix 3.2: List of germplasm constituting storage insect pest resistant S_4 and their respective origin and pedigrees

Storage pests resistant population							
Entry	Stock ID	Pedigree	Origin	Entry	Stock ID	Pedigree	Origin
1	SM-406-3	(CKSPL10001/KCB)-1-B-3	IR-KIB-11B-13-1-3	34	SM-406-636	(CKSPL10005/KCB)-9-B-3	IR-KIB-11B-13-70-3
2	SM-406-12	(CKSPL10001/KCB)-2-B-1	IR-KIB-11B-13-2-1	35	SM-406-650	(CKSPL10005/KCB)-10-B-1	IR-KIB-11B-13-71-1
3	SM-406-1215	(CKSPL10006/KCB)-9-B-2	IR-KIB-11B-13-120-2	36	SM-406-667	(CKSPL10005/KCB)-11-B-1	IR-KIB-11B-13-72-1
4	SM-406-41	(CKSPL10001/KCB)-6-B-1	IR-KIB-11B-13-5-1	37	SM-406-680	(CKSPL10005/KCB)-12-B-1	IR-KIB-11B-13-73-1
5	SM-406-56	(CKSPL10001/KCB)-8-B-1	IR-KIB-11B-13-6-1	38	SM-406-691	(CKSPL10005/KCB)-13-B-3	IR-KIB-11B-13-74-3
6	SM-406-87	(CKSPL10001/KCB)-12-B-1	IR-KIB-11B-13-9-1	39	SM-406-707	(CKSPL10005/KCB)-14-B-1	IR-KIB-11B-13-75-1
7	SM-406-109	(CKSPL10001/KCB)-19-B-1	IR-KIB-11B-13-11-1	40	SM-406-728	(CKSPL10005/KCB)-16-B-2	IR-KIB-11B-13-77-2
8	SM-406-128	(CKSPL10001/KCB)-21-B-1	IR-KIB-11B-13-13-1	41	SM-406-752	(CKSPL10005/KCB)-19-B-2	IR-KIB-11B-13-79-2
9	SM-406-155	(CKSPL10001/KCB)-23-B-1	IR-KIB-11B-13-15-1	42	SM-406-766	(CKSPL10005/KCB)-20-B-1	IR-KIB-11B-13-80-1
10	SM-406-166	(CKSPL10001/KCB)-24-B-1	IR-KIB-11B-13-16-1	43	SM-406-784	(CKSPL10005/KCB)-22-B-1	IR-KIB-11B-13-82-1
11	SM-406-180	(CKSPL10001/KCB)-25-B-1	IR-KIB-11B-13-17-1	44	SM-406-799	(CKSPL10005/KCB)-23-B-1	IR-KIB-11B-13-83-1
12	SM-406-195	(CKSPL10001/KCB)-26-B-3	IR-KIB-11B-13-18-3	45	SM-406-827	(CKSPL10005/KCB)-26-B-2	IR-KIB-11B-13-86-2
13	SM-406-203	(CKSPL10001/KCB)-27-B-1	IR-KIB-11B-13-19-1	46	SM-406-845	(CKSPL10005/KCB)-28-B-1	IR-KIB-11B-13-88-1
14	SM-406-223	(CKSPL10001/KCB)-29-B-1	IR-KIB-11B-13-21-1	47	SM-406-877	(CKSPL10005/KCB)-31-B-1	IR-KIB-11B-13-91-1
15	SM-406-229	(CKSPL10001/KCB)-30-B-1	IR-KIB-11B-13-22-1	48	SM-406-910	(CKSPL10005/KCB)-33-B-8	IR-KIB-11B-13-93-8
16	SM-406-238	(CKSPL10001/KCB)-31-B-1	IR-KIB-11B-13-23-1	49	SM-406-931	(CKSPL10005/KCB)-35-B-2	IR-KIB-11B-13-95-2
17	SM-406-249	(CKSPL10002/KCB)-1-B-1	IR-KIB-11B-13-24-1	50	SM-406-949	(CKSPL10005/KCB)-37-B-1	IR-KIB-11B-13-97-1
18	SM-406-302	(CKSPL10004/KCB)-1-B-1	IR-KIB-11B-13-29-1	51	SM-406-990	(CKSPL10005/KCB)-41-B-5	IR-KIB-11B-13-101-5
19	SM-406-360	(CKSPL10004/KCB)-9-B-1	IR-KIB-11B-13-37-1	52	SM-406-1004	(CKSPL10005/KCB)-43-B-1	IR-KIB-11B-13-103-1
20	SM-406-374	(CKSPL10004/KCB)-10-B-2	IR-KIB-11B-13-38-2	53	SM-406-1017	(CKSPL10005/KCB)-44-B-1	IR-KIB-11B-13-104-1
21	SM-406-401	(CKSPL10004/KCB)-13-B-1	IR-KIB-11B-13-41-1	54	SM-406-1030	(CKSPL10005/KCB)-45-B-1	IR-KIB-11B-13-105-1
22	SM-406-405	(CKSPL10004/KCB)-14-B-1	IR-KIB-11B-13-42-1	55	SM-406-1048	(CKSPL10005/KCB)-46-B-1	IR-KIB-11B-13-106-1
23	SM-406-411	(CKSPL10004/KCB)-15-B-1	IR-KIB-11B-13-43-1	56	SM-406-1332	(CKSPL10006/KCB)-24-B-1	IR-KIB-11B-13-134-1
24	SM-406-416	(CKSPL10004/KCB)-16-B-1	IR-KIB-11B-13-44-1	57	SM-406-1088	(CKSPL10005/KCB)-50-B-1	IR-KIB-11B-13-110-1
25	SM-406-423	(CKSPL10004/KCB)-18-B-1	IR-KIB-11B-13-46-1	58	SM-406-1100	(CKSPL10005/KCB)-51-B-1	IR-KIB-11B-13-111-1
26	SM-406-442	(CKSPL10004/KCB)-20-B-2	IR-KIB-11B-13-48-2	59	SM-406-1116	(CKSPL10006/KCB)-1-B-1	IR-KIB-11B-13-112-1
27	SM-406-450	(CKSPL10004/KCB)-21-B-1	IR-KIB-11B-13-49-1	60	SM-406-1131	(CKSPL10006/KCB)-2-B-1	IR-KIB-11B-13-113-1
28	SM-406-498	(CKSPL10004/KCB)-26-B-2	IR-KIB-11B-13-54-2	61	SM-406-1389	(CKSPL10007/KCB)-7-B-1	IR-KIB-11B-13-141-1
29	SM-406-1253	(CKSPL10006/KCB)-13-B-1	IR-KIB-11B-13-124-1	62	SM-406-1159	(CKSPL10006/KCB)-4-B-2	IR-KIB-11B-13-115-2
30	SM-406-553	(CKSPL10005/KCB)-1-B-1	IR-KIB-11B-13-62-1	63	SM-406-1177	(CKSPL10006/KCB)-6-B-1	IR-KIB-11B-13-117-1
31	SM-406-563	(CKSPL10005/KCB)-2-B-1	IR-KIB-11B-13-63-1	64	SM-406-1190	(CKSPL10006/KCB)-7-B-1	IR-KIB-11B-13-118-1
32	SM-406-1319	(CKSPL10006/KCB)-21-B-8	IR-KIB-11B-13-131-8	65	SM-406-1205	(CKSPL10006/KCB)-8-B-1	IR-KIB-11B-13-119-1
33	SM-406-582	(CKSPL10005/KCB)-4-B-3	IR-KIB-11B-13-65-3				

Appendix 4.1: Means of traits for hybrids with combined resistance *Chilo partellus* and storage insect pests

Entry	Cross	GYP	GYI	CTL	EXhLS	LLI	LDS	LuDkn	Lwtls	MLI	MuDkn	Mwtls	Oil	Protein	Starch
1	1×2	5.4	5.1	3.7	1.1	80.9	2.3	31.5	50.3	72.4	29.0	22.9	4.5	10.4	70.5
2	1×3	5.6	5.7	3.9	1.0	83.0	2.1	32.9	52.4	72.6	26.3	25.1	4.7	11.0	69.7
3	1×4	7.6	6.8	4.3	1.1	81.6	2.3	28.1	54.0	64.8	34.6	19.2	5.1	10.2	69.7
4	1×5	6.4	6.1	3.5	1.2	85.7	2.4	26.0	53.4	73.0	15.6	26.7	4.7	10.6	69.9
5	1×6	7.0	10.1	4.3	1.4	81.6	2.8	29.6	51.9	67.4	29.1	20.0	4.9	10.7	69.8
6	1×7	7.5	7.0	6.2	2.2	85.5	2.7	25.8	58.9	64.7	33.5	22.5	4.8	11.2	69.5
7	1×8	5.8	5.5	4.4	1.0	81.8	2.3	31.4	45.1	55.1	47.1	15.4	4.8	11.5	69.3
8	1×9	6.3	6.7	4.7	1.4	82.6	2.2	31.2	50.5	57.7	47.1	15.9	4.7	10.6	69.9
9	1×10	8.7	8.8	4.1	1.4	84.1	2.6	25.2	59.7	67.3	22.5	25.1	4.8	9.9	70.1
10	1×11	7.4	7.2	4.1	1.6	86.4	2.7	21.7	65.4	71.8	24.0	24.2	4.9	10.4	69.9
11	1×12	8.8	8.3	5.3	1.8	81.1	2.5	29.3	53.0	70.4	30.0	21.2	4.7	10.3	70.0
12	2×3	6.3	5.7	3.1	1.0	82.7	2.2	31.8	52.7	71.9	30.1	21.2	4.9	10.8	69.8
13	2×4	6.3	7.1	4.9	1.4	83.9	2.4	30.8	51.1	68.0	40.3	17.3	5.1	9.9	69.9
14	2×5	6.3	6.0	4.8	1.2	83.0	2.3	32.7	46.7	70.1	37.6	19.1	4.8	10.4	70.2
15	2×6	6.4	6.7	5.8	1.7	80.2	2.5	35.2	48.3	65.8	35.1	19.5	5.0	10.6	69.9
16	2×7	7.0	6.4	4.4	1.4	82.2	2.5	32.1	51.0	65.1	45.7	15.9	4.8	10.7	69.8
17	2×8	6.1	5.6	6.0	1.4	78.2	2.7	39.5	43.9	56.7	55.7	12.7	5.0	10.9	69.8
18	2×9	7.4	8.1	4.3	1.4	79.6	2.2	36.6	49.1	51.2	64.0	10.5	4.7	10.7	70.0
19	2×10	5.5	5.2	4.2	1.2	79.9	2.6	38.5	44.9	59.6	54.6	14.8	4.9	10.6	70.0
20	2×11	7.2	7.0	6.2	2.2	82.9	2.9	29.8	55.1	70.9	34.4	20.4	5.0	10.2	70.0
21	2×12	8.7	8.1	4.0	1.7	80.5	2.6	31.9	53.8	69.8	44.2	17.0	4.9	10.1	70.1
22	3×4	5.9	5.0	3.8	1.0	87.7	2.2	33.5	50.9	65.5	37.9	18.4	5.3	10.5	69.3
23	3×5	4.9	4.8	5.2	1.6	86.0	2.4	32.6	50.4	68.8	24.2	22.8	4.9	10.8	69.7
24	3×6	6.6	6.5	5.6	1.8	84.0	2.8	35.9	45.8	66.3	37.1	18.7	5.4	11.0	69.0

Apendix 4-1: Continued from page 119

Entry	Cross	GYP	GYI	CTL	EXhLS	LLI	LDS	LuDkn	Lwtls	MLI	MuDkn	Mwtls	Oil	Protein	Starch
25	3×7	6.0	5.6	6.2	1.8	81.0	2.4	37.1	48.1	65.6	40.2	17.6	5.1	11.2	69.0
26	3×8	4.6	4.9	4.5	1.4	72.9	2.4	46.2	36.0	51.5	53.5	12.9	5.5	12.0	68.2
27	3×9	6.5	6.3	3.6	1.1	74.8	2.2	41.8	41.1	62.4	53.0	13.6	5.0	11.4	69.3
28	3×10	6.0	5.7	4.4	1.4	84.2	2.3	40.5	41.1	51.7	59.9	11.6	5.2	11.8	68.8
29	3×11	7.1	6.1	3.8	1.3	81.9	2.4	29.7	52.4	67.8	27.9	21.9	5.0	10.7	69.7
30	3×12	7.9	7.2	4.7	1.3	82.6	2.6	30.2	53.2	65.1	38.5	19.1	5.0	10.6	69.7
31	4×5	6.6	6.4	4.3	1.3	82.2	2.7	34.0	50.2	68.2	34.8	18.8	5.3	10.3	69.5
32	4×6	8.0	7.9	5.1	1.2	83.9	2.6	33.5	50.4	60.2	42.0	16.0	5.4	10.3	69.4
33	4×7	8.6	8.1	6.3	2.1	84.6	2.5	31.1	51.7	60.2	44.8	16.2	5.2	10.4	69.3
34	4×8	6.8	6.2	4.6	1.2	78.5	2.4	37.6	44.8	48.9	54.1	11.7	5.3	11.2	68.9
35	4×9	8.4	8.3	4.1	1.0	80.9	2.2	36.0	47.0	57.6	50.7	15.0	5.3	10.8	69.3
36	4×10	7.1	7.6	4.4	1.4	83.3	2.6	33.5	51.4	59.6	50.8	13.5	5.4	10.4	69.2
37	4×11	8.3	8.6	5.6	1.5	82.8	2.6	25.4	58.9	61.3	37.6	19.0	5.0	10.1	69.9
38	4×12	10.5	9.2	4.3	1.3	82.6	2.6	30.2	54.0	69.3	34.5	18.4	5.3	9.9	69.3
39	5×6	7.8	6.9	6.3	1.7	83.1	2.9	32.0	51.7	64.9	39.8	16.5	5.2	10.5	69.5
40	5×7	6.7	5.9	5.3	1.4	79.2	2.6	34.5	50.0	59.3	44.1	17.5	5.0	11.0	69.2
41	5×8	5.9	8.2	5.5	1.5	74.9	2.4	36.9	43.6	53.4	56.6	11.9	5.1	11.2	69.3
42	5×9	6.2	6.6	4.1	1.5	78.0	2.5	41.7	38.1	56.8	50.7	15.8	4.8	11.2	69.7
43	5×10	5.9	6.3	4.8	1.4	79.8	2.6	35.1	45.9	53.5	55.4	14.0	5.1	10.7	69.7
44	5×11	7.4	7.2	5.8	2.0	83.1	2.9	25.2	59.8	77.8	18.5	24.9	5.2	10.3	69.6
45	5×12	8.7	8.3	4.7	1.4	84.3	2.6	27.7	55.0	70.2	28.0	20.9	4.9	10.0	70.0
46	6×7	7.2	7.0	5.9	1.9	86.4	3.0	28.7	54.6	68.4	34.6	19.0	5.1	10.8	69.6
47	6×8	7.8	6.7	4.9	1.3	83.0	3.0	32.0	51.2	67.8	38.9	17.0	5.4	11.1	68.7
48	6×9	6.9	9.1	6.3	2.3	81.2	2.8	39.3	43.0	56.7	50.3	14.6	5.0	10.9	69.6
49	6×10	5.7	5.0	8.8	3.5	76.9	3.4	36.7	46.0	55.6	47.2	15.8	5.2	10.8	69.4
50	6×11	8.5	7.6	6.2	2.0	83.7	3.0	25.4	58.3	70.0	32.4	21.6	5.4	10.8	69.2

Appendix 4-1: Continued from page 120

Entry	Cross	GYP	GYI	CTL	EXhLS	LLI	LDS	LuDkn	Lwtls	MLI	MuDkn	Mwtls	Oil	Protein	Starch
51	6×12	6.3	6.5	5.5	1.8	79.7	3.2	32.8	51.7	62.7	46.8	15.9	4.9	10.3	70.2
52	7×8	3.7	4.0	4.6	1.4	78.5	3.0	35.4	43.3	60.1	54.9	15.0	4.7	11.4	69.8
53	7×9	3.0	3.3	7.6	2.0	73.3	3.1	38.1	42.9	48.2	56.6	13.9	4.8	11.6	69.4
54	7×10	8.3	7.0	6.5	2.5	80.4	2.8	25.2	58.0	72.1	23.6	25.5	5.0	10.9	69.3
55	7×11	6.1	4.0	6.7	1.8	83.0	3.2	27.7	53.7	61.9	39.7	20.0	4.6	11.2	69.9
56	7×12	8.4	8.4	7.1	2.4	82.9	3.3	30.4	53.3	68.7	42.5	17.6	5.0	10.3	69.8
57	8×9	4.6	4.4	5.5	1.6	77.2	2.6	45.6	34.1	40.5	75.1	8.8	5.0	11.9	69.3
58	8×10	3.7	3.6	5.9	1.9	71.6	3.0	45.4	32.3	52.7	62.9	13.3	5.3	11.5	69.1
59	8×11	7.2	6.9	6.9	2.1	80.2	3.1	32.0	48.7	64.3	40.9	17.6	4.8	11.0	69.9
60	8×12	7.4	7.1	6.6	2.1	81.4	3.2	37.9	42.7	54.0	56.8	11.9	5.0	10.7	69.7
61	9×10	5.4	4.6	5.2	1.9	77.5	3.0	44.1	37.2	50.2	64.4	10.0	5.1	11.5	69.2
62	9×11	5.6	5.4	5.6	1.9	76.1	3.1	38.4	45.6	60.0	57.1	13.1	5.5	11.2	68.8
63	9×12	7.5	7.3	6.1	1.9	79.2	2.9	35.2	49.2	61.7	47.2	15.0	4.8	10.8	70.3
64	10×11	7.1	6.4	7.0	2.5	83.4	3.1	32.0	49.1	61.5	51.3	16.6	5.5	11.1	68.7
65	10×12	5.1	5.4	8.5	3.4	81.2	3.9	38.2	46.0	61.5	55.9	15.7	5.0	10.7	70.1
66	11×12	10.0	8.8	6.6	2.9	82.2	3.3	23.0	62.9	64.3	41.0	18.7	5.6	10.4	69.0
	Mean	6.8	6.6	5.3	1.7	81.2	2.7	33.3	49.5	62.7	42.7	17.4	5.0	10.8	69.6
	Max	10.5	10.1	8.8	3.5	87.7	3.9	46.2	65.4	77.8	75.1	26.7	5.6	12.0	70.5
	Min	3.0	3.3	3.1	1.0	71.6	2.1	21.7	32.3	40.5	15.6	8.8	4.5	9.9	68.2
	StdErr	0.7	0.8	1.3	0.4	2.4	0.2	2.3	2.8	3.8	4.9	2.1	0.1	0.2	0.3

Appendix 5.1: Female parents of used for estimating combined resistance in maize hybrids to *Chilo partellus* and *Sitophilus zeamais*

Entr	Stock ID	Name	Pedigree	Resistance	Parent
1	SM-176-34	CKSBL1003	P590 C7 Blancos F156-1-2-1-B-B-B-B-B-B	Cp(Res), Sz(Susc)	Female 1
2	SM-480-	CKSBL1001	CML311/MBR C3 Bc F43-2-1-1-B-B-B-B-B-B-B	Cp(Res), Sz(Susc)	Female 2
3	SM-390-44	CKSBL1002	MBR C6 Bc F299-2-B-#-1-1-B-B-B-B-B-B-B	Cp(Res), Sz(Susc)	Female 3
4	SM-480-	CKSBL1002	MBR C5 Bc F60-2-1-2-B-B-BxCML 384-B-1-2-B-B-B-B-B-B-B	Cp(Res), Sz(Susc)	Female 4
5	SM-480-	CKSBL1000	MBR C5 Bc F4-1-2-2-B-1-2-B-B-B-B	Cp(Res), Sz(Susc)	Female 5
11	SM-204-36	CKSBL1004	P590 C7 Blancos F206-1-1-2-B-B-B-B-B-B-B	*Cp*(Res), *Sz*	Female 6
12	SM-480-	CKSBL1000	CML 380xMBR/MDR C3 Bc F21-1-1-2-B-B-B-B-3-1-B-B-B-B-B-B-B	*Cp*(Res), *Sz*	Female 7
13	SM-480-	CKSBL1004	Pob.SEW-HG"B"c0F39-1-1-1-1xMBR C5 Bc F22-2-1-4-B-B-B-B-2-2-B-B-B-B-B-B-B	*Cp*(Res), *Sz*	Female 8
14	SM-480-	CKSBL1004	P591c4 F14-1-2-1-B-B-B-B-B-B-B	*Cp*(Res), *Sz*	Female 9
15	SM-480-	CKSBL1003	MBR-Et(W)/P590C3 F35-1-3-B-1x1760B G1 Bco x Comp.-B-2-B-1-1-B-B-B-B-B-B-	*Cp*(Res), *Sz*	Female
21	SM-480-	CKSBL1004	P590 C7 Blancos F27-1-1-2-B-B-B-B-B-B-B	*Cp*(Susc), *Sz*	Female
22	SM-480-	CKSBL1002	MBR E.T(W)C3 S5/SINTxMBR F41-1-1-1-B-B-B-B-B-B-B	*Cp*(Susc), *Sz*	Female
23	SM-480-	CKSBL1000	MBR/MDR C3 Bc F1-1-1-1-B-3-2-B-B-B-B	*Cp*(Susc), *Sz*	Female
24	SM-480-	CKSBL1000	CML 384xMBR/MDR C3 Bc F58-2-1-3-B-B-B-B-3-1-B-B-B-B-B-B-B	*Cp*(Susc), *Sz*	Female
25	SM-480-21	CKSBL1002	MBR C5 Bc F114-1-1-3-B-8-2-B-B-B-B-#-#-B-B-B	*Cp*(Susc), *Sz*	Female
31	SM-480-	CKSBL1000	CML 384xMBR/MDR C3 Bc F58-2-1-3-B-B-B-B-3-1-B-B-B-B-B-B-B	*Cp*(Susc), *Sz*	Female
32	SM-480-	CKSBL1002	MBR C5 Bc F108-2-3-1-B-5-2-B-B-B	*Cp*(Susc), *Sz*	Female
33	SM-480-	CKSBL1000	MBR/MDR C3 Bc F1-1-1-1-B-3-2-B-B-B-B	*Cp*(Susc), *Sz*	Female
34	SM-480-	CKSBL1002	MBR E.T(W)C3 S5/SINTxMBR F15-2-1-2-B-B-B-B-B-B-B	*Cp*(Susc), *Sz*	Female
35	SM-480-29	CKSBL1002	MBR C5 Bc F14-2-2-3-B-B-BxG16SeqC1F47-2-1-2-1-BBBBB-B-1-2-B-B-B-B-B-B	*Cp*(Susc),*Sz Sus*es)	Female

†Cp, *Chilo partellus*; Sz, *Sitophilus zeamais*; Res, resistance; Susc, Susceptible

Appendix 5.2: Male parents used in North Carolina design II, their pedigrees and resistance information

Entr	Stock ID	Name	Pedigree	Resistance	Parent
6	SM-482-	CKSPL10013	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-141-1-B-1-B-	Cp (Susc), Sz	Male
7	SM-482-	CKSPL10090	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-277-1-B-3-B-	Cp (Susc), Sz	Male
8	SM-482-	CKSPL10089	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-277-1-B-2-B-	Cp (Susc), Sz	Male
9	SM-482-	CKSPL10111	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-306-1-B-1-B-	Cp (Susc), Sz	Male
10	SM-482-	CKSPL10343	M37W/ZM607#bF37sr-2-3sr-6-2-X]-8-2-X-1-BB-B-xP84c1 F27-4-3-3-B-1-B] F29-1-2-2 x	Cp (Susc), Sz	Male
16	SM-390-64	CKSPL10081	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-230-1-B-5-B-	*Cp* (Susc), *Sz*	Male
17	SM-482-	CKSPL10028	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-160-1-B-2-B-	*Cp* (Susc), *Sz*	Male
18	SM-390-84	CKSPL10229	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-463-1-B-5-B-	*Cp* (Susc), *Sz*	Male
19	SM-390-68	CKSPL10088	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-277-1-B-1-B-	*Cp* (Susc), *Sz*	Male
20	SM-482-	CKSPL10224	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-463-1-B-4-B-	*Cp* (Susc), *Sz*	Male
26	SM-482-	CKSPL10186	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-420-1-B-7-B-	*Cp* (Res), *Sz*	Male
27	SM-442-24	P100C6-200-1-1-B***-#-#	P100C6-200-1-1-B***-#-#	*Cp* (Res), *Sz*	Male
28	SM-482-	CKSPL10036	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-160-1-B-5-B-	*Cp* (Res), *Sz*	Male
29	SM-442-74	LPSC7-F180-3-1-1-1-BB-	LPSC7-F180-3-1-1-1-BB-#-#	*Cp* (Res), *Sz*	Male
30	SM-157-8	CML440	CML440	*Cp* (Res), *Sz*	Male
36	SM-215-16	DTPWC9-F16-1-1-1-1-	DTPWC9-F16-1-1-1-1-BBB-#	*Cp* (Res), *Sz*	Male
37	SM-482-	CKSPL10230	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-466-1-B-2-B-	*Cp* (Res), *Sz*	Male
38	SM-482-	CKSPL10003	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-445-1-B-1-B-	*Cp* (Res), *Sz*	Male
39	SM-482-	CKSPL10113	(CUBA/GUAD C1 F27-4-3-3-B-1-Bx[KILIMA ST94A]-30/MSV-03-2-10-B-2-B-B)-306-1-B-3-B-	*Cp* (Res), *Sz*	Male
40	SM-442-4	CML159	CML159	*Cp* (Res), *Sz*	Male

†Cp, *Chilo partellus*; Sz, *Sitophilus zeamais*; Res, resistance; Susc, Susceptible.

Appendix 5.3: General combining ability effects for inbred lines used in North Carolina II analysis for combined stem borer and storage insect pest resistance

GCA												
		Female					Male					
Set	Entry	Gyp	Gyi	LDS	Mwtls	M-uDgrn	Entry	Gyp	Gyi	LDS	Mwtls	M-uDgrn
1	1	-0.83	-0.61	0.17	0.71	-2.56	21	0.17	0.24	0.15	2.24	-1.59
1	2	-0.95	-0.86	0.12	-1.07	-1.26	22	0.30	0.39**	-0.12	-2.76**	4.14
1	3	0.95	0.70**	-0.07	-0.46	1.66	23	-0.17	-0.18	-0.06	-0.62	-0.79
1	4	0.79	0.88**	-0.024	0.6	0.077	24	-0.58	-0.59	0.03	0.31	-0.92
1	5	-0.12	-0.26	-0.15	0.052	1.65	25	0.34	0.23	-0.01	0.6	-0.48
2	6	-0.25	-0.094	-0.36	2.76	-0.59	26	0.06	0.09	0.08	2.8	0.25
2	7	0.66	0.57**	0.18	-0.33	-4.15	27	-0.01	0.27*	-0.1	-1.18	1.23
2	8	0.36	0.44**	0.04	1.34	1.01	28	0.06	-0.03	0.14	-1.83	0.36
2	9	-0.40	-0.63	0.16	-1.47	2.83	29	0.04	-0.056	-0.21	0.28	-1.32
2	10	-0.37	-0.29	-0.016	-2.3**	0.89	30	-0.14	-0.27	0.08	-0.11	-0.52
3	11	-0.42	-0.29	0.28	0.27	0.4	31	-0.70	-0.46	0.09	-4.24**	3.28
3	12	0.84	0.7**	0.03	0.53	-2.82	32	0.85	0.45**	0.24	3.01	-3.08
3	13	-0.34	-0.28	0.026	-2.39**	2.34	33	-0.30	-0.41	-0.19*	-3.71**	0.048
3	14	0.34	0.24	-0.17	1.07	1	34	1.01	1.05**	-0.09	0.68	-2.79
3	15	-0.42	-0.38	-0.17	0.52	-0.92	35	-0.86	-0.63	-0.05	4.26	2.55

Appendix 5.3: Continued from page 124

GCA												
		Female					Male					
Set	Entry	Gyp	Gyi	LDS	Mwtls	M-uDgrn	Entry	Gyp	Gyi	LDS	Mwtls	M-uDgrn
4	16	0.07	-0.11	-0.3	0.23	4.81**	36	-2.51	-2.29	-0.04	2.2	7.05**
4	17	0.15	0.3*	-0.21	1.74	1.05	37	0.27	0.3*	-0.2	2.27	-2.43
4	18	-0.25	-0.15	0.14	-1.01	2.38	38	0.39	0.21*	-0.15	-3.08**	-0.74
4	19	0.56	0.27*	0.03	-2.21**	-1.27	39	-0.14	0.16	0.09	-1.96**	-5.84
4	20	-0.53	-0.31	0.36	1.25	-6.98	40	1.99	1.62**	0.29	0.58	1.95
5	1	-1.46	-1.23	0.27	2.35	-1.15	31	-0.72	-0.73	-0.06	-2.25	1.07
5	2	0.68	0.43*	0.14	0.63	-2.9	32	1.40	1.08	0.14	1.24	-1.75
5	3	0.69	0.57**	-0.28	-3.77**	1.94	33	-0.36	-0.17	-0.25	-4.81	-1.1
5	4	0.12	0.28*	-0.12	1.5	1.26	34	0.54	0.57**	0.077	0.51	0.93
5	5	-0.61	-0.54	0.1	0.23	0.4	35	-0.66	-0.58	0.077	4.6	0.28
6	6	0.15	-0.28	-0.013	1.79	1.44	36	-2.38	-2.4	0.13	1.41	7.6**
6	7	0.28	0.54	0.13	-1	-1.84	37	0.07	0.47**	-0.37*	2.65	-3.02
6	8	0.26	0.44	0.08	1.17	-1.5	38	0.39	0.17	-0.23	-2.98	-0.89
6	9	-0.40	-0.58	-0.055	-1.85	1.15	39	0.23	0.06	0.32	-1.94	-2.94
6	10	-0.29	-0.11	-0.13	-0.11	0.75	40	1.69	1.74***	0.15	0.87	-0.76

Appendix 5-3: Continued from page 125

GCA												
	Female						Male					
Set	Entry	Gyp	Gyi	LDS	Mwtls	M-uDgrn	Entry	Gyp	Gyi	LDS	Mwtls	M-uDgrn
7	11	-0.20	-0.15	0.24	-3.18	0.71	21	0.11	0.26**	0.105	0.94	-2.59
7	12	1.37	0.85**	0.16	-1.238	-2.41	22	-0.33	-0.47	-0.104	-0.3	0.349
7	13	-0.49	-0.29	0.053	-0.37	3.5**	23	-0.10	-0.21	-0.08	-1.28	0.88
7	14	0.03	-0.097	-0.34**	1.51	0.4	24	0.49	0.22**	0.013	-1.33	-1.15
7	15	-0.71	-0.32	-0.12	3.27	-2.2	25	-0.16	0.20**	0.07	1.97	2.52**
8	16	-0.25	-0.22	-0.095	1.O	0.92	26	-0.10	0.22**	-0.12	2.32	-3.88
8	17	-0.03	-0.19	0.04	-0.01	1.85	27	0.06	-0.17	-0.4*	-0.71	1.26
8	18	0.14	0.21**	0.028	-0.5	-1.39	28	-0.13	-0.08	0.36	-2.22*	-1.22
8							29	0.26	0.42**	-0.005	0.25	1.56
8							30	-0.09	-0.4	0.17	0.35	2.29

†Data: GYP/l, grain yield protected/infested; LDS, leaf damage scores; Mwtls, weight loss due to maize weevil: Significance: *** means data is significant at p ≤ 0.001, *** data is significant at p ≤ 0.01, * data is significant at p ≤ 0.05